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1 **Incremental predictive value of echocardiographic indices of right ventricular**
2 **function in the assessment of long-term prognosis in dogs with myxomatous**
3 **mitral valve disease**

4

5 **Short title:** Prognostic value of RV function in MMVD dogs

6

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19

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23

24

25

26 **Abstract**

27 **Introduction:** Few studies have evaluated the utility of echocardiographic indices of
28 right ventricular (RV) function in predicting prognosis in dogs with myxomatous mitral
29 valve disease (MMVD).

30 **Animals:** Sixty-seven client-owned dogs were diagnosed with MMVD.

31 **Materials and Methods:** Clinical cohort study. Dogs diagnosed with ACVIM stage B2,
32 C, or D between April 2014 and March 2017 were enrolled. Long-term outcomes were
33 assessed by telephone or from the medical record. The primary endpoint was defined
34 as cardiac-related death. Echocardiographic indices of RV function, including the RV
35 Tei index, free wall and septal RV longitudinal strain (RVLS), were obtained.
36 Univariable and multivariable Cox proportional hazard analyses were used to identify
37 variables predictive of cardiac-related death.

38 **Results:** Twenty-four dogs died during the follow-up period. The median follow-up
39 time was 482 days, and the median survival time for dogs with cardiac-related death
40 was 230 days. For cardiac-related death, peak early diastolic mitral inflow velocity,
41 ACVIM stage C or D, tricuspid regurgitation velocity, RV Tei index, and RV
42 end-diastolic area were predictors in univariable Cox proportional hazard analysis. In

43 multivariable Cox proportional analysis adjusted for the left atrial to aorta ratio, peak
44 early diastolic mitral inflow velocity, and ACVIM stage, an increase in the Tei index by
45 0.1 increased the hazard ratio of cardiac-related death by 33% (95% CI, 16 to 70%; p
46 = 0.002).

47 **Conclusions:** In dogs with MMVD, RV dysfunction assessed by the Tei index is an
48 independent predictor of cardiac-related death.

49

50 **Keywords:** Canine, Mitral regurgitation, Tei index, Strain, Speckle tracking

51 echocardiography

52

53 **ABBREVIATIONS**

DPD	dual pulsed-wave Doppler
E _{max}	peak early diastolic mitral inflow velocity
FAC	fractional area change
LA/Ao	left atrial to aorta root ratio
LVIDDN	left ventricular end-diastolic diameter normalized for body weight

MMVD	myxomatous mitral valve disease
MR	mitral regurgitation
PA	pulmonary artery
PH	pulmonary hypertension
RV	right ventricle
RVEDA	right ventricular end-diastolic area
RVESA	right ventricular end-systolic area
RVLS	right ventricular longitudinal strain
RV-SD4	standard deviation of the systolic shortening time of four mid-basal segments of right ventricular
STE	speckle tracking echocardiography
TAPSE	tricuspid annulus plane systolic excursion
TDI	tissue Doppler
TR	tricuspid regurgitation

55 **Introduction**

56 Myxomatous mitral valve disease (MMVD) is the most common heart disease in
57 dogs, and the natural history of these dogs is heterogeneous, with many dogs never
58 developing heart failure or cardiac-related death [1,2]. Therefore, the ability to identify
59 dogs at higher risk could be of clinical value. To date, several echocardiographic
60 indices (i.e., left atrial to aorta root ratio (LA/Ao) [1–3], peak early diastolic mitral
61 inflow velocity (E_{max}) [1,4], left ventricular (LV) end-diastolic diameter normalized for
62 body weight (LVIDDN) [3,5,6], left atrial function [7]), age [2,5,6], cardiac biomarkers
63 (i.e., N-terminal pro B-type natriuretic peptide [5,6] and cardiac troponin I [6]), and
64 classification of MMVD [2,3,8], have been demonstrated to be good prognostic
65 indicators for dogs with MMVD. In addition, pulmonary hypertension (PH) assessed
66 by a peak tricuspid regurgitation (TR) pressure gradient >55 mm Hg was reported to
67 be a predictor of worse outcome in dogs with MMVD [3].

68 Right ventricular (RV) dysfunction assessed by various modalities is an
69 independent predictor of poor outcome in human patients with left heart disease,
70 such as mitral regurgitation (MR) and dilated cardiomyopathy [9–13]. Several
71 echocardiographic indices of RV function, including tricuspid annulus plane systolic
72 excursion (TAPSE), fractional area change (FAC), and RV Tei index, have been

73 demonstrated to be independent prognostic factors in human patients with MR
74 [11–13]. Furthermore, recently, a novel quantitative method for the assessment of
75 myocardial deformation, speckle tracking echocardiography (STE), has been applied
76 for assessing RV function, and free wall RV longitudinal strain (RVLS) derived from
77 STE, which is an index of RV systolic function, has been reported to be related to
78 poor outcomes in human patients with cardiomyopathy and advanced systolic heart
79 failure [14–17]. However, the prognostic value of RVLS is unknown in human patients
80 with MR.

81 On the other hand, little is available on the prognostic value of echocardiographic
82 indices of RV function in dogs with MMVD [8]. We previously showed that the RV Tei
83 index is an independent predictor of cardiac-related death within one year in dogs
84 with MMVD [8]. However, in this previous study, the utility of other echocardiographic
85 indices of RV function, including RVLS, for assessing prognosis was not assessed.
86 Therefore, the objective of this study was to determine the prevalence of RV
87 dysfunction assessed by echocardiography and to estimate the survival and
88 prognostic value of echocardiographic indices of RV function, including RVLS, on
89 cardiac-related death in dogs with stage B2, C, and D MMVD.

90

91 **Animals, Materials and Methods**

92 **Animals**

93 Sixty-seven client-owned dogs were enrolled in this study. The population included
94 six dogs from our previous study [8]. Dogs were selected based on an
95 echocardiographic diagnosis of MMVD at the Hokkaido University Veterinary
96 Teaching Hospital between April 2014 and March 2017. Informed consent was
97 obtained from all owners involved in this study.

98

99 **Inclusion and exclusion criteria**

100 Dogs with ACVIM stage B2, C, or D MMVD who underwent physical examination,
101 thoracic radiography, and echocardiography were included in this study. Inclusion
102 criteria were the presence of a left apical systolic murmur (murmur intensity $\geq 3/6$) and
103 the presence of MR on Doppler echocardiography in conjunction with mitral valvular
104 lesions and LA/Ao ≥ 1.6 ; LVIDDN ≥ 1.7 ; vertebral heart score >10.5 [18]. Dogs were
105 classified as ACVIM stage B2, C, or D based on a previous report [18]. Based on
106 ACVIM consensus statement guidelines, the probability of PH was determined using
107 echocardiography [19]. Dogs were excluded if they had congenital heart disease,
108 dilated cardiomyopathy, or infective endocarditis from this study. Dogs with known

109 clinically important systemic or other organ-related diseases that were expected to
110 limit the dog's life expectancy, such as tumours, pneumonia, and severe chronic
111 kidney disease, were also excluded.

112

113 Echocardiographic measurements

114 Echocardiographic examinations were performed by one echocardiographer (KN)
115 using two ultrasound machines^{c, d} equipped with a 3–7 MHz sector probe^e and a 3–6
116 MHz sector probe^f. HI VISION Preirus^d was used to measure the RV Tei index by dual
117 pulsed-wave Doppler (DPD). An ECG trace (lead II) was recorded simultaneously
118 with echocardiographic imaging and automatically measured heart rate.

119 Left ventricular end-diastolic diameter was measured from M-mode
120 echocardiography with a right parasternal short-axis view. The normalized
121 dimensions were calculated according to the following equations [20]: Left ventricular
122 end-diastolic diameter normalized for body weight (LVIDDN) = Left ventricular
123 end-diastolic diameter/(body weight)^{0.294}. Left atrium and aorta root diameters and
124 pulmonary artery (PA) valve annulus diameter were obtained with a right parasternal
125 short-axis view, and the LA/Ao and PA/Ao ratios were calculated. Measurements of
126 transmitral E_{max} and peak late diastolic mitral inflow velocity were obtained with

127 pulsed-wave Doppler with a left apical four-chamber view, and then the E/A ratio was
128 calculated. Pulmonary artery flow was measured with a left parasternal short-axis
129 view. The acceleration time of PA flow was measured from the onset of the PA flow
130 signal to peak velocity, the ejection time was measured from the onset to the end of
131 the PA flow signal, and then the acceleration time/ ejection time ratio was calculated.
132 The peak TR velocity was measured from the echocardiographic view that provided
133 the highest velocity.

134 Peak systolic tricuspid annular velocity was determined by tissue Doppler (TDI) at
135 the lateral tricuspid annulus with an apical four-chamber view. Tricuspid annulus
136 plane systolic excursion was obtained by placing an M-mode cursor over the tricuspid
137 annulus with an apical four-chamber view and measuring its amplitude of motion
138 during systole, and then TAPSE was normalized by body weight as follows:
139 $\text{normalized TAPSE} = \text{TAPSE}/(\text{body weight})^{0.33}$ [21]. RV end-diastolic area (RVEDA)
140 and end-systolic area (RVESA) were obtained by tracing the RV endocardium in
141 systole and diastole from the annulus to the apex in the modified apical four-chamber
142 view, which included the RV apex, and then FAC was calculated as $(\text{RVEDA} -$
143 $\text{RVESA})/\text{RVEDA} \times 100\%$. Right ventricular end-diastolic area and RVESA were
144 normalized according to the following equation: normalized RVEDA (nRVEDA) and

145 RVEA (nRVEA) = RVEDA/body surface area and RVEA/body surface area. To
146 obtain a modified left apical four chamber view, the transducer was rotated until the
147 maximal plane of the RV basal diameter was obtained. The RV should not be
148 foreshortened, and visualization of the left ventricular outflow tract should be avoided.
149 The RV Tei index was calculated using DPD and TDI as the sum of the isovolumic
150 contraction time and isovolumic relaxation time divided by the ejection time. Tei index
151 was calculated after image acquisition. Tricuspid inflow and PA flow were measured
152 simultaneously using DPD with a left parasternal short-axis view, and the sum of the
153 isovolumic contraction time and isovolumic relaxation time was derived by subtracting
154 the ejection time from the time of cessation of the tricuspid valve peak late diastolic
155 mitral inflow velocity to the onset of the tricuspid valve E-wave in one image (Figure
156 1A) [22]. The right ventricular Tei index derived from TDI was calculated as the sum of
157 the isovolumic time derived by subtracting the peak systolic tricuspid annular velocity
158 duration from the time from the end of the late diastolic tricuspid annulus velocity to
159 the onset of the early diastolic tricuspid annulus velocity on the basis of TDI
160 recordings (Figure 1B) [23].

161 Speckle tracking echocardiography was performed using conventional greyscale
162 echocardiography with a modified apical four-chamber view. The frame rate was

163 optimized to >200 frames/s by narrowing the imaging sector and reducing the depth
164 to focus on the RV. Three consecutive cardiac cycles were stored on a hard drive,
165 and the images were analysed with offline softwareⁱ. The value for STE indices was
166 determined from the average of three cardiac cycles. Care was taken to obtain the
167 best visualization of the RV endocardial border from the base to apex. The
168 endocardial border was manually traced in an end-diastolic frame, and a region of
169 interest was generated followed by adjustments to incorporate the entire RV wall
170 myocardial thickness. The RV wall was divided into inner and outer layers, and the
171 RV-free and septal walls were divided into three segments (basal, middle, and apical).
172 Right ventricular longitudinal strain, which is a systolic index of RV, is defined as the
173 percentage shortening of a region of interest relative to its original length and is
174 expressed as a negative percentage [24]. Right ventricular longitudinal strain was
175 obtained for each segment at the highest peak of the software-generated strain
176 curves. Global RVLS was calculated by averaging values observed in four mid-basal
177 segments of the RV, and free wall and septal RVLS were calculated by averaging
178 values of two segments each along the entire RV (Figure 1C). Systolic shortening
179 time was calculated from the QRS onset to peak longitudinal strain of each of the 4
180 segments of the RV. To quantify RV intraventricular dyssynchrony, the standard

181 deviation of the systolic shortening time of four mid-basal segments of the RV
182 (RV-SD4) was calculated using online softwareⁱ (Figure 1D), and RV-SD4 was
183 corrected for the RR interval according to Bazett's formula: Corrected RV-SD4 =
184 $RV-SD4/\sqrt{RR}$ interval [25].

185

186 Follow-up

187 An investigator (TM) conducted telephone interviews with dog owners or referral
188 veterinarians or reviewed medical records to determine the outcomes and the cause
189 of death. The primary endpoint of this study was cardiac-related death.

190 Cardiac-related death was defined as spontaneous death due to progression of
191 clinical signs of heart failure or sudden death when no other cause was evident.

192 Euthanasia due to cardiac reasons was also defined as cardiac-related death, but no
193 dog was euthanized in this study. Death from any other cause was defined as

194 noncardiac death. Survival time was calculated as the time from the day of diagnosis
195 of MMVD to either the day of death or closing time of the study. Dogs were censored

196 in the survival analysis if they were alive at the end of the study period (November
197 2017) or if they died due to noncardiac-related causes.

198

199 Statistical analysis

200 Statistical analysis program^h was used. Power calculations for sample size
201 determination were made based on our previous report [8]. When a statistical power
202 is 80% and level of significance is 0.05, the necessary sample size is 62. The normal
203 distribution of the data was confirmed by the Shapiro Wilk test. All data was normality
204 distributed. Data are presented as the mean \pm standard deviation for normally
205 distributed continuous variables. Two-group comparisons for characteristics and
206 echocardiographic indices were performed with unpaired *t*-tests for continuous
207 variables with Bonferroni correction for multiple comparisons and with Fisher's exact
208 test for categorical variables. The relationships between TR velocity, LVIDDN,
209 nRVEDA and echocardiographic indices of RV function were assessed by Pearson's
210 correlation coefficient test with Bonferroni correction for multiple comparisons.

211 Univariable Cox proportional hazard analysis was used in survival analysis to
212 evaluate the hazard ratio of cardiac-related death. Variables with a *p*-value <0.2 on
213 univariable analysis were entered into a multivariable Cox proportional hazard
214 analysis using a forward selection stepwise method and Akaike information criteria
215 adjusted for ACVIM stage, LA/Ao, and Emax. The results of multivariable analysis
216 were considered significant when *p* <0.05.

217 The effects of the clinical and echocardiographic variables, such as ACVIM stage,
218 LA/Ao, Emax, and RV Tei index by DPD, on survival were determined with the
219 Kaplan-Meier method, and comparisons of cumulative event rates were performed
220 with the log-rank test. Cut-off values were determined based on the median value
221 (LA/Ao and Emax) or the cited veterinary literature (RV Tei index by DPD > 0.61 [8]).
222 Six dogs included our previous study for establishing cut-off value [8] were not
223 included in survival analysis with the Kaplan-Meier method using cut-off value of RV
224 Tei index.

225 A *p*-value of less than 0.05 was considered significant.

226

227 **Results**

228 **Characteristics**

229 Sixty-seven dogs from 14 breeds, with Chihuahua being the most frequently
230 represented (n = 15, 22.4%), followed by Shih Tzu (n = 10, 14.9%), Miniature
231 Schnauzer (n = 7, 10.4%), mixed breeds (n =6, 9.0%), and Cavalier King Charles
232 Spaniel (n = 6, 9.0%), were included in this study. Thirty-seven dogs (55.2%) were in
233 ACVIM stage B2, 28 dogs (41.8%) were in ACVIM stage C, and 2 dogs (3.0%) were
234 in ACVIM stage D of MMVD. Characteristics and echocardiographic variables in dogs

235 with MMVD at different stages are summarized in Table 1. Left atrial to aorta root ratio,
236 Emax, peak TR velocity, RV Tei index by DPD, RV Tei index by TDI, nRVEDA, and
237 nRVESA were significantly increased in dogs with ACVIM stage C or D MMVD. The
238 presence of ascites was significantly more common in dogs with ACVIM stage C or D
239 MMVD. At baseline, 35 dogs (52.2%, 15 stage B2; 18 stage C; 2 stage D) received
240 pimobendan, and 24 dogs (35.8%, 7 stage B2; 15 stage C; 2 stage D) received
241 diuretics.

242 Correlations between peak TR velocity, LVIDDN, and nRVEDA and
243 echocardiographic indices of RV function are summarized in Table 2. In contrast to
244 peak systolic tricuspid annular velocity and TAPSE, the FAC ($r = -0.44$, $P < 0.001$), RV
245 Tei index by DPD ($r = 0.57$, $p < 0.001$), and RV Tei index by TDI ($r = 0.55$, $P < 0.001$)
246 were significantly correlated with peak TR velocity. Tricuspid annulus plane systolic
247 excursion ($r = 0.48$, $p < 0.001$), and normalized TAPSE ($r = 0.46$, $p < 0.001$) were
248 significantly correlated with LVIDDN. Right ventricular Tei index by TDI ($r = 0.63$, p
249 < 0.001), RV Tei index by DPD ($r = 0.61$, $p < 0.001$), and septal RVLS ($r = 0.42$, p
250 < 0.001) were all significantly correlated with nRVEDA.

251

252 Survival analysis

253 The primary endpoint (cardiac-related death) occurred in 24 dogs (35.8%), with 19
254 deaths (28.4%) due to progression of clinical signs of heart failure and five sudden
255 deaths (7.5%). Eleven dogs (16.4%) were considered to have died of other causes,
256 and 32 dogs (47.8%) were still alive at the end of the study. The median survival time
257 for all-cause death was 616 days (95% CI, 364 to 730 days), and the median survival
258 time for cardiac-related death was 230 days (95% CI, 131 to 364 days). The median
259 follow-up time for dogs still alive was 482 days (95% CI, 444 to 652 days).

260 Characteristics and echocardiographic variables in dogs with cardiac-related death
261 and without cardiac-related death are summarized in Table 3. Dogs that experienced
262 cardiac-related death exhibited significantly increased Emax, peak TR velocity, RV
263 Tei index by DPD, RV Tei index by TDI, nRVEDA, and nRVESA compared to dogs
264 without cardiac-related death. In contrast, free wall RVLS was not different between
265 patients with and without cardiac-related death. Stage C or D MMVD was more
266 common in dogs with cardiac-related death.

267 Univariable Cox proportional hazard analysis of the predictive value of continuous
268 and categorical variables for cardiac-related death is shown in Table 4. In the
269 univariable analysis, descriptive statistics, including age, presence of ascites, and
270 ACVIM stage C or D, and echocardiographic indices, including LA/Ao, Emax, LVIDDN,

271 peak TR velocity, TAPSE, FAC, RV Tei index by DPD, RV Tei index by TDI, septal
272 RVLS, nRVEDA, nRVESA, and presence of septal flattening, were significantly
273 associated with cardiac-related death.

274 Exploratory Multivariable analysis demonstrated that the RV Tei index by DPD
275 (hazard ratio per 0.1 unit increase, 3.30; 95% CI, 1.57 to 6.95; $p = 0.002$) and TAPSE
276 (hazard ratio per 1 unit increase, 1.37; 95% CI, 1.05 to 1.781; $p = 0.019$) remained
277 significant predictors of cardiac-related death after adjusting for ACVIM stage, LA/Ao,
278 and Emax (Table 4).

279

280 Kaplan-Meier analysis

281 Figure 2 shows event-free survival in dogs with MMVD. The long-term outcomes
282 were worse for dogs with ACVIM stage C or D MMVD than for those with ACVIM
283 stage B2 MMVD (log-rank $p < 0.001$), as expected. Similarly, long-term outcomes for
284 dogs with LA/Ao ≥ 2.15 or Emax ≥ 1.1 m/s were worse than those with LA/Ao < 2.15
285 or Emax < 1.1 m/s (log-rank $p = 0.001$ and $p < 0.001$). In addition, dogs with a Tei
286 index of DPD ≥ 0.61 exhibited worse outcomes than those with a Tei index < 0.61
287 (log-rank $p < 0.001$).

288

289 **Discussion**

290 The present study demonstrates that the RV Tei index by DPD and TDI are
291 impaired in dogs with ACVIM stage C or D compared to those with ACVIM stage B2
292 MMVD, and RV dysfunction as evaluated by the RV Tei index derived from DPD was
293 associated with worse long-term outcomes for dogs with MMVD. In addition, the
294 combined assessment of the RV Tei index and previously reported prognostic indices
295 by echocardiography, such as LA/Ao and Emax, may further enhance the ability to
296 predict worse long-term outcomes in MMVD dogs.

297 Mitral regurgitation due to MMVD causes volume overload in the left heart, and the
298 onset of left heart failure depends on the severity of volume overload. Indeed, several
299 echocardiographic variables assessing volume overload, such as LA/Ao, Emax, and
300 LVIDDN, have been reported as good prognostic indices in dogs with MMVD
301 [1–3,5,6]. Furthermore, PH was common in dogs with ACVIM stage C, and PH
302 diagnosis assessed by a peak TR pressure gradient >55 mm Hg by
303 echocardiography has been shown to be an independent predictor of worse
304 outcomes in dogs with MMVD [3]. Similar to a previous study, our results showed that
305 peak TR velocity was associated with cardiac-related death on univariate Cox
306 proportional hazard analysis. On the other hand, multivariate Cox analysis revealed

307 that peak TR velocity was not an independent predictor of cardiac-related death. The
308 reason for this finding may be related to the limitation of peak TR velocity
309 measurement. The Doppler measurement of peak TR velocity has been reported to
310 be an inaccurate method for assessing the severity of PH due to several factors, such
311 as the effect of RV contractility, technical difficulties in obtaining an ideal alignment,
312 and the absence of TR [26].

313 In human patients with MR, RV systolic dysfunction is partially related to pulmonary
314 arterial pressure elevation and is an independent predictor of poor outcome [9].

315 Therefore, assessment of RV function may be needed to determine the prognosis in
316 dogs with MMVD. However, little is available on the effect of RV function on the
317 prognosis in dogs with MMVD.

318 The RV Tei index by DPD was impaired in dogs with cardiac-related death and was
319 associated with long-term outcome in dogs with MMVD in multivariate analysis. We
320 previously reported that the RV Tei index by DPD was an independent predictor of
321 cardiac-related mortality within one year in dogs with MMVD [8]. The present result
322 strengthens and expands our previous finding. The present study confirms the
323 prognostic significance of the RV Tei index by DPD in dogs with MMVD. In human
324 patients with PH, it was reported that not only RV systolic dysfunction but also

325 diastolic dysfunction occurred [27]. The RV Tei index has also been reported to
326 assess both RV systolic and diastolic function in healthy dogs and dog models of mild
327 RV pressure overload [28,29]. Given these findings, the RV Tei index by DPD may be
328 superior to other echocardiographic indices of RV function for assessing prognosis in
329 dogs with MMVD.

330 The RV Tei index by TDI was also impaired in dogs with cardiac-related death and
331 was significantly correlated with TR velocity. In addition, the RV Tei index by TDI was
332 related to long-term outcome in dogs with MMVD in univariate analysis. Therefore,
333 the RV Tei index by TDI is also useful for predicting worse long-term outcomes in
334 dogs with MMVD. However, in the multivariable analysis, there was no association
335 between the RV Tei index by TDI and long-term outcomes. The reason for differences
336 in echocardiographic methods may be related to differences in the measurement site.
337 The RV Tei index by TDI is measured only at the right ventricular inlet portion, in
338 contrast to that by DPD, which is measured at both the RV inlet and outlet portions;
339 therefore, the RV Tei index by TDI may be unrelated to global RV function. In addition,
340 we previously reported that values of the RV Tei index by TDI were higher than those
341 measured by DPD [22]. Therefore, RV Tei indices derived from different methods
342 should be interpreted with caution and not used interchangeably.

343 Free wall RVLS has been shown to be correlated with invasive haemodynamic
344 variables, and impaired RVLS has been strongly predicted to lead to a poor outcome
345 in human patients with left heart failure due to cardiomyopathy and advanced systolic
346 heart failure [14–16]. However, to date, the prognostic values of RVLS in human
347 patients with MR and dogs with MMVD have not been clarified. In the present study,
348 septal RVLS by STE was impaired, but free wall RVLS was not impaired in dogs with
349 cardiac-related death. In addition, only septal RVLS was related to long-term outcome
350 on univariate analysis. These results are consistent with some previous human
351 studies. Some groups have reported that global RVLS, including the septum and free
352 wall, was superior to free wall RVLS for assessing the prognosis in human patients
353 with left heart disease [15,17]. The superiority of septal RVLS can potentially be
354 explained by the fact that septal RVLS is not only related to RV function but also
355 reflects the influence of LV function. Therefore, septal RVLS can be considered a
356 marker of biventricular function. In addition, free wall RVLS may be affected by
357 hyperkinetic LV contraction due to LV volume overload via interventricular
358 interdependence. Our previous study demonstrated that free wall RVLS significantly
359 decreased in dogs with precapillary PH had the same degree of maximum TR velocity
360 compared to control dogs (median, -12.2%; interquartile range -16.1–8.5%) [30]. On

361 the other hand, free wall RVLS was enhanced in dogs with ACVIM stage B2 MMVD
362 compared to those with ACVIM stage B1 MMVD [31]. Indeed, while both free wall and
363 septal RVLS were significantly correlated with TR velocity, only free wall RVLS was
364 significantly correlated with LVIDDN in the present study. Hyperkinetic LV contraction
365 can mask a possible free wall RVLS impairment.

366 In contrast to the RV Tei index, other echocardiographic indices of RV function
367 were not impaired in dogs with ACVIM stage C or D or in those with cardiac-related
368 death. The TAPSE increase, which represents enhanced RV contractility, was
369 correlated with worse outcomes in dogs with MMVD in univariate and multivariate
370 analyses. This finding conflicts with a previous human study [11] in which decreased
371 TAPSE was an independent predictor of worse outcomes in human patients with
372 secondary MR due to dilated cardiomyopathy [11]. This discrepancy might be due to
373 the difference between primary and secondary MR or differences between dogs and
374 humans. Tricuspid annulus plane systolic excursion has been reported to be no
375 different among dogs with MMVD, regardless of the ACVIM stage or the severity of
376 PH [21,31]. Tricuspid annulus plane systolic excursion is affected by hyperkinetic LV
377 contraction caused by LV volume overload via interventricular interdependence [32]
378 and RV volume overload due to TR [33]. Indeed, there was a significant positive

379 correlation between normalized TAPSE and LVIDDN ($r = 0.46$), similar to a previous
380 study [21]. Therefore, the present result suggests that the increase in TAPSE reflects
381 more severe LV volume overload. Further studies are needed to validate the effect of
382 the severity of TR on echocardiographic indices of RV function.

383 There remain several limitations in the present study. The gold standard of
384 haemodynamic measurement, cardiac catheterization, was not performed. Another
385 limitation is the small number of dogs with cardiac-related death. Therefore, the
386 statistical power for determination of prognosis is low and because the number of
387 explanatory variables relative to deaths is high, the multivariable model might be
388 unstable and not accurately predictive of outcome in a larger population of dogs with
389 MMVD. In this study, instances of non-cardiac death were censored, and because
390 non-cardiac death is a competing event, this might have led to informative censoring
391 and over-estimation of cumulative incidence. Although stepwise forward selection
392 was used for multivariable Cox proportional hazard analysis in this study, there are
393 several limitations of stepwise selection such as bias in parameter estimation. In
394 addition, this study was a retrospective design. Retrospective studies increase the
395 risk of uncontrolled systematic errors, and some echocardiographic variables were
396 not available at baseline. The dogs were administered many medications, and it is

397 possible that medications influenced the echocardiographic indices and survival time.
398 Furthermore, it was difficult to standardize the treatment. In the present study RV
399 strain was measured using software for LV strain. Finally, although the inter- and
400 intra-observer repeatability of RV Tei index by DPD were higher than those by TD and
401 pulsed-wave Doppler in our previous study [22], DPD is a novel application of
402 ultrasonography that is available on only a few ultrasonographic systems. Therefore,
403 the utility of the Tei index by DPD may be limited in clinical settings.

404

405 **Conclusions**

406 The RV Tei index measured by DPD is an independent predictor of cardiac-related
407 death in dogs with ACVIM stage B2, C, or D MMVD. Therefore, assessment of the RV
408 Tei index in combination with traditional left heart assessments such as LA/Ao and
409 Emax will result in more accurate prediction of worse long-term outcomes and may
410 have clinical implications for better management of dogs with MMVD.

411

412 **Conflicts of interest**

413 None of the authors have any financial or personal relationship that would
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415

416 **Footnotes**

417 c. Artida, Toshiba Medical Systems Corp., Tochigi, Japan.

418 d. HI VISION Preirus, Hitachi Aloka Medical Ltd., Tokyo, Japan.

419 e. PST-50BT, Toshiba Medical Systems Corp., Tochigi, Japan.

420 f. EUP-S52, Hitachi Aloka Medical Ltd., Tokyo, Japan.

421 g. 2D Wall Motion Tracking, Toshiba Medical Systems Corp., Tochigi, Japan.

422 h. JMP, version 13.0, SAS Institute Inc., Cary, NC, USA.

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426 **References**

- 427 [1] Borgarelli M, Crosara S, Lamb K, Savarino P, Rosa G La, Tarducci A,
428 Häggström J. Survival characteristics and prognostic variables of dogs with
429 preclinical chronic degenerative mitral valve disease attributable to
430 myxomatous degeneration. *J Vet Intern Med* 2012;26:69–75.
- 431 [2] Borgarelli M, Savarino P, Crosara S, Santilli RA, Chiavegato D, Poggi M,
432 Bellino C, Rosa G La, Zanatta R, Häggström J, Tarducci A. Survival
433 characteristics and prognostic variables of dogs with mitral regurgitation
434 attributable to myxomatous valve disease. *J Vet Intern Med* 2008;22:120–8.
- 435 [3] Borgarelli M, Abbott J, Braz-Ruivo L, Chiavegato D, Crosara S, Lamb K,
436 Ljungvall I, Poggi M, Santilli RA, Häggström J. Prevalence and prognostic
437 importance of pulmonary hypertension in dogs with myxomatous mitral valve
438 disease. *J Vet Intern Med* 2015;29:569–74.
- 439 [4] Sargent J, Muzzi R, Mukherjee R, Somarathne S, Schranz K, Stephenson H,
440 Connolly D, Brodbelt D, Fuentes VL. Echocardiographic predictors of survival in
441 dogs with myxomatous mitral valve disease. *J Vet Cardiol* 2015;17:1–12.
- 442 [5] Moonarmart W, Boswood A, Fuentes VL, Brodbelt D, Souttar K, Elliott J.

443 N-terminal pro B-type natriuretic peptide and left ventricular diameter
444 independently predict mortality in dogs with mitral valve disease. *J Small Anim
445 Pract* 2010;51:84–96.

446 [6] Hezzell MJ, Boswood A, Chang Y-M, Moonarmart W, Souttar K, Elliott J. The
447 combined prognostic potential of serum high-sensitivity cardiac troponin I and
448 N-terminal pro-B-type natriuretic peptide concentrations in dogs with
449 degenerative mitral valve disease. *J Vet Intern Med* 2012;26:302–11.

450 [7] Nakamura K, Osuga T, Morishita K, Suzuki S, Morita T, Yokoyama N, Ohta H,
451 Yamasaki M, Takiguchi M. Prognostic value of left atrial function in dogs with
452 chronic mitral valvular heart disease. *J Vet Intern Med* 2014;28:1746–52.

453 [8] Nakamura K, Morita T, Osuga T, Morishita K, Sasaki N, Ohta H, Takiguchi M.
454 Prognostic value of right ventricular Tei index in dogs with myxomatous mitral
455 valvular heart disease. *J Vet Intern Med* 2016;30:69–75.

456 [9] Tourneau TL, Deswarte G, Lamblin N, Foucher-Hossein C, Fayad G,
457 Richardson M, Polge A-S, Vannesson C, Topilsky Y, Juthier F, Trochu J-N,
458 Enriquez-Sarano M, Bauters C. Right ventricular systolic function in organic
459 mitral regurgitation: impact of biventricular impairment. *Circulation*
460 2013;127:1597–608.

- 461 [10] Doesch C, Dierks DM, Haghi D, Schimpf R, Kuschyk J, Suselbeck T,
462 Schoenberg SO, Borggrefe M, Papavassiliu T. Right ventricular dysfunction,
463 late gadolinium enhancement, and female gender predict poor outcome in
464 patients with dilated cardiomyopathy. *Int J Cardiol* 2014;177:429–35.
- 465 [11] Dini FL, Conti U, Fontanive P, Andreini D, Banti S, Braccini L, Tommasi SM De.
466 Right ventricular dysfunction is a major predictor of outcome in patients with
467 moderate to severe mitral regurgitation and left ventricular dysfunction. *Am*
468 *Heart J* 2007;154:172–9.
- 469 [12] Ye Y, Desai R, Vargas Abello LM, Rajeswaran J, Klein AL, Blackstone EH,
470 Pettersson GB. Effects of right ventricular morphology and function on
471 outcomes of patients with degenerative mitral valve disease. *J Thorac*
472 *Cardiovasc Surg* 2014;148:2012–20.
- 473 [13] Kammerlander AA, Marzluf BA, Graf A, Bachmann A, Kocher A, Bonderman D,
474 Mascherbauer J. Right ventricular dysfunction, but not tricuspid regurgitation, is
475 associated with outcome late after left heart valve procedure. *J Am Coll Cardiol*
476 2014;64:2633–42.
- 477 [14] Cameli M, Lisi M, Righini FM, Tsioulpas C, Bernazzali S, MacCherini M, Sani G,
478 Ballo P, Galderisi M, Mondillo S. Right ventricular longitudinal strain correlates

- 479 well with right ventricular stroke work index in patients with advanced heart
480 failure referred for heart transplantation. *J Card Fail* 2012;18:208–15.
- 481 [15] García-Martín A, Moya-Mur J-L, Carbonell-San Román SA, García-Lledó A,
482 Navas-Tejedor P, Muriel A, Rodriguez-Muñoz D, Casas-Rojo E,
483 Jimenez-Nacher J-J, Fernandez-Golfín C, Zamorano J-L. Four chambers right
484 ventricular longitudinal strain vs right free wall longitudinal strain. Prognostic
485 value in patients with left heart disease. *Cardiol J* 2015;23:189–94.
- 486 [16] Cameli M, Righini FM, Lisi M, Bennati E, Navarri R, Lunghetti S, Padeletti M,
487 Cameli P, Tsioulpas C, Bernazzali S, Maccherini M, Sani G, Henein M,
488 Mondillo S. Comparison of right versus left ventricular strain analysis as a
489 predictor of outcome in patients with systolic heart failure referred for heart
490 transplantation. *Am J Cardiol* 2013;112:1778–84.
- 491 [17] Motoki H, Borowski AG, Shrestha K, Hu B, Kusunose K, Troughton RW, Tang
492 WHW, Klein AL. Right ventricular global longitudinal strain provides prognostic
493 value incremental to left ventricular ejection fraction in patients with heart failure.
494 *J Am Soc Echocardiogr* 2014;27:726–32.
- 495 [18] Keene BW, Atkins CE, Bonagura JD, Fox PR, Häggström J, Fuentes VL,
496 Oyama MA, Rush JE, Stepien R, Uechi M. ACVIM consensus guidelines for the

- 497 diagnosis and treatment of myxomatous mitral valve disease in dogs. *J Vet*
498 *Intern Med* 2019;33:1127–40.
- 499 [19] Reiner C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, Williams
500 K, Abbott J, Borgarelli M, Scansen BA. ACVIM consensus statement guidelines
501 for the diagnosis, classification, treatment, and monitoring of pulmonary
502 hypertension in dogs. *J Vet Intern Med* 2020;34:549–73.
- 503 [20] Cornell CC, Kittleson MD, Torre P Della, Häggström J, Lombard CW, Pedersen
504 HD, Vollmar A, Wey A. Allometric scaling of M-mode cardiac measurements in
505 normal adult dogs. *J Vet Intern Med* 2004;18:311–21.
- 506 [21] Poser H, Berlanda M, Monacoli M, Contiero B, Coltro A, Guglielmini C.
507 Tricuspid annular plane systolic excursion in dogs with myxomatous mitral
508 valve disease with and without pulmonary hypertension. *J Vet Cardiol*
509 2017;19:228–39.
- 510 [22] Morita T, Nakamura K, Osuga T, Lim SY, Yokoyama N, Morishita K, Ohta H,
511 Takiguchi M. Repeatability and reproducibility of right ventricular Tei index
512 valves derived from three echocardiographic methods for evaluation of cardiac
513 function in dogs. *Am J Vet Res* 2016;77:715–20.
- 514 [23] Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K,

515 Solomon SD, Louie EK, Schiller NB. Guidelines for the echocardiographic
516 assessment of the right heart in adults:A report from the American society of
517 echocardiography. *J Am Soc Echocardiogr* 2010;23:685–713.

518 [24] Reisner SA, Lysyansky P, Agmon Y, Mutlak D, Lessick J, Friedman Z. Global
519 longitudinal strain: a novel index of left ventricular systolic function. *J Am Soc*
520 *Echocardiogr* 2004;17:630–3.

521 [25] Bazett HC. An analysis of the time-relations of electrocardiograms. *Heart*
522 1920;7:353–70.

523 [26] Soydan LC, Kelliham HB, Bates ML, Stepien RL, Consigny DW, Bellofiore A,
524 Francois CJ, Chesler NC. Accuracy of Doppler echocardiographic estimates of
525 pulmonary artery pressures in a canine model of pulmonary hypertension. *J Vet*
526 *Cardiol* 2015;17:13–24.

527 [27] Murch SD, Gerche AL, Roberts TJ, Prior DL, Andrew I, Burns AT, Murch SD,
528 Gerche A La, Roberts TJ, Prior DL, Macisaac AI, Burns AT. Abnormal right
529 ventricular relaxation in pulmonary hypertension. *Pulm Circ* 2015;5:370–5.

530 [28] Teshima K, Asano K, Iwanaga K, Koie H, Uechi M, Kato Y, Kutara K, Edamura
531 K, Hasegawa A, Tanaka S. Evaluation of right ventricular Tei index (index of
532 myocardial performance) in healthy dogs and dogs with tricuspid regurgitation.

- 533 J Vet Med Sci 2006;68:1307–13.
- 534 [29] Morita T, Nakamura K, Osuga T, Yokoyama N, Morishita K, Sasaki N, Ohta H,
535 Takiguchi M. Changes in right ventricular function assessed by
536 echocardiography in dog models of mild RV pressure overload.
537 Echocardiography 2017;34:1040–9.
- 538 [30] Morita T, Nakamura K, Osuga T, Morishita K, Sasaki N, Ohta H, Takiguchi M.
539 Right ventricular function and dyssynchrony measured by echocardiography in
540 dogs with precapillary pulmonary hypertension. J Vet Cardiol 2019;23:1–14.
- 541 [31] Chapel EH, Scansen BA, Schober KE, Bonagura JD. Echocardiographic
542 estimates of right ventricular systolic function in dogs with myxomatous mitral
543 valve disease. J Vet Intern Med 2018;32:64–71.
- 544 [32] Haddad F, Hunt SA, Rosenthal DN, Murphy DJ. Right ventricular function in
545 cardiovascular disease, part I: anatomy, physiology, aging, and functional
546 assessment of the right ventricle. Circulation 2008;117:1436–48.
- 547 [33] Hsiao SH, Lin SK, Wang WC, Yang SH, Gin PL, Liu CP. Severe tricuspid
548 regurgitation shows significant impact in the relationship among peak systolic
549 tricuspid annular velocity, tricuspid annular plane systolic excursion, and right
550 ventricular ejection fraction. J Am Soc Echocardiogr 2006;19:902–10.

552

553 **Table 1. Comparison of clinical and echocardiographic variables in dogs with**554 **ACVIM B2 and C or D of MMVD.**

Variables	Stage B2 (n = 37)	Stage C or D (n = 30)	<i>p</i> value
Number	B2 (37)	C (28), D (2)	
Age (year)	10.9 ± 2.4	12.6 ± 1.9	0.003
Body weight (kg)	5.1 ± 2.6	5.3 ± 2.0	0.806
Sex (male/female)	19 / 18	20 / 10	0.465
Heart rate (bpm)	138 ± 24	130 ± 24	0.169
Mean blood pressure (mmHg)	114 ± 11	115 ± 18	0.847
High probability of PH	12 (32%)	21 (70%)	0.005
Ascites (Yes/No) *	0 / 37 (0% / 100%)	5 / 25 (17% / 83%)	0.001
Pimobendan (Yes/No)	15 / 22 (41% / 59%)	20 / 10 (67% / 33%)	0.032
Diuretics (Yes/No) *	7 / 30 (19% / 81%)	17 / 13 (57% / 43%)	0.001
LA/Ao *	2.01 ± 0.30	2.43 ± 0.43	<0.001
E _{max} (m/s) *	1.01 ± 0.25	1.35 ± 0.37	<0.001
LVIDDN	1.88 ± 0.34	1.99 ± 0.28	0.168
TR velocity (m/s) *	3.1 ± 0.6	3.8 ± 0.7	<0.001
S' _{TV} (cm/s)	13.9 ± 3.9	14.1 ± 3.4	0.793
TAPSE (mm)	11.8 ± 2.6	12.2 ± 2.6	0.507
Normalized TAPSE	0.70 ± 0.15	0.74 ± 0.15	0.250
FAC (%)	41.1 ± 7.5	39.3 ± 10.3	0.431
Tei index by DPD *	0.38 ± 0.12	0.65 ± 0.23	<0.001
Tei index by TDI *	0.53 ± 0.09	0.73 ± 0.18	<0.001
Free wall RVLS (-1%)	24.1 ± 6.5	26.0 ± 5.3	0.175
Septal RVLS (-1%)	20.5 ± 5.2	18.8 ± 5.0	0.183
RV-SD4 (msec)	22.6 ± 10.2	27.1 ± 9.5	0.068
AT/ET	0.36 ± 0.10	0.30 ± 0.09	0.016
nRVEDA (cm ² /m ²) *	15.2 ± 2.9	19.3 ± 5.2	<0.001
nRVESA (cm ² /m ²) *	9.0 ± 2.2	11.9 ± 4.3	<0.001
Septal flattening (Yes/No)	2 / 32 (6% / 94%)	5 / 19 (21% / 79%)	0.086

555 AT/ET, acceration time to ejection time ratio; DPD, dual pulsed-wave Doppler; E,

556 peak early diastolic mitral inflow velocity; FAC, fractional area change; LA/Ao, left
557 atrial to aorta root ratio; LVIDDN, normalized left ventricular end-diastolic diameter;
558 nRVEDA, normalized right ventricular end-diastolic area; nRVESA, normalized right
559 ventricular end-systolic area; PH, pulmonary hypertension; RVLS, right ventricular
560 longitudinal strain; RV-SD4, standard deviation of the systolic shortening time of right
561 ventricular 4segment corrected for RR interval according to Bazett's formula; S'_{TV},
562 peak systolic tricuspid annular velocity; TAPSE, tricuspid annular plane systolic
563 excursion; TDI, tissue Doppler; TR, tricuspid regurgitation .
564 * $p < 0.002$ were statistically significant after Bonferroni correlation.

565 **Table 2. Correlation between TR velocity, LVIDDN, and nRVEDA and**
 566 **echocardiographic indices of RV function.**

Variables	TR velocity		LVIDDN		nRVEDA	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
S' _{TV} (cm/s)	0.03	0.849	0.23	0.059	-0.14	0.238
TAPSE (mm)	0.09	0.540	0.48*	< 0.001	0.18	0.157
Normalized TAPSE	0.03	0.842	0.46*	< 0.001	0.22	0.073
FAC (%)	-0.45*	< 0.001	-0.13	0.292	-0.27	0.025
Tei index by DPD	0.57*	< 0.001	0.39	0.002	0.61*	< 0.001
Tei index by TDI	0.55*	< 0.001	0.20	0.102	0.63*	< 0.001
Free wall RVLS (%)	0.40	0.003	-0.26	0.034	0.15	0.232
Septal RVLS (%)	0.33	0.014	-0.07	0.583	0.42*	< 0.001

567 DPD, dual pulsed-wave Doppler; FAC, fractional area change; LVIDDN, normalized
 568 left ventricular end-diastolic diameter; nRVEDA, normalized right ventricular
 569 end-diastolic area; RVLS, right ventricular longitudinal strain; S'_{TV}, peak systolic
 570 tricuspid annular velocity; TAPSE, tricuspid annular plane systolic excursion; TDI,
 571 tissue Doppler; TR, tricuspid regurgitation.

572 * *p* < 0.002 were statistically significant after Bonferroni correlation.

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576 **Table 3. Comparison of clinical and echocardiographic variables in dogs with**577 **cardiac-related death and without cardiac-related death.**

Variables	Dogs with cardiac-related death (n = 24)	Dogs without cardiac-related death (n = 43)	p value
Age (year)	12.0 ± 2.0	11.4 ± 2.5	0.298
Body weight (kg)	5.3 ± 2.5	5.2 ± 2.1	0.841
Sex (male/female)	15 / 9	24 / 19	0.853
Heart rate (bpm)	139 ± 24	130 ± 24	0.158
Mean blood pressure (mmHg)	114 ± 14	115 ± 17	0.735
Ascites (Yes/No)	4 / 20 (17% / 83%)	2 / 41 (5% / 95%)	0.107
ACVIM stage (B/C or D) *	7 / 17 (29% / 71%)	30 / 13 (70% / 30%)	0.001
High probability of PH	18 (75%)	15 (35%)	0.002
Pimobendan (Yes/No)	15 / 9 (63% / 37%)	20 / 23 (47% / 53%)	0.207
Diuretics (Yes/No)	12 / 12 (50% / 50%)	12 / 31 (28% / 72%)	0.072
LA/Ao	2.39 ± 0.47	2.09 ± 0.34	0.004
E _{max} (m/s) *	1.38 ± 0.38	1.04 ± 0.27	<0.001
LVIDDN	2.07 ± 0.29	1.85 ± 0.30	0.004
TR velocity (m/s) *	3.8 ± 0.8	3.1 ± 0.5	<0.001
S _{TV} (cm/s)	14.3 ± 3.5	13.8 ± 3.8	0.562
TAPSE (mm)	13.0 ± 2.8	11.4 ± 2.4	0.016
Normalized TAPSE	0.78 ± 0.16	0.68 ± 0.14	0.015
FAC (%)	38.2 ± 9.5	41.5 ± 8.3	0.155
Tei index by DPD *	0.68 ± 0.23	0.41 ± 0.15	<0.001
Tei index by TDI *	0.74 ± 0.18	0.55 ± 0.13	<0.001
Free wall RVLS (-1%)	24.1 ± 7.4	25.7 ± 4.9	0.291
Septal RVLS (-1%)	18.0 ± 5.7	20.8 ± 4.6	0.032
RV-SD4 (msec)	25.4 ± 10.6	24.2 ± 9.9	0.647
AT/ET	0.31 ± 0.10	0.35 ± 0.10	0.078
nRVEDA (cm ² /m ²) *	19.7 ± 5.6	15.6 ± 3.0	<0.001
nRVESA (cm ² /m ²) *	12.3 ± 4.2	9.2 ± 1.5	<0.001
Septal flattening (Yes/No)	5 / 14 (26% / 74%)	2 / 37 (5% / 95%)	0.025

578 AT/ET, acceration time to ejection time ratio; DPD, dual pulsed-wave Doppler; E,
579 peak early diastolic mitral inflow velocity; FAC, fractional area change; LA/Ao, left
580 atrial to aorta root ratio; LVIDDN, normalized left ventricular end-diastolic diameter;
581 nRVEDA, normalized right ventricular end-diastolic area; nRVESA, normalized right
582 ventricular end-systolic area; PH, pulmonary hypertension; RVLS, right ventricular
583 longitudinal strain; RV-SD4, standard deviation of the systolic shortening time of right
584 ventricular 4segment corrected for RR interval according to Bazett's formula; S'_{TV},
585 peak systolic tricuspid annular velocity; TAPSE, tricuspid annular plane systolic
586 excursion; TDI, tissue Doppler; TR, tricuspid regurgitation.

587 * $p < 0.002$ were statistically significant after Bonferroni correlation.

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591 **Table 4.** Univariate and multivariate Cox proportional hazard analysis of the
 592 predictive value for cardiac-related death in dogs with MMVD.

Variables	Univariate		† Multivariate	
	Hazard ratio (95% CI)	<i>p</i>	Hazard ratio (95% CI)	<i>p</i>
Age (per 1 year increase) *	1.23 (1.01–1.52)	0.035		
Body weight (per 1kg increase)	1.01 (0.84–1.21)	0.886		
Sex (male)	1.18 (0.52–2.83)	0.691		
Heart rate (per 10 bpm increase)	5.33 (0.60–52.7)	0.136		
Mean blood pressure (per 10mmHg increase)	1.28 (0.78–1.35)	0.834		
Ascites (Yes) *	4.23 (1.19–11.87)	0.028		
ACVIM stage (C or D) *	4.66 (2.00–12.10)	<0.001	2.64 (0.30–23.25)	0.382
Pimobendan (Yes)	1.37 (0.61–3.27)	0.449		
Diuretics (Yes)	1.69 (0.75–3.82)	0.204		
LA/Ao (per 0.1 increase) *	1.19 (1.08–1.31)	<0.001	0.13 (0.01–1.88)	0.138
E _{max} (pre 0.1 m/s increase) *	1.30 (1.16–1.45)	<0.001	1.60 (0.21–12.07)	0.646
LVIDDN (per 0.1 increase) *	1.20 (1.06–1.36)	0.005		
TR velocity (per 0.1 m/s increase) *	1.10 (1.05–1.17)	<0.001		
S ₁ TV (per 1 cm/s increase)	1.04 (0.93–1.15)	0.532		
TAPSE (per 1 mm increase) *	1.22 (1.05–1.42)	0.012	1.37 (1.05–1.78)	0.019
Normalized TAPSE (per 0.1 increase) *	1.48 (1.11–1.98)	0.008		
FAC (per 1% increase) *	0.94 (0.88–0.99)	0.016		
Tei index by DPD (per 0.1 increase) *	1.81 (1.47–2.26)	<0.001	3.30 (1.57–6.95)	0.002
Tei index by TDI (per 0.1 increase) *	1.65 (1.34–2.00)	<0.001		
Free wall RVLS (per -1% increase)	0.95 (0.87–1.03)	0.194		
Septal RVLS (per -1% increase) *	0.91 (0.94–0.98)	0.017		
RV-SD4 (per 1 msec increase)	1.01 (0.97–1.05)	0.663		

AT/ET (per 0.1 increase)	0.66 (0.43–1.01)	0.057
nRVEDA (per 1 cm ² /m ² increase) *	1.17 (1.09–1.26)	<0.001
nRVESA (per 1 cm ² /m ² increase) *	1.22 (1.11–1.33)	<0.001
Septal flattening (Yes) *	5.78 (1.76–16.93)	0.006

593 † Adjusted for ACVIM stage, LA/Ao, and Emax.

594 AT/ET, acceration time to ejection time ratio; DPD, dual pulsed-wave Doppler; E,
595 peak early diastolic mitral inflow velocity; FAC, fractional area change; LA/Ao, left
596 atrial to aorta root ratio; LVIDDN, normalized left ventricular end-diastolic diameter;
597 nRVEDA, normalized right ventricular end-diastolic area; nRVESA, normalized right
598 ventricular end-systolic area; PH, pulmonary hypertension; RVLS, right ventricular
599 longitudinal strain; RV-SD4, standard deviation of the systolic shortening time of right
600 ventricular 4segment corrected for RR interval according to Bazett's formula; S'_{TV},
601 peak systolic tricuspid annular velocity; TAPSE, tricuspid annular plane systolic
602 excursion; TDI, tissue Doppler; TR, tricuspid regurgitation.

603 * $p < 0.05$.

604

605 **Figure Legends**

606 Figure 1. Echocardiographic images illustrating the technique used to measure the
607 right ventricular (RV) Tei index and speckle tracking echocardiographic indices. (A)
608 The RV Tei index was calculated using dual-pulsed Doppler as follows: $(a - b)/b$. (B)
609 The RV Tei index was calculated using tissue Doppler at the lateral tricuspid annulus
610 with an apical four-chamber view as follows: $(a - b)/b$. (C) The RV free wall and
611 septum were each automatically divided into three segments (apical, middle, and
612 basal). The free wall and septal right ventricular longitudinal strain (RVLS) were
613 calculated by averaging the peak longitudinal strain values in each two mid-basal
614 segments of the RV with a modified apical four-chamber view. This image shows the
615 septal RVLS. (D) RV-SD4 was calculated as the standard deviation of systolic
616 shortening time of the four mid-basal segments of RV and corrected for the R-R
617 interval (Bazett's formula). The coloured arrows indicate each segmental systolic
618 shortening time. AL = apical lateral free wall; AS = apical septum; BL = basal lateral
619 free wall; BS = basal septum, ML = middle lateral free wall; MS = middle lateral
620 septum; RV-SD4 = standard deviation of the systolic shortening time of four mid-basal
621 segments of right ventricle corrected for RR interval according to Bazett's formula.

622

623 Figure 2. Kaplan-Meier curves for long-term survival in dogs with MMVD. (A)
624 Kaplan-Meier analysis revealed that long-term outcomes for patients with ACVIM
625 stage C or D (median survival time, 364 days; 95% CI, 168 days to not applicable)
626 were worse than those for patients with ACVIM stage B2 MMVD (median survival
627 time, not reached; 95% CI, 629 days to not applicable). (B) Dogs with LA/Ao ≥ 2.15
628 (median survival time, 486 days; 95% CI, 231 days to not applicable) exhibited worse
629 outcomes than those with LA/Ao < 2.15 (median survival time, not reached; 95% CI,
630 616 days to not applicable). (C) Dogs with Emax ≥ 1.1 m/s (median survival time, 364
631 days; 95% CI, 168 to 616 days) showed worse outcomes than those with Emax < 1.1
632 m/s (median survival time, not reached; 95% CI, 730 days to not applicable). (D) The
633 long-term outcome for patients with a right ventricular Tei index by DPD ≥ 0.61
634 (median survival time, 237 days; 95% CI, 131 to 486 days) was worse than for
635 patients with a right ventricular Tei index by DPD < 0.61 (median survival time, not
636 reached; 95% CI, 629 days to not applicable).

637



