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Clinical findings using echocardiography and plasma cardiac troponin I and pathological findings in dogs with hypertrophic cardiomyopathy: A retrospective study

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Abstract

Background: Hypertrophic cardiomyopathy (HCM) is considered rare in dogs, and there is a lack of clinical data. Cardiac troponin I (cTnI) is a biomarker of cardiomyocyte damage and necrosis and can be used to diagnose cat and human HCM.

Aim: We investigated whether the presence of cTnI in clinical data can be used in conjunction with echocardiography to diagnose canine HCM.

Methods: This study comprised client-owned dogs with clinical evidence of concentric hypertrophy on echocardiographic images, serum total thyroxine levels of ≤5 µg/dl, systolic blood pressure of ≤180 mmHg, and absence of aortic stenosis. All cases were necropsied.

Results: Cardiomyocyte hypertrophy (mean diameter, 18.3 ± 1.8 µm), myocardial fiber disarray (70%), interstitial fibrosis (80%), and small vessel disease (100%) were assessed. In dogs with HCM, the left ventricles were concentric, almost symmetrical, and hypertrophied above the aortic diameter. The end-diastolic interventricular septum normalized to body weight [intraventricular septal thickness in diastole (IVSDN)] was 0.788 [interquartile range (IQR), 0.7–0.92], which exceeded the normal range (5%–95%, IQR: 0.33–0.52). In total, 70% of the dogs with HCM had syncope and dyspnea, and all dogs had high cTnI levels (median, 3.94 ng/ml), exceeding the upper limit of normal (0.11 ng/ml) and indicating cardiomyocyte damage. IVSDN and serum cTnI levels were correlated ($\rho = 0.839$, $p = 0.01$).

Conclusion: Ventricular wall thickening and high serum cTnI levels can provide a presumptive diagnosis of HCM and prompt the initiation of treatment or additional diagnostic investigations.

Keywords: Biomarker, Cardiac troponin I, Dog, End-diastolic thickness of the interventricular septum, Hypertrophic cardiomyopathy.

Introduction

Hypertrophic cardiomyopathy (HCM) is a common disease in humans and domestic cats, with genetic mutations being the most frequently identified underlying etiology (Freeman *et al*., 2017; Ueda and Stern, 2017; Luis Fuentes *et al.*, 2020). Despite being a common cause of sudden death in humans (Richardson *et al*., 1996) and the leading cause of feline heart disease (Fox *et al*., 2018), there are limited reports of HCM in dogs (Washizu *et al*., 2003; Schober *et al*., 2022). However, HCM has been identified after anesthesia-related accidents or sudden cardiac death (SCD) in dogs (Liu *et al*., 1979b).

HCM is characterized by concentric hypertrophy of the left ventricular wall with a normal or small left ventricular size. The diagnosis of HCM requires the exclusion of systemic hypertension, hyperthyroidism, congenital aortic stenosis, and less common causes of wall hypertrophy, such as multicentric lymphoma and pseudohypertrophy secondary to dehydration (Schober *et al*., 2022). HCM has been reported in male and female dogs aged 1–14 years (Liu *et al*., 1979a; Washizu *et al.*, 2003; Pang *et al*., 2005; Schober *et al*., 2022). Previous studies have reported HCM in all dog sizes, including German shepherds, Doberman pinschers, Airedale terriers, Great Danes, Boston terriers, poodles, bulldogs (Liu *et al*., 1979b), corgis (Marks, 1993), Shih-Tzu, and terriers (Washizu *et al*., 2003; Schober *et al.*, 2022). Typical historical and physical examination findings in dogs with HCM include cardiac murmur, exercise intolerance, and convulsive seizures (Schober *et al*., 2022). B-mode echocardiography in cases of HCM may

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demonstrate moderate enlargement of the left atrium, with M-mode echocardiography revealing the excess motion of the left ventricular free wall and ventricular septum (Washizu *et al*., 2003). Systolic anterior motion of the mitral valve and left ventricular diastolic dysfunction are frequently observed on echocardiography in dogs with HCM (Schober *et al*., 2022).

Pathological findings, such as myocardial fiber hypertrophy, intricate myocardial alignment, plexiform fibrosis, and coronary arteriosclerosis have been reported in HCM in cats (Fox, 2003), dogs (Liu *et al*., 1979b; Washizu *et al*., 2003), and humans (Fujiwara *et al*., 1982). Cardiac troponin I (cTnI) has been used as a biomarker to assess damage and necrosis of cardiomyocytes (Adams *et al*., 1994; Fonfara *et al*., 2010; Kociol *et al*., 2010; White, 2011; Langhorn and Willesen, 2016). Normal cTnI concentrations in dogs have been reported as <0.1 (Porter *et al*., 2016), <0.07 (Sleeper *et al*., 2001), and <0.03 ng/ml (with an upper 95th percentile of 0.11 ng/ml) (Oyama and Sisson, 2004). In recent years, cTnI levels in HCM have been measured in humans (Cambronero *et al*., 2009; Kubo *et al*., 2010, 2011; McGorrian *et al*., 2013) and cats (Langhorn *et al*., 2014; Hori *et al*., 2018; Hertzsch *et al*., 2019; Luis *et al*., 2020) and are expected to have utility in diagnosis and prognosis.

HCM is typically diagnosed via echocardiography (Anwar and TenCate, 2021; Schober *et al*., 2022). The cutoff value for left ventricular wall hypertrophy when diagnosing HCM is ³6 mm in cats (Payne *et al*., 2013) and ³15 mm in humans (Gersh *et al*., 2011; Enriquez and Goldman, 2014); however, there is currently no accepted cutoff value in dogs given the significant variations in body size between dog breed. Additionally, reference data for dogs are limited. Therefore, the assessment of concentric hypertrophy requires the use of standardized echocardiographic values (Schober *et al*., 2022). However, diagnosing HCM by evaluating left ventricular wall thickening alone can be challenging (Schober *et al*., 2022). Therefore, pathological diagnosis (Fox, 2003; Maron and Fox, 2015; Ueda and Stern, 2017) is still considered a common method of diagnosing HCM.

Given the above, there is a need for novel diagnostic methods for HCM that can be used in conjunction with the use of echocardiography for assessing myocardial wall hypertrophy. This study included only cases with left ventricular wall hypertrophy that underwent necropsy. Dogs with confirmed HCM were evaluated for echocardiography and common clinical signs. In particular, we investigated whether clinical data cTnI can help diagnose HCM in dogs.

Materials and Methods

Animals

All dogs included in this study were raised and cared for by their owners and screened for heart disease at Ando Animal Hospital (Hyogo Prefecture, Japan). Dogs with HCM (n = 10) were examined between 2007 and 2017. HCM was suspected based on clinical examinations. All dogs were definitively diagnosed after death based on histopathological examinations.

Clinical examination and diagnosis

Presumptive diagnoses of HCM were based on the results of general physical examinations, blood examinations, electrocardiography (ECG), thoracic radiography, and ultrasonography. Holter monitoring and angiography were also performed in dogs requiring close monitoring for arrhythmias or close examination of the left heart chamber, respectively. Information on general conditions, including previous history of syncope, was obtained by interviewing the owners at the hospital. Physical examinations were performed to assess heart and respiratory symptoms, such as cough and heart murmurs, using the Levine scale (Silverman and Wooley, 2008). Blood testing was performed to determine serum thyroid hormone and cTnI levels. Additionally, ECG was performed to determine the presence of premature ventricular contractions and atrioventricular or bundle branch blocks. Thoracic radiography was performed to measure the vertebral heart size (VHS) in the lateral view (Buchanan, 2000).

Echocardiography

A single veterinarian performed all echocardiographic evaluations. Three cardiac cycles were monitored in each measurement, with the mean of three cycles calculated. Relative myocardial wall thickness was measured using M-mode echocardiography in the right parasternal long-axis view. Measurements were calculated using the following formulas for standardization (Cornell *et al*., 2004; Schober *et al*., 2022): end-diastolic interventricular septum (IVSd) normalized for body weight (BW; IVSDN = IVSd, in cm/$BW^{0.241}$) and end-diastolic left ventricular posterior wall normalized for body weight (LVPWDN = LVPWd, in cm/$BW^{0.232}$). The end-diastolic left ventricular internal dimension normalized for body weight (LVIDDN = LVIDd, in cm /$BW^{0.294}$) was calculated from measurements in the two-dimensional (2D) long-axis view of the outflow tract from the right parasternal location by applying an ultrasonic beam vertically to the left ventricular wall at the level of the tendinous cords in M-mode (Bodh *et al*., 2019). The type of concentric or eccentric left ventricular hypertrophy was determined using the following equation in long- and short-axis views: 0.53 < (IVSd + LVWd)/LVDd (Borgarelli *et al*., 2007; Schober *et al*., 2022). Left ventricular fractional shortening (FS) was calculated using the following formula: (end-diastolic left ventricular internal dimension—end-systolic left ventricular internal dimension) × 100. In addition, the left atrium-to-aorta (LA/Ao) ratio was calculated by comparing the left atrium's internal diameter in maximum diastole to the aorta's internal diameter (i.e., the measured length between the aortic leaflets) based on the 2D short-axis view from the right parasternal

location at the level of the heart base during the diastolic phase (Rishniw and Erb, 2000). The ratio of the E wave to the A wave was calculated by measuring the early diastolic and atrial contraction velocities at the approximate center of the mitral valve where the mitral leaflets meet using pulsed-wave Doppler based on the 2D four-chamber view of the apex from the left parasternal location (Bodh *et al*., 2019).

Survival duration was defined as the period from the day of clinical HCM diagnosis to that of death owing to heart failure. At the time of the clinical diagnosis of HCM, signs of heart failure in dogs were classified according to the modified New York Heart Association (NYHA) classification (Atkins *et al*., 2009) as follows: class I, asymptomatic heart disease; class II, heart disease displaying clinical signs only during strenuous exercise; class III, heart disease displaying clinical signs during routine activities or mild exercise; and class IV, heart disease showing severe clinical signs, even at rest.

Histopathological examinations

Histopathological examinations were performed by a veterinary pathologist at the Laboratory of Pathology of Osaka Prefecture University. Death was confirmed at the hospital before pathological dissection. Hearts were cut vertically from the base to the apex to allow observation of the aorta and the four chambers of the heart. The thicknesses of the IVSd and left ventricular free wall were measured. The left ventricular outflow tract diameter was measured. Photography of the septum and left ventricular outflow tract was performed to obtain macroscopic images. Sections were fixed in 10% formalin before histopathological examinations. Morphological observation of the myocardium was performed by cross-sectioning (3 mm) the IVSd and left ventricular free wall from the endocardium to the pericardium at the base and apex while avoiding the papillary muscle. The sections were embedded in paraffin and stained with hematoxylin and eosin. Myocardial disarray and changes in cardiomyocytes and small-to-medium-sized arteries were subsequently evaluated. Myocardial fibrosis was assessed using paraffin-embedded sections stained with Azan. Myocyte hypertrophy was evaluated by calculating the mean short diameter of 40–100 myocytes measured at sites where the longitudinal section contained nuclei using micrographs in three fields of view at ×200 magnification (Hoshino *et al*., 1983). Myocyte disarray (Fox, 2003) was assessed and scored according to the degree of abnormality in cardiomyocyte arrangement. When hypertrophied, myocytes typically display a pinwheel configuration or herringbone pattern that can be scored using the following system: 0, none; 1+, mild; 2+, moderate; and 3+, severe. A comprehensive histopathological examination, including the thorough evaluation of cardiomyocyte hypertrophy (cutoff, >15 µm/100 cells) (Hoshino *et al*., 1983) and disarray, intimal thickening, cavity narrowing in small vessels, focal necrosis, and fibrosis was required for the definitive diagnosis of HCM. The pattern of left ventricular hypertrophy was determined based on visual estimation and was classified as symmetrical or asymmetrical according to the difference in wall thickness, with 50% considered the cutoff value. As Langhorn and Willesen (2016) reported that plasma cTnI levels may also be high in myocarditis, myocarditis was excluded by pathological examination in this study.

Plasma cTnI measurements

Blood testing to measure cTnI levels was performed at the time of clinical diagnosis of HCM in all cases. Blood samples (1 ml) were collected from the saphenous or cephalic veins, placed in heparinized tubes, and centrifuged at 3,000 rpm for 5 minutes. Separated plasma was then measured using i-STAT® 1 (Abbott Laboratories, Princeton, NJ) equipped with a dedicated cartridge (i-STAT® cTnI test cartridge) that uses a two-site enzyme-linked immunosorbent assay based on a monoclonal antibody. This method has a measurable range of serum cTnI values in dogs of 0.00–50.00 ng/ml (Porter *et al*., 2016) and a detection limit of 0.02 ng/ml in humans (Apple *et al*., 2004).

Statistical analyses

Data distribution normality was assessed using the c^2 goodness-of-fit test. Normally distributed data are presented as means ± standard deviations. Nonnormally distributed data are presented as medians and interquartile ranges (IQR). Additionally, Spearman's rank correlation coefficient (r) was used to assess correlative relationships in dogs with HCM. The log-rank test was used to test bivariate associations between each prognostic factor and survival duration. The cutoff value of the clinical index used for the log-rank test was the mean or median in dogs with HCM. All statistical analyses were performed using statistical software (Statcel on Excel 3rd ed., OMS Ltd., Tokyo, Japan). p-values of <0.05 were considered statistically significant.

Ethical approval

This retrospective study complied with the Regulations for Animal Experiments and Related Activities at Osaka Prefecture University (Sakai, Japan).

Results

Table 1 presents the characteristics of dogs with HCM (n = 10; mean age, 9.8 ± 4.5 years; mean BW, 12.7 ± 7.6 kg). Most dogs with HCM were male (n = 7; 70%). Dogs with HCM were categorized by breed as follows: mixed breed (n = 5; 50%), miniature dachshund (n = 2; 20%), bulldog (n = 1; 10%), Shiba inu (n = 1; 10%), and Chihuahua (n = 1; 10%).

Most dogs (n = 9; 90%) with HCM had clinical signs, including syncope (n = 7) and dyspnea (n = 7). Most dogs (n = 8; 80%) had arrhythmia or abnormal wave morphologies on ECG. Arrhythmias were attributable to an atrioventricular block in two dogs and premature ventricular contractions in one dog. Abnormal wave

Table 1. Characteristics of dogs with HCM.

Case no.			1	2	3	4	5	6	7	8	9	10	
General information	Age	Years	5	12	5	14	3	15	15	7	10	12	9.8 ± 4.5*
	Sex		m/c	m/c	m/c	m	m	f	f	f	m	m	m: 70***
	Weight	kg	23.4	17.2	24.3	5.92	6.5	17.9	1.9	11.3	11	8	12.7 ± 7.6*
	Breed		Bulldog	Mix	Mix	Miniature dachshund	Shiba inu	Mix	Chihuahua	Mix	Mix	Miniature dachshund	
	NYHA		4	2	4	4	4	2	3	1	4	4	4 (2–4)**
Clinical signs	Survival time	Days	9	77	1	65	9	1621	2309	40	2	1	24.5 (2–77)**
	Syncope		+	–	–	+	+	+	+	–	+	+	70***
	Dispnea		+	–	+	–	+	+	–	+	+	+	70***
Blood examination	cTnI	ng/ml	18.3	3.57	28.93	0.42	17.05	0.131	4.31	0.12	4.31	0.23	3.94 (0.23–17.05)**
	GOT		>2000	21	55	58.8	62	41	61	22	38	74	56.9 (38–62)**
	T4	µg/dL	1.5	2.3	2.2	0.8	1.2	0.9	1.4	1.7	1.6	2.2	1.58 ± 0.6*
X-ray	VHS	Vertebrae	11	8.7	9.8	8.7	11	10.5	8.7	9.4	9.2	10.3	9.7 ± 0.9*
ECG	HR	bpm	95	104	155	94	124	105	115	162	115	166	123.5 ± 27.5*
			bbb	bbb	bbb	pvc + avb	bbb	bbb	non	bbb + avb	non	bbb	80***

NYHA: New York Heart Association; cTnI: plasma cardiac troponin I; VHS: vertebral heart size; bbb: bundle branch block; avb: atrioventricular block; pvc: premature ventricular contraction. *Mean ± SD, ** Median (IQR), ***present (%).

morphology was attributable to a bundle branch block in seven dogs. The mean VHS on thoracic radiography was 9.7 ± 0.9 v, an almost normal value (Buchanan and Bücheler, 1995), except for two dogs (dogs one and five) with a mean VHS of 11.0 v.

Dogs 3, 4, and 8 were treated with β-adrenergic blockers (4,8), angiotensin-converting enzyme inhibitors (3,4,8), furosemide (4,8), spironolactone (4,8), aspirin (4), clopidogrel (3,8), or cardiac glycosides (4,8) based on clinical signs. The other dogs were untreated.

Echocardiography

Echocardiography has been used to evaluate abnormal ventricular wall thickening in previous studies (Morrison *et al*., 1992; Gonçalves *et al*., 2002; Cornell *et al*., 2004). As left ventricle morphological features are affected by BW, Cornell's standardized formula was used (Cornell *et al*., 2004). Dogs with HCM had a median IVSd and IVSDN of 13.7 (IQR, 11–15.8 mm) and 0.788 (IQR, 0.7–0.92), respectively, which were higher than those observed in clinically normal dogs (a 95% prediction interval of 0.29–0.52 is typically used for IVSDN using M-mode variables) (Cornell *et al*., 2004). Moreover, dogs with HCM had a median LVIDd and LVIDDN of 17.1 ± 10.3 mm and 0.849 (IQR, 0.57–1.15), respectively, lower than those observed in clinically normal dogs (a 95% prediction interval of 1.35–1.73 is typically used for LVIDDN using M-mode variables) (Cornell *et al*., 2004). Dogs with HCM had a median LVWd and LVPWDN of 10.7 (IQR, 9.6–12.3 mm) and 0.595 (IQR, 0.54–0.84), respectively (Tabl 2), higher than those observed in clinically normal dogs (a 95% prediction interval of 0.29–0.53 is typically used for LVPWDN using M-mode variables) (Cornell *et al*., 2004). Echocardiography revealed concentric cardiac hypertrophy in all cases [(IVSd + LVWd)/LVDd > 0.53] (Tables 2 and 3).

In dogs with HCM, the mean LA/Ao ratio (1.6 ± 0.5) and FS (57.7% ± 15.5%) were higher than those in clinically normal dogs (95% prediction intervals of 0.8–1.3 for LA/Ao and 25%–44% for FS are typically used for indexing M-mode variables) (Cornell *et al*., 2004).

Pathology and histopathology

Dogs with HCM demonstrated symmetrically hypertrophied ventricular septa (mean, 13.5 mm; range, 10–17 mm) and left ventricular free walls (mean, 14.5 mm; range, 9–19 mm). Greater concentric hypertrophy was observed compared with the ventricular cavity. The left ventricular wall was thickened compared with the aorta (9.2 mm) and protruded into the left ventricular outflow tract, which macroscopically appeared to be narrowed by >50% (Table 3, Figure 1A).

Histopathological examination revealed hypertrophy of cardiomyocytes in all dogs (mean, 18.3 µm; range, 15.1–20.3 µm) compared with the normal range (≤13.0 ± 0.7 µm) (Fujiwara *et al*., 1982). The proportions of dogs with cardiomyocyte disarray (70%), interstitial

Table 2. Echocardiographic findings with HCM in dogs.

Measurement		1	2	3	4	5	6	7	8	9	10	
IVSd	mm	22.2	15.8	19.8	11	13.4	14	12.8	11	13.9	8.5	13.7 (11–15.8)**
LVIDd	mm	40.9	21.3	20.8	10.3	14.6	8	4.6	20.6	10.2	19.7	17.1 ± 10.3*
LVWd	mm	11.6	9.8	12.3	9	13.6	15	9.7	9.4	18.6	9.6	10.7 (9.6–12.3)**
LA/Ao		1.41	1	0.7	1.8	1.59	1.8	2.5	2	1.6	1.6	1.6 ± 0.5*
FS	%	53.8	63.5	53.5	58.1	87.7	80	37.5	42	59.1	51.4	57.7 ± 15.5*
IVSDN		1.04	0.80	0.92	0.72	0.85	0.70	1.10	0.61	0.78	0.52	0.788 (0.7–0.92)**
LVIDDN		1.62	0.92	0.78	0.67	0.93	0.40	0.39	1.15	0.57	1.19	0.849 (0.57–0.15)**
LVWDN		0.53	0.51	0.59	0.60	0.88	0.77	0.84	0.54	1.07	0.59	0.595 (0.54–0.84)**

IVSd: interventricular septum thickness at end-diastole; LVIDd: left ventricular internal dimension at end-diastole; LVWd: Left ventricular posterior wall dimension at end-diastole; LA/Ao: left atrium-to-aorta ratio; FS: fractional shortening; IVSDN: intraventricular septal thickness in diastole, normalized for body weight; LVIDDN: left ventricular internal dimension at end-diastole, normalized for body weight; LVWDN: left ventricular free wall thickness in diastole, normalized for body weight. *Mean ± SD, ** Median (IQR).

fibrosis (80%), and small vessel disease (100%) are shown in Table 3 and Figure 1B.

Statistical analyses

Table 1 presents laboratory data from serum sampling. (Table 2 presents the echocardiography results. In dogs with HCM, the median plasma cTnI level (3.94 ng/ml; IQR, 0.23–17.05) was greater than that in healthy dogs (Langhorn and Willesen, 2016). Survival duration decreased as NYHA class increased ($r = -0.676$, $p = 0.04$; Table 4). NYHA classification and serum cTnI levels were weakly correlated ($r = 0.629$, $p = 0.06$), IVSDN and serum cTnI levels were strongly correlated ($r = 0.839$, $p = 0.01$), and serum cTnI levels and the LA/Ao ratio were weakly correlated ($r = -0.639$, $p = 0.06$; Table 4). There was no correlation between IVSDN and the LA/Ao ratio (Table 4).

Discussion

Owing to the limited previous studies in dogs with HCM (Liu *et al*., 1979a; Marks, 1993; Washizu *et al*., 2003; Pang *et al*., 2005; Schober *et al*., 2022), there is a lack of information regarding the clinical signs of HCM in dogs compared with cats (Payne *et al*., 2013; Maron and Fox, 2015) and humans (Gersh *et al*., 2011; Enriquez and Goldman, 2014). The results of this study demonstrate correlations between clinical, echocardiographic, and serological biomarker findings. All dogs with HCM in this study had a thickened left ventricular wall [median IVSd, 13.7 mm (IQR, 11–15.8 mm)] and postmortem pathological findings confirming HCM.

Echocardiography allows the measurement of wall hypertrophy for the diagnosis of HCM in cats (³6 mm) (Payne *et al*., 2013) and humans (³15 mm) (Gersh *et al*., 2011; Enriquez and Goldman, 2014). However, the results of the M-mode echocardiography in healthy dogs vary greatly according to BW. Therefore, a formula was devised to correct left ventricular wall measurements for BW (Cornell *et al*., 2004); however, these values cannot be used to differentiate between heart diseases (Cornell *et al*., 2004). Similarly, 2D echocardiography values can be weight-corrected (Schober *et al*., 2022). Dogs with HCM confirmed on postmortem pathology had IVSDN values [median, 0.788 (IQR, 0.7–0.92)] above the upper limit of normal (0.52, 95% prediction interval) (Cornell *et al*., 2004). Additional work to confirm or rule out HCM should be considered when echocardiography reveals ventricular wall hypertrophy in dogs (Schober *et al*., 2022).

Previous studies have reported factors associated with secondary cardiac hypertrophy; however, there are limited studies describing factors that worsen HCM in dogs (Liu *et al*., 1979b). The pathological findings in this study of dogs with HCM revealed the greater thickness of the ventricular septum (mean, 13.5 ± 2.5 mm) compared to the left ventricular outflow tract diameter (mean, 9.2 mm ± 2.1), indicating narrowing of the outflow tract. When this occurs, environmental factors, such as intensive exercise, high temperatures, and high humidity, can further overload the heart, potentially worsening concentric cardiac hypertrophy over time. In some cases, mild mitral regurgitation can cause eccentric cardiac hypertrophy attributable to volume overload.

Previous studies on clinical signs in cats with HCM (Payne *et al*., 2013) have reported syncope, dyspnea, SCD, and thrombosis. In this study, syncope (70%), dyspnea (70%), and SCD (30%) were observed. These results are similar to the previously reported findings in cats. Exercise intolerance and syncope were the most common clinical signs reported in previous studies of canine HCM (Schober *et al*., 2022).

HCM reportedly causes atrial fibrillation and ventricular arrhythmia in humans based on electrocardiographic

Table 3. Autopsy findings in dogs with HCM.

Case no.			1	2	3	4	5	6	7	8	9	10	
Macroscopic lesions	VS	mm	16	16	13	11	14	15	10	12	17	11	13.5 ± 2.5*
	LVW	mm	19	15	17	12	11	18	9	13	17	14	14.5 ± 3.3*
	LVOTD	mm	11	10	8	9	8	10	5	8	13	10	9.2 ± 2.1*
Cardiomyocyte histopathology	Diameter	µm	18.9 ± 5.4	20.0 ± 5.2	15.7 ± 3.8	15.1 ± 5.1	20.2 ± 5.5	20.3 ± 6.0	18.4 ± 4.9	18.8 ± 5.6	17.4 ± 4.5	18.0 ± 5.0	18.3 ± 1.8*
	Disarray	grade	0	0	1+	1+	2+	1+	2+	3+	0	1+	70**
	Interstitial fibrosis	grade	2+	0	1+	2+	1+	2+	1+	2+	0	1+	80**
	Small vessel disease	grade	3+	3+	1+	2+	1+	1+	1+	2+	3+	2+	100**

VS: ventricular septum; LVW: left ventricular wall; LVOTD: left ventricular outflow tract diameter. The cardiomyocyte diameter was measured as the mean short diameter at sites where the longitudinal section contained the nucleus. Cardiomyocyte disarray was scored according to the degree of abnormality in the arrangement of cardiomyocytes (hypertrophied, pinwheel configuration, or herringbone pattern) using the following scoring system: 0, none; 1+, mild; 2+, moderate; and 3+, severe. *Mean ± SD, **present (%).

findings (Enriquez and Goldman, 2014). We observed ventricular arrhythmia (10%) but were unable to confirm atrial fibrillation in dogs with HCM. Additionally, abnormal waveforms (80%) were observed on ECG; however, these ECG findings were not associated with the incidence or severity of HCM in dogs.

Histopathological examination is essential for diagnosing HCM in cats and humans. Characteristic histopathological findings include cardiomyocyte disarray (Fox, 2003) and myocyte hypertrophy (Hoshino *et al*., 1983). We performed necropsies on all dogs with HCM and observed histopathological findings characteristic of HCM, including cardiomyocyte hypertrophy (mean, 18.3 ± 1.8 µm), cardiomyocyte disarray (70%), interstitial fibrosis (80%), and small vessel disease (100%). These results demonstrate that dogs with HCM, similar to cats with HCM, had less cardiomyocyte disarray compared with humans with HCM (Lombardi and Betocchi, 2002).

In this study, left ventricular hypertrophy was characteristically concentric with nearly symmetrical wall thickening between the IVSd and left posterior ventricular wall (70%) (Schober *et al*., 2022). Ventricular wall thickening beyond the aortic diameter was observed leading to obstruction of the outflow tract (Figure 1). Additionally, we observed cardiomyocyte hypertrophy as a histological feature of HCM; however, no association was identified between cardiomyocyte hypertrophy and myocardial wall thickening. Although cardiomyocyte hypertrophy is posited to result from pressure overload, myocardial wall thickening is strongly associated with increased fat and fibrotic connective tissue (Kociol *et al*., 2010; Kubo *et al*., 2011). Accordingly, cardiomyocyte hypertrophy may not be the only factor contributing to the pathogenesis of HCM.

Numerous retrospective studies on cats and humans have reported risk factors for HCM. Many cats with HCM have an enlarged LA (Linney *et al*., 2014), which is believed to affect disease severity (Payne *et al*., 2013); however, other studies have reported that 34% of cats with HCM exhibit LA enlargement (Duler *et al*., 2019). LA enlargement was reported in only 22% of dogs with HCM (Schober *et al*., 2022); however, seven dogs in this study had apparent left atrium enlargement as indicated by an LA/Ao ratio of ≥1.6. However, no association was observed between left atrium enlargement (LA/Ao ratio ≥ 1.6) and prognostic factors. As this study's cohort was relatively small and many dogs had severe disease, further studies are warranted to validate these findings.

To the best of our knowledge, this is the first study to report serum cTnI levels in dogs with HCM. Serum cTnI levels were substantially higher (median, 3.94 ng/ml; IQR, 0.23–17.05) than those previously reported in healthy dogs (median, 0.03 ng/ml; range, 0.02–0.15 ng/ml) (Oyama and Sisson, 2004), dogs with dilated

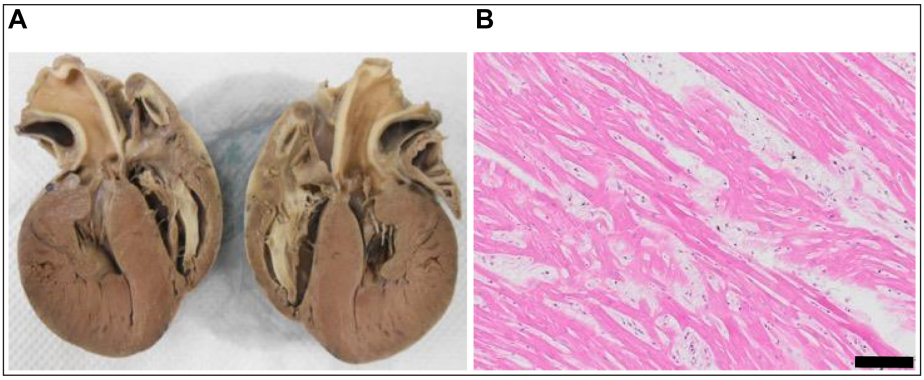

Fig. 1. Histopathological findings of HCM in dogs (A) 1 and (B) 5. (A) 2D long-axis view of the heart. Prominent left ventricular wall thickening, left ventricular cavity narrowing, and left ventricular outflow tract dysfunction are observed. (B) Staining of the IVSd myocardium with hematoxylin and eosin demonstrating cardiomyocyte disarray (black bar, 100 μm).

Table 4. Spearman's correlation coefficient between data with HCM in dogs.

Measurement		Correlation coefficient RS	Equal rank correction *p*-value
Survival time	NYHA	−0.676	0.04
	IVSDN	0.159	0.63
	LA/Ao	0.555	0.10
	cTnI	0.367	0.27
NYHA	IVSDN	0.233	0.48
	LA/Ao	−0.428	0.20
	cTnI	0.629	0.06
IVSDN	LA/Ao	−0.293	0.38
	cTnI	0.839	0.01
LA/Ao	cTnI	−0.639	0.06
cTnI	Age	−0.561	0.09
	Body weight	0.225	0.50
	Heart rate	−0.134	0.69
	LVIDDN	0.055	0.87
	LVWDN	0.067	0.84
	Cardiomyocyte diameter	−0.164	0.62

NYHA: New York Heart Association; IVSDN: intraventricular septal thickness in diastole, normalized for body weight; LA/Ao: left atrium-to-aorta ratio; cTnI: plasma cardiac troponin I; LVIDDN: left ventricular internal dimension at end-diastole, normalized for body weight; LVWDN: left ventricular posterior wall dimension at end-diastole, normalized for body weight. The cardiomyocyte diameter was measured as the mean short diameter at sites where the longitudinal section contained the nucleus. Significance of correlation coefficient ($p < 0.05$) 0.648, ($p < 0.01$) 0.794.

cardiomyopathy (median, 0.22 ng/ml) (Wess *et al*., 2017), dogs with mitral valve disease (median, 0.11 ng/ml) (Oyama and Sisson, 2004), dogs with congenital subaortic stenosis (median, 0.08 ng/ml) (Oyama and Sisson, 2004), and dogs with severe congestive heart failure (1–2 ng/ml) (Langhorn and Willesen, 2016). Various factors, including cardiac and noncardiac diseases, can increase serum cTnI levels (Kociol *et al*., 2010). Plasma cTnI levels have also been shown to be increased in cases of mild myocardial ischemia or stress due to leakage of free troponin (Hickman *et al*., 2010). The serum cTnI levels in dogs with HCM included in this study were highly variable (range, 0.12–28.93 ng/ml). The leakage of cTnI is attributable to myocardial injury and necrosis (Bertinchant *et al*., 1996). In this study, serum cTnI levels in dogs 1, 3, and 5 were

extremely high (range, 17.05–28.93 ng/ml). This finding may be attributable to severe myocardial ischemia, injury, and necrosis in dogs with acute exacerbation of NYHA class IV HCM-induced heart failure. Conversely, reversible myocardial injury or stress without cardiomyocyte necrosis or severe damage is reportedly correlated with low serum cTnI levels (Kubo *et al*., 2010). Interestingly, serum cTnI levels in the other seven dogs with HCM in this study were relatively low (range, 0.12–4.31 ng/ml). These lower serum cTnI levels may indicate ischemia or excessive loading rather than cardiomyocyte necrosis.

A correlation was observed between IVSDN and plasma cTnI levels in dogs with HCM (r = 0.839, p = 0.01) (Korraa *et al*., 2012). Elevated serum cTnI levels indicate myocardial ischemia, injury, or necrosis. Thus, elevated IVSDN levels in dogs with HCM may be attributable to myocardial injury or necrosis. The pathological analysis in this study revealed high rates of interstitial fibrosis (80%) and small vessel disease (100%) (Fox, 2003; Lombardi and Betocchi, 2004). These findings may indicate myocardial cell damage or ischemia (Pop *et al*., 2006; Belerenian *et al*., 2021), with myocardial cell damage known to cause cTnI leakage (Zhou *et al*., 2019).

Our results should be considered within the context of several limitations. First, the statistical analysis may have decreased accuracy given the small sample size in this study. Further, dogs with HCM are rare in clinical practice and pathological examinations are infrequently performed. Thus, the cases included in this study may have been skewed toward particular breeds or dogs with specific pathological conditions. Second, this study included only clinical cases. Accordingly, enrolled dogs may have had varying levels of cardiomyocyte damage as dogs with asymptomatic, chronic, and terminal stages of heart failure were included. Similarly, some dogs were treatment-naive, whereas others had previously been treated for cardiac diseases. Thus, some dogs included in this study may have exhibited the side effects of medications. Dogs with acute exacerbations of heart failure may have experienced additional cardiomyocyte damage owing to myocardial ischemia and excess loading. These effects may explain the observed variability in the levels of serum cTnI. Additionally, we did not quantify the areas of myocardial ischemia or necrosis. Serum cTnI levels were used to identify factors associated with myocardial ischemia and necrosis; however, only qualitative evaluations were possible. Novel methods for quantifying the area of necrosis in myocardial injury or ischemia are required. Future prospective and well-powered studies are warranted to overcome the limitations of this study and increase the generalizability of its findings.

In conclusion, IVSDN measurements have utility in assessing left ventricular wall hypertrophy in dogs. IVSDN values of >0.52 and increased serum cTnI levels are characteristic of HCM in dogs and may have utility in informing the clinical diagnosis of HCM or monitoring disease progression.

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Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

Dr. Takeki Ando conceptualized the study, collected the data, analyzed the data, and wrote the manuscript. Dr. Takeshi Izawa performed the pathological diagnosis. Dr. Hidetaka Nishida proofread the manuscript. Dr. Hideo Akiyoshi provided general guidance on the structure of this research and the proofreading of the manuscript.

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