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RESEARCH ARTICLE

Diagnostic Value of Heart to Single Vertebra Ratio-T6 in Feline Hypertrophic Cardiomyopathy: Comparison with Vertebral Heart Score

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ABSTRACT

The heart-to-single vertebra ratio measured at the sixth thoracic vertebra (HSVRT6) is a recently described radiographic index developed to simplify the assessment of cardiac silhouette size in cats. This prospective method-comparison study evaluated the diagnostic performance of HSVRT6 in feline hypertrophic cardiomyopathy (HCM), compared it with vertebral heart score (VHS), and assessed the ability of both measurements to differentiate ACVIM stages. A total of 124 cats were included: 48 control cats and 76 cats with HCM classified as ACVIM B1 (n=23), B2 (n=23), or C (n=30). Right lateral thoracic radiographs and echocardiographic examinations were obtained, and HSVRT6 and VHS were calculated. HSVRT6 values were lower in control cats than in all HCM groups and showed a progressive numerical increase across ACVIM stages (control: 7.35±0.70; B1: 8.07±0.70; B2: 8.51±0.96; C: 8.72±0.87). HSVRT6 was strongly correlated with VHS (r=0.835, P<0.001). For differentiating cats with HCM from control cats, HSVRT6 yielded an AUC of 0.803, with 88.9% sensitivity and 58.8% specificity, whereas VHS yielded an AUC of 0.752, with 75.6% sensitivity and 67.6% specificity. HSVRT6 showed particularly high sensitivity for distinguishing control cats from ACVIM B1 cats, while neither HSVRT6 nor VHS reliably discriminated against adjacent ACVIM stages. Overall, HSVRT6 appears to be a useful radiographic screening aid for cats suspected of having HCM, particularly when deciding whether echocardiography is warranted. However, its limited specificity and poor discrimination between adjacent stages indicate that echocardiography remains necessary for definitive diagnosis and staging.

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the leading myocardial disorder diagnosed in cats and may progress without obvious clinical signs for a prolonged period. Although echocardiography is required for definitive diagnosis and phenotypic classification, thoracic radiography continues to be widely used in first-line clinical assessment because it is accessible, rapid, and routinely available in many veterinary practices (Mark, 2000; Bradley, 2013). However, radiography cannot reliably distinguish HCM from other cardiomyopathies and is useful for detecting secondary cardiopulmonary changes such as cardiac silhouette enlargement, pulmonary edema, pleural effusion, or marked left atrial enlargement (Mark, 2000; Luis Fuentes *et al.*, 2020;).

The vertebral heart score (VHS) provides a quantitative way to estimate cardiac size on thoracic radiographs by relating the cardiac long (LA) and short (SA) axes to vertebral length (Litster and Buchanan, 2000). In cats, VHS has been used as an objective indicator of cardiac silhouette enlargement; nevertheless, its diagnostic sensitivity may be limited in diseases characterized by concentric myocardial thickening, such as feline HCM. In addition, technical factors, body conformation, vertebral abnormalities, and observer-related variation may influence the measurement (Hansson *et al.*, 2005; Jepsen-Grant *et al.*, 2013; Rungpupradit and Sutthigran, 2020; Souza *et al.*, 2020; Sak and Pazvant, 2021; Bagardi *et al.*, 2022; Mutlu *et al.*, 2024).

More recently, the heart to single vertebra ratio (HSVR) has been proposed as an alternative radiographic index that uses a single vertebral reference rather than

cumulative vertebral units in dogs (Costanza *et al.*, 2023) and cats (Costanza *et al.,* 2025). In cats, HSVRT6 showed the strongest agreement with VHS (CCC=0.95; 95% CI: 0.91–0.97), while HSVR measurements demonstrated excellent interobserver agreement (ICC 0.95–0.96) and good-to-excellent interobserver agreement (ICC 0.75–0.94). Notably, the interobserver ICC values reported for HSVR were higher than that obtained for VHS, which was attributed partly to elimination of the vertebral transposition and conversion steps required for conventional VHS measurement (Costanza *et al.,* 2025). These findings establish the repeatability of the HSVR method in cats and support evaluation of its clinical diagnostic performance in specific cardiac diseases.

The present study therefore aimed to determine the diagnostic value of HSVRT6 in cats with HCM staged according to ACVIM criteria. We aimed to compare HSVRT6 with VHS, evaluate its ability to distinguish cats with HCM from control cats, and assess its performance in pairwise comparisons between disease stages.

MATERIALS AND METHODS

Experimental Design and Animals: This prospective method-comparison study initially included 127 cats examined at the Department of Internal Medicine, Faculty of Veterinary Medicine, Ondokuz Mayıs University, between June 2025 and May 2026. Seventy-eight cats were diagnosed with HCM and classified as ACVIM B1, B2, or C. Cats with radiographs in which assessment of the cardiac silhouette was difficult due to thoracic or technical factors were excluded from the study. These factors include plural or pericardial effusion, pulmonary edema, pneumothorax, thoracic neoplasia, improper positioning, superimposition, artifacts, or spinal abnormalities such as deformities affecting the T6 vertebra, spondylitis, kyphosis, or other lesions that could interfere with spinal measurements.

Three cats that did not meet the criteria Control (ACVIM A) (n=1), Group B2 (n=1), Group C (n=1)] were excluded from the study. One cat, each from groups Control and B2 was excluded because pleural effusion prevented clear interpretation of the cardiac silhouette, while one cat from group C was excluded due to superimposed radiographic images. As a result of excluding these three cats, the study population consisted of 124 cats: 48 healthy cats and 76 cats diagnosed with HCM. Clinically healthy cats had no clinical or echocardiographic evidence of cardiovascular disease and were not considered to have a recognized predisposition to HCM. ACVIM Stage A cats had no echocardiographic evidence of cardiomyopathy but belonged to breeds considered predisposed to HCM. Both subgroups were phenotypically free of HCM and were therefore combined as the control group for the primary analyses. Of these cats, 48 control cats, comprising 20 clinically healthy cats and 28 ACVIM stage A cats (Group HC), 23 were Stage B1 (ACVIM B1), 23 were Stage B2 (ACVIM B2), and 30 were Stage C (ACVIM C).

Methods: All cats underwent echocardiographic examination for diagnosis and clinical classification. Hypertrophic cardiomyopathy and ACVIM stage allocation were determined according to the 2020 ACVIM consensus recommendations. Standard right parasternal long-axis and short-axis views were obtained using B-mode and M-mode echocardiography. Measurements included interventricular septal thickness (IVS), left ventricular internal diameter (LVID), left ventricular posterior wall thickness (LVPW) during diastole and systole, ejection fraction and fraction shortening (EF and FS), aortic diameter (Ao), left atrial diameter (LA), and the LA/Ao ratio. Echocardiographic examinations were performed using a Vetus 9 ultrasound device (Mindray®, Shenzhen, China) which is equipped with a 2.3-8.0 MHz phased cardiac probe (P8-2s, Mindray®, Shenzhen, China). Cats were classified according to their cardiomyopathy stages as Healthy and ACVIM A (48 cats), ACVIM B1 (23 cats), ACVIM B2 (23 cats), and ACVIM C (n=30). Cats with pathologies other than HCM were excluded from the study.

Radiographic imaging of the cats was performed without administering any sedative or anesthetic agents. Radiographic imaging was performed in right latero-lateral position and using a computerized radiography system (838 UHF100, Hasvet®, Antalya, Turkey). LA, SA, T6, Vertebral Heart Score, and LA+SA/T6 (HSVRT6) measurements were performed as described before (Litster and Buchanan, 2000; Costanza *et al.*, 2025). The cardiac long axis was drawn from the apex to the cardiac base at the level of the tracheal bifurcation, whereas the short axis was placed perpendicular to the long axis at the widest part of the cardiac silhouette. VHS was calculated by transposing these two axes onto the vertebral column from the cranial border of T4. For HSVRT6, the combined length of the long and short cardiac axes was divided by the length of the T6 vertebral body, including the caudal intervertebral disc space (Fig. 1-5).

Statistical analysis: Statistics were conducted using IBM SPSS Statistics version 21. The distribution of continuous variables was evaluated with Shapiro–Wilk and Kolmogorov–Smirnov tests. Group comparisons were performed using one-way analysis of variance, followed by Bonferroni correction for post-hoc pairwise comparisons. Diagnostic performance VHS and HSVRT6 were assessed by ROC curve analysis. For each ROC model, the AUC, 95% confidence interval, p value, optimal threshold, sensitivity, specificity, and Youden index were calculated. Because HSVRT6 and VHS were measured in the same cats, their correlated ROC curves were directly compared using DeLong's test. Data are expressed as mean ± standard deviation with 95% confidence intervals, and statistical significance was set at $P<0.05$.

RESULTS

Of the 127 cats initially assessed, three were excluded because radiographic assessment of the cardiac silhouette was not reliable. One cat from the control group and one ACVIM B2 cat were excluded because pleural effusion impaired cardiac silhouette evaluation, and one ACVIM C cat was excluded because of superimposed radiographic images. The final study population comprised 124 cats: 48 control cats, including 20 clinically healthy cats and 28 cats classified as ACVIM stage A, and 76 cats with HCM classified as ACVIM B1 (n=23), ACVIM B2 (n=23), or ACVIM C (n=30).

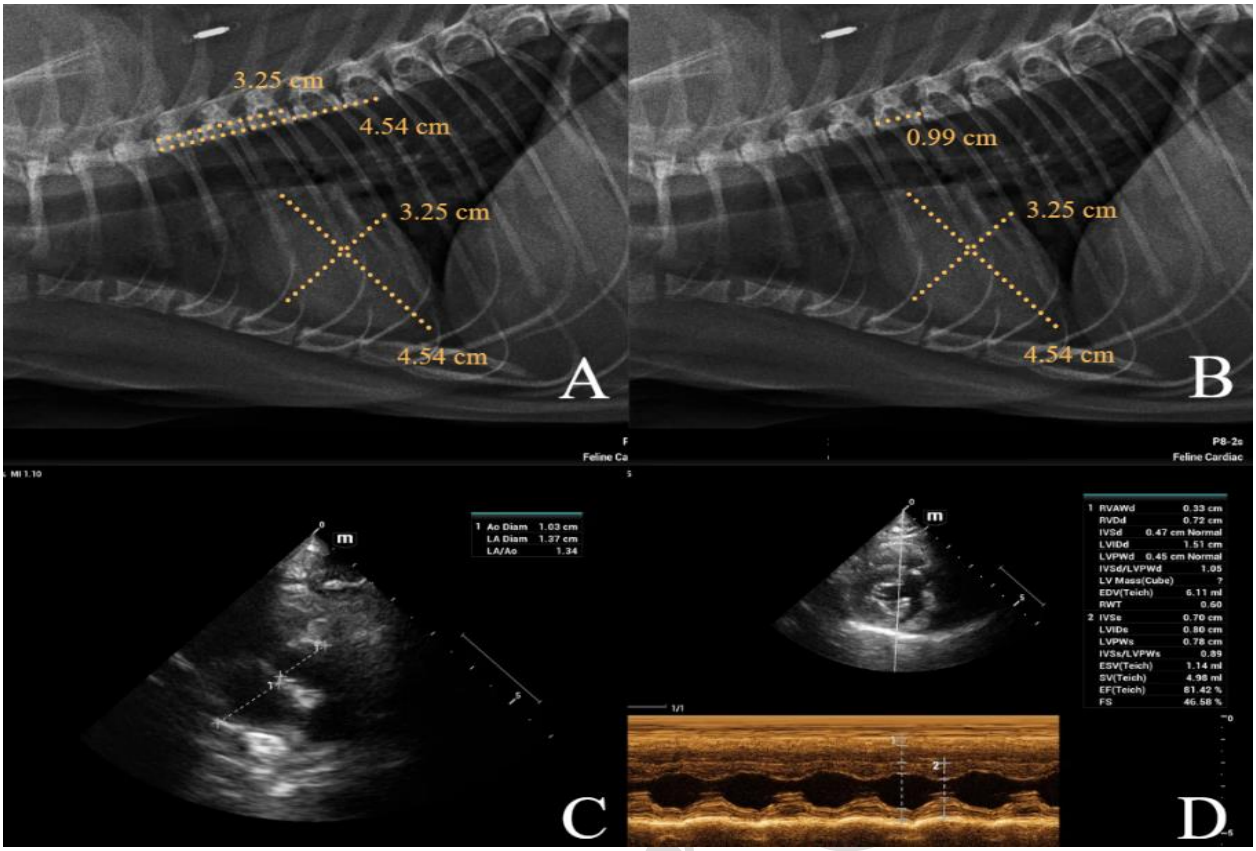

Fig. 1: Imaging of a Group HC cat. Yellow dotted lines indicate the cardiac long and short axes used for VHS (A) and HSVRT6 (B) measurements, and the T6 vertebral reference length used for HSVRT6 calculation (B), on a right lateral thoracic radiograph. Echocardiographic measurement of LA/Ao ratio (C) and M-mode measurement (D) were obtained from the right parasternal short-axis view.

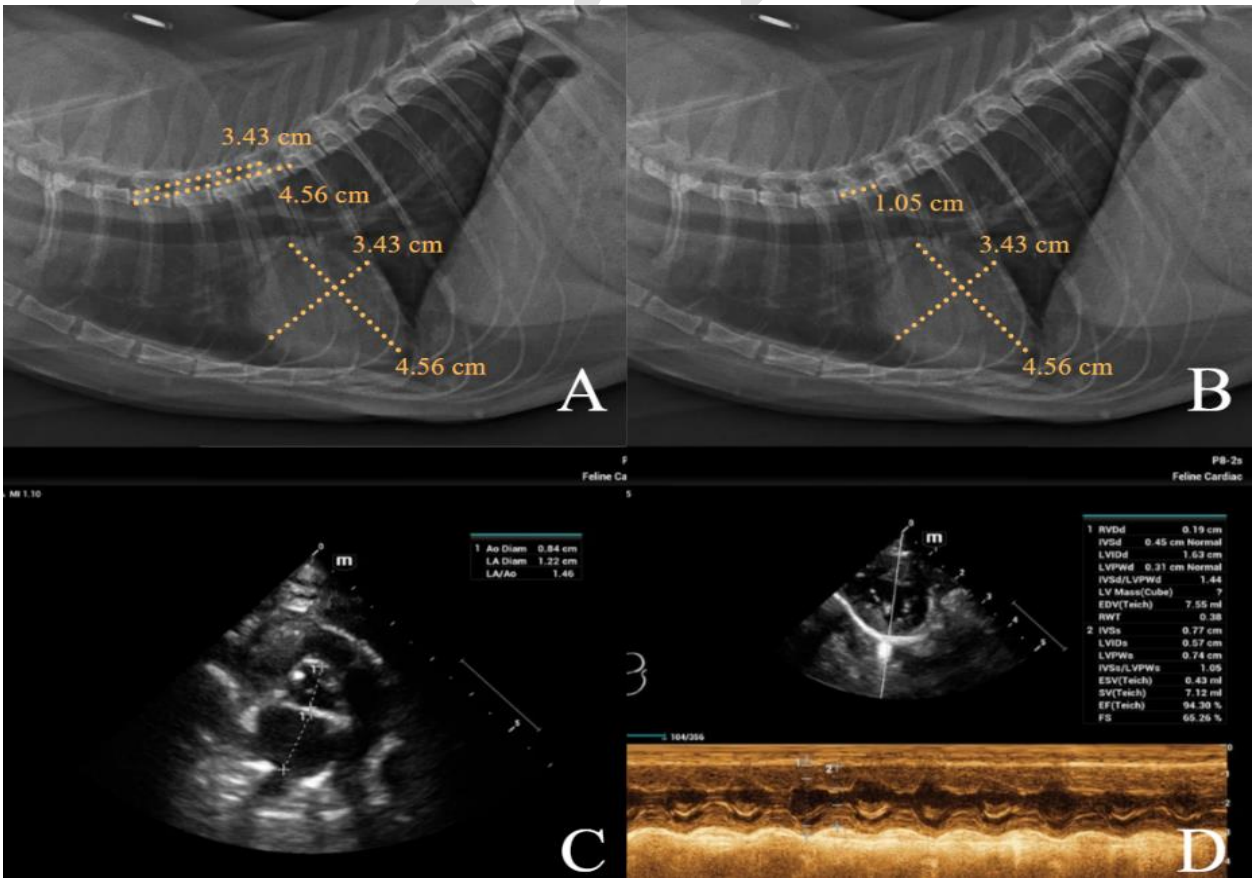

Fig. 2: Imaging of an ACVIM Stage A cat. Yellow dotted lines indicate the cardiac long and short axes used for VHS (A) and HSVRT6 (B) measurements, and the T6 vertebral reference length used for HSVRT6 calculation (B), on a right lateral thoracic radiograph. Echocardiographic measurement of LA/Ao ratio (C) and M-mode measurement (D) were obtained from the right parasternal short-axis view.

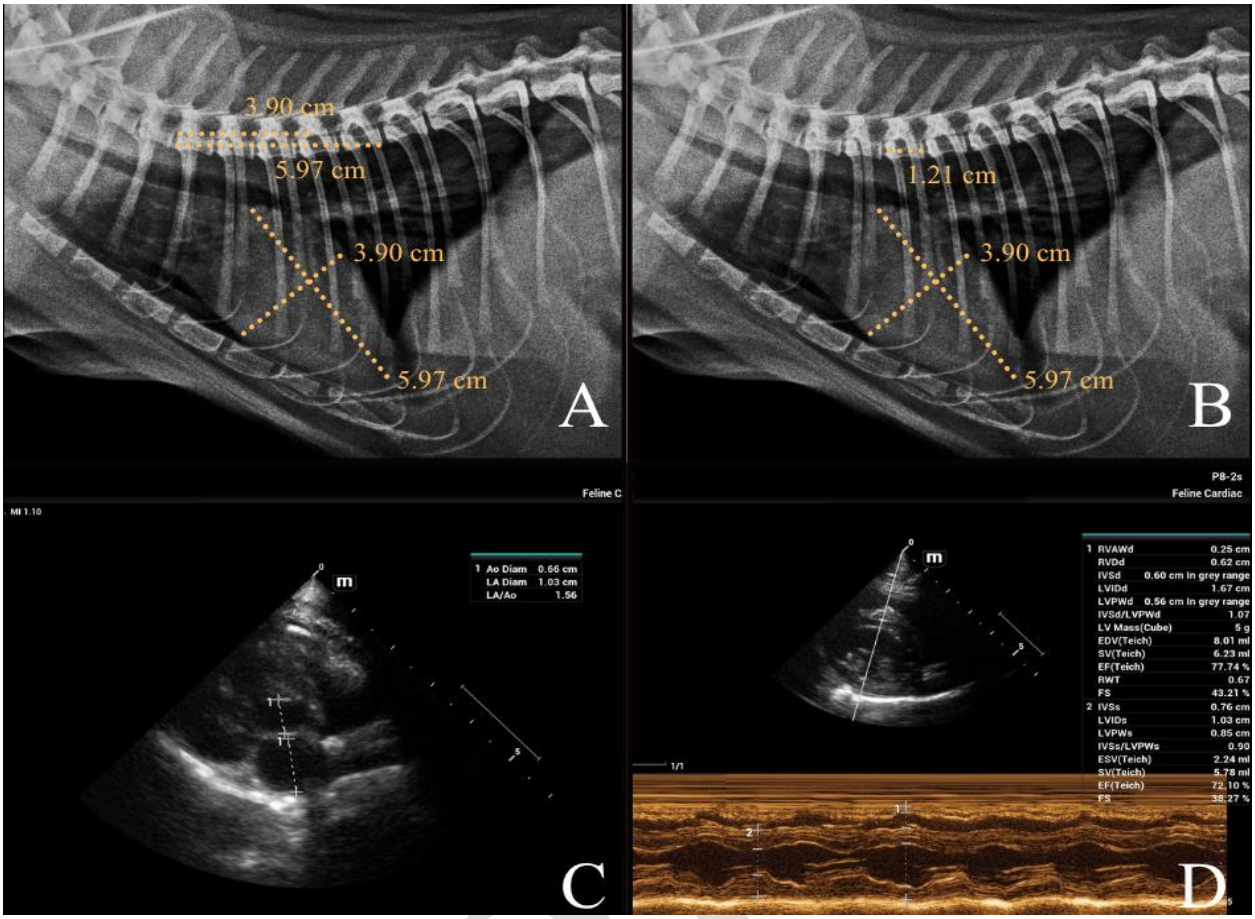

Fig. 3: Imaging of Stage B1 HCM cat. Yellow dotted lines indicate the cardiac long and short axes used for VHS (A) and HSVRT6 (B) measurements, and the T6 vertebral reference length used for HSVRT6 calculation (B), on a right lateral thoracic radiograph. Echocardiographic measurement of LA/Ao ratio (C) and M-mode measurement (D) were obtained from the right parasternal short-axis view.

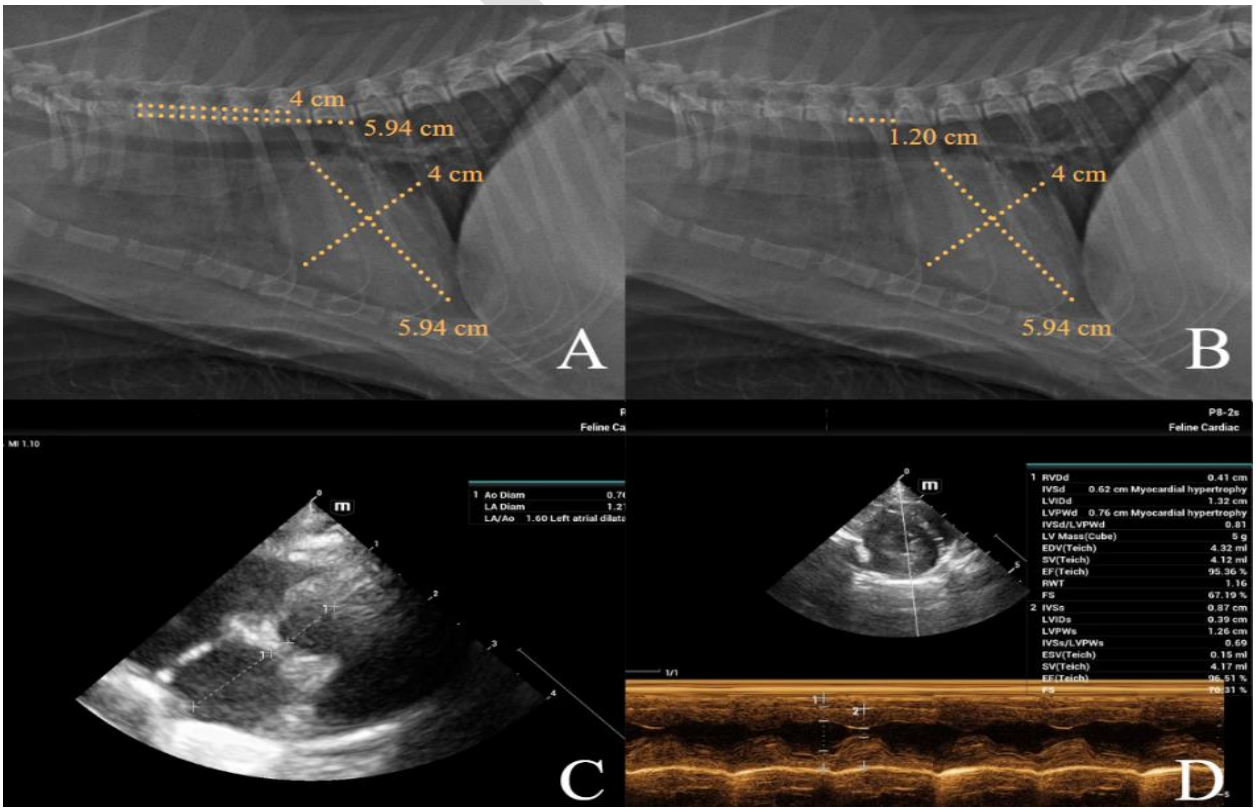

Fig. 4: Imaging of a Stage B2 HCM cat. Yellow dotted lines indicate the cardiac long and short axes used for VHS (A) and HSVRT6 (B) measurements, and the T6 vertebral reference length used for HSVRT6 calculation (B), on a right lateral thoracic radiograph. Echocardiographic measurement of LA/Ao ratio (C) and M-mode measurement (D) were obtained from the right parasternal short-axis view.

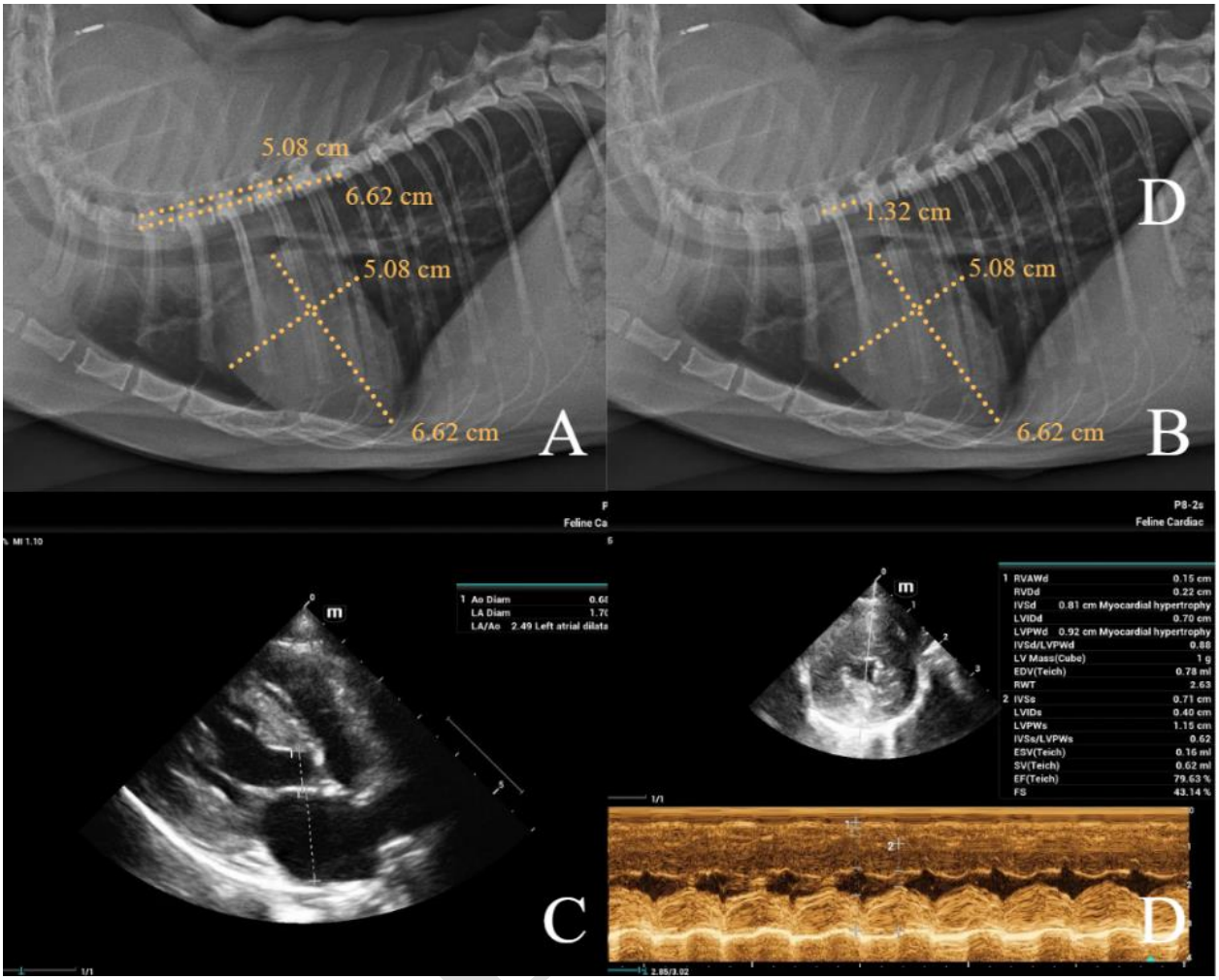

Fig. 5: Imaging of a Stage C HCM cat. Yellow dotted lines indicate the cardiac long and short axes used for VHS (A) and HSVRT6 (B) measurements, and the T6 vertebral reference length used for HSVRT6 calculation (B), on a right lateral thoracic radiograph. Echocardiographic measurement of LA/Ao ratio (C) and M-mode measurement (D) were obtained from the right parasternal short-axis view.

The mean age was 47.54±34.82 months in the control group, 57.04±33.54 months in ACVIM B1 cats, 61.83±34.22 months in ACVIM B2 cats, and 51.03±30.77 months in ACVIM C cats. No statistically significant difference in age was detected among groups. Overall, the study population comprised 48 males (30 intact and 18 neutered) and 76 females (60 intact and 16 spayed). The control group included 18 males and 30 females, whereas the ACVIM B1, B2, and C groups included 15, 9, and 6 males and 8, 14, and 24 females, respectively. British Shorthair (47/124, 37.9%), domestic shorthair (42/124, 33.9%), and Scottish Fold cats (22/124, 17.7%) were the most frequently represented breeds. The remaining cats comprised Persian (n=5), Chinchilla (n=2), Siamese (n=2), Turkish Angora (n=2), Norwegian Forest (n=1), and Exotic Shorthair cats (n=1), with their group-specific distributions presented in Table 1.

Echocardiographic comparisons are summarized in Table 2. Interventricular septal thickness during diastole and systole, as well as LVIDs, differed between the control group and all HCM groups (P<0.05), whereas no significant differences were observed among ACVIM B1, B2, and C cats for these variables. LVPW thickness during diastole and systole was greater in all HCM groups than in control cats (P<0.05), and ACVIM C cats had higher values than ACVIM B1 and B2 cats (P<0.05).

Table 1: Demographic data of cats included in the study.

Parameter/Group	HC	ACVIM B1	ACVIM B2	ACVIM C
Number of cats	48	23	23	30
Intact/Neutered Male	11/7	11/4	4/5	4/2
Intact/Spayed Female	26/4	4/4	11/3	19/5
Age (Months) Mean±Standard Deviation (95% CI)	47.54±34.82 (37.43-57.65)	57.04±33.54 (42.54-71.55)	61.83±34.22 (47.03-76.62)	51.03±30.77 39.54-62.52
Breed				
Domestic Shorthair	21	7	9	5
British Shorthair	12	10	10	15
Scottish Fold	11	5	1	5
Chinchilla	2			
Siamese		1		1
Norwegian Forest	1			
Exotic Shorthair				1
Persian	1		1	3
Turkish Angora			2	

LVIDd was lower in ACVIM B1 and B2 cats than in control cats (P<0.05), whereas ACVIM C cats did not differ significantly from the other groups. Left atrial diameter and LA/Ao were higher in ACVIM B2 cats than in control cats and were highest in ACVIM C cats, which differed significantly from the control, ACVIM B1, and ACVIM B2 groups (P<0.05). ACVIM B1 cats showed overlapping left atrial diameter and LA/Ao values with both the control and ACVIM B2 groups. No significant differences were observed among groups for EF, FS, or aortic diameter (P<0.05) (Table 2).

Table 2: Echocardiographic parameters of cats according to clinical stage.

Variable/Group	HC	ACVIM B1	ACVIM B2	ACVIM C
IVSd	0.46 ± 0.07[a] (0.44–0.48)	0.61 ± 0.11[b] (0.56–0.66)	0.65 ± 0.13[b] (0.59–0.70)	0.69 ± 0.18[b] (0.63–0.76)
LVIDd	1.47 ± 0.17[a] (1.42–1.52)	1.28 ± 0.22[b] (1.19–1.38)	1.24 ± 0.31[b] (1.11–1.37)	1.34 ± 0.26[ab] (1.25–1.44)
LVPWd	0.42 ± 0.07[a] (0.40–0.44)	0.62 ± 0.18[b] (0.54–0.69)	0.69 ± 0.16[b] (0.62–0.76)	0.88 ± 0.21[c] (0.80–0.95)
IVSs	0.70 ± 0.11[a] (0.66–0.73)	0.82 ± 0.17[b] (0.75–0.89)	0.89 ± 0.14[b] (0.83–0.95)	0.84 ± 0.22[b] (0.76–0.93)
LVIDs	0.66 ± 0.15[a] (0.61–0.70)	0.52 ± 0.20[b] (0.43–0.60)	0.56 ± 0.19[b] (0.47–0.64)	0.60 ± 0.21[b] (0.53–0.68)
LVPWs	0.76 ± 0.11[a] (0.73–0.79)	0.90 ± 0.22[b] (0.81–1.00)	0.98 ± 0.18[b] (0.90–1.06)	1.18 ± 0.22[c] (1.10–1.26)
EF%	87.38 ± 7.53[a] (85.19–89.56)	89.31 ± 8.79[a] (85.52–93.11)	87.49 ± 10.12[a] (83.11–91.86)	86.47 ± 10.09[a] (82.71–90.24)
FS%	55.21 ± 8.82[a] (52.65–57.77)	59.70 ± 13.30[a] (53.95–65.45)	57.24 ± 11.11[a] (52.43–62.05)	55.29 ± 12.57[a] (50.60–59.98)
AO Diameter (cm)	0.83 ± 0.11[a] (0.79–0.86)	0.81 ± 0.08[a] (0.78–0.85)	0.77 ± 0.11[a] (0.72–0.81)	0.75 ± 0.16[a] (0.69–0.81)
LA Diameter (cm)	1.08 ± 0.14[a] (1.04–1.12)	1.16 ± 0.14[ab] (1.10–1.22)	1.24 ± 0.16[b] (1.17–1.31)	1.60 ± 0.32[c] (1.49–1.72)
LA/Ao	1.31 ± 0.13[a] (1.27–1.35)	1.43 ± 0.13[ab] (1.37–1.48)	1.63 ± 0.17[b] (1.56–1.70)	2.19 ± 0.47[c] (2.01–2.37)

Note: Data are presented as Mean ± standard deviation (95% confidence interval). Cats were classified as controls (HC) or according to ACVIM stages B1, B2, and C. Echocardiographic measurements, including left atrial and ventricular parameters, systolic function indices, and LA/Ao ratio, are summarized for each group. Different superscript letters within a row indicate significant pairwise differences after Bonferroni correction (P<0.05).

Radiographic measurements are summarized in Table 3, and their distributions across groups are illustrated in Fig. 6. The mean HSVRT6 values were 7.35±0.70 in the control group, 8.07±0.70 in ACVIM B1 cats, 8.51±0.96 in ACVIM B2 cats, and 8.72±0.87 in ACVIM C cats. Corresponding VHS values were 7.50±0.56, 7.94±0.70, 8.48±0.88, and 8.68±0.97, respectively. Both HSVRT6 and VHS values were lower in the control group than in all HCM groups. In addition, ACVIM B1 cats had lower HSVRT6 and VHS values than ACVIM C cats; however, no significant differences were detected between ACVIM B1 and B2 cats or between ACVIM B2 and C cats (Table 3; Fig. 6). The cardiac long axis increased significantly across all groups, whereas T6 diameter did not differ among groups. Short-axis measurements were significantly higher in ACVIM C cats than in control and ACVIM B1 cats, while the ACVIM B2 group overlapped with the other groups (Table 3). HSVRT6 and VHS showed a strong positive correlation (Pearson's r=0.835, P<0.001; Fig.7).

Table 3: Radiographic measurements, HSVRT6, and VHS across study groups.

Variable	HC	ACVIM B1	ACVIM B2	ACVIM C
Long Axis (cm)	4.66 ± 0.60[a] (4.48–4.84)	5.19 ± 0.45[b] (4.99–5.39)	5.78 ± 0.77[c] (5.44–6.13)	5.90 ± 0.76[d] (5.60–6.21)
Short Axis (cm)	3.34 ± 0.45[a] (3,21–3.47)	3.56 ± 0.45[a] (3.36–3.76)	3.76 ± 0.40[ab] (3.58–3.94)	3.98 ± 0.62[bc] (3.73–4.23)
T6 Diameter (cm)	1.09 ± 0.09[a] (1.06–1.12)	1.09 ± 0.08[a] (1.06–1.13)	1.13 ± 0.12[a] (1.07–1.18)	1.14 ± 0.11[a] (1.10–1.19)
HSVRT6	7.35 ± 0.70[a] (7.14–7.55)	8.07 ± 0.70[b] (7.77–8.38)	8.51 ± 0.96[bc] (8.10–8.93)	8.72 ± 0.87[c] (8.39–9.05)
VHS	7.50 ± 0.56[a] (7.34–7.66)	7.94 ± 0.70[b] (7.63–8.24)	8.48 ± 0.88[bc] (8.10–8.86)	8.68 ± 0.97[c] (8.32–9.04)

Note: Different superscript letters within a row indicate significant pairwise differences after Bonferroni correction (P<0.05).

In ROC analysis, HSVRT6 differentiated cats with HCM from control cats with an AUC of 0.803 (95% CI: 0.717–0.889; P<0.001). At a cut-off value of ≥7.375, sensitivity and specificity were 88.9 and 58.8%, respectively. VHS showed an AUC of 0.752 (95% CI: 0.661–0.844; P<0.001), with 75.6% sensitivity and 67.6% specificity at a cut-off value of ≥7.614. Direct comparison of the correlated ROC curves using DeLong's test demonstrated that the AUC of HSVRT6 was significantly greater than that of VHS (P<0.05) (Fig. 8).

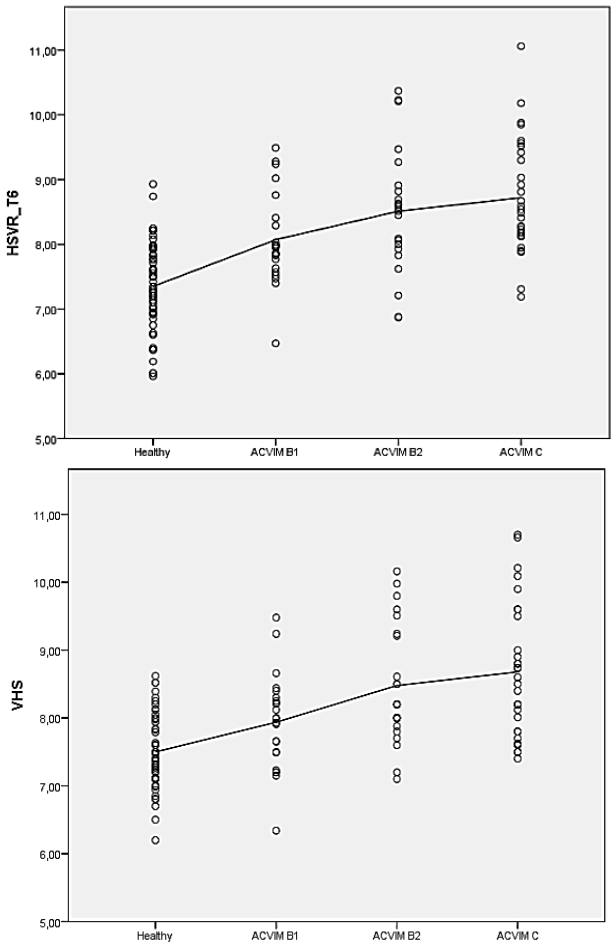

Fig. 6: A graph showing the distribution of HSVRT6 and VHS scores among the HC, ACVIM B1, ACVIM B2, and ACVIM C groups.

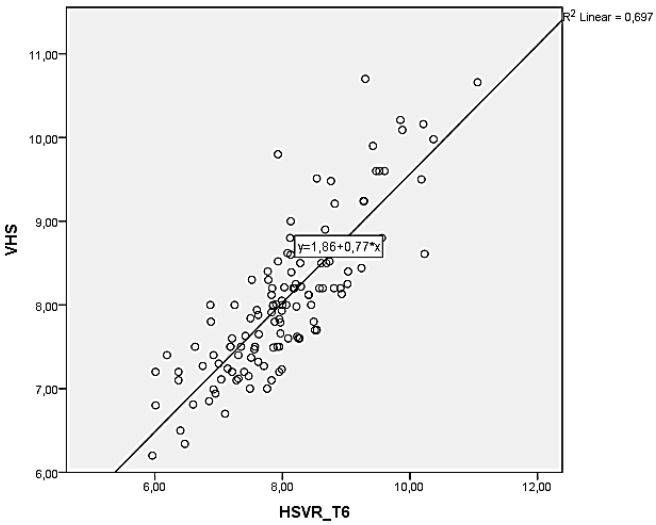

Fig. 7: Scatter plot of the linear relationship between VHS and HSVRT6. The graph shows that VHS is positively and significantly correlated with HSVRT6 for values obtained from 124 cats (Pearson r=0.835, P<0.001).

For differentiation between control cats and ACVIM B1 cats, HSVRT6 yielded an AUC of 0.770 (95% CI: 0.658–0.883; P<0.001), with 95.7% sensitivity and 50.0% specificity at a cut-off value of ≥7.375. VHS yielded an AUC of 0.693 (95% CI: 0.560–0.826; p = 0.009), with 69.6% sensitivity and 66.7% specificity at a cut-off value of ≥7.640 (Table 4; Fig. 9). Neither HSVRT6 nor VHS significantly differentiated ACVIM B1 from B2 cats (HSVRT6: AUC=0.661, p=0.062; VHS: AUC=0.657, p=0.068) or ACVIM B2 from C cats (HSVRT6: AUC=0.557, p=0.478; VHS: AUC=0.559, p=0.467) (Table 4; Fig. 10).

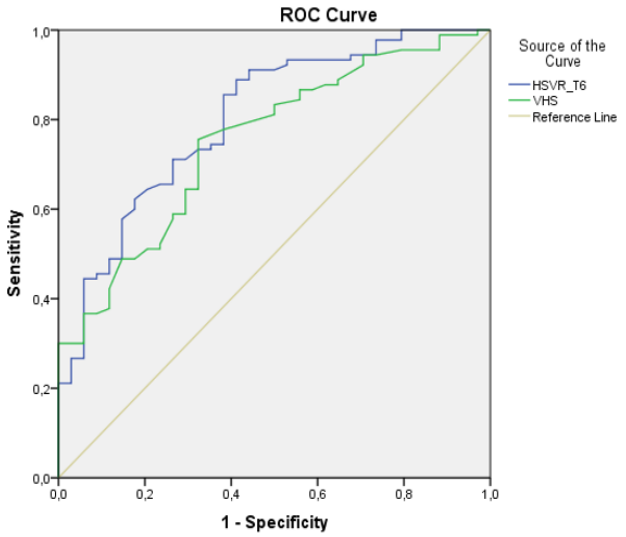

Fig. 8: ROC analysis showed that HSVRT6 discriminated cats with HCM from control group with good discriminatory performance (AUC = 0.803, 95% CI: 0.717-0.889, P<0.001). At a cut-off 7.375, sensitivity and specificity were 88.9% and 58.8%, respectively. VHS showed an AUC 0.752 (95% CI: 0.661-0.844, P<0.001), with a cut-off 7.614 yielding a sensitivity of 75.6% and a specificity of 67.6%. Direct comparison of the ROC curves using DeLong's test showed a significantly greater AUC for HSVRT6 than for VHS (P<0.05).

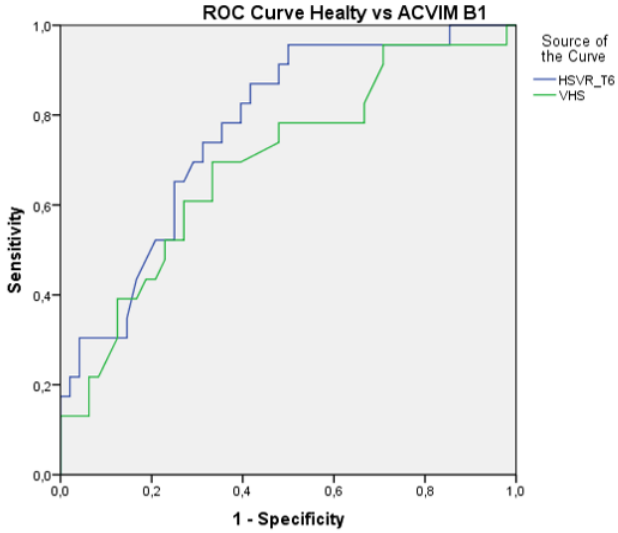

Fig. 9: ROC analysis demonstrated that HSVRT6 discriminated cats with ACVIM stage B1 from control cats with mild-to-moderate accuracy (AUC=0.770; 95% CI: 0.658–0.883; P<0.001). The optimal cut-off value was ≥7.375, yielding a sensitivity of 95.7% and a specificity of 50.0%. In comparison, VHS showed lower discriminatory performance (AUC=0.693; 95% CI: 0.560–0.826; p=0.009), with an optimal cut-off value of ≥7.64, yielding a sensitivity of 69.6% and a specificity of 66.7%.

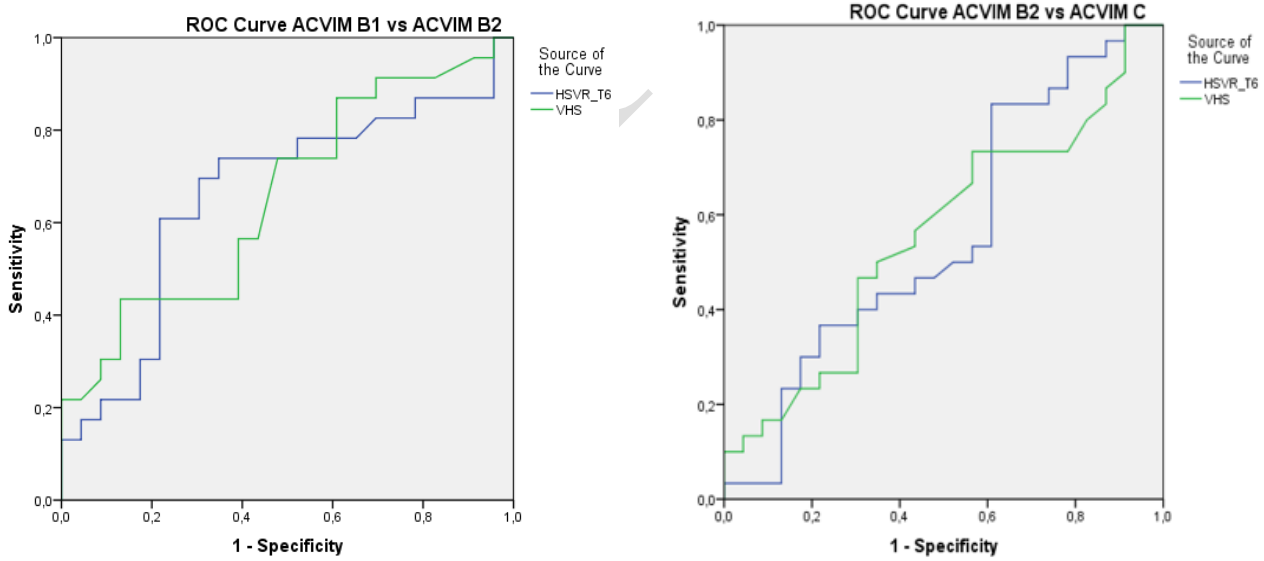

Fig. 10: ROC analysis showed that neither HSVRT6 nor VHS provided statistically significant discrimination between ACVIM stages B1 and B2 or between stages B2 and C.

Table 4: Pairwise ROC curve analysis of HSVRT6 and VHS across HC and ACVIM subgroups

Comparison	Positive /Negative n	Parameter	AUC	95% CI	p value	Optimal cut-off	Sensitivity (%)	Specificity (%)	Youden index
HC vs ACVIM B1	23/48	HSVRT6	0.770	0.658-0.883	<0.001	≥7.375	95.7	50.0	0.457
HC vs ACVIM B1	23/48	VHS	0.693	0.560-0.826	0.009	≥7.640	69.6	66.7	0.363
ACVIM B1 vs ACVIM B2	23/23	HSVRT6	0.661	0.497-0.824	0.062	≥8.430	60.9	78.3	0.392
ACVIM B1 vs ACVIM B2	23/23	VHS	0.657	0.498-0.815	0.068	≥8.470	43.5	87.0	0.305
ACVIM B2 vs ACVIM C	30/23	HSVRT6	0.557	0.396-0.718	0.478	-	-	-	-
ACVIM B2 vs ACVIM C	30/23	VHS	0.559	0.401-0.716	0.467	-	-	-	-

Note: (-), not reported because the ROC curve was not statistically significant (P>0.05); therefore, the corresponding optimal cut-off value, sensitivity, specificity, and Youden index were not considered clinically interpretable.

DISCUSSION

This study examined the effectiveness of HSVRT6 in diagnosing HCM in cats, its ability to distinguish between healthy cats and cats with HCM, and its use as an alternative to or in conjunction with VHS.

It has been reported in the literature that the average age increases as the stages of HCM progress in cats and that age is associated with HCM (Luis Fuentes *et al.*, 2020). Although the higher average age in HCM cats, particularly in ACVIM stages B1 and B2, suggests that increased age may be associated with HCM, the fact that HSVRT6 shows significant differences between stages despite a similar age distribution in healthy and early-stage groups strongly supports that this measurement is not age-dependent but rather a structural marker specific to HCM.

In the ACVIM consensus statement, cats with HCM are reported to be more likely to be male compared with healthy cats; however, in the present study, female cats were more frequently represented in both groups, and no statistically significant difference was observed (Luis Fuentes *et al.*, 2020). Similarly, sex distribution was not significantly different among groups, although females were more common in the present population as reported before (Costanza *et al.*, 2025). British Shorthair and Scottish Fold cats are found to be represented at a higher rate in advanced stages (ACVIM B1, B2 and ACVIM C). This finding is consistent with these breeds' predisposition to hypertrophic cardiomyopathy (Luis Fuentes *et al.*, 2020). However, the consistent performance of HSVRT6 across different breeds, in line with findings reported in the ACVIM consensus, demonstrates that this measurement is independent of genetic characteristics and is a clinically reliable parameter for staging (Luis Fuentes *et al.*, 2020).

Radiographic imaging measurements are commonly used methods for evaluating heart diseases in cats. The most used method is VHS measurement. VHS in cats was proposed by Litster and Buchanan in 2000 (Litster and Buchanan, 2000). VHS, which is an effective method either in addition to or as a standalone alternative to echocardiographic imaging is frequently preferred in cats today due to its practicality, speed, and cost-effectiveness (Bradley, 2013; Sleeper *et al.*, 2013; Kim *et al.*, 2023). Despite these advantages, VHS method is particularly inadequate in the early diagnosis of cardiomyopathies, and that measurements are affected in the presence of certain breeds, age, obesity and pathologies in the thoracic spine (Schober *et al.*, 2007; Johnson *et al.*, 2008; Guglielmini *et al.*, 2014; de Oliveira *et al.*, 2014; Schober *et al.*, 2014; Sak and Pazvant, 2021; Ozdil *et al.*, 2025). Studies conducted by Costanza *et al.* which proposed HSVR measurement in dogs in 2023 and in cats in 2025, have shown that the variation of HSVR measurement taken at the T6 level could be an alternative to VHS (Costanza *et al.*, 2023; Costanza *et al.*, 2025). The HSVRT6 value obtained using T6 showed the highest degree of reliability and accuracy with the VHS method. T6 provided the highest consistency from an anatomical and measurement perspective. Furthermore, HSVR and VHS measurements were compared in the study, and statistical consistency between the measurements was demonstrated (Costanza *et al.*, 2025).

A strong positive correlation was observed in HSVRT6 and VHS values were compared. Our findings support the data found in the literature (Costanza *et al.*, 2025). Studies comparing VHS with other measurement methods are available in the literature. In a study by Bilgiç *et al.* (2025), VHS was compared with manubrium heart score (MHS) measurement, no correlation was observed, and MHS was found to be insufficient for the diagnosis of heart disease (Bilgiç *et al.*, 2025). When VHS measurement is used and set as the standard in the diagnosis of heart disease, both the studies by Costanza *et al.* and this study demonstrate the adequacy of HSVRT6. The correlation between VHS and HSVRT6 support HSVR as a new method to diagnose heart diseases.

Feline hypertrophic cardiomyopathy is widely regarded as the predominant myocardial disease in cats, with reported prevalence rates generally ranging between 10 and 15% depending on the population evaluated (Riesen *et al.*, 2007; Paige *et al.*, 2009; Payne *et al.*, 2015; Sukumolanan and Petchdee, 2020; Kittleson and Côté, 2021; Bazzo da Costa *et al.*, 2025; Maurer *et al.*, 2025). Echocardiography remains the only gold standard method for HCM diagnosis and staging (Luis Fuentes *et al.*, 2020; Sukumolanan and Petchdee, 2020; Kittleson and Côté, 2021). In contrast, radiographic indices and circulating biomarkers may provide supportive information during initial assessment or screening, but they cannot independently establish the diagnosis of HCM or reliably separate cats into ACVIM stages.

Auscultation has limited reliability for identifying cardiac disease in cats, as physiological and mild-to-moderate murmurs can cause false results, and in the study by Paige *et al.* (2009) auscultated murmurs showed low sensitivity (31%) but relatively high specificity (87%) for diagnosing cardiomyopathy, leading to many missed cases. Electrocardiography (ECG) can also be used in the evaluation of cardiac diseases; however, the current literature shows that ECG does not provide sufficient accuracy in diagnosing HCM and distinguishing between stages of HCM (Häggström *et al.*, 2015; Gaia de Sousa *et al.*, 2025). It has been reported that cats with HCM can have ECG findings like those of healthy cats (Häggström *et al.*, 2015).

One of the biomarkers used in the diagnosis of HCM is cardiac troponin I (cTnI). In the literature, the adequacy of the cTnI value in establishing the diagnosis of HCM has been effectively reported. If other causes of cardiac damage are ruled out, plasma cTnI concentration is highlighted as being useful in predicting the severity of HCM in cats (Hemdon *et al.*, 2002; Connolly *et al.*, 2003; Borgeat *et al.*, 2014; Langhorn *et al.*, 2014; Hori *et al.*, 2018; Hanås *et al.*, 2022). In a study conducted by Hori *et al.* (2018), when comparing cTnI values in healthy and heart-diseased cats, it was suggested that cTnI values below <0.163 ng/mL rule out HCM diagnosis, while cTnI values ≥0.234 ng/mL or higher may represent severe HCM stage. Another biomarker is NT-proBNP. Hsu *et al.* (2009) ranked the stages of HCM from mild to severe in their study. NT-proBNP shows greater diagnostic performance in severe HCM, with reported sensitivity and specificity of 90 and 83%, respectively, whereas its sensitivity decreases markedly in moderate disease. Overall, its reported sensitivity and specificity for

distinguishing cats with HCM from healthy cats are approximately 58 and 86%, respectively (Hsu *et al.*, 2009; Laudhittirut *et al.*, 2020).

The effectiveness of HSVRT6 in diagnosing HCM has been demonstrated. The present findings indicate that HSVRT6 has potential clinical value as a radiographic screening index for HCM. For differentiation of cats with HCM from control cats, HSVRT6 yielded an AUC of 0.803, with a sensitivity of 88.9 and a specificity of 58.8%. Its high sensitivity suggests that HSVRT6 may be useful for identifying cats that warrant further echocardiographic evaluation; however, its moderate specificity indicates a considerable potential for false-positive results. This limitation was even more evident in the comparison between control and ACVIM B1 cats, in which sensitivity reached 95.7% but specificity was only 50.0%. Therefore, HSVRT6 should not be interpreted as a stand-alone diagnostic test but rather as a screening or referral-support parameter.

VHS is an established and readily available radiographic method for assessing cardiac silhouette enlargement in cats; however, its diagnostic performance may be limited in subclinical or early HCM, in which marked cardiomegaly may not yet be present (Guglielmini *et al.*, 2014; Kim *et al.*, 2023). The values found are valid in cases of cardiomegaly, but their accuracy is limited in subclinical cases or cases where HCM is not severe. In this regard, VHS is considered a screening tool for cardiac disease, cardiomegaly, etc., rather than for diagnosing HCM. In contrast to VHS, in our study, HSVRT6 measurement showed significant diagnostic differentiation in terms of HCM diagnosis. Although VHS remains a rapid preliminary assessment tool, it is not reliable on its own, particularly in early-stage HCM cases, due to its low sensitivity. When these studies are evaluated, it is seen that the HSVRT6 showed higher sensitivity in diagnostic accuracy for HCM diagnosis compared to VHS.

A VHS measurement >9.3 was found to be specific for heart disease (Sleeper *et al.*, 2013). In the study conducted by Guglielmini *et al.* the cut-off VHS measurement was found to be >7.9. In cats with left atrial enlargement, the specificity was reported as 82% and the sensitivity as 91%. Based on this measurement, its diagnostic power was reported as moderate in distinguishing healthy cats from cats with heart disease, in cats with left-sided heart disease, and in distinguishing cats with left atrial enlargement (Guglielmini *et al.*, 2014). In the review conducted by Laudhittirut *et al.* it was observed that the specificity and sensitivity values of VHS varied according to the cut-off cats with measurements >8, the sensitivity value was 44.74% and the specificity value was 65%, while in cats with measurements >8.5, the sensitivity value was 26.32% and the specificity value was 85%. The study indicates that VHS alone may be insufficient, particularly in detecting mild/early disease (Laudhittirut *et al.*, 2020). The clinical implication is that VHS alone can cause false positive or false negative results. It has been reported that VHS differs between HCM and healthy cat groups, but in some HCM-CHF cases, VHS can remain within normal limits (Kim *et al.*, 2023). The measurement of the HSVR ratio using the T6 variation yielded highly significant results in distinguishing cats with HCM from healthy cats.

An additional objective was to assess whether HSVRT6 could contribute not only to the identification of cats with HCM but also to differentiation among ACVIM stages. HSVRT6 values showed a progressive increase across the study groups, from 7.35 ± 0.70 in the control group to 8.07±0.70 in ACVIM B1, 8.51±0.96 in ACVIM B2, and 8.72±0.87 in ACVIM C. The echocardiographic profile of the study groups was consistent with the expected structural phenotype of feline HCM, with greater ventricular wall thickness in HCM groups and more pronounced left atrial remodeling in ACVIM C cats. Because echocardiographic findings contributed to ACVIM classification, these observations should be interpreted as a description of the cohort phenotype rather than as independent validation of HSVR_T6.

Pairwise comparisons showed that HSVRT6 values were lower in the control group than in all HCM groups. In addition, ACVIM B1 and B2, as well as ACVIM B2 and C, showed overlapping values. These group-level findings suggest that HSVRT6 reflects the overall increase in cardiac silhouette dimensions associated with progression of HCM. However, statistically significant differences between group means should not be interpreted as evidence that HSVRT6 can accurately classify individual cats into adjacent ACVIM stages.

Indeed, ROC analysis showed that HSVRT6 did not achieve statistically significant discrimination between ACVIM B1 and B2 cats or between ACVIM B2 and C cats. VHS showed comparable limitations in these comparisons, with AUC values of 0.657 for ACVIM B1 versus B2 and 0.559 for ACVIM B2 versus C. Therefore, although both HSVRT6 and VHS values increased with disease severity at the population level, substantial overlap between adjacent stages limited their utility as individual stage-classification tools.

This finding is clinically plausible because ACVIM staging is not based solely on the magnitude of cardiac silhouette enlargement. The classification of feline cardiomyopathy incorporates echocardiographic phenotype, left atrial remodeling, estimated risk of congestive heart failure or arterial thromboembolism, and the presence or previous occurrence of clinical signs. Consequently, cats in adjacent ACVIM stages may have overlapping radiographic cardiac dimensions despite clinically meaningful differences in disease risk and echocardiographic findings (Luis Fuentes *et al.*, 2020).

The higher diagnostic performance of HSVRT6 in distinguishing control cats from ACVIM B1 cats is particularly noteworthy. In this comparison, HSVRT6 showed an AUC of 0.770, with a sensitivity of 95.7% at a cut-off value of ≥7.375. This result suggests that HSVRT6 may be sensitive to early changes in the cardiac silhouette and may help identify cats that warrant echocardiographic evaluation. However, its lower specificity (50.0%) indicates that it should be regarded as a screening or referral-support tool rather than as a stand-alone diagnostic or staging test.

Although inter- and intraobserver repeatability of HSVRT6 has previously been demonstrated in cats, observer agreement was not reassessed within the present HCM cohort. Future multicenter studies including observers with diverse levels of radiographic experience could further confirm the reproducibility of HSVRT6

under disease-specific clinical conditions. Published radiographic studies similarly suggest that thoracic radiography has greater value for detecting cardiac enlargement, HCM-associated structural changes, or congestive heart failure than for accurately defining individual disease stages. VHS may remain within conventional reference ranges in cats with subclinical HCM, whereas more marked abnormalities are observed in cats with severe disease or congestive heart failure (Kim *et al.*, 2023). The previously reported feline HSVR study demonstrated that HSVR is a simple and reproducible radiographic method with good agreement with VHS; however, it was not designed to validate HSVR as an independent ACVIM staging test (Costanza *et al.*, 2025).

A similar limitation is reported for circulating cardiac biomarkers. NT-proBNP may support the identification of cats with HCM and may increase with disease severity; however, its performance varies according to the severity of myocardial remodeling and the selected diagnostic threshold. Its ability to distinguish mild, equivocal, or adjacent disease categories is less consistent than its performance in advanced disease (Hsu *et al.*, 2009; Luis Fuentes *et al.*, 2020). Taken together, these data indicate that staging of feline HCM requires an integrated approach, with echocardiography remaining the primary method for disease classification and clinical risk assessment.

Conclusions: The current results indicate that HSVRT6 is useful for distinguishing healthy control cats from cats with HCM, particularly cats in ACVIM B1. Nevertheless, HSVRT6 and VHS should not be used alone to discriminate between adjacent ACVIM stages. Larger, stage-balanced, multicenter studies including cats with ACVIM stage D disease are needed to establish whether HSVRT6 can provide additional prognostic or staging value when interpreted together with echocardiographic variables and cardiac biomarkers.

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