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1 **Evaluation of Red Blood Cell Distribution Width in Cats with Hypertrophic Cardiomyopathy**

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8 **Short title:** RDW in cats with hypertrophic cardiomyopathy

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22 **Abstract:**

23 **Background:** Red Blood Cell Distribution Width (RDW) is a measurement of variability in circulating
24 erythrocytes volume and has recently been shown to correlate with prognosis in a variety of human diseases,
25 including acute and chronic heart failure.

26 **Objective:** To determine if RDW differs between healthy controls, cats with hypertrophic cardiomyopathy
27 (HCM) without congestive heart failure (CHF) and cats with HCM and CHF and evaluate whether RDW values at
28 presentation can provide useful prognostic information in cats with HCM.

29 **Animals:** Retrospective single-centre study. Seventy-three cats diagnosed with HCM by echocardiography and
30 30 healthy controls presented to a veterinary teaching hospital between October 2006 and April 2013 were
31 included. Physical examination, haematology and echocardiographic data obtained on one single visit were
32 retrospectively reviewed and compared between three groups: controls, cats with HCM without CHF and cats
33 with HCM and CHF. Outcome data was obtained from clinical records or referring veterinarians. Univariable
34 and multivariable survival analyses were performed.

35 **Results:** RDW was significantly greater in cats with HCM and CHF compared to cats with HCM without CHF and
36 controls. RDW was also significantly associated with all-cause mortality in univariable survival analysis and this
37 association remained significant in multivariable survival analysis after controlling for the effect of CHF, left
38 atrial size, left ventricular systolic function, haematocrit and pro-thrombotic state.

39 **Conclusions:** RDW increases may be seen in cats with CHF and is an independent predictor of all-cause death
40 in cats with HCM without concurrent non-cardiac related illness.

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43 **Keywords:** Feline, Congestive Heart Failure, Prognosis, Biomarker

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46 **Abbreviation:**

ATE	Arterial Thromboembolism
CBC	Complete Blood Count
CHF	Congestive Heart Failure
HCM	Hypertrophic Cardiomyopathy
LVFS%	Left ventricular fractional shortening
Max LVWd	Maximal 2D end-diastolic left ventricular septal or free wall thickness
LA:Ao	Ratio of diastolic left atrial diameter to aortic root diameter
RDW	Red Blood Cell distribution Width

47

48 **Introduction**

49 Red Blood Cell Distribution Width (RDW) is a measurement of the heterogeneity of red blood cell size
50 distribution data and is routinely reported by automated haematology analysers.¹ RDW is defined as the
51 coefficient of variation of the red blood cell size; it has historically been used for the classification of anaemia.
52 Recently, however, RDW has been correlated with prognosis in a variety of different human diseases, including
53 acute and chronic heart failure,³⁻¹⁰ with an increase in RDW values associated with a decrease in survival time.
54 The proposed mechanisms for the alteration of RDW in these patients include: inflammatory stress, nutritional
55 deficiencies, impaired iron metabolism, inadequate production of erythropoietin, and the impact of
56 comorbidities.^{8, 11, 12}

57 Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats, and several negative
58 prognostic factors associated with decreased survival time have been identified, including the presence of:
59 arterial thromboembolism, congestive heart failure, left atrial dilation, left ventricular and left atrial systolic
60 dysfunction, extreme ventricular hypertrophy and elevation in cardiac biomarkers.¹³⁻¹⁷

61 RDW has been investigated in veterinary patients as an index of regenerative anaemia^{18, 19} and in dogs with
62 mitral valve disease²⁰ and pulmonary hypertension.^{b, 21} However, no association between RDW and outcome
63 has been established in these studies. To date there have been no publications evaluating RDW as a prognostic
64 indicator in feline patients.

65 The aims of this study are to determine if RDW differs between healthy controls, cats with HCM and cats with
66 HCM in congestive heart failure (CHF) and whether RDW values at presentation can provide useful prognostic
67 information in feline patients with HCM. The hypothesis was that RDW would be higher in cats with HCM
68 compared with the controls, and that higher RDW would be independently associated with cardiac death.

69

70 **Animals, materials and Methods**

71 The electronic medical record system of a veterinary teaching hospital was retrospectively searched for feline
72 patients diagnosed with HCM between October 2006 and April 2013. Patients were selected if they had a full
73 echocardiographic examination and haematology analysis submitted during the same visit or hospitalization
74 period. Data collected from medical record included signalment, presenting clinical signs, physical examination
75 findings, results of serum biochemistry, haematology analysis and thoracic radiographs when available.

76

77 HCM was defined by two-dimensional echocardiography as an end-diastolic left ventricular wall thickness ≥ 6
78 mm^{22, 23} on two-dimensional (2D) echocardiography in the absence of haemodynamic or metabolic causes of
79 hypertrophy (such as systemic hypertension, fixed aortic stenosis, hyperthyroidism, acromegaly) based on
80 appropriate tests performed during the diagnostic or treatment regime. Echocardiographic measurements
81 were performed at the time of presentation to the clinic by a diplomate cardiologist or a cardiology resident
82 under supervision and later reviewed by a single cardiologist (DJC). Data collected from echocardiography
83 included maximal 2D end-diastolic left ventricular wall thickness (Max LVWd), ratio of left atrium to aortic root
84 diameter (LA:Ao), left ventricular fractional shortening (LVFS%) and presence of spontaneous echo-contrast or
85 a thrombus within the left atrium or auricular appendage. LA:Ao was measured from a 2D short axis view at
86 the heart base, optimised for the left atrium and aortic valve, in the frame before aortic valve opening (end-
87 ventricular diastole) using an inner edge to inner edge technique.²⁴ M-mode measurements LV fractional

88 shortening (LVFS%) were made using a leading edge to leading edge method.¹⁶ Congestive heart failure was
89 defined as present if the cat had radiographic or ultrasonographic evidence of cardiogenic pulmonary oedema
90 or pleural effusion in the presence of left atrial dilation (LA:Ao \geq 1.5)²⁴ or tachypnoea responsive to furosemide
91 in the presence of left atrial dilation (LA:Ao \geq 1.5).²⁴ If another potential cause of pleural effusion (e.g.
92 intrathoracic neoplasia) was present the cat was excluded from the study.

93

94 Arterial Thromboembolism (ATE) was defined as an acute onset of lower motor neuron deficits in one or more
95 limbs associated with signs of regional hypoperfusion including pallor, cold extremities, and absence of
96 peripheral pulses.²⁵

97

98 Due to the potential to affect the RDW value, patients were also excluded if they presented with concurrent
99 systemic disease specifically neoplasia, endocrine, renal, inflammatory, infectious diseases or porto-systemic
100 shunt; had evidence of recent blood loss or underwent surgical procedures or blood transfusions within the
101 previous 3 months. This was established based on review of the history, physical examination, clinical
102 progression, available laboratory and imaging findings and final diagnosis by the attending clinician.

103 A single automated haematology analyser, Cell-Dyn 3500^c routinely used in the Royal Veterinary College
104 Diagnostic Laboratories for the analysis of feline haematology samples,^{26,27} was used for all the RDW and
105 haematocrit measurements. The Cell-Dyn 3500 reports a relative RDW equivalent to a coefficient of variation
106 in percentage. The RDW is derived from the RBC histogram using the 20th and 80th percentiles. Quality
107 control was performed on the Cell-Dyn 3500 every day and consisted of low, normal and high reference
108 materials; if these materials were out of range, appropriate remedial action was taken. Haematology analyser
109 histograms and blood smears were assessed for all patients at the time of the initial sample analysis. The
110 assessment was performed by an experienced veterinary technician and reviewed by a board-certified or
111 board eligible clinical pathologist, where abnormalities were identified according to the authors' institution
112 diagnostic laboratory protocols. For the purpose of the present study, all haematology reports were
113 retrospectively reviewed and patients were excluded if they had a haematocrit <28% or poor separation of red
114 blood cell and platelets populations on histograms was reported. Presence of platelet clumping on blood
115 smear examination was noted.

116 The study end-point was survival time associated with all-cause mortality. Cardiac mortality was defined as
117 death or euthanasia due to clinical signs of CHF (i.e. worsening respiratory distress) or ATE, or sudden death
118 unrelated to known systemic disease. Non-cardiac mortality was defined as death or euthanasia following
119 clinical signs not related to cardiac disease. The end of the study period was the 1st of July 2014. Referring
120 veterinarians were contacted to obtain missing follow-up data, using a protocol that conformed to good
121 research practice policy at the authors' institution.

122

123 A control group was established with blood donor cats that underwent a full physical examination by either a
124 cardiology or emergency and critical care diplomat and had a blood sample submitted for haematology
125 analysis and serum biochemistry as part of their pre-donation screening. Controls were included if they had
126 not donated in the previous 3 months, if no abnormalities were identified on medical history and physical
127 examination and if results of haematology and serum biochemistry were unremarkable. Laboratory results
128 were considered unremarkable if all parameters were within reference intervals, or, if outside these intervals,
129 they were judged to be not clinically significant by 2 of the authors (GS, DJC). The controls were excluded if
130 they had evidence of blood loss or underwent surgical procedures in the 3 months preceding the blood
131 sample. Controls were also excluded if a murmur, gallop sound or arrhythmia was identified at the time of
132 donation.

133 ***Statistical analysis***

134 Normality of data was evaluated by visual inspection of histograms and the Shapiro-Wilk test. Normally and
135 non-normally distributed data were reported as mean (s/ standard deviation) or as median (interquartile
136 range), respectively. Differences between two groups of continuous data were tested with independent
137 samples t-test for normally distributed data or the Mann-Whitney U test for non-normally distributed data.
138 Differences between more than two groups of continuous data were tested with one-way ANOVA for normally
139 distributed data or a Kruskal-Wallis test for non-normally distributed data. Post hoc, all pairwise comparisons
140 were performed for statistically significant results and a Bonferroni correction was applied. Adjusted p-values
141 are presented. Categorical variables were compared using a Chi-squared test. Linear correlation between
142 continuous variables was tested with Spearman's rank correlation (ρ_s).

143

144 The median survival times and associated 95% Confidence Intervals (CI) were estimated via the KaplanMeier
145 method. Both univariable and multivariable time-to-event models were built and Hazard Ratios (HR) with CI
146 were calculated using Cox Proportional Hazards Analysis. The end point of survival analysis was cardiac death,
147 including both spontaneous death and euthanasia. For the purpose of survival analysis, each continuous
148 variable was initially ranked in tertiles and assessed via the KaplanMeier method: variables that presented an
149 ordinal increase or decrease in survival for each tertile were evaluated in the univariable analysis as
150 continuous. For variables that did not fit these criteria, a suitable cut-off was selected and they were assessed
151 in the univariable analysis as categorical. Variables that had a significant association with outcome in the
152 univariable analysis were carried forward in the multivariable analysis. The validity of the proportional hazard
153 assumption was tested by visual assessment of the log minus log survival plots and scaled Schoenfeld
154 residuals. Commercial statistical software was used to perform all the analyses.^d Level of significance was set
155 at 0.05.

156 **Results**

157 Seventy-three cats were eligible for inclusion between October 2006 and April 2013. Median (IQR) age on
158 presentation was 5.78 (3.2-10.14) years. The majority of cats were male (77%), neutered (98%) and non-
159 pedigree (75%). Patients of 11 different pedigree breeds were included, with British Short Hair (n=4), Persian
160 (n=3), Bengal (n=2) and Maine Coon (n=2) being the most represented breeds. On initial presentation 42/73
161 (58%) cats were in CHF and 5/73 (7%) cats were diagnosed with ATE. Three cats had concurrent CHF and ATE.
162 By June 2014 44/73 (60%) cats had died, 8/73 (11%) were alive and 21/73 (29%) were lost to follow up.
163 Patients lost to follow-up or still alive at the end of the study period were right-censored for the purpose of
164 survival analysis. Median (IQR) survival time was 188 (47-677) days, with a range of 0-2431 days.
165 Platelet clumping was reported in 33/73 (45%) cats. Echocardiography identified evidence of spontaneous
166 echo-contrast in 30/73 (41%) cats and a thrombus within the left atrium or left auricular appendage in 6/73
167 (8%) cats.
168 Thirty controls were included over the same period. Median (IQR) age of controls was 4.86 (3.17-5.86) years.
169 The majority of controls were male (67%), neutered (90%) and non-pedigree (87%). Four pedigree breeds were
170 represented among controls with one patient for each of the following: Bengal, Birman, Burmese and Maine
171 Coon.

172 For the initial statistical analysis 3 groups were compared: controls, cats with HCM without CHF (HCM non-
173 CHF) and cats with HCM and CHF (HCM+CHF). A summary of demographic, haematological and
174 echocardiographic data for the 3 groups is presented in table 1.

175

176 No statistically significant differences were present in sex ($P = .363$), breed ($P = .327$) or age ($P = .224$) between
177 these 3 groups. A statistically significant difference was present in RDW between HCM+CHF and controls ($P =$
178 $.03$) and between HCM+CHF and HCM non-CHF ($P = .003$) (figure 1). A statistically significant difference was
179 also present in haematocrit between controls and HCM non-CHF ($P = .005$) (figure 2). No difference in the
180 proportion of platelet clumping was present between the 3 groups ($P = .094$). To verify whether platelet
181 clumps could affect RDW determination, RDW values of patients with and without platelet clumping were
182 compared: no significant difference was identified ($P = .235$).

183

184 Echocardiographic measurements were compared between HCM non-CHF and HCM+CHF groups. A
185 statistically significant difference was present in LA:Ao ($P = .006$) and LVFS% ($P = .016$), but not in Max LVWd ($P =$
186 $.349$).

187

188 For the purpose of testing for correlations and survival analysis the controls were excluded and all HCM
189 affected cats were evaluated together. The presence of ATE, left atrium or auricular appendage thrombus or
190 spontaneous echo-contrast was analysed as a single categorical variable identifying a pro-thrombotic state. No
191 significant difference was present in RDW between patients with and without a pro-thrombotic state ($P =$
192 $.094$).

193

194 Correlation between RDW and other continuous variables (Age, haematocrit, LA:Ao, Max LVWd and LVFS%)
195 was tested. The only significant, although weak, positive correlation was present between RDW and Max
196 LVWd (ρ_s correlation coefficient = $.288$, $P = .014$).

197

198 Based on Kaplan-Meier curves RDW and LA:Ao appeared to have an ordinal decrease in survival for each
199 tertile, therefore they were analysed as continuous variables (see an example in figure 3). Age, Max LVWd,
200 LVFS% and haematocrit did not fulfil the requirements to be analysed as continuous variables, and were

201 therefore transformed into dichotomous categorical variables. As cut-offs for Max LVWd and LVFS% we used
202 ≥ 9 mm and $\leq 30\%$, respectively. These cut-offs were selected based on their clinical relevance: they
203 respectively define extreme hypertrophy and left ventricular systolic dysfunction and have been found to be
204 independent predictors of decreased survival time in a recent study.¹⁶ The median patient age (5.78 years) was
205 used as a cut-off for the age variable. The intermediate tertile for haematocrit ($32.7\% < \text{haematocrit} < 37.2\%$)
206 appeared to be associated with a worse outcome compared to the upper and lower tertiles. The upper and
207 lower tertiles were therefore pooled and used as a reference for comparison with the intermediate tertile.
208 The results of the univariable survival analysis are summarized in table 2. Univariable predictors of increased
209 risk of death were RDW, LA:Ao, CHF, left ventricular systolic dysfunction, pro-thrombotic state and an
210 haematocrit between 32.7% and 37.2%. Age, sex, breed and extreme hypertrophy were not significantly
211 associated with outcome in univariable analysis.

212

213 The six variables that were significant in the univariable survival analysis were carried forward to multivariable
214 survival analysis. Multivariable survival analysis was performed to ascertain if the association between RDW
215 and increased risk of death would remain statistically significant after taking into account the effect of other
216 control variables. Of the 6 predictor variables tested only RDW and LV systolic dysfunction remained
217 statistically significant (Table 3).

218

219 **Discussion**

220 In this study RDW was significantly greater in HCM+CHF cats with compared to HCM non-CHF cats and control
221 cats. However there was major overlap between the groups limiting its potential use as a diagnostic test
222 especially when more established cardiac biomarkers such as cardiac troponin I and N-terminal pro-B type
223 natriuretic peptide have proven efficacy.^{17, 28, 29} Of more clinical relevance however is our finding that RDW
224 provides useful prognostic information since a greater RDW value at presentation was associated with a
225 significantly higher risk of death in cats with HCM. This remained statistically significant following correction
226 for the presence of congestive heart failure, pro-thrombotic state, systolic dysfunction, left atrial size or
227 haematocrit. This shows that RDW remains an independent predictor of all-cause death in cats with HCM
228 without concurrent non-cardiac illness, even when previously established and robust prognostic indicators are
229 accounted for.¹⁶ Each single percentage point increase in RDW was associated with a 1.34 increase in the risk

230 of death in our study population. To the authors' knowledge this is the first study to report an association
231 between RDW and prognosis in veterinary patients. Previous studies have investigated RDW in dogs with
232 pulmonary hypertension and mitral valve disease, but failed to identify an association between RDW and
233 outcome.

234

235 These findings are in agreement with what has been reported in human patients, where RDW is an
236 independent prognostic factor across a variety of conditions, including acute and chronic heart failure.³⁻¹⁰ The
237 pathophysiology of this association has not been fully elucidated. Potential mechanisms that have been
238 proposed to explain the relationship between RDW and heart failure include inflammatory stress, nutritional
239 deficiencies, impaired iron metabolism, inadequate production of erythropoietin, and the impact of
240 comorbidities such as liver and renal dysfunction.^{8, 11, 12} Most of these processes share common pathways with
241 anaemia of chronic disease³⁰ and anaemia of critical illness.³¹ RDW might therefore represent an integrative
242 measure of different pathological processes occurring during heart failure and contributing to the clinical
243 outcome. Given the practical difficulties associated with measuring these underlying processes, RDW has been
244 proposed as a "barometer" of cardiovascular health that provides the sum of these multiple complex
245 interactions.^{2, 8, 12}

246

247 Of note: RDW was not significantly different between control cats and cats with HCM non-CHF. A possible
248 explanation is that in the context of HCM, RDW may be a late marker of severity that does not rise until the
249 disease has reached a more advanced stage. However, this finding may also be influenced by insufficient
250 statistical power due to the small sample size or choice of control population. The control population used in
251 this study was formed of blood donor cats, and although cats had not donated for at least 3 months before
252 blood sampling, an increase in RDW associated with previous blood donations could not be completely ruled
253 out. Furthermore, although all control cats received a careful physical examination by either a cardiology or
254 emergency and critical care diplomat only a small percentage of them had an echocardiogram performed at
255 time of donation.

256 Age, sex and breed distributions of our population were similar to that reported in previous studies.¹³⁻¹⁶

257 However, the median (range) survival time for mortality in our population (188, IQR 47-677 days) was shorter
258 than previously reported (709-1276 days).¹³⁻¹⁵ This probably reflects a higher percentage of patients in CHF in

259 our population (58%) compared to previous reports (33-46%),¹⁴⁻¹⁶ since the majority of the affected cats in
260 this study were presented as emergencies. In our institution these cats are more likely to have a blood sample
261 submitted for haematological analysis compared to asymptomatic cats presenting for a routine appointment.
262 Interestingly the haematocrit values of the HCM non-CHF population were significantly lower than those of the
263 controls. This might be associated with the development of anaemia of chronic disease or the effect of neuro-
264 humoral systems response to the cardiomyopathy causing an increase in circulating volume without overt CHF
265 resulting in dilutional anaemia. The absence of a significant difference in haematocrit between controls and
266 HCM cats in CHF may reflect the small sample size or the effect of treatments such as furosemide.

267

268 This study contains numerous limitations mainly due to its retrospective nature. Different diagnostic and
269 treatment protocols were used. Not all patients had thyroid hormone levels analysed to definitively rule out
270 hyperthyroidism in the absence of a palpable goitre. The small population size limited the study statistical
271 power and prevented the evaluation of the effect of other possible confounding variables such as treatment.
272 Time from sample collection to processing could not be retrospectively evaluated and aging of the sample
273 could have affected haematological variables.²⁶ Follow-up data obtained by referring veterinarians were also
274 inadequate to accurately classify patients' cause of death or euthanasia as cardiac or non-cardiac. For this
275 reason we elected to consider only all-cause mortality for the purpose of survival analysis.

276

277 Only one haematology analysis and one echocardiographic examination were evaluated for each patient at the
278 time of presentation. A progressive increase in RDW values over time is associated with a worse outcome in
279 human heart failure and may provide additional information compared to a single determination.³²

280 Assessment of the prognostic value of RDW in cats with HCM at different time points during disease
281 progression should be evaluated in future studies.

282

283 The RDW values can vary depending on the analytical technique used to measure erythrocyte volume and the
284 algorithm that calculates it based on the erythrocyte volume distribution data.³³ Therefore, the results of the
285 current studies cannot be generalised to RDW measured with different methodologies.

286

287 Aggregation of platelets into large clumps is common in cats and may cause them to be counted as one large
288 cell by automated haematology analyser,³⁴ falsely altering haematological variables such as RDW. However, in
289 our study population, RDW did not appear to be significantly affected by platelet clumping. All haematological
290 analyses were performed with a Cell-Dyn 3500. Most of the human literature is based on the use of more
291 modern haematology analysers that could provide better discrimination between different cell populations
292 thus providing more accurate data. This would also permit us to better elucidate the role of platelet numbers
293 and platelet clumping in the overall RDW determination.

294

295 **Conclusions**

296 Red blood cell distribution width is a simple, inexpensive and ubiquitously available laboratory parameter.
297 Greater RDW values were independently associated with an increased risk of cardiac mortality in cats with
298 HCM. Given the retrospective nature of this study and the small sample size, the results should be interpreted
299 as a promising foundation for further prospective studies evaluating the clinical value of RDW as a prognostic
300 indicator in this disease.

301

302 **Conflict of Interest**

303 The authors declare no conflict of interest.

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311

312 **Footnotes**

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317 Washington;
- 318 c. Cell-Dyn 3500 Abbot Abbott Laboratories, Abbott Park, Illinois, USA with system operator manual;
- 319 d. IBM SPSS Statistics for Windows, Version 22.0, IBM Corp., Armonk, NY, USA.

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419 Table legend

420 Table 1: Comparison of demographic, haematological and echocardiographic data between controls, cats with
 421 hypertrophic cardiomyopathy without congestive heart failure (HCM non-CHF) and cats with hypertrophic
 422 cardiomyopathy and congestive heart failure (HCM + CHF). Data are presented as number present (%), Mean ±
 423 SD or Median (IQR).

	Controls	HCM(a) non-CHF(b)	HCM + CHF	p-Value
Number of cats	30	31	42	–
Age, years (range)	4.9 (3.2–5.9)	6.0 (2.6–10.1)	5.7 (3.2–8.5)	0.224
Male, n (%)	20/30 (67%)	22/31 (71%)	34/42 (81%)	0.363
Non-pedigree, n (%)	26/30 (87%)	22/31 (71%)	33/42 (79%)	0.327
RDW (c) % (range)	19.2 (18.6–20.6)**	19.0 (18.3–20.1)*	20.3 (19.5–22.6)*	0.002
HCT (d) (%)	37.8 ± 4.3*	34.1 ± 3.9*	36.4 ± 4.8	0.006
Platelet clumping, n (%)	17/30 (57%)	10/31 (32%)	23/42 (55%)	0.094
Pro-thrombotic state, n (%)	–	13/31 (42%)	24/42 (57%)	0.241
LA:Ao (e) (range)	–	1.87 (1.29–2.21)	2.15 (1.84–2.63)	0.006
Max LVWd, (f) mm (range)	–	8.03 (7.20–9.89)	7.83 (6.48–9.07)	0.349
LVFS, (g) % (range)	–	46.5 (37.0–59.0)	40.0 (29.0–48.2)	0.016

424 * and ** indicate significant differences between groups following post hoc pairwise comparisons.

- 425 a. Hypertrophic cardiomyopathy.
- 426 b. Congestive heart failure.
- 427 c. Red blood cell distribution width.
- 428 d. Haematocrit.
- 429 e. Left atrium to aortic diameter ratio.
- 430 f. Maximal 2D left ventricular free or septal wall thickness.
- 431 g. Left ventricular fractional shortening.

432 Table 2: Results of univariable Cox proportional hazards analysis evaluating the association of individual
 433 variables with a shorter time to cardiac death.

	p-Value	Hazard ratio	95.0% CI(a) for hazard ratio	
			Lower	Upper
RDW(b)(%)	0.00006	1.346	1.164	1.557
Age (years)				
≤5.8	Reference			
>5.8	0.326	1.369	0.731	2.565
Sex				
Female	Reference			
Male	0.105	1.862	0.877	3.952
Breed				
Non-pedigree	Reference			
Pedigree	0.587	0.825	0.389	1.708
CHF(c)				
No	Reference			
Yes	0.022	2.095	1.112	3.947
LA:Ao(d)	0.0003	2.303	1.458	3.637
LVFS%(e)				
>30%	Reference			
≤30%	0.00001	4.744	2.353	9.562
Max LVWd(f)				
<9 mm	Reference			
≥9 mm	0.967	1.015	0.496	2.078
Pro-thrombotic state				
No	Reference			
Yes	0.004	2.557	1.36	4.808
Haematocrit				
HCT(g) ≤32.7% and ≥37.2%	Reference			
32.7% < HCT < 37.2%	0.005	2.441	1.315	4.532

- 434 a. 95% Confidence Interval.
 435 b. Red blood cell distribution width.
 436 c. Congestive heart failure.
 437 d. Left atrium to aortic diameter ratio.
 438 e. Left ventricular fractional shortening.
 439 f. Maximal 2D left ventricular free or septal wall thickness.
 440 g. Haematocrit.
 441

442 Table 3: Results of multivariable Cox proportional hazards analysis using parameters identified as
 443 significant in the univariable analysis.

			95.0% C(a) for hazard ratio	
	p-value	Hazard ratio	Lower	Upper
RDW (b)(%)	0.001	1.337	1.127	1.585
>30%	Reference			
≤30%	0.0004	4.872	2.023	11.73
LA: Ao (d)	0.714	1.147	0.549	2.396
CHF (e)				
No	Reference			
Yes	0.547	1.262	0.591	2.695
Pro-thrombotic state				
No	Reference			
Yes	0.099	1.753	0.901	3.414
Haematocrit				
HCT (f) ≤32.7% and ≥37.2%	Reference			
32.7% < HCT < 37.2%	0.11	1.716	0.884	3.329

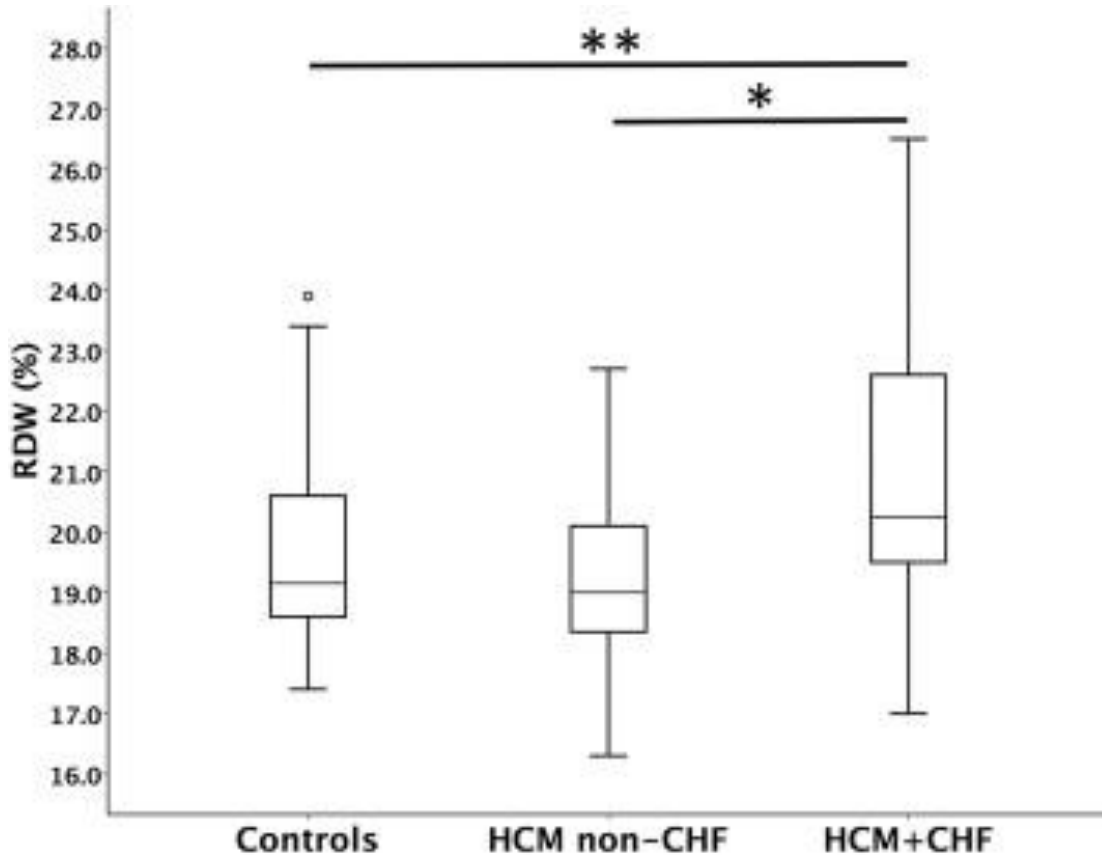
444

- 445 a. 95% Confidence Intervals.
- 446 b. Red blood cell distribution width.
- 447 c. Left ventricular fractional shortening.
- 448 d. Left atrium to aortic diameter ratio.
- 449 e. Congestive heart failure.
- 450 f. haematocrit.

451

452 **Figure captions**

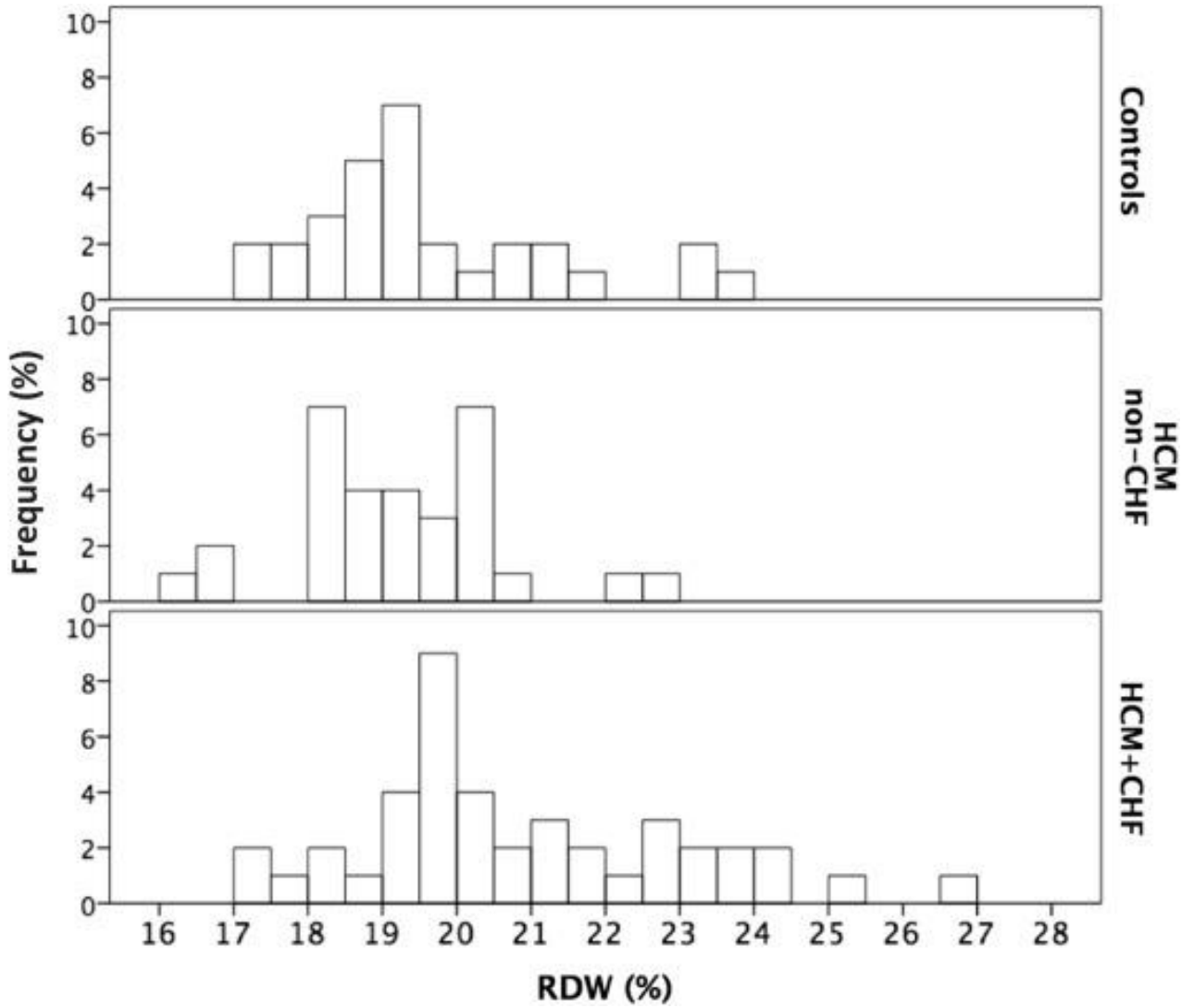
453 **Figure 1.** Boxplot graph comparing Red Blood Cell Distribution Width (RDW) values between controls, cats with
454 Hypertrophic Cardiomyopathy (HCM) without Congestive Heart Failure (CHF) and cats with HCM and CHF. *
455 adjusted P value = .003; ** adjusted P value = .03.



456

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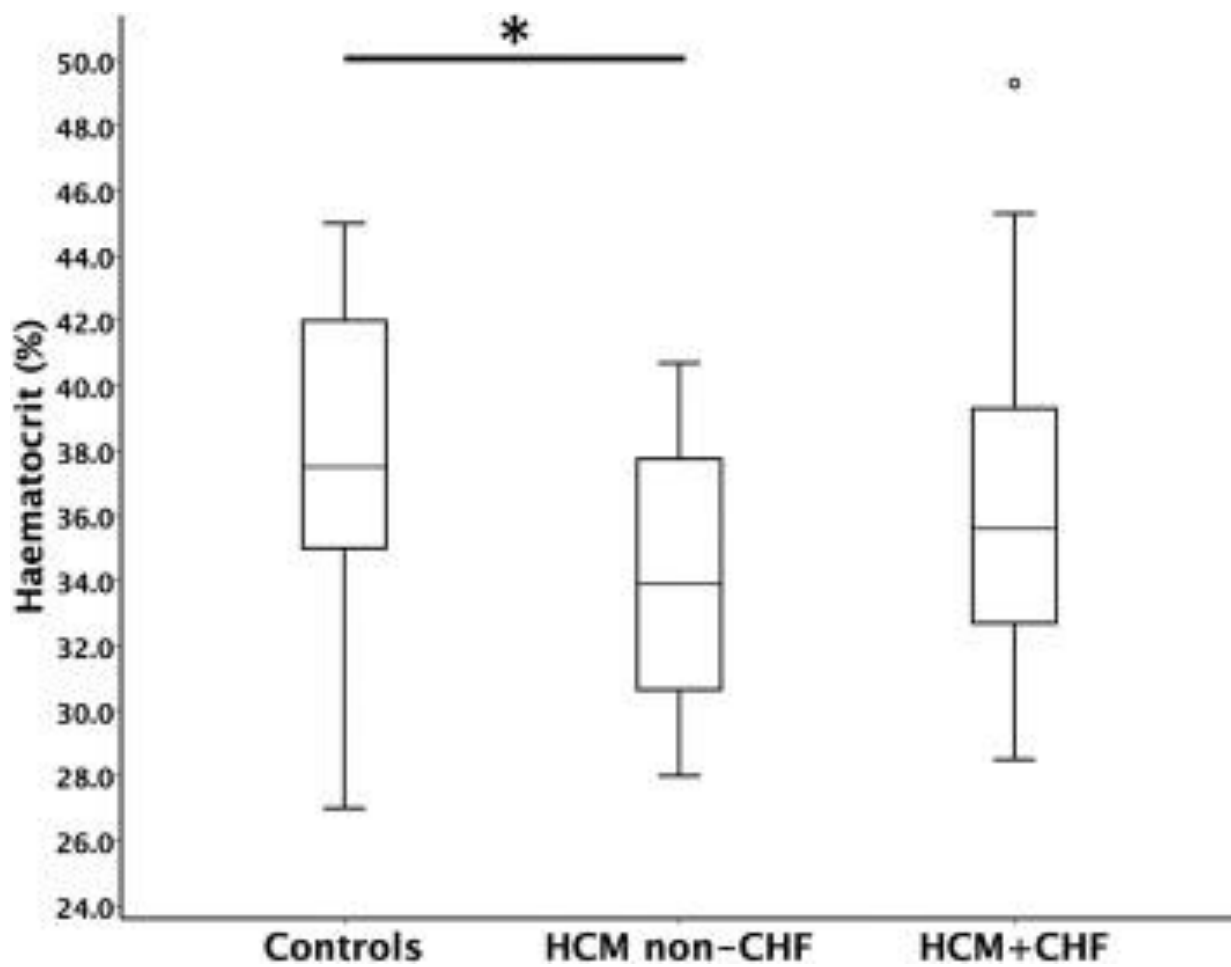
458 **Figure 2.** Boxplot graph comparing Haematocrit values between controls, cats with Hypertrophic
459 Cardiomyopathy (HCM) without Congestive Heart Failure (CHF) and cats with HCM and CHF. * adjusted P value
460 = .005.



461

462

463 **Figure 3.** Kaplan-Meier curves to show differences in survival associated with each Red Blood Cell Width (RDW)
464 tertile.



465