1   **Running Title: Retrospective survival evaluation study in dogs affected by DMVD**

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3   **MULTIPLE RETROSPECTIVE ANALYSIS OF SURVIVAL AND EVALUATION OF**

4   **CARDIAC DEATH PREDICTORS IN A POPULATION OF DOGS AFFECTED BY**

5   **DEGENERATIVE MITRAL VALVE DISEASE IN ACVIM CLASS C TREATED**

6   **WITH DIFFERENT THERAPEUTIC PROTOCOLS**

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25   Conflict of interest

26   The authors declare no conflict of interests.

Abstract:

Clinical records of dogs with spontaneous degenerative mitral valve disease (DMVD) with clinical signs related to congestive heart failure (CHF) that had been recruited during routine clinical practice, between 2001 and 2018 at the Cardiology Unit of the Veterinary Teaching Hospital (University of Milan) were included in this retrospective cohort study. Baseline echocardiographic data were evaluated. Median survival times (MSTs) were calculated. Data on therapeutic treatment, ISACHC or ACVIM classes were reviewed based on the inclusion period and type of endpoint (i.e. cardiac death or death for other causes). The main goal of this data review was to retrospectively evaluate 259 clinical records of subjects belonging to ACVIM C class examined between 2001 to 2018 together with the 202 examined between 2010 to 2018. The MSTs of these subjects was 531 d (2001-2018) and 335.5 d (2010-2018), respectively. Univariate survival regression analysis for subjects included from 2010 to 2018 showed the following variables as being significantly related to cardiac death (CD): LA/Ao ratio (HR 2.754, p=0.000), E wave (HR 2.961, p=0.000), E/A ratio (HR 1.372, p=0.000), EDVI (HR 1.007, p=0.000), ESVI (HR 1.012, p=0.026), Allo(d) (HR 4.018, p=0.000) and Allo(s) (HR 2.674, p=0.049), age (HR 1.006, p=0.009) and PH severity (HR=1.309, p=0.012). Multivariate analysis, adjusted for age, showed that the only variable that determined a statistically significant difference in MST was PH severity (HR 1.334, p=0.033). The type of therapeutic treatment within this class was not significant for the MST of the subjects.

Keywords: DMVD, dog, MST, therapeutic protocols, prognosis.

Introduction

Degenerative mitral valve disease is the most common heart disease in middle-old aged and medium-sized dogs. It is characterized by a slow progression over years, and in many affected dogs, because of the age of onset, does not always progress to clinical signs of congestive heart failure (CHF) [1,2,3]. Some authors have reported a survival time of between 5 to 14 months after the onset of clinical signs [4,5]. Although DMVD has been studied for more than 40 years, its treatment remains a challenge for the clinician.

Many advances have been made regarding diagnosis, imaging, medical and surgical therapy, however few studies have evaluated the natural history and prognostic factors of the disease in dogs [3,6,7]. Furthermore, to the best of our knowledge, no studies have evaluated MST in relation to different types of multiple therapeutic treatment within the same ACVIM C class severity class.

The aim of DMVD treatment is to modulate hemodynamic and neurohormonal disorders, including high venous pressures, reduce the systolic function, activate the sympathetic and renin-angiotensin-aldosterone systems, and also release cytokines and vasopressin [1,2]. Treatment of CHF due to DMVD consists of a diuretic (loop diuretic, ++ furosemide) and additional agents (angiotensin-converting enzyme inhibitors, inodilators and aldosterone receptor antagonists) [8,9]. Angiotensin-converting enzyme inhibitors (ACE-Is), benazepril or enalapril, combined with furosemide improve the quality of life [4,10]. The administration of pimobendan, an inodilator, has been evaluated in dogs with CHF due to DMVD [5,11].

Several studies have compared symptomatic dogs with DMVD that have received pimobendan and furosemide versus dogs treated with an ACE-I (ramipril or benazepril) and furosemide [5,12]. Dogs that received pimobendan and furosemide showed an improvement in clinical signs and quality of life, and a reduction in the probability of developing an adverse cardiac event [5,12]. VetSCOPE and QUEST reported that dogs receiving pimobendan

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77 survived longer than dogs that did not [5,13]. Improved survival and reduction of risk for a

78 cardiac event have also been shown in dogs affected by DMVD and CHF treated with

79 spironolactone [14,15].

80 The main goal of this study was to retrospectively investigate the survival time of a

81 population of dogs affected by DMVD belonging to ACVIM class C and treated with

82 different combinations of drugs including furosemide, ACE-I (benazepril or enalapril),

83 pimobendan, and spironolactone.

84 The effects of the different therapeutic protocols on MST, and the prognostic value of the

85 echocardiographic variables were also evaluated.

86

Materials and methods

Study design

89 This is a retrospective cohort study. The clinical records of dogs affected by DMVD

90 examined at the Cardiology Unit of the Veterinary Teaching Hospital (University of Milan)

91 between 2001 and 2018 were reviewed. Owner consent was routinely requested before the

92 first examination of each dog. It was not necessary to obtain authorization from the Ethics

93 Committee because this is a retrospective study carried out on data collected in subjects

94 routinely brought to a clinical examination by the owners.

95 From the beginning of the study to 2009, the admitted dogs were classified according to

96 ISACHC classes [16], and from 2010 to 2018 according to the ACVIM classification [9]. In

97 order to compare the subjects, a univocal classification was needed, and the patients classified

98 in ISACHC classes II, IIIa and IIIb were reallocated to ACVIM class C. Data obtained from

99 clinical records from 2001 to 2018 were then statistically analysed.

100 Later, in order to avoid inclusion bias due to the reallocations, statistical analyses were

101 performed on a selection of subjects belonging to ACVIM class C, recruited from 2010 to

102 2018.

103 Specific attention was focused on the therapy changes, as well as detailed information on any

104 change in ACVIM class and the causes of death reported in clinical records of subjects

105 included from 2010 to 2018 were studied.

Inclusion criteria

107 The clinical records were selected according to the following inclusion criteria: complete

108 clinical findings including signalment, history, physical examination, thoracic radiographs,

109 electrocardiogram (ECG) and a diagnosis of DMVD ACVIM class C based on

110 echocardiographic, and Doppler evaluation associated with clinical signs (increased resting

111 respiratory rate, cough, dyspnoea, ascites) [17]. In all subjects the presence and severity of PH

112 were evaluated, based on the TRV. The PH was classified as reported in the literature [18,19].

113 The accepted administered drugs were a combination of diuretics (furosemide), ACE-I

114 (benazepril, enalapril and ramipril), inodilator (pimobendan), and spironolattone. The

115 therapeutic protocol applied was clearly reported on the clinical record from the first to the

116 last examiner, as well as whether the owner was willing to be interviewed by telephone. In

117 this study all genders, weights and breeds were included, except for Cavalier King Charles

118 Spaniel [8,20,21]. The clinical records of subjects for whom cardioactive therapy had

119 previously been set up were also included.

Exclusion criteria

121 Clinical records of subjects affected by any other heart disease apart from DMVD and/or with

122 concurrent congenital heart disease and acquired cardiovascular disorders that could affect the

123 mitral valve or its functions (bacterial endocarditis, myocardial disease, arrhythmias) were

124 excluded. Subjects with primary hypertension were not included in the study [22].

5

125 Incomplete clinical records or with missing information on the therapeutic protocol adopted

126 were also excluded.

Echocardiography

128 Echocardiographic examinations were performed on conscious dogs by specialists in

129 cardiology, and in accordance with the guidelines of the American Society of

130 Echocardiography using the leading edge-to-leading edge method for M-mode measurements

131 and Hansson's method for 2-dimensional (2D) measurements of the left atrial (LA) and aortic

132 root (Ao) diameters [23,24].

Follow-up and endpoints

134 A single investigator (M.B.) conducted telephone interviews with dog owners to determine

135 the clinical outcome of each dog. For this the following information was obtained: was the

136 dog dead or alive, had the dog been euthanized or did it die spontaneously, and reasons for

137 euthanasia or cause of death. The date and cause of death (either spontaneous death or

138 euthanasia) were recorded. Dogs euthanized for severe refractory heart failure were

139 considered as cardiac-related deaths. Sudden deaths were counted as cardiac-related if no

140 other cause of death was obvious.

141 Dogs still alive, dead or euthanized for reasons unrelated to cardiac disease were removed

142 from the statistical analysis; subjects lost to follow-up were included in the survival analysis

143 up to the last time point at which they were known to be alive and were then removed from

144 the analysis.

145 The survival analysis was performed considering different end points such as death due to

146 other causes (OC), death related to the studied heart pathology (CD – cardiac death), first and

147 following therapy changes and moving to more advanced gravity class. The survival time in

148 the CD group was also analysed in relation to the therapeutic scheme.

6

149 The selected clinical records had to report the date and the medications prescribed as well as

150 any variation in therapy during the follow-up.

151 Median survival time (days) was calculated from the admission date to death or to the last

152 contact with the owner (lost to follow-up). The MST of each patient was subsequently related

153 to the combination of medicines (groups 1, 2 and 3) and the echocardiographic data (LA/Ao

154 ratio, E wave, E/A ratio, FE%, FS%, EDVI, ESVI, Allo(d), Allo(s), TRV and PH) at the time

155 of inclusion.

Statistical analysis

157 The data obtained for the analysis were compiled on an Excel spreadsheet and then processed

158 with SPSS^{TM} 25.0 (IBM, SPSS, USA). The statistical analysis was performed in two different

159 steps. In the first part of the study, which included the analysis of the reallocated ACVIM C

160 class population between 2001 and 2018, the statistical analysis was essential to verify the

161 presence of correlations and their significance between the MST and therapy group and

162 echocardiographic parameters such as LA/Ao ratio, E wave, E/A ratio, FE%, FS%, EDVI,

163 ESVI, Allo(d) and Allo(s).

164 The analysis thus included clinical records of subjects belonging to the ACVIM C class

165 included from 2010 to 2018 with the same inclusion criteria as the previous analysis. The

166 prognostic CD values of echocardiographic measurements, presence and severity of PH, and

167 the modulation of therapeutic protocols for each patient over the follow up time were

168 estimated.

169 A descriptive analysis of the sample was performed in terms of mean and standard deviation

170 or median and interquartile range (IQR) for normally or non-normally distributed variables,

171 respectively. Differences between variables were assessed by the appropriate test (Student t-

172 test for independent or paired normal variables, Mann Whitney U-test for independent non-

173 normal variables, and Wilcoxon signed ranks test; and the sign test for paired non-normal

7

174 variables). The therapeutic groups were compared by ANOVA and Tukey's HSD test. Median

175 survival times were compared by the log rank test.

176 The correlation between variables was investigated by the Pearson correlation or the Kendall

177 tau test, as appropriate. The Kolmogorov-Smirnov test for independent samples was used to

178 compare distributions.

179 The influence of individual physical and echocardiographic parameters on the MST was

180 assessed by univariate and multivariate survival analysis. Correlations between variables were

181 calculated to: (a) determine the possible predictors in the multivariate regression model, (b)

182 select only uncorrelated variables as predictors. The hazard ratio (HR) for each variable was

183 also evaluated.

184 A confidence interval (CI) of 95% was considered. The differences were considered as

185 statistically significant with $p < 0.05$.

186

187 # Results

188 ## Results ACVIM C class (2001-2018)

189 Six hundred and thirty-one clinical records from 2001 to 2018 reported a diagnosis of DMVD

190 with varying levels of gravity. After the reclassification (conversion from ISACHC to

191 ACVIM), the following 259 clinical records fulfilled the inclusion criteria and were thus

192 included: 135 (52%) intact male dogs, 29 (11%) neutered males, 60 (23%) sterilized females,

193 and 35 (13%) intact females. The median weight was 11.07 Kg (CI=5.8-14), the median age

194 was 11.89 (CI=10.37-13.87) years. Any breed of dog was included of which: 125 (48.2%)

195 were mixed breed, 20.5 (7.9%) Poodle, 20 (7.7%) Yorkshire Terrier, 13 (5.1%) Dachshund, 7

196 (2.8%) Shi-Tzu, 6 (2.6%) Pinscher, as well as lower percentages of other breeds .

197 The therapeutic groups considered in the first analysis are reported in Table 1. Table 2 reports

198 the average, median, standard deviation, minimum and maximum age, weight, LA/Ao ratio, E

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199   wave, E/A ratio, FE%, FS%, EDVI, ESVI, Allo(d) and Allo(s) of all subjects included in the

200   analysis belonging to C ACVIM class.

201   Class ACVIM C included 259 subjects, 136 of which (52.5%) died of CD with an MST of

202   531 days, and 123 dogs (47.5%) were still alive at the end of the study or died of OC. The

203   MST was 318 days (Table 3).

204   Univariate regression analysis showed the following variables to be statistically significant:

205   LA/Ao ratio, E wave, E/A ratio, EDVI, ESVI, Allo(d), Allo(s), Age and administration of

206   spironolactone. All the variables analysed were statistically significant for survival and their

207   increment was found to be related to an increase in the risk of death, except for the

208   administration of spironolactone (HR < 1).

209   Multivariate analysis highlighted that the LA/Ao ratio correlated negatively to MST and

210   significantly increased the risk of death (by 2.5 times) (Table 3).

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Table 1: Therapeutic treatments considered from 2001 to 2018 – subjects ACVIM class C			
Group 1	No. 168	44.8%	Furosemide + ACE-I
Group 2	No. 96	25.6%	Furosemide + ACE-I + Pimobendan
Group 3	No. 34	9.1%	Furosemide + ACE-I + Pimobendan + Spironolactone

212   Table 1: Classification of therapeutic treatments considered in the first analysis (subject in

213   ACVIM class C from 2001 to 2018). Clinical records of dogs for whom cardioactive therapy

214   had previously been arranged were also included.

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Table 2: Physical and echocardiographic parameters of all dogs belonging to ACVIM class C (2001-2018)											
CLASS		Weight (Kg)	Age	LA/Ao	E	E/A	FE %	FS%	EDVI	ESVI	Allo diastolic	Allo systolic
C	Average	11.27	12.02	2.29	1.33	1.65	73.89	43.56	156.77	40.86	2.02	1.16
	No.	259.00	179.00	257.00	127.00	121.00	255.00	255.00	257.00	257.00	258.00	258.00
	SD	8.51	2.90	0.49	0.38	0.71	10.41	9.51	60.92	25.22	0.33	0.26
	Median	8.00	12.49	2.22	1.36	1.47	76.00	44.00	148.02	36.10	2.02	1.15
	Interval	51.00	17.37	3.10	2.32	4.04	65.00	87.00	377.20	205.45	2.34	1.86
	Minimum	2.00	0.14	1.30	0.48	0.15	28.00	13.00	6.22	3.83	0.58	0.49
	Maximum	53.00	17.51	4.40	2.80	4.19	93.00	100.00	383.42	209.28	2.92	2.34

216 Table 2: Summary of the physical and echocardiographic parameters assessed in the statistical analysis of all the dogs included in the first

217 analysis belonging to ACVIM class C. All these parameters had a normal distribution. SD: standard deviation.

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Table 3: Clinical records analysis from 2001 to 2018				
Results ACVIM C class (2001-2018)				
ACVIM Class	No. of subjects	No. and % of CD	No. and % of alive or dead due to OC	MST days (CI 95%) for CD
C	259	136 (52.5%)	123 (47.5%)	531 (440.812-621.188)
Univariate and multivariate analysis in ACVIM class C				
Predictors	Univariate analysis HR (95% CI)	p value	Multivariate analysis HR (95% CI)	p value
LA/Ao ratio	2.473 (1.784-3.429)	0.000	2.473 (1.784-3.429)	0.000
E wave	2.560 (1.489-4.010)	0.001	-	-
E/A ratio	1.687 (1.222-2.329)	0.001	-	-
EDVI	1.004 (1.001-1.007)	0.002	-	-
ESVI	1.006 (1.001-1.011)	0.021	-	-
Allo(d)	2.197 (1.282-3.763)	0.004	-	-
Allo(s)	2.058 (1.126-3.764)	0.019	-	-
Age	1.080 (1.005-1.162)	0.037	-	-

Administration of spironolactone	0.623 (0.403-0.963)	0.033	-		-

Analysis of the groups of treatment in ACVIM class C					
Therapeutic groups	n. of subjects	n. and % of CD	n. and % of alive or death due to OC	MST days (CI 95%)	
1	n. 145	66 (45.4%)	79 (54.6%)	665 (532.724-794.276)	
2	n. 80	45 (56.2%)	35 (43.8%)	487 (306.392-667.608)	
3	n. 34	25 (73.3%)	9 (26.7%)	447 (190.470-703.530)	

Univariate analysis of the groups of treatment in ACVIM class C					
Predictors	Univariate analysis HR (95% CI)	p value	Multivariate analysis HR (95% CI)	p value	
LA/Ao ratio	2.773 (1.978-3.880)	0.000	-	-	
administration of therapy (yes/no)	1.295 (1.020-1.644)	0.033	-	-	
	2.686 (1.571-4.594)	0.000	-	-	
E wave	1.669 (1.200-2.321)	0.002	-	-	
E/A ratio	1.005 (1.002-1.008)	0.000	-	-	

EDVI	1.006 (1.001-1.012)	0.014	-	-
ESVI	2.821 (1.607-4.952)	0.000	-	-
Allo(d)	2.222 (1.218-4.054)	0.009	-	-
Allo(s)				

Table 3: Univariate and multivariate analysis of clinical records from 2001 to 2018. Parameters not reaching statistical significance are not reported in the table.

Analysis of the groups of treatment in ACVIM class C (2001-2018)

234

235 The therapy groups in the ACVIM class C were 1, 2 and 3 (Table 1).

236 Group 1 included 145 subjects, 66 (45.4%) died of CD, and 79 (54.6%) died of OC, and the

237 others were still alive at the end of the study. Group 2 included 80 dogs, 45 (56.2%) died of

238 CD and 35 (43.8%) died of OC or were still alive at the end of the study. Group 3 was

239 comprised of 34 dogs, 25 of which (73.3%) died of CD and 9 died (26.7%) of OC or were still

240 alive at the end of the study.

241 The MST of subjects that died of CD was 665 days in group 1, 487 days in group 2, and 447

242 days in group 3.

243 The univariate analysis revealed a positive correlation among the LA/Ao ratio, administration

244 of therapy (yes or no), E wave, E/A ratio, EDVI, ESVI, Allo(d) and Allo(s)), and CD. The

245 univariate analysis of the aforementioned variables was statistically significant for MST,

246 whose increase, increased the risk of death (Table 3).

247 The multivariate analysis for subjects in class ACVIM C showed that only LA/Ao led to a

248 statistically significant difference in MST, and significantly increased (2.5 times) the risk of

249 CD, as described in Table 3.

250 MSTs were also evaluated together with the influence of individual parameters on the MST

251 using univariate analysis and ANOVA tests between subjects with different types of therapy.

252 Using Tukey HSD tests, a multiple comparison was performed between the therapeutic

253 classes listed above. The log-rank method also showed that there was no statistically

254 significant difference (p=0.091) between the MSTs for the CD of dogs undergoing different

255 cardioactive therapies. Univariate analysis for subjects in the ACVIM class C with different

256 cardioactive therapies showed that the LA/Ao ratio (p=0.000), E wave (p=0.000) and EDVI

257 (p=0.01) led to a statistically significant difference in MST, some of which significantly

258 increased the risk of CD, as did some of the other variables considered (Table 3).

259   The multiple comparisons among therapeutic classes 1, 2 and 3 executed by ANOVA were

260   confirmed by Tukey's HSD test. The LA/Ao ratio, E wave and EDVI were statistically

261   significant (p<0.001) between therapeutic groups 1 and 2, 1 and 3, and not between groups 2

262   and 3. The statistical analysis was then carried out with the log-rank method between groups 1

263   and 2, in order to confirm the results. The MSTs of patients in groups 1 and 2 were assessed.

264   This analysis did not highlight any statistically significant difference (p>0.05) between the

265   MST of dogs who underwent different cardio-active therapies, and CD as end point.

266   Analysis of the groups of treatment in ACVIM class C (2010 to

267   2018)

268   Two hundred and two dogs (130 males, 72 females), median age 12.54 years (IQR=3.69),

269   median weight 8.18 Kg (IQR=8.46) were included. One hundred and twenty-one dogs died

270   (59,9%) and 91 (75%) died of CD. The therapy groups and the MST of each dog are reported

271   in Table 4.

272   The MST was: group 1=318 d (IQR 698), group 2=339 d (IQR 415), and group 3=408 d (IQR

273   480).

274   In 70 subjects PH was found, of which 38 (54.3%) were mild, and 32 were moderate-severe

275   (45.7%).

276   The Kendall tau test revealed a positive correlation between CD and the presence of PH

277   (R=0.2, p=0.005); CD and PH severity (R=0.2, p=0.003); as well as CD and the presence of a

278   moderate-severe PH grade (R=0.18, p=0.011).

279   The relationship between PH and CD was then investigated in each therapeutic group.

280   A weak and positive correlation was found in group 1 between the severity of PH and CD

281   (Kendall association coefficient R=0.24, p=0.023), while in groups 2 and 3, the correlation

282   was not statistically significant (R=0.13 and R=0.24, respectively).

283 The distribution of PH severity among the three groups of dogs who died of CD is reported in

284 Table 5. The Kolmogorov-Smirnov test for independent samples highlighted a statistically

285 significant difference of PH severity distribution only between therapeutic groups 1 and 3

286 (p=0.033).

287 The variables considered for survival regression were: sex, therapy, PH severity, weight, age,

288 LA/Ao ratio, E wave, E/A ratio, FE%, FS%, Allo(d) and Allo(s), EDVI, ESVI, and tricuspid

289 regurgitation velocity. Based on the correlation with CD, the chosen predictors were: PH

290 severity, age, LA/Ao ratio, E wave, E/A ratio, Allo(d), and Allo(s). The final model only

291 contained age (HR=1.009, p=0.003) and PH severity (HR=1.316, p=0.032). After adjusting

292 for age, PH severity was a risk factor for CD.

293 The regression model applied to each group of therapy evidenced different significant

294 correlations. In group 1 the following correlated significantly to CD: E wave, E/A ratio,

295 FS%, Allo(d) and Allo(s) and PH severity. In group 2 the following correlated significantly to

296 CD: LA/Ao ratio, E wave, E/A ratio, EDVI, ESVI and Allo(d) and Allo(s), In group 3 these

297 variables were LA/Ao ratio, EDVI, Allo(d). In groups 1 and 2 considered together as a single

298 group, the variables correlating significantly to CD were LA/Ao ratio, E wave, E/A wave,

299 EDVI, ESVI, Allo(d) and Allo(s) and PH severity.

300 We found a significant model only in therapeutic group 3, containing predictor LA/Ao

301 (HR=5.867, p=0.014) adjusted for age.

302 An analysis of the three different endpoints (EP) was performed for each group: CD, first and

303 following therapy changes and moving to more advanced gravity class.

304 The final step of the survival analysis entailed comparing the clinical findings and the

305 echocardiographic variables at the first and at the last visit for each subject. There was no

306 statistically significant difference between the first and last visit (p>0.05), except for the

307 echocardiographic variable related to the TRV (sign test, p=0.008).

308   The difference in the tricuspid regurgitation velocity between the first and last visits was the

309   only parameter to be statistically significant (p=0.008) in animals subjected to a therapeutic

310   change in ACVIM C.

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312

Therapeutic treatments considered from 2010 to 2018					
Groups	No.	%	Therapy	MST	IQR
Group 1	85 dogs	42.1	Furosemide + ACE-I	318 days	698 days
Group 2	76 dogs	37.6	Furosemide + ACE-I + Pimobendan	339 days	415 days
Group 3	41 dogs	20.3	Furosemide + ACE-I + Pimobendan + Spironolactone	408 days	480 days

Table 4: Therapeutic groups and MST of subjects in the ACVIM C class included in the second analysis (2010-2018).

Distribution of PH severity in CD related to therapeutic class (2001-2018)						
	Therapeutic Groups					
	1		**2**		**3**	
PH severity	Frequency	%	Frequency	%	Frequency	%
Absent	26	63.4	16	57.1	8	36.4
Mild	10	24.4	7	25	3	13.6
Moderate	3	7.3	3	10.7	6	27.3
Severe	2	4.9	2	7.1	5	22.7
Total	41	100	28	100	22	100

Table 5: distribution of PH severity in Cardiac Death (CD).

Discussion

313 DMVD is a progressive disease with a slow onset of clinical signs, and many affected animals

314 die of unrelated diseases [25]. Several studies have reported MSTs and prognostic indicators

315 in dogs with this pathology. However, these studies focused on specific breeds and did not

316 include large breed dogs [26,27,28] or focused on specific aspects of the disease, such as the

317 influence on survival after chordal rupture [29] and the effect of therapy on survival time

318 [30,31]. To the best of our knowledge, there are no studies on the evaluation of MST within

319 the same severity class in relation to the various therapeutic combinations.

320 The demographic data of the studied population were in line with the data reported in the

321 literature, concerning breed, weight and age [5,25,32].

322 The literature reports a median survival time of between 5 and 14 months once CHF develops

323 [4,5,30,33]. In our study, the MST between the diagnosis of mitral disease in ACVIM stage C

324 and CD was 531 days (17.7 months) for subjects included from 2001 to 2018, and 335.5 days

325 (11.2 months) for subjects included only from 2010 to 2018. The difference in MSTs between

326 the two populations can be explained by the different classification applied during the study.

327 In veterinary medicine, in order to improve the diagnostic and therapeutic approach to CHF,

328 two classification schemes have been proposed: the ISACH classification and the ACVIM

329 classification [9,16,34]. In this study, it was assumed that for the records included from 2010

330 to 2018, there was a more standardised classification, not affected by conversion errors.

331 While the majority of dogs died or were euthanized because of worsening heart failure,

332 multiple factors other than the underlying cardiac disease can impact survival time in

333 veterinary medicine, including medication adherence, financial issues, and owner compliance.

334 Knowledge of MST and prognostic factors could assist clinicians in communicating the

335 prognosis to owners of dogs with advanced heart failure because of DMVD. We believe it is

336 important to understand the long-term outcome and the influence of certain clinical and

338   echocardiographic variables and of the therapeutic scheme on survival in a large series of

339   dogs.

340   The aim of this retrospective study was to investigate the MST of dogs affected by DMVD

341   belonging ACVIM class C and treated with different combinations of drugs. In addition, the

342   effects of the different therapeutic protocols on the MST and the prognostic values of the

343   echocardiographic data were evaluated.

344   The clinical records were analysed, the MST was calculated, and the various pharmacological

345   treatments and the changes in class during the follow-up period as well as the prognostic

346   factors were evaluated.

347   In this study the MST of ACVIM C patients belonging to different therapeutic groups was in

348   accordance with those reported in the literature, although the more complex therapeutic

349   scheme (groups 2 and 3) was associated with a shorter survival time [5,13,35,36,37]. This is

350   despite the fact that patients with more advanced DMVD need more complex cardioactive

351   therapy.

352   Our study highlighted that with the same severity level of DMVD (subjects in ACVIM class

353   C included from 2001 to 2018), the MST of dogs who died of CD was longer than the MST of

354   those who died of OC. This could mean that cardioactive therapies play a pivotal role in

355   maintaining a good quality of life and in increasing the probability of a longer survival if no

356   other superimposed pathology occurs. Today, DMVD alone is a less frequent cause of death

357   in dogs than in the past.

358   In line with the literature, we found that LA/Ao and E wave velocity are predictors of CD

359   [35]. The increase in LA/Ao ratio and E-wave values corresponds to an increased risk of CD.

360   The univariate analysis also revealed the E/A ratio, EDVI, ESVI, Allo(d), Allo(s), age and

361   spironolactone administration as predictors of CD. However, only the LA/Ao ratio proved to

362   be significantly correlated in the multivariate analysis, as reported in the literature [35,37].

363   The univariate analysis of the LA/Ao ratio, E wave, E/A ratio, EDVI, ESVI, Allo(d) and

364   Allo(s) of patients categorized into different therapeutic groups (1, 2 and 3) showed a

365   negative and statistically significant correlation with MST and a significant association with

366   an increased risk of CD. The differences in MSTs among the therapeutic groups were

367   evaluated and an increase in LA/Ao ratio, E wave and EDVI was negatively related to

368   survival.

369   To the best of our knowledge, no other retrospective study has analysed the MST in different

370   groups of therapy patients belonging to the ACVIM C class. The analysis highlighted that in

371   dogs who died of CD, there was no significant difference in the MST between cardio-active

372   therapy groups, which means that the MST of patients in the ACVIM C class is not related to

373   the therapy group.

374   The correlation was evaluated between CD and PH in subjects belonging to the ACVIM C

375   class, included from 2010 to 2018, in each therapeutic group. Only in group A was there a

376   positive correlation between severity of PH and risk of CD. The multivariate regression

377   analysis was applied in order to highlight the predictor factors among the clinical and

378   echocardiographic variables, and the uncorrelated variables were selected. Only PH severity

379   and age were positively related to CD. Adjusting for age, the PH severity was shown to be a

380   risk of factor of CD.

381   The same approach was carried out in each therapy group. Multivariate regression within

382   therapeutic groups showed that only LA/Ao adjusted for age in therapeutic group C was a

383   predictor of CD, and no other references were found in the literature regarding this.

384   Regarding subjects included from 2010 to 2018, different EPs were considered for each

385   therapeutic group: CD, first and following therapy changes and moving to more advanced

386   severity class. Between the first and last visits, none of the normally distributed variables

387   considered (weight, E wave, E/A ratio, EDVI and Allo(d)) were statistically different. Even

388   for not normally distributed variables (LA/Ao ratio, FS%, ESVI, Allo(s) and TRV), there was

389   no significant difference between the first and last visits, except for TRV. The differences in

390   TRV correlated positively to CD in ACVIM C dogs undergoing therapeutic changes.

391   As far as the limitations of our study are concerned, this study was performed retrospectively

392   on a population of dogs affected by spontaneous DMVD, and recruited over a long period of

393   time (2001 – 2018), when many changes in diagnostic procedures, therapies and patient

394   classification systems have occurred.

395   The inclusion criteria of the patients were very strict. However, this is a retrospective study,

396   thus biases cannot be as well controlled as in a well-designed prospective study. Patients who

397   had already been treated with cardioactive therapy were recruited which justifies the

398   variability in therapeutic groups of the overall population.

399   The echocardiographic values, associated with ACVIM class of DMVD, were useful from a

400   prognostic point of view, and to answer any of the owners' questions.

401   The PH severity also correlated strongly to CD and therapeutic groups. In addition, we

402   believe that our study indicates that data regarding therapeutic choices and any variations after

403   the initial diagnosis should be monitored in clinical practice in order to assess the prognosis

404   and modulate the treatment of animals with DMVD.

405   The retrospective evaluation of the medical records of patients visited over a very long period

406   of time suggests that the classification of DMVD needs revisiting. The ACVIM classification

407   does not include any possible reclassifications into less severe classes of mitral disease, due to

408   the effects of cardioactive therapy.

409   The lengthening of MST and a good quality of life (QoL) are significant aspects of the

410   therapeutic strategy, and both are very important for the owners. The achievement of a longer

411   MST in our study compared to the literature might be explained by the good compliance of

412   the owners over time, even given the complex protocols [38]. Prospective studies are needed

413   to investigate the compliance effects of owners and the influence of more standardized

414   therapeutic protocols on the QoL and the survival of dogs with DMVD.

415

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