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Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio in Dogs With Various Degrees of Myxomatous Mitral Valve Disease

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MYXOMATOUS MİTRAL VALVE DİSEASE (MMVD) is the most common cause of left-sided congestive heart failure (CHF) in dogs and is characterised by a chronic progression. The Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been proposed as promising inflammatory markers of various heart diseases in humans. Nevertheless, there is limited research on these parameters in dogs with MMVD. The study aimed to evaluate the clinical and diagnostic role of the NLR and PLR in dogs with various degrees of MMVD. This study included 15 healthy dogs and 60 dogs with MMVD classified based on the American College of Veterinary Internal Medicine (ACVIM) guidelines (Stage A-D). Complete blood count variables were determined for all dogs. NLR and PLR were calculated using the neutrophil, platelet, and lymphocyte counts. Stage D (with CHF) had significantly higher WBC, neutrophil count, NLR and PLR values than healthy dogs and other ACVIM stages. The degree of MMVD positively correlated with NLR and PLR. The best cut-off value of NLR to predict CHF was 6.84 with 81.12% sensitivity and 75.51%, and the best cut-off value of PLR to predict CHF was 280.80 with 63.64% sensitivity and 83.67%. In conclusion, the NLR and PLR could be used to determine the inflammatory state in dogs with MMVD. Also, both parameters may be a valuable aid to other conventional diagnostic methods for predicting CHF.

Keywords: Biomarker, Canine, Cardiovascular disease, Complete blood count, Heart failure, İnflammation.

Introduction

Cardiovascular system diseases are common in veterinary medicine and account for approximately 10 % of all medical cases [1,2]. Myxomatous mitral valve disease (MMVD) is the most common form of acquired heart disease in small-breed dogs, and MMVD causes about 75 % of heart disease cases in these dogs [3-6]. MMVD is characterised by mitral valve degeneration and valvular insufficiency leading to volume overload, eccentric left ventricular hypertrophy and left atrial enlargement [7]. Slow-progressing valve diseases start from the first half of life (e.g., 2 to 3 years) [8]. However, clinical findings usually develop after middle age, and disease progression leads to congestive heart disease (CHF) in late old age. Although MMVD may be seen in dogs of all sizes, it is typically seen in small-breed dogs (less than 20 kg). In particular, certain breeds are predisposed to MMVD, such as the Cavalier King Charles Spaniel (CKCS) [9,10].

The aetiology and pathophysiology of MMVD are complex and incompletely understood. However, it is characterised by

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progressive changes in cellular constituents and the intercellular matrix of the mitral valve leaflets [3]. These progressive changes lead to thickening, deformation and dysfunction of the valve and chordae tendineae. The pathological changes give rise to mitral regurgitation leading to a gradually increasing chronic volume load on the left side of the heart, resulting in CHF and cardiac remodelling [2,11,12]. Although MMVD is commonly considered a non-inflammatory condition, studies have found increased circulating inflammatory cytokines in dogs and humans with CHF due to MMVD [7,13-15]. While the presence of proinflammatory cytokines such as TNF-α, IL-1 and IL-6 may contribute to the pathogenesis of cardiac remodelling and systolic/diastolic dysfunction, systemic inflammation also plays a role in the development and progression of CHF [14,15]. Several clinical studies have demonstrated significant alterations in some inflammatory markers such as C reactive protein (CRP), white blood cell (WBC) count and tumour necrosis factor α in canine and human cardiovascular patients [13,14,16-19].

The complete blood count (CBC) is the most commonly used and quickly performed blood test in human and veterinary medicine. It is often performed as part of a medical evaluation and can be used to detect a range of disorders and monitor numerous diseases. The CBC offers a quick evaluation of alterations in different blood cells (e.g., leukocytes, neutrophils, lymphocytes and platelets) [20,21]. Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are novel inflammatory markers derived from CBC and easily performed in daily routines [20,22]. These parameters have also been reported as potential diagnostic/prognostic biomarkers in inflammatory, neoplastic and cardiovascular diseases [22-24].

Although there is much research in human and veterinary medicine regarding the role of NLR and PLR in various diseases, there are limited studies on these parameters' clinical and diagnostic role in dogs with MMVD [25,26]. Therefore, this study aimed to reveal the clinical and diagnostic role of NLR and PLR in dogs with varying degrees of MMVD and to evaluate the relationship between these parameters and the clinical severity of the disease.

Material and Methods

Animals and inclusion criteria

This Prospective study was conducted with the approval of the Aydin Adnan Menderes University Animal Experiments was following Local Ethics Committee (protocol number 64583101/2016/199). The study was carried out from 2017 to 2020, and data were collected at the Aydın Adnan Menderes University Veterinary Faculty Animal Hospital Small Animal Clinics. Seventy-five dogs, including 60 with MMVD of varying severity and 15 healthy, were included in this study. All dogs were client-owned, and their owners were informed about the study. The dogs were of different breeds, ages and sexes. The study did not include dogs with varying heart diseases (congenital or acquired). In addition, dogs with another cardiovascular disease (congenital or acquired), concomitant non-cardiac diseases, including other illnesses and chronic inflammation (e.g., infection, cancer, chronic renal failure, and pancreatitis) were excluded study. The criteria were determined based on medical history, clinical examination, laboratory analysis and imaging techniques.

Cardiological examination

Routine cardiological examination protocol (Auscultation, X-ray, electrocardiography and echocardiography) was applied to the dogs (Figure 1). Radiographic images of the thorax were taken with routine radiographic techniques (right lateral and ventral-dorsal position). Vertebral heart size was measured using a vertebral unit system, comparing the cardiac dimensions with the length of the thoracic vertebrae [27]. The six-lead electrocardiographic examinations were performed on the right lateral recumbency in a quiet place. An echocardiographic examination was performed using standard imaging planes. Faculty members (BU, GET) performed echocardiography using an ultrasonography system (Esaote MyLab 30, Genova, Italy). For this purpose, 4,0 – 7.5 MHz phased array transducers (Esaote PA 122 Vet, Genova, Italy) were used. Ventricular end-diastolic dimension (LVIDd), left ventricular (LV) end-systolic dimension (LVIDs), LV free wall thickness in diastole (LVWd) and systole (LVWs), end-diastolic interventricular septum dimension (IVSd), end-systolic interventricular septum dimension (IVSs) and left atrial to aortic root ratio (LA/Ao) were measured [28]. Also, The LV end-diastolic internal diameter (LVIDDN) normalised for body weight was determined as previously reported [LVIDd (cm)/ body weight (kg)$^{0.294}$] by Keene et al. [4] Relative MR severity was assessed based on the area of the regurgitant jet projecting into the left atrium

and the jet density of the signal using the colour Doppler [14].

Classification

The severity of the MMVD was assessed according to the American College of Veterinary Internal Medicine (ACVIM) based on the data obtained from the clinical and diagnostic evaluation [4]. Sixty dogs with MMVD were divided into four stages (Stages A-D). Predispose dogs (small breeds dogs, e.g. CKCS) with normal echocardiogram and no auscultatory heart murmur were included in Stage A, asymptomatic dogs were included in Stage B, dogs with clinical manifestations of heart failure and compensated by treatment were included in Stage C. In stage D, dogs with end-stage disease and refractory to standard therapy were included.

Laboratory analysis

Blood samples from dogs were collected via cephalic vein puncture into serum separator tubes and anticoagulant tubes containing K3-ethylenediaminetetraacetic acid (EDTA). Routine serum biochemistry parameters were analysed on an autoanalyser device (Sinnova D 280, China). CBCs were performed with an automated haematology analyser (Abacus Vet 5, Diatron). WBC count, differential count and percentages of the WBCs (Neutrophil and lymphocyte counts/ percentages) and platelet count were extracted from the routine hematologic profile. Abnormal results in CBC were verified using a manual differential from a blood smear stained with May Grunwald Giemsa [29]. Neutrophil, platelet and lymphocyte counts were extracted from the CBC. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count [20]. The PLR was defined as the platelet count divided by the absolute lymphocyte count [20].

Statistical analysis

All data were analysed using the commercial statistical software package (SPSS, version 19.0, IBM Corporation). The distribution of data was evaluated using Shapiro-Wilk tests. Normally distributed data were compared by one-way analysis of variance (ANOVA). For non-normal data, the non-parametric Kruskal Wallis test was used. The associations between two parameters and disease severity were evaluated Spearman correlation coefficient (rho). Receiver operating characteristic (ROC) curves were constructed to determine the optimal diagnostic cut-off values. Differences in $p<0.05$ value were considered statistically significant.

Results

Baseline characteristics of the dogs

The 15 dogs were considered healthy according to clinical and laboratory examinations and diagnostic imaging methods. The healthy group include several breeds such as CKCS, Maltese Terrier, Pointer, Yorkshire Terrier, Miniature Pinscher, French Bulldog, Pug and Golden Retriever. The 60 dogs with MMVD included 15 breeds. The Maltese Terrier was the most common breed, followed by Crossbreed dogs, French Bulldog, CKCS, Yorkshire Terrier, Pekingese, Golden Retriever, Pug and Pomeranian. The characteristics and some echocardiographic variables of dogs in different ACVIM stages are shown in Table 1. Some dogs in ACVIM groups depending on the severity of clinical findings and the type of arrhythmias, have received one or more cardiac medications. The cardiac medicines used and their distribution according to ACVIM groups are also presented in Table 1.

Complete Blood Count Variables

Stage D had a significantly higher mean WBC count in comparison to the healthy group ($p < 0.001$), Stage A ($p < 0.001$), Stage B ($p < 0.05$) and Stage C ($p < 0.05$) (Table 2). Also, Stage B and Stage C had significantly higher WBC counts than the healthy group ($p < 0.01$, $p \leq 0.05$, respectively) and Stage A ($p \leq 0.001$, $p < 0.5$, respectively). Although Stage B mean lymphocyte counts were significantly higher than the healthy group ($p < 0.\ 0.05$) and Stage D ($p < 0.05$), the mean lymphocyte count of all groups was within normal reference ranges [24]. The mean lymphocyte percentage of Stage D was significantly lower than other ACVIM stages ($p < 0.001$). There was no statistical significance between the groups ($p > 0.05$) in the PLT count. The mean neutrophil count and percentage of Stage D were significantly higher than the healthy group and other ACVIM stages ($p < 0.001$; $p < 0.001$, respectively). Also, Stage C had a significantly higher neutrophil count than the healthy group ($p \leq 0.05$) and Stage A ($p < 0.05$) (Table 2).

A significantly higher NLR was found in Stage D (median 13.83; IQR 6.87-23.49) compared to the healthy group ($p < 0.01$; median 5.04; IQR 4-5.99), Stage A ($p \leq 0.001$; median 4.13; IQR 3.22-5.10), Stage B ($p < 0.01$; median 3.20; IQR 1.85-5.56) and Stage C ($p < 0.01$; median 4.32; IQR 2.28-9.55) (Figure 2). The median PLR of Stage D (median 416.67; IQR 114.91-1578.95) was significantly higher than the healthy group

($p \leq 0.001$; median 198.06; IQR 161.14-283), Stage A ($p < 0.001$; median 156.10; IQR 139.24-269.23), Stage B ($p < 0.001$; median 131.74; IQR 50.63-209.55) and Stage C ($p \leq 0.001$; median 200; IQR 156.73-274.92) (Figure 3). The Stage of disease positively correlated with NLR (rho = 0.407; $p \leq 0.001$) and PLR (rho = 0.254; $p \leq 0.05$) in dogs with MMVD. The optimal cut-off point of NLR for the predicted CHF (Stage D) was 6.84, with 81.12 % sensitivity and 75.51 % specificity ($p \leq 0.001$, AUC 0.851, 95 % Cl 0.723 - 0.979) (Figure 4). The optimal cut-off point of PLR for predicting CHF (Stage D) was 280.80 with sensitivity and specificity 63.64 % and 83.67 %, respectively ($p < 0.05$; AUC 0.722, 95 % Cl 0.524 - 0.920) (Fig. 4).

Discussion

Cardiovascular diseases are a significant reason for morbidity and mortality worldwide in human and veterinary medicine. Mitral valve disease is characterised by a chronic progression and is the most common cause of congestive heart failure in dogs [3]. NLR and PLR are used in human medicine as inflammatory response biomarkers and potential diagnostic/prognostic biomarkers in many diseases, such as cardiovascular diseases [19]. Recently, many studies on NLR and PLR have also been conducted in veterinary medicine. [20,24,30-33]. There is limited study on these parameters in dogs with heart disease [25,26]. Therefore, the study aimed to evaluate the possible clinical and diagnostic significance of the NLR and PLR in dogs with various degrees of MMVD.

Total leukocyte counts and subtypes are widely used in clinical practice to detect the inflammatory process [34]. Studies in humans and dogs have demonstrated the relationship between heart failure and WBC count and subtypes [17]. Human studies have revealed that increased WBC count is a predictive biomarker of cardiovascular disease, acting independently of other well-known risk factors and being a marker of inflammation [22]. Also, different WBC subtypes are believed to have a more decisive role in predicting cardiovascular disease risk than the total WBC count [19]. There are few studies on WBC and its subtypes in dogs with different cardiovascular diseases. Hamilton-Elliott et al. [35] reported that dogs with CHF have significantly higher WBC and neutrophil counts than the control group. Two other studies on dogs classified by the Small Animal Cardiac Health Council (ISACHC) revealed that dogs in ISACHC III (dogs with CHF) had significantly higher WBC and neutrophil counts than the control group and different ISACHC groups [17,18]. Also, Domanjko Petrič et al. [17] reported that the WBC and neutrophil counts of dogs in ISACHC III were above normal reference values. Similarly, in this study, dogs in Stage D (with CHF) had a significantly higher mean WBC and neutrophil count/percentage than dogs in the healthy group and other ACVIM stages. The mean WBC and neutrophil count of Stage D were above the reference values reported for the dogs. There is much evidence that inflammatory processes play a role in the pathophysiology of heart failure, and the blood concentrations of inflammatory parameters increase with the severity of the disease [15,36]. WBC and neutrophils are also among these inflammatory parameters and secrete most of the cytokines associated with the development of heart failure [17,35]. Increased WBC and neutrophil count/percentage in Stage D in this study may be according to the severity of the disease and due to systemic inflammation associated with disease progression, as mentioned above. Also, the physiological stress associated with their diseases may have contributed to this increase.

Alteration in the immune system can also play an important role in heart disease progression [18]. The severity of congestion or the systemic release of cytokines induced by acute decompensation episodes can lead to lymphocyte apoptosis in patients with heart failure [37]. Also, higher cortisol and catecholamine levels due to physiological stress in these patients may play a crucial act in the number and function of lymphocytes [38]. Human studies have demonstrated a significant reduction in lymphocyte count in people with CHF and that this low total lymphocyte count can be used as an independent predictor of mortality. In contrast to human studies, no significant lymphopenia was found in the limited number of studies in dogs with CHF [18,35,39]. In line with these studies, the mean lymphocyte count and percentage of all groups were within reference ranges in our study. Although the dogs in Stage D had a relatively lower mean lymphocyte count than the control group and other ACVIM stages, there was no statistically significant difference ($p>0.05$). Also, the Stage D lymphocyte percentage was significantly lower than the other ACVIM

stages (Table 2). One of the most important components of the inflammatory response is adaptive immunity, which consists mainly of T lymphocytes and B lymphocytes. It has been previously shown that both CD4+ and CD8+ lymphocyte percentages are reduced in dogs with heart failure [18,39]. The fact that the lymphocyte percentages of dogs in Stage D were lower in this study than in the other groups may be related to the decrease in these lymphocyte subtypes due to inflammation associated with heart failure. Also, as noted above by the researchers, lymphocyte apoptosis or physiological stress seen in patients with heart failure may have contributed to this condition.

The neutrophil-to-lymphocyte ratio is one of the inflammatory biomarkers used to evaluate the severity and prognosis of various diseases in humans and animals [20,23,24,30,31,33,37,40-44]. In addition to determining the inflammatory response, some of these studies revealed NLR's diagnostic and predictive importance. In human medicine, NLR has been studied extensively in patients with cardiovascular problems such as ischemic heart diseases, valvular diseases, heart failure, and arrhythmias [19,45]. Various studies have reported that patients with decompensated HF have significantly higher NLR values than healthy groups, and NLR is a useful parameter in predicting the mortality and presence of heart failure [37,38]. The relationship between the degree of the disease and the NLR values has been revealed [19,37,38]. There are two studies on NLR in dogs with heart disease [25,26]. These studies showed that NLR was higher in dogs with CHF than in the control and asymptomatic MMVD groups and changed with treatment. The current study found that the median NLR of Stage D was significantly higher than healthy dogs and other ACVIM stages. Also, similar to the human studies, the degree of disease fairly positively correlated with NLR (rho = 0.420; $p \leq 0.001$). Heart failure and systemic inflammation are interconnected processes that continually reinforce each other. The NLR is a proportion of different (neutrophil and lymphocyte) but complementary immune parts. Thus, NLR combines the detrimental effects of neutrophils responsible for non-specific active inflammation and the change in lymphocyte count, a marker of poor general health and physiological stress [19,37,38]. In the current study, the increase in NLR of Stage D may be related to the activation of the mentioned specific pathophysiological pathways.

The platelet-to-lymphocyte ratio is an easy, cheap, and fast inflammatory parameter like NLR that can be used as a diagnostic and prognostic marker in various diseases [46-48]. In the limited number of studies on PLR in dogs, the diagnostic and prognostic role of PLR has not been fully understood [20,23,24,31,49]. In parallel with these studies, the role of PLR could not be demonstrated in the few studies conducted in dogs with MMVD [25, 26]. However, many human studies have revealed that PLR is a useful predictor of morbidity and mortality in different heart diseases [19,37,47,48]. In this study, the median PLR value of dogs in Stage D was significantly higher than the healthy group and other ACVIM stages (Figure 3). Also, the degree of disease was poorly positively correlated with the PLR value (rho = 0.254; p = 0.050). Changes in lymphocytes and platelet counts can explain this situation and various inflammatory processes induced by heart failure [47,48,50]. The platelet count increases with an inflammatory stimulus, and platelets secrete cytokines, thus acting like inflammatory cells. Also, serum cortisol levels rise during systemic stress, decreasing lymphocyte count [47,48]. Therefore, the current study's elevated PLR levels in Stage D may indicate the inflammation and/or progression of heart disease.

This study had some limitations that should be considered in interpretation. First, the number of dogs in the study groups was comparatively small. Many dogs with MMVD were excluded from the study because of concurrent disease (e.g. another cardiovascular problem, metabolic or inflammatory disease) and lost data. Second, dogs with MMVD and healthy were not strictly matched for breed, age, and gender. Although most dogs were small breeds, a few large and medium-breed dogs were in the healthy dogs and study group. Third, blood samples were taken from dogs once brought to the clinic, so NLR and PLR were based on a single measurement. Fourth, preanalytical, analytical and post-analytical factors may affect haematological parameters. Finally, although concurrent diseases were determined based on medical history, clinical examination, laboratory analysis, and imaging technique, there is still the possibility that we could not identify some subclinical conditions.

Conclusion

In conclusion, NLR and PLR were significantly higher in dogs with Stage D than in the healthy group and other ACVIM stages. There was a positive correlation between the degree of MMVD and NLR and PLR values. This condition can be used in determining the severity of the inflammatory state. It has also been demonstrated that both parameters can help predict CHF. However, these markers are nonspecific and should be used with other specific diagnostic methods. Further warrant studies are needed to explore the diagnostic and predictive value of NLR and PLR in dogs with MMVD.

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Conflict of interest

The author declare that have no conflict of interest.

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Contribution of authors

Gulten Emek TUNA: Conceptualisation, formal analysis, funding acquisition, investigation, methodology, project administration, validation, writing-original draft, writing-review & editing

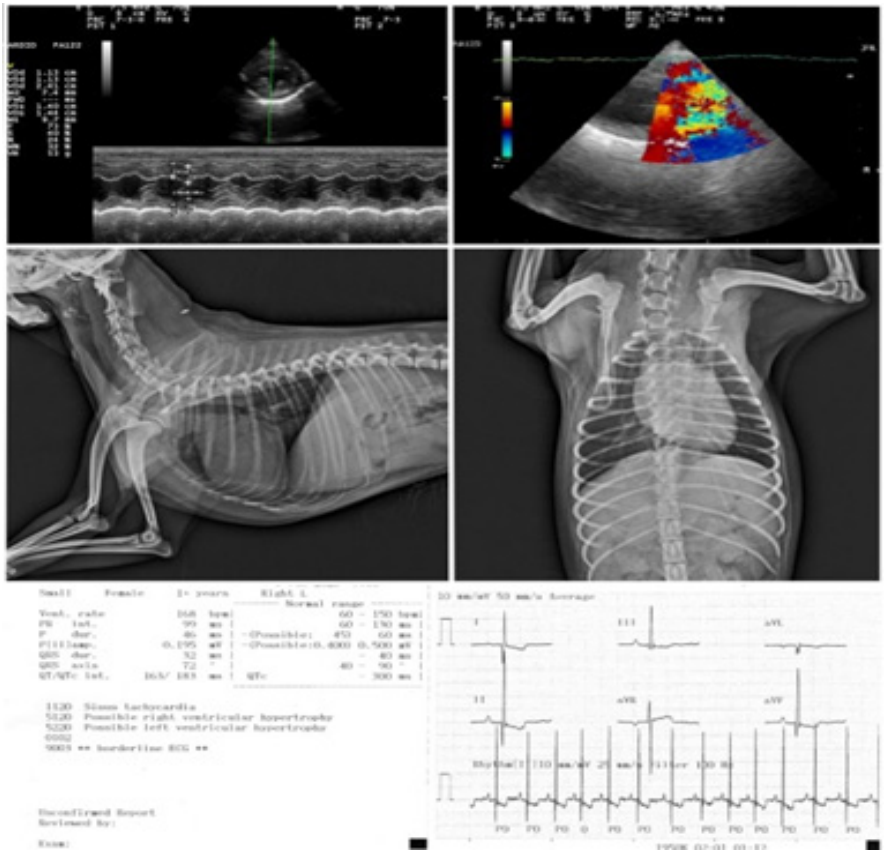

Fig.1. X-ray, electrocardiography and echocardiography samples from the cardiological examination protocol performed in the study

TABLE 1. Baseline characteristics data and some echocardiographic variables of dogs in different ACVIM stages.

	Stage A n=15	Stage B n=15	Stage C n=19	Stage D n=11	Healthy n=15
Breed (n)					
Maltese Terrier	4	4	5	3	3
Crossbreed dogs	-	3	5	2	-
Cavalier King Charles Spaniel	6	2	-	1	3
French Bulldog	2	3	3	1	1
Yorkshire Terrier	3	-	3		2
Pekingese	-	-	2	2	-
Golden Retriever	-	-	1	2	1
Pug	-	2	-	-	1
Pomeranian	-	1	-	-	-
Pointer	-	-	-	-	2
Miniature Pinscher	-	-	-	-	2
Sex (n)					
Female	9	12	4	2	8
Male	6	3	15	9	7
Age (years)	3.33 ± 0.97	4.90 ± 1.27	10.24 ± 2.52	12.91 ± 2.77	3.47 ± 1.04
Bodyweight (kg)	6.04 ± 2.73	7.12 ± 3.93	8.50 ± 5.98	10.10 ± 10.71	9.39 ± 8.66
VHS	<10.5	<10.5	>10.5	>10.5	<10.5
MR (%)	-	20-40	> 40	> 40	-
LA (cm)	1.53 ± 0.18	1.62 ± 0.38	2.40 ± 0.34	2.55 ± 0.45	1.64±0.33
LA/Ao	1.33 ± 0.12	1.55 ± 0.23	2.20 ± 0.38	2.51 ± 0.43	1.46±0.14
LVIDDN	1.54 ± 0.07	1.61 ± 0.12	1.98 ± 0.07	2.01 ± 0.11	1.48±0.13
Treatment (n)					
ACE inhibitor	-	1	18	11	-
Pimobendan	-	1	2	4	-
Diuretic	-	-	19	11	-
Calcium channel blockers	-	-	6	3	-
Beta-blockers	-	-	4	2	-
Cardiac glycoside	-	-	1	5	-

Abbreviation: ACE inhibitör, angiotensin-converting enzyme inhibitors; LA, left atrium; LA/Ao, the ratio of the left atrium to aortic root; LVIDDN, left ventricular end-diastolic diameter normalised for body weight; MR, mitral regurgitation.

TABLE 2. Selected complete blood count variables of dogs with healthy and various degrees of MMVD.

Parameters		Healthy Dogs	Stage A	Stage B	Stage C	Stage D	p
WBC ($\times10^3/\mu L$)	mean±SD	9.66±2.46^c	9.22±1.25^c	13.02±3.73^b	13.47±6.42^b	17.65±4.44^a	< 0.001
	Min-Max	6.15-14.60	6.48-11.10	6.89-20.99	4.49-28.50	11.49-25.68	
Neutrophil ($\times10^3/\mu L$)	mean±SD	7.35±2.02^c	6.66±1.25^c	9.36±2.88^{bc}	10.70±6.25^b	15.32±4.25^a	< 0.001
	Min-Max	4.73-10.64	4.42-8.90	4.82-13.25	2.78-26.10	9.16-22.11	
Neutrophil (%)	mean±SD	75.06±3.11^b	71.75±10.80^b	71.95±10.04^b	76.12±10.98^b	86.26±6.94^a	≤ 0.001
	Min-Max	67.38-79.39	54.33-88.73	58.04-89.09	59.65-92.03	69.62-94.77	
Lymphocyte ($\times10^3/\mu L$)	mean±SD	1.63±1.17^b	1.86±0.81^{ab}	2.94±1.72^a	2.11±1.10^{ab}	1.41±0.95^b	< 0.05
	Min-Max	0.11-5.42	0.99-3.99	0.40-7.02	0.62-4.42	0.22-2.95	
Lymphocyte (%)	mean±SD	15.08±3.47^a	20.15±8.78^a	22.18±9.82^a	18.97±10.56^a	8.23±6.31^b	≤ 0.001
	Min-Max	10.30-23.40	9.91-41.13	3.89-34.10	2.53-32.29	1.13-22.35	
Platelet ($\times10^3/\mu L$)	mean±SD	318.93±80.4	311±115.95	292.87±131.23	458.73±227.63	440.36±313.23	< 0.05
	Min-Max	204-448	105-525	103-610	149-910	152-1001	

Abbreviation: Min, minimum; Max, maximum; WBC, White blood cell; SD, standard deviation. Within the same row, values with different letters in the superscript indicate a significant difference between groups in post hoc tests (P<0.05). p, ANOVA or Kruskal–Wallis derived P-value.

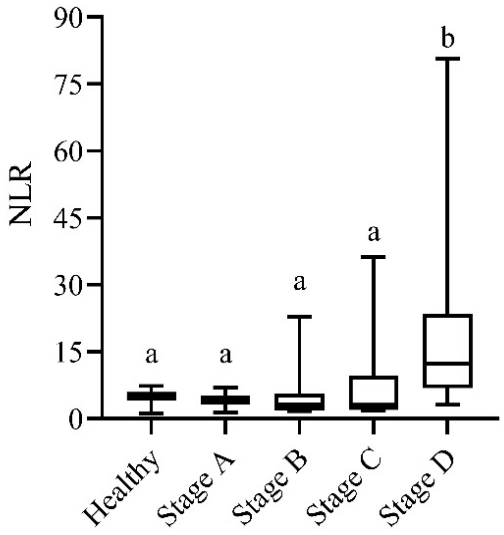

Fig. 2. A box and whisker plot showing neutrophil-to-lymphocyte ratio in healthy dogs and dogs with various degrees of MMVD.

Abbreviation: NLR, neutrophil-to-lymphocyte ratio. Different letters indicate statistically significant differences at $p < 0.05$.

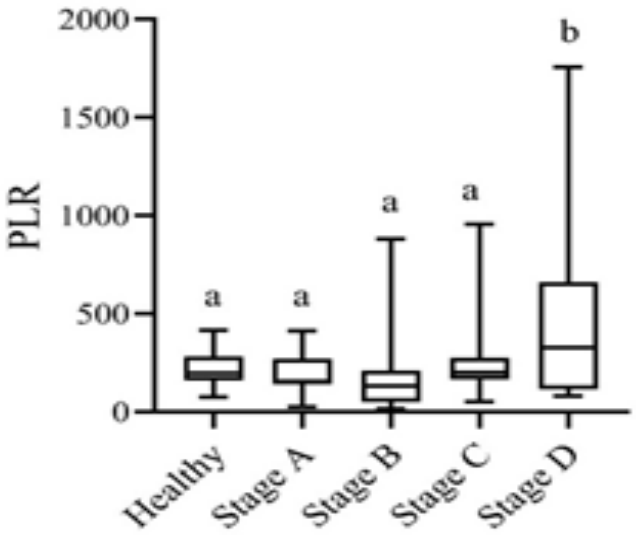

Fig. 3. A box and whisker plot showing platelet-to-lymphocyte ratio in healthy dogs and dogs with various degrees of MMVD.

Abbreviation: PLR, platelet-to-lymphocyte ratio. Different letters indicate statistically significant differences at $p< 0.05$.

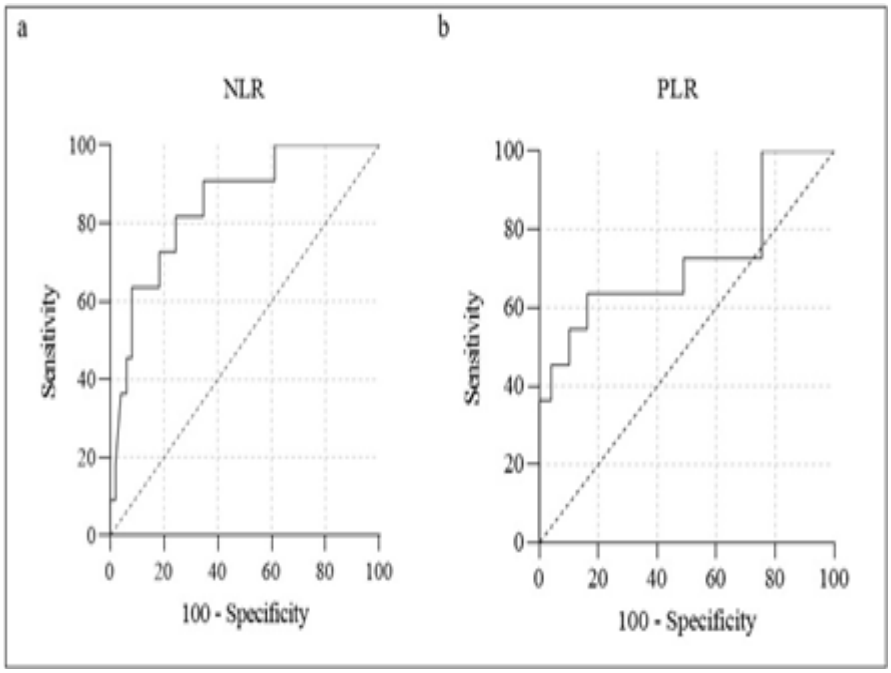

Fig. 4. The receiver operating characteristic of NLR (a) and PLR (b) for predicting the CHF in dogs with MMVD. a) AUC = 0.851; 95% CI 0.723 - 0.979 b) AUC = 0.722; 95% CI 0.524 - 0.920.

Abbreviation: AUC, area under the curve; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio

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