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Galectin-3 and cardiac biomarkers in cats with cardiomyopathy[1]

Özge Şahin[2], Öznur Aslan[3]*

ABSTRACT.- Şahin Ö, Aslan Ö. **Galectin-3 and cardiac biomarkers in cats with cardiomyopathy**. *Pesquisa Veterinária Brasileira 46:e07839, 2026*. Department of Internal Medicine, Faculty of Veterinary Medicine, Erciyes University, 38280 Talas, Kayseri, Türkiye. E-mail: oznuratalay@gmail.com

Galectin-3 (GAL-3) is a serum-detectable biomarker involved in inflammatory and fibrotic processes and has been associated with the development of cardiovascular diseases in human medicine. Therefore, this study aimed to evaluate GAL-3 levels in cats with different types and stages of cardiomyopathy. A total of 48 cats were included in the study, comprising a healthy control group (Group I, n = 12) and cats diagnosed with cardiomyopathy classified according to the American College of Veterinary Internal Medicine staging system (Class B2 cats to Group II, n = 12; Class C cats to Group III, n = 12; and Class D cats to Group IV, n = 12). All cats underwent echocardiographic, electrocardiographic, and radiographic examinations, and serum NT-proBNP, cTn-I, and GAL-3 levels were evaluated. Serum NT-proBNP and cTn-I levels differed significantly among the groups. NT-proBNP concentrations in Group IV were significantly higher than those in Group I and Group II, while NT-proBNP levels in Group III were significantly higher than those in Group I and Group II. The cTn-I concentration in Group IV was significantly higher than in Group I, Group II, and Group III. Additionally, Group III exhibited significantly higher cTn-I levels compared with Group I. No statistically significant difference in galectin-3 concentrations was detected between the control group and cats with cardiomyopathy. NT-proBNP and cardiac troponin I levels were associated with disease stage and demonstrated diagnostic importance in feline cardiomyopathies. Although no significant difference in GAL-3 levels was observed according to cardiomyopathy classification in this study, further large-scale studies using different classifications are warranted.

INDEX TERMS: Cardiomyopathy, cats, cTn-I, Galectin-3, NT-proBNP.

RESUMO.- [Galectina-3 e biomarcadores cardíacos em gatos com cardiomiopatia.] A galectina-3 (GAL-3) é um biomarcador detectável no soro, envolvido em processos inflamatórios e fibróticos, e tem sido associada ao desenvolvimento de doenças cardiovasculares em humanos. Portanto, este estudo teve como objetivo avaliar os níveis de GAL-3 em gatos com diferentes tipos e estágios de cardiomiopatia. Um total de 48 gatos foram incluídos no estudo, compreendendo um grupo controle saudável (Grupo I, n = 12) e gatos diagnosticados com cardiomiopatia, classificados de acordo com o sistema de estadiamento do American College of Veterinary Internal Medicine (gatos da Classe B2 para o Grupo II, n = 12; gatos da Classe C para o Grupo III, n = 12; e gatos da Classe D para o Grupo IV, n = 12). Todos os gatos foram submetidos a exames ecocardiográficos, eletrocardiográficos e radiográficos, e os níveis séricos de NT-proBNP, cTn-I e GAL-3 foram avaliados. Os níveis séricos de NT-proBNP e cTn-I diferiram significativamente entre os grupos. As concentrações de NT-proBNP no Grupo IV foram significativamente maiores do que nos Grupos I e II, enquanto os níveis de NT-proBNP no Grupo III foram significativamente maiores do que nos Grupos I e II. A concentração de cTn-I no Grupo IV foi significativamente maior do que nos Grupos I, II e III. Além disso, o Grupo III apresentou níveis de cTn-I significativamente maiores em comparação com o Grupo I. Não foi detectada diferença estatisticamente significativa nas concentrações de GAL-3 entre o grupo controle e os gatos com cardiomiopatia. Os níveis de NT-proBNP e troponina I cardíaca estiveram associados ao estágio da doença e demonstraram importância diagnóstica em cardiomiopatias felinas. Embora nenhuma diferença significativa nos níveis

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[2] Department of Veterinary Internal Medicine, Institute of Health Science, Erciyes University, 38280 Talas, Kayseri, Türkiye.
[3] Department of Internal Medicine, Faculty of Veterinary Medicine, Erciyes University, 38280 Talas, Kayseri, Türkiye. *Corresponding author: oznuratalay@gmail.com

de GAL-3 tenha sido observada de acordo com a classificação da cardiomiopatia neste estudo, estudos adicionais em larga escala utilizando diferentes classificações são necessários.

TERMOS DE INDEXAÇÃO: Cardiomiopatia, cTn-I, Galectina-3, gatos, NT-proBNP.

INTRODUCTION

Heart disease is common in cats, affecting approximately 10–15% of the population (Romito et al. 2018). Cardiomyopathy is defined as an acquired heart disease characterized by myocardial damage and can lead to heart failure associated with cardiac morbidity and mortality in cats (Fox et al. 2011, Filipe et al. 2015, Kittleson & Côté 2021a). Structural and functional changes in the heart muscle result in reduced cardiac output and an inability to adequately meet the metabolic needs of tissues. While some affected cats remain asymptomatic, cases of congestive heart failure or sudden death may develop in others (Kiatsilapanan & Surachetpong 2020). In the 2020 guidelines published by the American College of Veterinary Internal Medicine (ACVIM), cardiomyopathies are classified as: hypertrophic (HCM), restrictive (RCM), dilated (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and unclassified cardiomyopathy (UCM) (Luis Fuentes et al. 2020). Of these, HCM and RCM are the most common types of cardiomyopathy in cats. HCM is characterized by concentric left ventricular hypertrophy and diastolic dysfunction and is the most common myocardial disorder (Jung & Kittleson 2011, Kittleson & Côté 2021b). RCM, on the other hand, is a heterogeneous myocardial disease characterized by impaired ventricular filling and reduced myocardial compliance (Chetboul et al. 2019). DCM is characterized by systolic dysfunction and left ventricular dilation. UCM is a cardiomyopathy that does not fit into other classifications based on echocardiographic and pathological criteria, and it presents with severe biventricular enlargement, normal or mild left ventricular hypertrophy, and normal or mildly reduced systolic function. ARVC, on the other hand, is defined as a cardiac muscle disorder characterized by severe right atrial and ventricular dilation, frequent arrhythmias, and significant tricuspid regurgitation due to distortion of the tricuspid valve (Luis Fuentes et al. 2020).

Echocardiography is considered the gold standard for the diagnosis of cardiomyopathies (Harris et al. 2017). However, because echocardiography requires specialized equipment and expertise and is not always readily available in clinical practice, the need for alternative methods in diagnosis and follow-up has increased (Klein et al. 2022). In this context, cardiac biomarkers are important tools for detecting heart disease, monitoring treatment, and assessing prognosis (Boswood 2009, Pierce et al. 2017).

N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTn-I) are biomarkers commonly used to assess cardiac stress and myocardial damage in cats. Various studies have demonstrated that these biomarkers reflect the presence and severity of heart disease (Fox et al. 2009, Oyama et al. 2013, Gavazza et al. 2021).

In recent years, it has been reported that galectin-3 (GAL-3), a β-galactoside-binding lectin, is associated with cardiac fibroblast proliferation, collagen deposition, and ventricular dysfunction (Lok et al. 2013, Filipe et al. 2015). While GAL-3, which plays a role in inflammation and fibrosis processes, is noted to be associated with the development and progression of heart failure, it is also stated that it is not specific to cardiac diseases and may increase in various systemic diseases as well (Kim et al. 2007, Erdevir 2021, Merino-Merino et al. 2021). However, studies on the clinical significance of GAL-3 in cardiomyopathies in cats are limited.

The primary objective of this study is to evaluate serum GAL-3 levels in cats diagnosed with cardiomyopathy and to determine how GAL-3 levels change across different stages of the disease.

MATERIALS AND METHODS

Ethical approval. This study was approved by the Erciyes University Local Ethics Committee for Animal Experiments under project application no. 22/232 dated November 3, 2022, and all procedures were conducted in accordance with animal ethics and welfare guidelines.

The number of cats to be included in the study and in each group was calculated with an effect size of 0.50, $\alpha = 0.05$, test power $(1-\beta) = 0.80$, and a group size of 4, and was determined to be at least 48. The cats included in the study were divided into a healthy control group (Group I, n = 12) and an experimental group (n = 36).

The study population consisted of cats brought to the clinic for routine check-ups or presenting with signs of heart disease (such as dyspnea, tachypnea, fainting, aortic thromboembolism, rapid fatigue, or coughing), and in which a murmur or arrhythmia was detected during auscultation. In the study, the weight, age, breed, and sex of all healthy and sick cats were recorded. After routine clinical examinations, all cats included in our present study underwent complete blood count, blood gas, and serum biochemical analyses, as well as radiographic, electrocardiographic, and echocardiographic examinations. Cats whose values were within the reference range were included in the study as healthy controls. Care was taken to ensure that the healthy control cats were of a similar age and breed to the cats in the patient group whenever possible. Echocardiographic examinations were performed according to established standard imaging protocols and techniques (Fox et al. 2009, Boon 2011, Petrič et al. 2012). The cats included in the study were divided into a healthy control group (Group I, n = 12) and an experimental group (n = 36). Cats in the experimental group were classified according to the ACVIM classification following echocardiographic examinations: cats with cardiomyopathy showing heart muscle problems and cardiac remodeling, with a left atrial (LA) long-axis diameter > 16 mm and/or an left atrium to aorta ratio (LA/Ao) ratio ≥ 1.6, and without clinical symptoms (asymptomatic) were classified as B2 (Group II = 12); cats with cardiomyopathy that had developed cardiac remodeling (vertebral heart score – VHS ≥ 8.1, LA/Ao ≥ 2), exhibited clinical symptoms (respiratory distress, thromboembolism), and showed effusion/pulmonary edema and/or pleural effusion/pericardial effusion on radiographic and ultrasonographic examinations were classified as C (Group III = 12), and cats with cardiomyopathy exhibiting the same symptoms as Group III patients but resistant to standard treatment (use of > 6 mg/kg/day of furosemide) and experiencing recurrent symptoms were classified as Group IV (Group IV = 12) (Luis Fuentes et al. 2020). Patients with non-cardiac diseases such as lung diseases (bronchitis, neoplasia, asthma, etc.) that could cause respiratory problems other than cardiac disease, chronic diseases, infectious diseases, endocrinological, kidney, liver or spleen problems, and congenital heart diseases were excluded from the study. Additionally, cats using any heart disease medication or medications that could alter the values of the cardiac biomarkers

being examined in the study (nonsteroids, steroids, antibiotics, etc.) were excluded.

Echocardiographic examination (2D, M-Mode, DDG). Without sedation, the animals included in the study underwent echocardiographic examinations using standard techniques, including 2D (B-mode), M-mode, color Doppler, continuous wave (CW), pulsed wave (PW), and DDG. Echocardiographic examinations were performed using a 4–12 MHz multi-frequency phased array probe (Philips Affiniti 50, Philips Healthcare, Andover/MA, USA). Measurements were repeated at least three times. Patients with an LA/Ao ratio greater than 1.5 were diagnosed with LA dilatation (Abbott & MacLean 2006). Clinical symptoms such as pain, cyanosis in the legs, paralysis, absent pulse, and decreased limb temperature that developed in patient groups (Levels C and D) were classified as cases of thromboembolism (Guillaumin 2024). Furthermore, thromboembolism was confirmed in patients during echocardiographic examination by the presence of clots and a smoke-like appearance inside the heart.

Radiographic examination. VHS measurements were performed on all cats included in the study using X-rays taken in the right lateral (LL) and dorsoventral (DV) positions (Browiner, Beatle 05 Vet HF; Mindray Vetipad M1 Plus DR). In the lateral radiograph, the lengths of the heart's long axis (from the apex of the heart to the base of the heart where it intersects the tracheal bifurcation) and short axis (perpendicular to the long axis measurement, at the point of maximum heart width, usually where the silhouette of the heart intersects the ventral border of the caudal vena cava) were measured in centimeters. The number of thoracic vertebrae was determined by placing each measurement from the cranial to the caudal aspect of T4. Vertebral heart score was obtained by summing the number of long-axis and short-axis thoracic vertebrae. The normal VHS range was accepted as 7.5 ± 0.32 (6.7–8.1) (Litster & Buchanan 2000). They were evaluated for pleural effusion, pulmonary edema, and left atrial (LA) enlargement, which can cause respiratory symptoms (Luis Fuentes et al. 2020)

Electrocardiographic examination (ECG). ECG recordings were performed on all patients in the right lateral recumbent position. The ECG trace was recorded at 50 mm/sec, with each amplitude calibrated at 10 mm/mV. Bipolar I, II, and III limb leads, as well as unipolar aVR, aVL, and aVF limb leads, were automatically printed when the optimal analysis was displayed on the device screen. The electrocardiographic examination was evaluated using a 6-channel electrocardiography device (Carewell, 1103L Vet) operating with a three-lead system (I, II, III, aVR, aVL, aVF) and 25–50 mV settings.

Biochemical analyses. Blood samples (3 mL) were collected from the saphenous vein of the cats included in the study into gel-filled tubes without anticoagulant for biochemical analysis. The samples collected for biochemical analysis were centrifuged at 3,000 rpm for 5 minutes to separate the serum. Serum samples for GAL-3 analysis were stored in Eppendorf tubes at -20 °C until the time of analysis. Quantitative measurements of feline NT-proBNP (50–1500 pmol/L) and feline cTn-I (0.01–20 ng/mL) concentrations were performed immediately on the collected serum samples using the Vcheck V200 immunofluorescence model (Bionote®, Korea: ELK Diagnostics). At the end of the study, serum GAL-3 levels (Grup I = 8, Grup II = 10, Group III = 10, Grup IV = 12) were measured in duplicate using a feline GAL-3 (4.5–1000 pg/mL) ELISA kit (EF1RB-USA) in accordance with the manufacturer's procedures and read with an ELISA reader (Biotek uQuant Plate Reader; Winooski/VT, USA) set to 450 nm.

Statistical analyses. IBM SPSS Statistics 22 was used to perform statistical analyses to evaluate the study's findings. The normality of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and it was found that the data did not follow a normal distribution. In evaluating the study data, descriptive statistical methods (median, interquartile range – IQR) were used, along with the Kruskal-Wallis test to compare quantitative data and conduct intergroup comparisons of parameters. Spearman's rho correlation analysis was used to examine the relationships between parameters. Statistical significance was assessed at the $p < 0.05$ level.

RESULTS

Of the 48 cats included in the study, three were Scottish Folds, 32 were mixed-breed, 10 were British Shorthairs, one was a Siamese cat, and two were Persian cats; the median age of the cats was eight (1–19), with 22 females and 26 males. Of these, Group I consisted of eight Domestic Shorthair, two Persian cats, one British Shorthair, and one Scottish Fold cats; aged 1–12 years, with eight females and four males; Group II consisted of eight Domestic Shorthair, two British Shorthair, and two Scottish Fold cats, aged 2–14 years, six males and six females; Group III consisted of eight Domestic Shorthair, four British Shorthair, aged 4–12, with 10 males and two females; and Group IV consisted of eight Domestic Shorthair, one Siamese cat, and three British Shorthair, aged 4–19, with six males and six females.

During the clinical examination of the animals in Group II, no symptoms such as exercise intolerance, respiratory distress, or thromboembolism were observed. However, a heart murmur was detected in eight cats. All cats in which a murmur was detected were classified as having hypertrophic obstructive cardiomyopathy (HOCM). All cats in Group III were presented with symptoms of acute respiratory failure, and all exhibited exercise intolerance (n = 12) and respiratory distress (n = 12). Pleural effusion (n = 6), pulmonary edema (n = 12), and pericardial effusion (n = 5) were observed. Thromboembolism was observed in only one of the Group III patients (n = 1), while a "smoke" (spontaneous echocardiographic contrast) appearance was noted in the left atrium of some patients (n = 9). Additionally, atrial fibrillation (AF, n = 8) and premature ventricular complexes (PVC, n = 9) were observed in Group III patients. In all cats in Group IV, recurrent signs of respiratory distress (pericardial effusion, n = 8; pleural effusion, n = 10; pulmonary edema, n = 12) were observed, and thromboembolism was detected in eight cats. In addition, recurrent ascites was observed in five patients. While AF and PVCs were observed in all patients, left bundle branch block was additionally identified in three patients. The types of heart muscle disease in the cats included in Groups II, III, and IV were determined based on echocardiographic findings. Among the 12 cats in Group II, eight were diagnosed with HOCM, one with eRCM, and three with HCM. Of the 12 cats in Group III, seven had HCM, three had DCM, and two had eRCM. Among the 12 cats in Group IV, three had mRCM, two had DCM, one had eRCM, four had HOCM, and two had HCM (Table 1).

Serum cardiac biomarker findings

The data on the significance of NT-proBNP, cTn-I, and GAL-3 biomarkers in healthy cats and cats with varying degrees of heart disease evaluated in this study are presented in Table 2. Although there was an increase in Group IV compared to Group I, no statistically significant difference was observed between the groups in terms of serum GAL-3 levels ($p = 0.119$). When evaluated in terms of serum NT-proBNP levels, a significant

difference was observed between Groups I and II and Groups III and IV (p = 0.001). In contrast, for serum cTn-I levels, significant differences were observed between Group I and Groups III and IV, and between Group II and Group IV (p = 0.001). No correlation was found among the groups between serum NT-proBNP, cTn-I, and GAL-3 levels (Table S1).

Vertebral heart score measurement findings

The vertebral heart scores of the cats included in the study were evaluated to determine heart size (Table 3). A statistically significant difference in VHS values was observed between the groups (p = 0.001). The results of the Kruskal-Wallis test, conducted to determine which groups contributed to the significance, showed that Group I's VHS value was significantly lower than those of Group II (p = 0.026), Group III (p = 0.001), and Group IV (p = 0.001). Group II's VHS value was significantly lower than that of Group IV (p = 0.006). There was no statistically significant difference among the other groups (p > 0.05).

Table 1. Types of heart muscle diseases observed in the group of cats under study

Patient group	Total number of patients	Conditions	Number of patients
Group II	12	HOCM	8
		eRCM	1
		HCM	3
Group III	12	HCM	7
		DCM	3
		eRCM	2
		mRCM	3
Group IV	12	DCM	2
		eRCM	1
		HOCM	4
		HCM	2

HOCM = Hypertrophic obstructive cardiomyopathy, eRCM = endomyocardial restrictive cardiomyopathy, HCM = hypertrophic cardiomyopathy, DCM = dilated cardiomyopathy, mRCM = myocardial restrictive cardiomyopathy.

Electrocardiographic findings

Echocardiographic examinations were performed to assess waveform changes and rhythms. No abnormal rhythms or waveform changes were observed in healthy cats. In the patient groups, PVCs were observed in three patients in Group II, and a left bundle branch block was identified in only one of these patients. In Group III, AF and PVCs were observed in eight patients; in another patient, AF was not present, but PVCs were identified in only one patient. In all patients in Group IV, AF and PVCs were observed, and left bundle branch block was additionally observed in three of these patients.

Echocardiographic findings

For all cats (both patients and healthy controls) included in the study, echocardiographic measurements were performed by a single operator using M-mode, 2D, pulsed-wave (PW), continuous-wave (CW), and tissue Doppler techniques through the right parasternal short-axis, right parasternal long-axis, left apical 4-chamber, and left apical 5-chamber views, and the statistical analyses of the data are presented in Table S2 and S3.

A statistically significant difference in interventricular septal diastolic thickness (IVSd) was observed between the groups (p = 0.034). According to the results of the Kruskal-Wallis test, the IVSd level in Group I was significantly lower than in Group II (p = 0.005) and Group III (p = 0.031). No significant difference was observed between the other groups (p > 0.05). A statistically significant difference was also found between the groups in terms of left ventricular posterior wall diastolic thickness (LVPWd) (p = 0.018). In the post hoc analysis, Group I's LVPWd level was significantly lower than those of Group II (p = 0.029) and Group IV (p = 0.044). No statistically significant differences were found between the other groups (p > 0.05). There was a significant difference among the groups in terms of left ventricular end-systolic diameter (LVIDs) (p = 0.007). As a result of the Kruskal-Wallis test, Group I's LVIDs level was significantly lower than that of

Table 2. Levels of significance of cardiac biomarkers between groups

	Group I	Group II	Group III	Group IV	p
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	
NT-proBNP (pmol/L)	n = 12 45 (45–54.88)[a]	n = 12 374.7 (183.5–494.88)[a]	n = 12 1500 (999.1–1500)[b]	n = 12 1500 (1500–1500)[b]	0.001*
GAL-3 (pg/mL)	n = 8 781.72 (635.71–913.69)	n = 10 813.16 (612.71–906.26)	n = 10 760.83 (507.58–825.28)	n = 12 869.21 (812.49–958.22)	0.119
cTn-I (ng/mL)	n = 12 0.01 (0.01–0.06)[a]	n = 12 0.05 (0.01–0.4)[ab]	n = 12 0.36 (0.06–2.24)[b]	n = 12 4.36 (2.08–8.88)[c]	0.001*

IQR = Interquartile range, n = number; * p < 0.05; [a,b,c] Different letters in the same row indicate differences between groups.

Table 3. Comparison of VHS measurements by group

	Group I	Group II	Group III	Group IV	p
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	
VHS	(7.5) (7.2–7.98)[a]	(8.35) (8–8.88)[b]	(9.5) (8.78–9.65)[bc]	(9.9) (9.5–10.5)[c]	0.001*

IQR = Interquartile range, VHS = vertebral heart scores; *p < 0.05; [a,b,c] Different letters in the same row indicate differences between groups.

Group III ($p = 0.019$) and Group IV ($p = 0.038$). No significant differences were found among the other groups ($p > 0.05$).

A statistically significant difference was observed between the groups in terms of left atrial diameter (LA Diam) ($p = 0.001$). Accordingly, the LA Diam levels in Group III and Group IV were significantly higher than in Group I ($p = 0.001$ for both) and Group II ($p = 0.039$ and $p = 0.034$, respectively). No significant differences were observed in other group comparisons ($p > 0.05$). A statistically significant difference was detected between the groups in terms of the LA/Ao ratio ($p = 0.001$). In the post hoc analysis, it was found that Group I's LA/Ao ratio was significantly lower than that of Group II ($p = 0.014$), Group III ($p = 0.001$), and Group IV ($p = 0.001$); and that Group II's LA/Ao ratio was significantly lower than that of Group III ($p = 0.029$) and Group IV ($p = 0.015$). No significant difference was observed between Group III and Group IV ($p > 0.05$).

A statistically significant difference in left ventricular ejection fraction (LV EF) was observed between the groups ($p = 0.005$). According to the results of the Kruskal-Wallis test, the LV EF level in Group I was significantly higher than in Group III ($p = 0.002$) and Group IV ($p = 0.014$). No significant differences were observed between the other groups ($p > 0.05$). Statistically significant differences were also observed between the groups in left ventricular fractional shortening (LV FS) values ($p = 0.006$). In the subsequent analysis, it was found that Group III's LV FS level was significantly lower than those of Group I ($p = 0.002$) and Group II ($p = 0.014$). No statistically significant difference was observed between the other groups ($p > 0.05$). No statistically significant differences were observed in the other echocardiographic measurements evaluated across the groups ($p > 0.05$).

DISCUSSION

Cardiomyopathies are among the most common heart diseases in cats and, according to the ACVIM consensus statement (2020), are classified as HCM, DCM, RCM, ARVC, and UCM based on structural and functional myocardial changes (Luis Fuentes et al. 2020). In the present study, HCM was the most common type of cardiomyopathy in the patient group, consistent with prevalence data reported in the literature. (Kittleson & Côté 2021a, Çolakoğlu et al. 2022).

According to the ACVIM classification, clinical findings such as congestive heart failure, effusion, and thromboembolism are expected to emerge in stages C and D (Luis Fuentes et al. 2020). Similarly, in the present study, the higher incidence of dyspnea, pleural/pericardial effusion, and thromboembolism in symptomatic groups is consistent with the literature (Ferasin & DeFrancesco 2015, Rohrbaugh et al. 2020). Furthermore, the widespread observation of atrial fibrillation and ventricular premature complexes in advanced-stage groups is consistent with studies reporting a relationship between left atrial enlargement and the development of arrhythmias (Côté et al. 2004, Bartoszuk et al. 2019).

Studies involving electrocardiographic examinations have reported detecting PVCs in cats with HCM (Bartoszuk et al. 2019) and AF in cats with left atrial enlargement (Côté et al. 2004). The detection of AF in 20 patients and PVCs in 21 patients in this study is consistent with previous reports.

The vertebral heart score is a reliable method used for the objective radiographic assessment of heart size (Litster & Buchanan 2000). In our present study, the mean VHS of the healthy group was consistent with reference values reported in the literature, and VHS was significantly higher in patient groups, supporting the notion that VHS is an important parameter in assessing cardiomegaly and disease severity. In particular, the detection of higher VHS values in symptomatic groups demonstrates the relationship between disease progression and radiographic findings.

Although echocardiography is considered the gold standard for the diagnosis and monitoring of cardiac diseases, the use of cardiac biomarkers has become increasingly important in clinical practice because echocardiographic examination requires costly equipment and experienced operators (Laudhittirut et al. 2020). In feline medicine, N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) are the most widely used biomarkers for the diagnosis and assessment of cardiac diseases (Oyama 2013, Szarková et al. 2022). NT-proBNP is a biomarker released in response to myocardial stress and is widely used in the diagnosis and staging of heart diseases (Connolly et al. 2008, Fox et al. 2011). Previous studies have reported that NT-proBNP levels are positively correlated with the presence and severity of cardiac disease (Tominaga et al. 2011, Oyama et al. 2013, Machen et al. 2014). This study demonstrated that NT-proBNP levels were lowest in the healthy group and significantly higher in stages C and D, supporting prior literature. However, the interpretation of the present findings should take into account the potential influence of a ceiling effect. In particular, one possible explanation for the lack of statistically significant differences in NT-proBNP concentrations between C and D stages, representing cats with advanced cardiomyopathy, is the presence of a ceiling effect. As a substantial proportion of the measurements approached the assay's upper limit, the ability of NT-proBNP to discriminate between individuals with more advanced disease stages may have been diminished. Consequently, the observed lack of statistically significant differences may reflect limitations in the analytical measurement range rather than the absence of underlying biological differences.

cTn-I is considered a specific marker of myocardial cell damage, and elevated serum levels are associated with a poor prognosis (Langhorn & Willesen 2016, Gavazza et al. 2021). Numerous studies have demonstrated that cTn-I levels in cats with HCM are significantly higher than in healthy cats (Herndon et al. 2002, Connolly et al. 2003, Borgeat et al. 2014). In the present study, the marked increase in cTn-I levels, particularly in stage D, suggests that myocardial damage increases in advanced stages of the disease and is consistent with the literature.

Galectin-3 is a biomarker that plays a role in inflammatory and fibrotic processes and is associated with heart failure (Filipe et al. 2015, Ding et al. 2020). However, it has been reported that GAL-3 levels may also increase in various systemic diseases beyond cardiac pathologies (Merino-Merino et al. 2021). Therefore, cats with diseases such as infection, liver failure, lung disease, malignancy, and hyperthyroidism were excluded from this study. Although studies in the veterinary field are limited, Stack et al. (2023) reported that GAL-3 levels were elevated in cats with HCM compared with healthy cats; however, no significant differences were found between disease stages. Similarly, Sakarin et al. (2016) detected elevated GAL-3 levels in dogs with mitral valve disease but noted no significant differences between disease stages.

Another study by Klein et al. (2022) showed that GAL-3 levels were not associated with the presence or stage of degenerative mitral valve disease. In the present study, no statistically significant difference in GAL-3 levels was observed between patient groups with different stages of cardiomyopathy and the healthy control group, and no significant correlation was found between GAL-3 and NT-proBNP or cTn-I. These findings suggest that GAL-3 may have limited discriminatory power in reflecting disease severity in feline cardiomyopathies. However, the lack of statistically significant differences in GAL-3 concentrations among cats at different stages of cardiomyopathy may be attributable to the heterogeneity of the study groups, which may have obscured phenotype-specific effects. Furthermore, factors such as sample size, different cardiomyopathy phenotypes, and storage of serum samples at -20 °C until testing should be considered, as they may affect the results.

The present study has several limitations. First, the upper detection limit of the NT-proBNP assay was 1500 pmol/L, which may have introduced a ceiling effect and limited discrimination among cats with advanced disease. Second, sample sizes differed between groups because the GAL-3 assay was performed in duplicate, resulting in the exclusion of some samples. Finally, serum samples used for GAL-3 analysis were stored at -20 °C before testing, which may have influenced biomarker stability and should be considered when interpreting the results.

CONCLUSION

While the clinical usability of NT-proBNP and cTn-I in cardiomyopathy staging and disease severity assessment was confirmed, galectin-3 (GAL-3) showed limited diagnostic and prognostic value in this study. Further studies should be conducted with larger sample groups, focused on specific subtypes of cardiomyopathy to gain a clearer understanding of the effects of new biomarkers, such as GAL-3, on diagnosis and staging.

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