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

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Short title: RDW in cats with hypertrophic cardiomyopathy

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Abstract:

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

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

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

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

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

Keywords: Feline, Congestive Heart Failure, Prognosis, Biomarker

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

Introduction

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

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

RDW has been investigated in veterinary patients as an index of regenerative anaemia[18, 19] and in dogs with mitral valve disease[20] and pulmonary hypertension.[b, 21] However, no association between RDW and outcome has been established in these studies. To date there have been no publications evaluating RDW as a prognostic indicator in feline patients.

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

Animals, materials and Methods

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

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

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

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

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

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

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

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

Statistical analysis

Normality of data was evaluated by visual inspection of histograms and the ShapiroeWilk test. Normally and non-normally distributed data were reported as mean (þ/ standard deviation) or as median (interquartile range), respectively. Differences between two groups of continuous data were tested with independent samples t-test for normally distributed data or the ManneWhitney U test for non-normally distributed data. Differences between more than two groups of continuous data were tested with one-way ANOVA for normally distributed data or a KruskaleWallis test for nonnormally distributed data. Post hoc, all pairwise comparisons were performed for statistically significant results and a Bonferroni correction was applied. Adjusted p-values are presented. Categorical variables were compared using a Chisquared test. Linear correlation between continuous variables was tested with Spearman's rank correlation (ps).

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

Results

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

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

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

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

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

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

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

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

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

Discussion

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

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

These findings are in agreement with what has been reported in human patients, where RDW is an independent prognostic factor across a variety of conditions, including acute and chronic heart failure.[3-10] The pathophysiology of this association has not been fully elucidated. Potential mechanisms that have been proposed to explain the relationship between RDW and heart failure include inflammatory stress, nutritional deficiencies, impaired iron metabolism, inadequate production of erythropoietin, and the impact of comorbidities such as liver and renal dysfunction.[8, 11, 12] Most of these processes share common pathways with anaemia of chronic disease[30] and anaemia of critical illness.[31] RDW might therefore represent an integrative measure of different pathological processes occurring during heart failure and contributing to the clinical outcome. Given the practical difficulties associated with measuring these underlying processes, RDW has been proposed as a "barometer" of cardiovascular health that provides the sum of these multiple complex interactions.[2, 8, 12]

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

Age, sex and breed distributions of our population were similar to that reported in previous studies.[13-16] However, the median (range) survival time for mortality in our population (188, IQR 47-677 days) was shorter than previously reported (709-1276 days).[13-15] This probably reflects a higher percentage of patients in CHF in

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

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

Only one haematology analysis and one echocardiographic examination were evaluated for each patient at the time of presentation. A progressive increase in RDW values over time is associated with a worse outcome in human heart failure and may provide additional information compared to a single determination.[32] Assessment of the prognostic value of RDW in cats with HCM at different time points during disease progression should be evaluated in future studies.

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

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

Conclusions

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

Conflict of Interest

The authors declare no conflict of interest.

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Footnotes

a. Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, UK, AL9 7TA;

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c. Cell-Dyn 3500 Abbot Abbott Laboratories, Abbott Park, Illinois, USA with system operator manual;

d. IBM SPSS Statistics for Windows, Version 22.0, IBM Corp., Armonk, NY, USA.

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Table legend

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

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

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

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

			95.0% CI**(a)** for hazard ratio	
	p-Value	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

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

Table 3: Results of multivariable Cox proportional hazards analysis using parameters identified as 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

a. 95% Confidence Intervals.

b. Red blood cell distribution width.

c. Left ventricular fractional shortening.

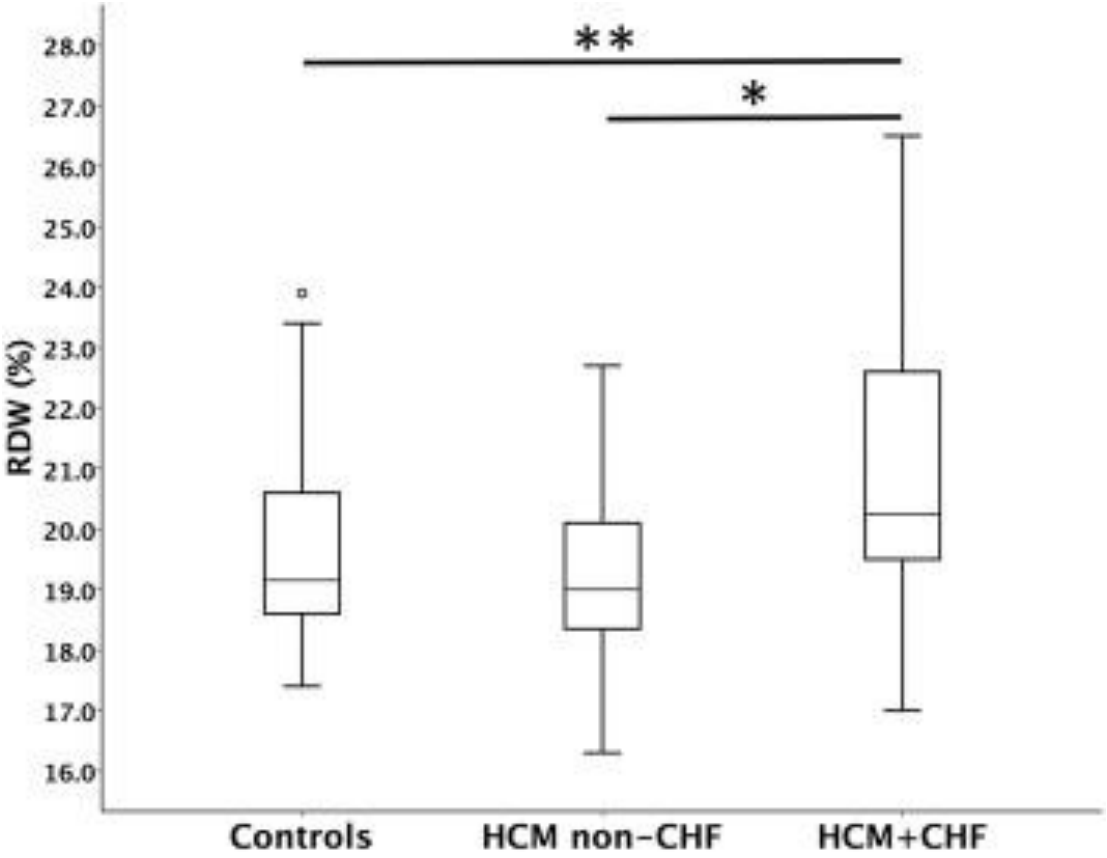
d. Left atrium to aortic diameter ratio.

e. Congestive heart failure.

f. haematocrit.

Figure captions

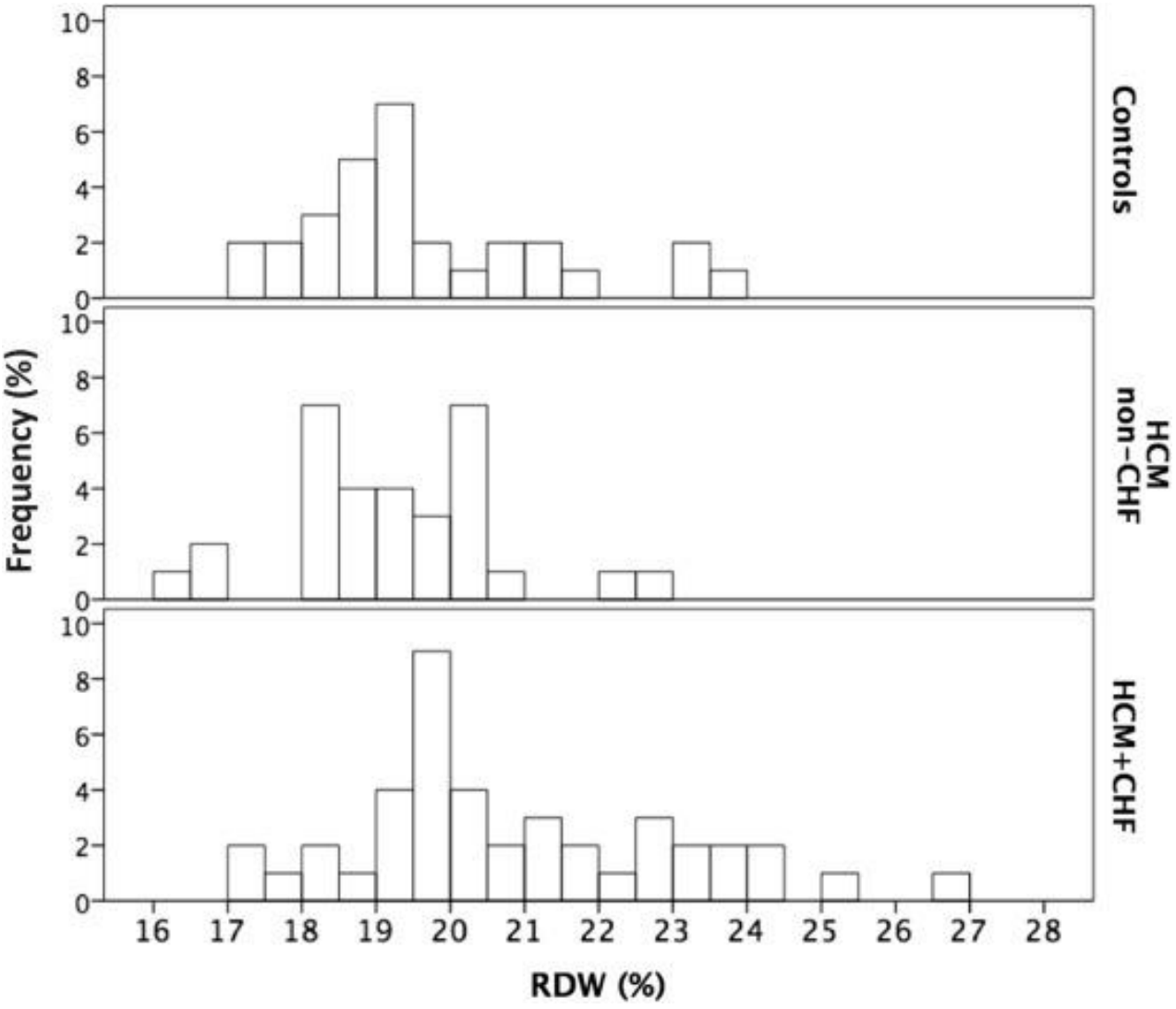
Figure 1. Boxplot graph comparing Red Blood Cell Distribution Width (RDW) values between controls, cats with Hypertrophic Cardiomyopathy (HCM) without Congestive Heart Failure (CHF) and cats with HCM and CHF. * adjusted P value = .003; ** adjusted P value = .03.



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.



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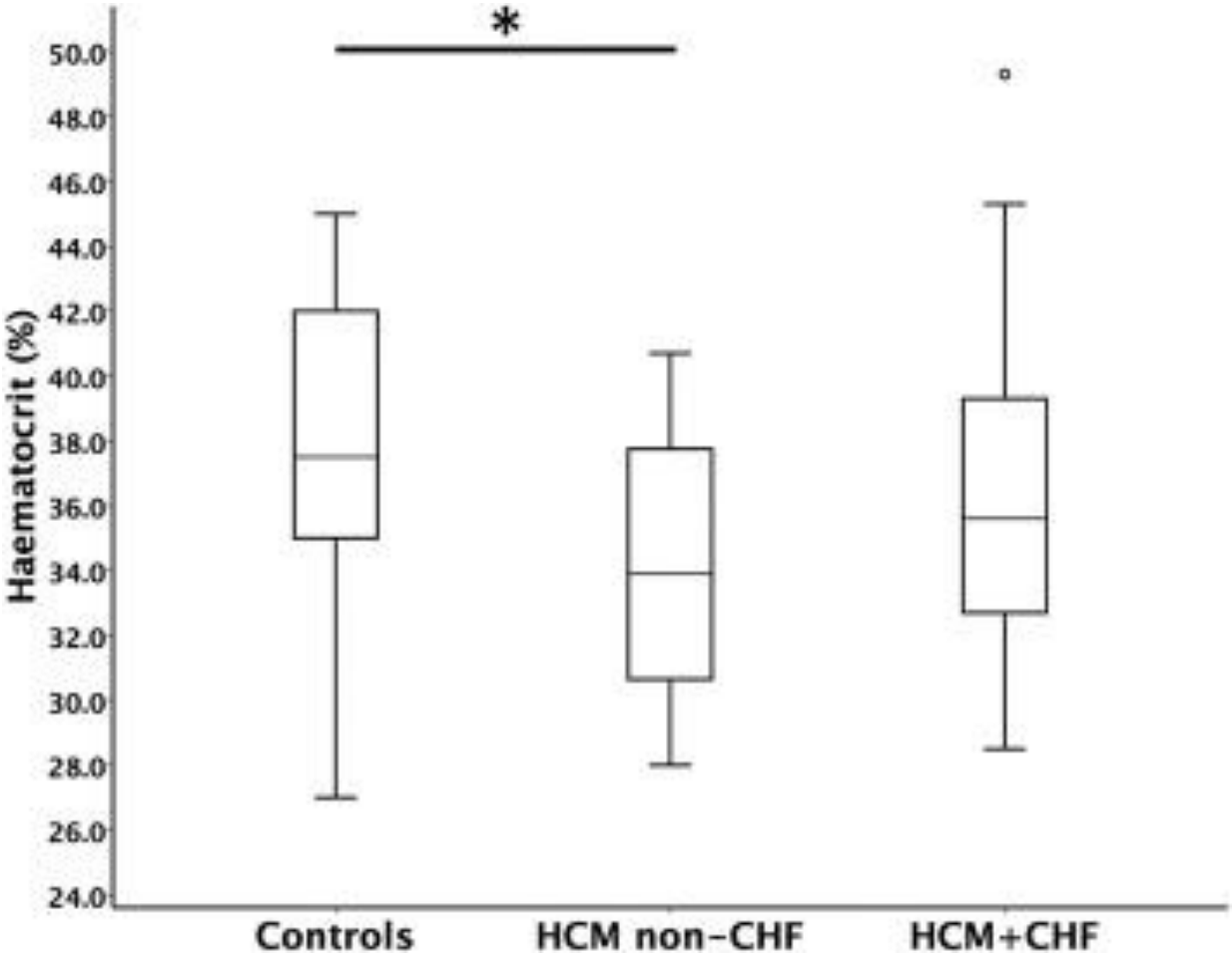
462

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

464 tertile.



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