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Changes in reticulocyte values and reticulocyte lymphocyte ratio in cats with hypertrophic cardiomyopathy

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a common heart disease in cats and can lead to hypoxia, leading to deterioration of cardiac function. In response to hypoxia, the bone marrow increases reticulocyte production to enhance oxygen-carrying capacity. In this study, reticulocyte count (RET), immature reticulocyte fraction (IRF), low fluorescence ratio (LFR), medium fluorescence ratio (MFR), high fluorescence ratio (HFR) values and reticulocyte/lymphocyte (RET/LYM) ratio were evaluated in cats with HCM without anemia. The study group consisted of 30 cats diagnosed with HCM, and the control group consisted of 30 healthy cats. According to the American College of Veterinary Internal Medicine classification manual, cats with left ventricular wall thickness <5 mm were classified as normal, while those with wall thickness ≧6 mm were classified as having HCM. In addition, cats with a left atrium/aorta ratio <1.5 were classified as normal, while cats with a left atrium/aorta ratio ≧of 1.6 were classified as having HCM. Physical, radiographic, echocardiographic examination and hematological analysis were performed on the cats in both groups. Following detailed echocardiographic and radiographic examinations, red blood cell, LYM, RET, IRF, LFR, MFR, and HFR values were measured. RET, IRF, MFR, HFR, RET/LYM values were significantly higher in cats with HCM than in healthy controls ($P<0.05$). LYM and LFR values were significantly lower in cats with HCM than healthy controls ($P<0.05$). In cats with HCM, non-anemic reticulocytosis due to hypoxia and decreased lymphocyte count due to cardiac dysfunction were observed. In addition, an increased RET/LYM ratio was observed in cats with HCM.

Key words: cats; hypertrophic cardiomyopathy; reticulocyte; reticulocyte/lymphocyte ratio; lymphocyte

Introduction

Hypertrophic cardiomyopathy (HCM) is the most prevalent type of cardiomyopathy found in cats, and can impact up to about 15% of the domestic cat population, usually manifesting as a subclinical condition (KITTLESON and CÔTÉ, 2021). HCM is characterized by concentric left ventricular hypertrophy (LVH), where the left ventricular (LV) wall becomes thickened, without the presence of any other cardiac or systemic condition that could

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cause such significant hypertrophy (OMMEN et al., 2020). In HCM, the thickening of the left ventricular wall reduces the volume of blood entering the ventricle. This results in decreased oxygen delivery to the body and inadequate cardiac output. Over time, the cardiac muscle cells, which are continuously working to pump blood, become damaged and replaced by fibrous tissue. Consequently, the heart begins to contract more forcefully during diastole. This increased contraction causes blood to accumulate in the atrium, leading to atrial enlargement. The elevated pressure consequently triggers a cardiac remodeling process due to the expansion of the atrium (CHONG, 2023).

In HCM, the heart muscle becomes abnormally thickened, impairing the heart's ability to pump blood. This makes it harder for the body to deliver enough oxygenated blood to organs and tissues, causing hypoxia (KITZ et al., 2019). In addition, the increase in left ventricular pressure is transmitted to the left atrium and the pulmonary vessels. The pulmonary capillaries, which are the most permeable structures in the vascular system, filter fluid from the vascular space into the lung parenchyma. When hydrostatic pressure in the pulmonary capillaries becomes significantly elevated, pulmonary edema occurs due to fluid accumulation. As a result of this condition, dyspnea and hypoxia occur (ITKIN et al., 2021).

Reticulocytes are non-nucleated, immature erythrocytes, with lengthy RNA molecules inside. After spending around two days in the bone marrow, reticulocytes are discharged into the bloodstream and undergo a two-day differentiation process to become adult erythrocytes (CHOI et al., 2022). Reticulocytosis refers to an increase in the number of reticulocytes in the blood. It is a quantitative indicator used to differentiate regenerative anemia, where erythrocyte production is increased, from non-regenerative anemia. An elevated reticulocyte count in non-anemic circumstances is referred to as reticulocytosis in the absence of anemia (PATTULLO et al., 2015). Earlier studies have shown that in non-anemic states, the reticulocyte count may be elevated as a result of an inflammatory response, splenic contraction, or a physiological response to stimulation (HORVATH et al., 2014; PATTULLO et al., 2015). Reticulocytosis in hypoxic patients usually occurs as an attempt by the body to increase its oxygen-carrying capacity. In hypoxia, the bone marrow produces more erythrocytes, and this process increases the number of reticulocytes (BAGWELL and STEWARD, 2019). In conditions of hypoxia, the bone marrow increases the production of erythropoietin. Erythropoietin stimulates the production of red blood cells. This increase also leads to a rise in the number of reticulocytes, as reticulocytes are immature red blood cells circulating in the body. Hypoxic conditions can be a prominent indicator of reticulocytosis, which is considered an adaptive response, aimed at improving the body's oxygen-carrying capacity (JI, 2023).

A significant contribution to the field of veterinary medicine would be the investigation of hypoxic reticulocytosis in the lack of anemia in particular disorders, given the increasing interest in reticulocytosis in the absence of anemia (FUCHS et al., 2018). The aim of this study was to determine the prevalence of reticulocytosis, and to determine the reticulocyte-lymphocyte ratios in cats with HCM but without anemia.

Materials and methods

Patient selection. The study group consisted of 30 cats brought with complaints of exercise intolerance, respiratory distress and cough, which were diagnosed with HCM. The control group consisted of 30 healthy cats brought for vaccination, antiparasitic agents application or general examination. The cats were examined for non-cardiac diseases that could lead to HCM, and cats with hyperthyroidism, renal failure, diabetes, anemia or infection were excluded from the study.

Diagnosis of HCM. The combination of clinical signs (dyspnea, abnormal lung and cardiac sounds), radiographic findings (increased pulmonary opacity due to unstructured or mixed interstitial alveolar pattern), and echocardiographic studies led to the diagnosis of hypertrophic cardiomyopathy (HCM).

Echocardiographic examination was performed using a Mindray®, VETUS 9 ultrasound device with a phased array cardiac probe. The cats were positioned in right lateral and left lateral recum-

bency on a specially prepared echocardiography table for parasternal short-axis and long-axis echocardiographic evaluations (KUO et al., 2024). No anesthesia was administered to the patients for the examination. For preparation, the fur on the chest wall was shaved to include the 3rd to 6th intercostal spaces, and ultrasound gel was applied to the area (ST JOHN and DURHAM, 2017). Echocardiograph- ic assessment included evaluation of left atrial and ventricular dimensions, contraction and relaxation, and the thickness of the interventricular septum and the left ventricular free wall. Additionally, the presence of regurgitant flow across the mitral and tricuspid valves was assessed using color Doppler ultrasound (BOON, 2010). Doppler techniques were used to examine the presence of regurgitant flow across the aortic and pulmonary valves, and to assess pulmonary arterial flow. M-mode echocardiographic measurements included sequential assessment of the right ventricular diastolic diameter, interventricular septal diastolic dimension, left ventricular diastolic diameter, left ventricular free wall diastolic dimension, interventricular septal systolic dimension, left ventricular systolic diameter, and left ventricular systolic wall dimension, with ejection fraction percentage and fractional shortening percentage recorded (GUGJOO et al., 2014). A 2D right parasternal short-axis image was used in M-mode echocardiography to obtain the aortic and left atrial diameters and the left atrium/aorta ratio. According to the published American College of Veterinary Internal Medicine (ACVIM) classification guidelines (LUIS FUENTES et al., 2020), left ventricular wall thickness at end-diastole was measured over three cardiac cycles, with cats having wall thickness <5 mm classified as normal, and those with wall thickness ≧6 mm classified as having hypertrophic cardiomyopathy.

Diagnosis of reticulocytosis in the absence of anemia. Red blood cell (RBC), lymphocyte (LYM), reticulocyte count (RET), immature reticulocyte fraction (IRF), low fluorescence ratio (LFR), medium-fluorescence ratio (MFR), and highly fluorescent ratio (HFR) values were measured using the Mindray®, BC60R, VET Hi-End laser and a fluorescence hematology analyzer.

Statistical analysis. The data were analyzed using the IBM SPSS Statistics for Windows program (IBM Corp., Armonk, N.Y., USA). Quantitative data were assessed for normality using the Shapiro-Wilk test. While the RBC and LYM values had a normal distribution, the RET, IRF, LFR, MFR HFR and RET/LYM values did not have a normal distribution. Where the data were normally distributed, before using the independent t-test, the two groups were assessed for equality of variances. For this purpose, Levene's test was used to determine whether the standard deviation of the control group was expected to be the same as the standard deviation of the study group. Since the RBC value had a normal distribution and also showed equality of variance, the independent t-test was used. Although the LYM value had a normal distribution, it did not show a homogeneous distribution, so the Welch t-test was used. The non-parametric Levene's test was used to verify the equality of variances in samples with non-normal distribution (RET, IRF, LFR, MFR HFR and RET/LYM). For this purpose, three new variables were automatically created through the SPSS program: ranked data, group mean rankings, and deviations from mean rankings. As a result of this test, it was determined that all non-parametric data had variance equality. Therefore, the Mann-Whitney U test was applied to data that did not have a normal distribution. Means and standard deviation were obtained for the continuous variables when normally distributed. Also, the median and interquartile range were obtained when the distribution was non-normal. P values less than 0.05 were considered significant.

Results

The hematological data are given in Table 1. No significant difference was found between the groups in terms of RBC (P=0.47). There was a significant difference (P<0.05) in LYM between the control and study groups. When the RET was compared between the two groups, it was higher in the study group than the control group, and this was found to be a significant difference (P<0.05) (Fig. 1a). IRF (Fig. 1b), MFR (Fig. 1c), LFR (Fig. 1d), and HFR (Fig. 1e) values are different indicators of reticulocyte parameters, and these values

Table 1. Hematology data in the control (n=30) and study groups (n=30)

Hematologic parameters	Control group (n=30)			Study group (n=30)			P-value
	Mean±SD	**Median**	**Interquartile range**	**Mean±SD**	**Median**	**Interquartile range**	
RBC (10^{12}/L)	8.86±1.65			9.18±1.78			=0.47
LYM (10^9/L)	4.14±1.08			1.37±0.61			**<0.05**
RET (10^9/L)	25.42±9.79	23.22	12.27	64.95±27.24	61.30	44.20	**<0.05**
IRF (%)	11.98±5.84	10.36	5.34	29.64±13.41	31.10	25.43	**<0.05**
MFR (%)	8.09±4.31	7.15	4.93	23.03±7.53	23.20	12.33	**<0.05**
LFR (%)	86.30±6.73	89.80	11.60	67.43±13.81	65.50	24.05	**<0.05**
HFR (%)	0.79±0.76	0.6	1.50	5.07±4.98	3.35	8	**<0.05**
RET/LYM	6.56±2.99	6.24	4.70	56.33±33.09	52.85	40.38	**<0.05**

RBC: Red blood cell; LYM: Lymphocyte; RET: Reticulocyte; IRF: Immature reticulocyte fraction; MFR: Medium fluorescence ratio; LFR: Low fluorescence ratio; HFR: High fluorescence ratio

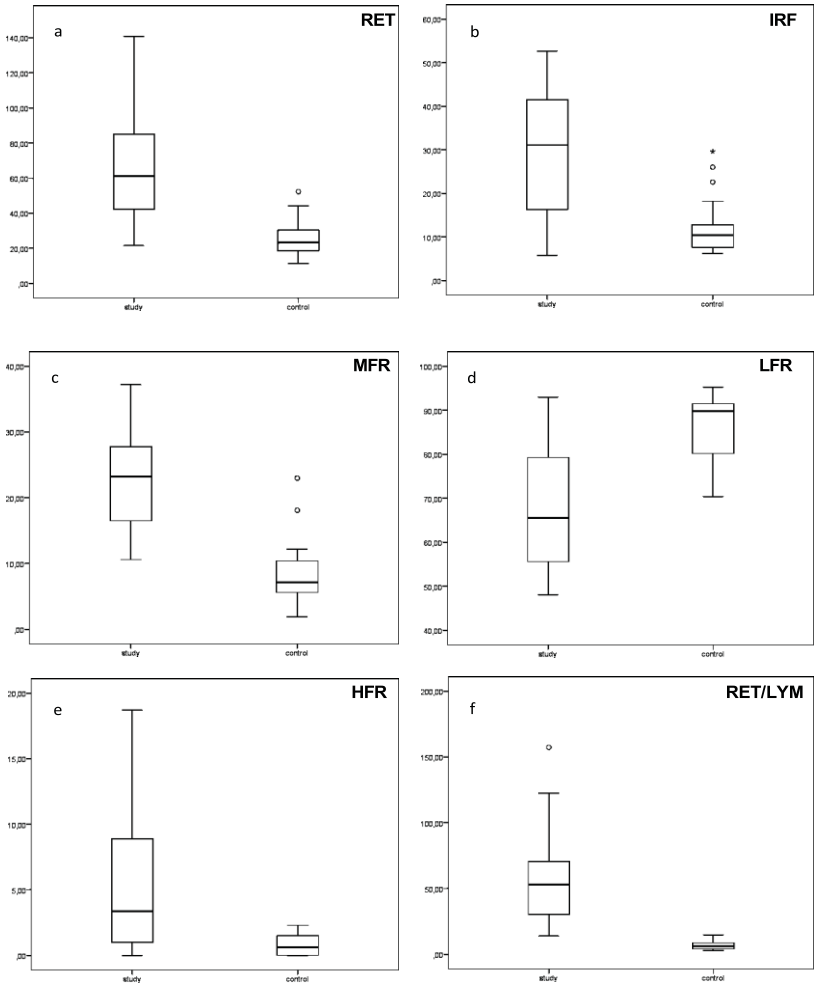

Fig. 1. Box-and-whiskers plot of RET (a), IRF (b), MFR (c), LFR (d), HFR (e), and RET/LYM (f) in 60 cats
For each plot, the box represents the interquartile range, the horizontal line in each box represents the median, and the whiskers denote the range

were higher in the study group than in the control group. This difference was statistically significant ($P<0.05$). When the RET/LYM (Fig. 1f) was examined in the two groups, it was higher in the control group than the study group, and this was found to be a significant difference ($P<0.05$).

Discussion

The reticulocyte lymphocyte ratio has been shown to be a major predictive factor in cats with HCM. The reticulocyte lymphocyte ratio is the number of reticulocytes divided by the number of lymphocytes. Decreases in the quantity of lymphocytes have been reported in cardiac, neoplastic, and inflammatory diseases in cats. Under physiological stress, endogenous cortisol and catecholamines will reduce the number of lymphocytes (NAITO et al., 2021; FRIES et al., 2022). Cats suffering from congestive heart failure due to cardiomyopathies, such as HCM, have shown elevated levels of tumor necrosis factor alpha and serum amyloid A in their plasma (VAN HOEK et al., 2020). Tumor necrosis factor alpha may be a catalyst for peripheral lymphocyte death, which in turn may exacerbate lymphopenia, according to the available data (TAKANO et al., 2007). Myocardial infiltrates of inflammatory cells, particularly neutrophils and lymphocytes, have been found in cats with mild preclinical HCM (KHOR et al., 2015). This situation also explains the decrease in the number of lymphocytes in cardiac disorders.

An elevated reticulocyte count in non-anemic circumstances is known as reticulocytosis in the absence of anemia (PATTULLO et al., 2015). Prior research has demonstrated that, in non-anemic circumstances, reticulocyte counts may be elevated by the body's response to excitement, splenic constriction, or inflammatory response (HORVATH et al., 2014; PATTULLO et al., 2015). Additionally, some studies have shown that patients with reticulocytosis in the absence of anemia have a worse prognosis (ZHANG et al., 2021). Examining reticulosis in the lack of hypoxic anemia in certain disorders will therefore be crucial to veterinary medicine, especially given the growing interest in reticulosis in the absence of anemia (CHOI et al., 2022). According to a recent study, reticulocytosis in animals without anemia is becoming more common each year, and hypoxia is the cause of reticulocytosis in animals without anemia (PATTULLO et al., 2015). Later, a study using a larger sample determined that reticulocytosis in dogs in the absence of anemia was caused by heart disease or respiratory disease (i.e., hypoxia) (FUCHS et al., 2018). HCM is the most common heart disease in cats, and can cause respiratory signs of hypoxia and pulmonary edema progression (WARE et al., 2021). HCM in cats may be primary, or secondary to some diseases. Therefore, non-cardiac diseases that can cause HCM in cats should be distinguished. The most important of these is hyperthyroidism. In cats with hyperthyroidism this results in excessive production of thyroxine (T4) and triiodothyronine (T3). These are powerful hormones that accelerate metabolism and affect various systems in the body (CARNEY et al., 2016). As a result of the increase in these hormones, tachycardia, shortening of isovolumic systolic time, an increase in cardiac output and a decrease in vascular resistance may occur. As a result of all these events, secondary HCM may occur (KITTLESON and CÔTÉ, 2021). Therefore, in cats with hyperthyroidism, cardiac troponin and NT-proBNP levels may increase (SANGSTER et al., 2014). Hyperthyroidism must be distinguished in these patients.

Reticulocytes in the blood rise on days 2-4 and peak on days 5-7 in the event of hypoxia (MOSQUEIRA et al., 2012). Reduced partial pressure of oxygen is detected by the renal cortex's peritubular interstitial cells, which then deactivate oxygen-dependent prolyl hydroxylase. Thus, erythropoietin synthesis is stimulated by hypoxia-inducible factors that remain undamaged. Reticulocytosis in the absence of anemia typically results in an increase in the immature reticulocyte fraction (IRF), medium-fluorescence ratio (MFR) and highly fluorescent ratio (HFR). This is because the bone marrow responds to a stimulus (such as increased erythropoietin levels) by producing more reticulocytes, including immature forms. Therefore, in cases of reticulocytosis without anemia, an elevated IRF would be expected because of the greater proportion of young reticulocytes released from the bone marrow into the circulation (ADANE and ASRIE, 2021). In the absence of anemia, the low fluo-

rescence ratio (LFR) is usually reduced in the presence of reticulocytosis. This is because the LFR reflects the presence of immature reticulocytes, and it tends to decrease as the proportion of immature reticulocytes decreases, particularly with increased production of more mature reticulocytes (ADANE and ASRIE, 2021).

One of the processes of hypoxic reticulocytosis in the absence of anemia is erythropoiesis, which is increased by erythropoietin, which drives erythroid progenitor cell division and differentiation in the bone marrow (MOSQUEIRA et al., 2012; CHOI et al., 2022).

Conclusions

This study has some limitations and it was an observational study. For example, neither ante-mortem evaluation of myocardial inflammation using cardiac magnetic resonance imaging, nor the measurement of circulating inflammatory cytokines were examined in these investigations. However, even with these data, it is clear that non-anemic reticulocytosis occurs due to hypoxia in cats with HCM, and the lymphocyte count decreases due to cardiac dysfunction. Finally, the reticulocyte-lymphocyte ratio was observed to be increased in cats with HCM. To our knowledge, this is the first study to assess reticulocytosis in the absence of anemia in cats with HCM. A study with a larger sample and longer study period is necessary to determine whether reticulocytosis in the absence of anemia can be used as a prognostic factor for HCM in cats.

Ethics approval

This study was approved by the X University Animal Experiments Local Ethics Committee (protocol number E-68489742-604.01-2400218615).

Declaration of competing interest

The authors declare no conflict of interest

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SAŽETAK

Hipertrofijska kardiomiopatija (HCM) česta je bolest srca u mačaka, koja može uzrokovati hipoksiju i dovesti do pogoršanja srčane funkcije. Kao odgovor na hipoksiju, koštana srž povećava proizvodnju retikulocita kako bi povećala kapacitet prijenosa kisika. Istraživanje je provedeno na mačkama s hipertrofijskom kardiomiopatijom bez anemije, a procjenjivani su broj retikulocita (RET), nezrela frakcija retikulocita (IRF), udio retikulocita niske fluorescencije (LFR), udio retikulocita srednje fluorescencije (MFR), udio retikulocita visoke fluorescencije (HFR) i omjer retikulocita i limfocita (RET/LYM). U istraživanoj skupini bilo je 30 mačaka s dijagnozom HCM-a, a u kontrolnoj skupini 30 zdravih mačaka. Prema klasifikacijskom priručniku American College of Veterinary Internal Medicine, mačke s debljinom stijenke lijeve klijetke <5 mm svrstane su u skupinu zdravih mačaka, dok su mačke s debljinom stijenke ≧6 mm svrstane u skupinu mačaka s HCM-om. Nadalje, mačke s omjerom lijevog atrija i aorte <1,5 svrstane su u skupinu zdravih mačaka, dok su mačke s omjerom lijevog atrija i aorte ≧1,6 svrstane u skupinu mačaka s HCM-om. U objema skupinama učinjen je klinički pregled, radiografske i ehokardiografske pretrage te hematološke pretrage. Nakon detaljnih radiografskih i ehokardiografskih pretraga izmjerene su vrijednosti eritrocita, limfocita, retikulocita, IRF, LFR, MFR i HFR. Vrijednosti retikulocita, IRF, MFR, HFR te omjer retikulocita i limfocita bili su znakovito veći ($P<0,05$) u mačaka s HCM-om nego u zdravih mačaka u kontrolnoj skupini. Vrijednosti retikulocita i limfocita bile su znakovito manje ($P<0,05$) u mačaka s HCM-om nego u mačaka u kontrolnoj skupini. U mačaka s HCM-om uočene su neanemijska retikulocitoza uzrokovana hipoksijom i smanjenim brojem limfocita, što je posljedica srčane disfunkcije. Osim toga, u mačaka s HCM-om uočen je povećan omjer retikulocita i limfocita.

Ključne riječi: mačke; hipertrofijska kardiomiopatija; retikulociti; omjer retikulocita i limfocita; limfociti