Original Article

The evaluation of eccentric cardiac hypertrophy due to volume overload using radiographic cardiac indices in rats

Yusuf Sinan ŞİRİN[1] Mehmet Alper ÇETİNKAYA[2] Mahir KAYA[3*]

Abstract

The purpose of this study was to determine the diagnostic values of the vertebral heart score (VHS), radiographic left atrial dimension (RLAD) and vertebral left atrial size (VLAS) in the radiographic evaluation of eccentric cardiac hypertrophy due to volume overload (VO) in rats. Rats underwent either needle aortocaval shunting or sham operations. Six weeks later, VHS, RLAD, and VLAS were measured from radiographs of all surviving rats (20 control and 54 VO). Measurements were performed by two observers blinded to the study data. Heart weight (HW) and HW: body weight (HBW) ratio were determined for all rats after sacrifice. Correlations between HBW and VHS, RLAD and VLAS values were estimated, as well as their sensitivity and specificity for detecting cardiomegaly and left atrial (LA) enlargement. Receiver operating characteristic (ROC) curves were used to estimate the optimal decision criteria for each radiographic index. A strong positive correlation was observed between these indices and both HW and HBW. The sensitivity and specificity of these indices for detecting cardiomegaly and LA enlargement when evaluated at the optimal cut-off values were 100% and 95%, respectively, at 8.7 vertebrae (v) for RL-VHS, 98.4% and 96.6% at 8.8v for DV-VHS, 97.6% and 90% at 1.6v for RLAD and 92.7% and 89.1% at 2.1v for VLAS. Both intra- and inter-observer measurements revealed good to excellent agreement for these indices. The study results indicate that VHS, RLAD and VLAS are simple, repeatable and useful radiographic measurements for predicting cardiomegaly and LA enlargement in rats with eccentric cardiac hypertrophy.

Keywords: Eccentric cardiac hypertrophy, volume overload, VHS, RLAD, VLAS, rat

[1]*Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Surgery, 15030, Burdur, Türkiye*
[2]*Laboratory Animal Research and Application Center, Hacettepe University, 06100; Sihhiye-Ankara, Türkiye*
[3]*Medical Imaging Techniques Program, Health Services Vocational College, Akdeniz University, 07070 Antalya, Türkiye*
***Correspondence**: kayamahir@gmail.com* (M. KAYA)

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Introduction

Small exotic mammals such as rodents, including ferrets, rabbits, and rats, laboratory animals, have also recently begun being kept as pets, for which reason their importance and numbers as veterinary patients are both growing. Cardiac diseases also occur in pet rats (Sharp and Villano, 2012; Dias *et al*., 2017), similarly to other exotic mammals (Orcutt and Malakoff, 2020). Spontaneous cardiomyopathy is more frequent in older rats as a component of the ageing process. Cardiomyopathy has also been linked to left atrial (LA) and ventricular thrombosis in elderly rats (McInnes, 2012). Mesquita *et al*. (2020), also demonstrated that aged rats developed left ventricular hypertrophy, LA enlargement, diastolic dysfunction and pulmonary congestion.

One of the most widespread causes of cardiac hypertrophy is volume overload (VO). This is caused by anemia, heart block, regurgitant mitral or aortic valves, atrial or ventricular septal defects or other congenital diseases and results in eccentric hypertrophy (Carabello BA., 2002, Cantor *et al*., 2005). Aortocaval shunting (ACS) for VO is a regularly employed model for the induction of eccentric hypertrophies (Garcia and Diebold, 1990).

Echocardiography is frequently used to assess cardiac structure and functions in both human and veterinary medicine and experimental studies in the context of the diagnosis of cardiac diseases (Coatney, 2001). However, it cannot be routinely employed in pet rats because, due to these animals' small body mass and high heart rates, a special ultrasonography probe and software are required, as well as a considerable degree of operator experience (Dias *et al*., 2021). Thoracic radiography permits heart size and dimension to be objectively evaluated using radiographic cardiac indices. These include vertebral heart score (VHS) (Buchanan and Bucheler, 1995), vertebral left atrial size (VLAS) (Malcom *et al*., 2018), and radiographic left atrial dimension (RLAD) (Sanchez Salguero *et al*., 2018) and have previously been defined for healthy dogs and cats and those with heart disease. VHS, RLAD, and VLAS have also been investigated in healthy Sprague–Dawley (Dias *et al*., 2021) and Wistar albino (Çetinkaya and Kaya, 2022, Kaya et al., 2022, Dogan et al., 2022) rats. However, no values have yet been published for rats with eccentric cardiac hypertrophy.

The purpose of this study was to determine the diagnostic value of VHS, RLAD and VLAS in the radiographic evaluation of eccentric cardiac hypertrophy developing due to ACS-induced VO within rats.

Materials and Methods

Animals: Eighty adult male Wistar albino rats (average weight 340 g) were housed in the Akdeniz University Experimental Research and Application Center (Türkiye) at 50-60% humidity and 20-21°C in a 12-h dark/light cycle. All rats were given standard rat chow and permitted ad libitum access to drinking water. The research was performed in compliance with the national guidelines for the care and use of laboratory animals. Approval for the experimental protocol was granted by the Akdeniz University animal care ethics committee on the use of animals (No. B.30.2.AKD.0.05.07.00/27). Physical examinations were performed on all animals prior to the surgical procedures. No cardiovascular or pulmonary abnormalities (such as murmur, arrhythmia and abnormal respiratory sounds) were detected in any animals and their hydration status was evaluated as normal. Rats were randomly divided into two groups, control (n = 20) and VO (n = 60), before surgery.

Experimental VO Model: VO was induced using the ACS method, as described elsewhere (Garcia and Diebold 1990). Laparotomy was performed on all rats using a combination of intraperitoneal ketamine (75 mg/kg, Ketasol, Richter Pharma-Interhas, Türkiye) and xylazine HCl (5 mg/kg, Xylazin Bio, Bioveta-Interhas, Türkiye). Blunt dissection between the renal arteries and the iliac bifurcation was applied in order to expose the abdominal aorta and caudal vena cava (CaVC). Next, a shunt was created by inserting an 18-gauge needle into the exposed aorta at a 45° angle and propelling this into the CaCV. The resulting puncture was sealed with cyanoacrylate (Leukosan®Adhesive, BSN Hamburg, Germany). In case of successful VO, oxygenated blood from the abdominal aorta was observed mixing in the CaCV. The sham-operated animals represented the control group and these underwent the same surgical procedure but without the shunt. The surgical procedure was carried out with the assistance of an operating microscope (OPMI Visu 150®, Carl Zeiss Meditec, Germany). The housing conditions described above were maintained unaltered throughout the six-week study period. The mortality rate due to VO at the end of this period was 10% (n = 6). No mortality occurred in the control group. The study was thus completed with 20 controls and 54 rats exposed to VO.

Radiographic procedures: At the end of the six-week study period, all surviving rats were weighed and re-anesthetized using a combination of ketamine and xylazine. A contrast medium was administered by means of intravenous access in the tail vein. Each animal's thoracic region was imaged in right lateral (RL) and dorsoventral (DV) projections (X-ray tube: ORIX-65, Ardet®, Istanbul, Türkiye. Parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance).

For RL projection, the rats were placed on their right sides on the X-ray cassette. The thoracic legs were then extended in a cranial direction. The beam was centered at the level of the thorax between the scapulohumeral joint and the last rib. RL contrast radiographs were captured using a bolus injection into the tail vein of 0.5 ml non-ionic opaque contrast agent (300 mg I/ml Iohexol, Omnipaque®, Opakim, Türkiye). Exposure was performed immediately at the end of the contrast agent injection (Çetinkaya and Kaya, 2022). In case of DV projection, the rats were immediately placed on another cassette in the sternal position. The thoracic region was then imaged under identical exposure conditions.

Radiographic images captured by means of a computed radiography reader (FCR Prima T2®, FujiFilm, Tokyo, Japan) were stored for subsequent retrieval. These radiographic images were anonymized and randomized before being evaluated by two observers on commercially available computer software (Image Intelligence™, FujiFilm, Tokyo, Japan). Both observers possessed more than 20 years' veterinary medicine experience and were blinded to the study groups and the animals' body and heart weights. The observers were permitted to manipulate the images as required by altering the window width, window level and magnification. Measurements were made of the radiographic cardiac indices. Intraobserver agreement for all radiographic measurements was determined with one investigator (MK) performing measurements on two separate occasions on 10 rats randomly selected from each group. Interobserver variability was established by two observers (MK and MAÇ), who completed all radiographic measurements for the same 20 animals.

***Measurements of radiographic cardiac indices*:** VHS (Buchanan, 2000), RLAD (Sanchez Salguero *et al*., 2018) and VLAS (Malcom *et al*., 2018) values were determined in a manner consistent with the previously published methods and on the same computer software. VHS, RLAD, and VLAS were measured from RL views, and VHS from DV views, as described below and also shown in Figure 1.

For VHS, the cardiac long (L) and short (S) axes were measured from both RL (Figure 1A2 and 1B2) and DV (Figure 1A3 and 1B3) views. On RL views, the L axis was measured between the tracheal bifurcation and the cardiac apex. The S axis was determined by calculating the distance between the intersection of the caudal border of the heart silhouette and the dorsal border of the CaCV to the cranial margin of the heart. A 90-degree rotation was applied between L and S axes using commercially available software. On the DV view, the L axis was determined by measuring the distance between the intersection of the right mediastinal and heart silhouettes to the apex. The widest measurement obtained perpendicular to the L axis was defined as the S axis. These two axes obtained from RL and DV views were then superimposed over the thoracic vertebrae from the cranial edge of T4 in RL view, parallel to the vertebral column. Lengths were determined based on the number of thoracic vertebrae (v), to the nearest 0.1v. RL-VHS and DV-VHS were expressed as the sum of vertebral numbers of L and S axes obtained from RL and DV views, respectively.

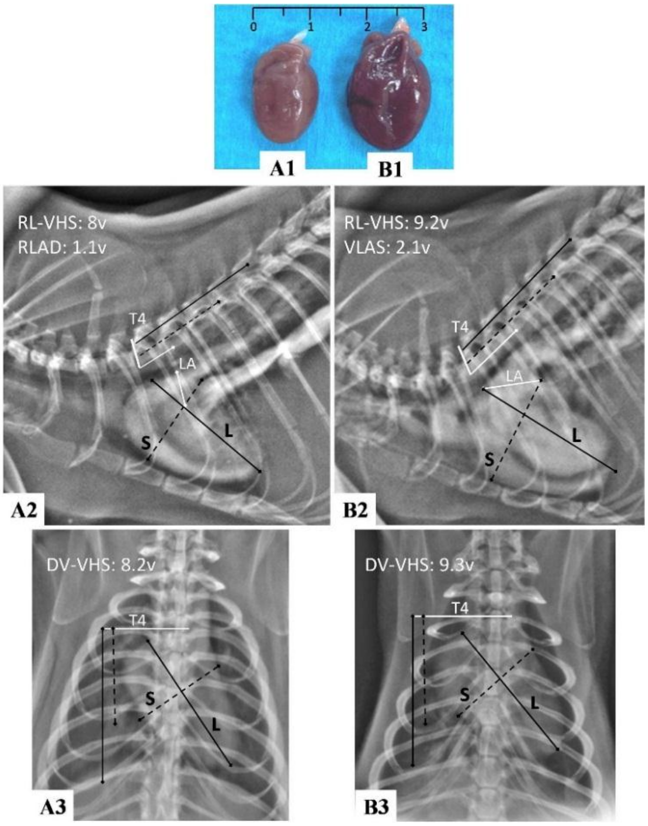

Figure 1 Heart sizes (ruler scale in cm) and contrast right lateral (RL) and dorsoventral (DV) thoracic radiographs (parameters: 65 kVp, 8 mA, 0.1 s, 30-cm film-focus distance) at the postoperative sixth weeks from two rats in the (A) control and (B) volume overload (VO) groups. A1 and B1. Heart size has increased in the VO group. A2. Demonstration of vertebral heart score (VHS) and radiographic left atrial dimension (RLAD) measurements on an RL image: The black line (L) represents the long axis of the heart and the dashed black line (S) the short axis. The VHS value obtained from total L and S values converted into a vertebral value is 8vertebrae (v). The RLAD value yielded by converting the measurement from the white line (LA) from the intersection of the L and S axes at an angle of 45^{o} to the dorsal wall of the left atrium into a vertebral value is 1.2v. B2. Demonstration of measurement of vertebral left atrial size (VLAS) from an RL image. The white line (LA) represents the distance from the ventral of the tracheal bifurcation to the intersection of the caudal edge of the left atrium with the caudal vena cava and the VLAS value yielded by converting this to a vertebral value is 2.1v. The VHS value of this rat with eccentric hypertrophy was also 9.2v. A3 and B3. Demonstration of DV-VHS measurement: After measuring the cardiac long (L, black line) and short (S, dashed black line) axes on the DV image, these two values were converted to vertebral values. DV-VHS was obtained from the sum of the two values (8.2v for control, 9.3v for VO).

For RLAD (Figure 1A2), a line bisecting the 90° angle at the junction of the RL-VHS, L and S axes was extended from that location as far as the radiographic projection of the dorsal edge of the LA. The same computer software was then applied to create a 45° angle between this line and the junction of the L and S axes. Similarly to VHS, this length was next normalized to v, starting from the cranial edge of the T4 and to the nearest 0.1v. This was subsequently used as the RLAD value. In case of difficulty in differentiating the dorsal anatomical margins of the LA from the pulmonary veins in this region, the most dorsal aspect of the contrast opacities identified at this level was used during all calculations.

For VLAS (Figure 1B2), a line was measured from the tracheal bifurcation to the most caudal aspect of the LA at the junction with the dorsal border of the CaVC. This was subsequently normalized to v, starting from the cranial edge of T4 and to the nearest 0.1v and was adopted as VLAS during all analyses

Heart weight:body weight ratio (HBW): Following the radiographic cardiac index measurements, all rats under anesthesia were sacrificed by making a thoraco-abdominal incision. The hearts were extracted, washed in ice-cold saline and finally weighed. HBW was calculated by dividing heart weight (HW) by terminal body weight (BW) at the sixth week postoperatively.

Statistical analysis: Statistical analysis was performed on commercial software (IBM SPSS Statistics 22.0, SPSS Inc., USA). Descriptive statistics were generated, and the Shapiro-Wilk test was applied to evaluate the normality of the distribution of continuous data. BW, HW, HBW, RL-VHS, DV-VHS, RLAD, and VLAS values were presented as median and interquartile ranges (IQR). Comparisons between the control and VO groups were performed using the Mann-Whitney U test. Spearman's rank-order correlation coefficient (rS) was applied to evaluate correlations between HW, HBW and radiographic cardiac indices (RL-VHS, DV-VHS, RLAD and VLAS). Correlation coefficient values of 0.1-0.3, 0.4-0.6, 0.7-0.9 or 1 were regarded as indicating weak, moderate, strong or perfect correlation, respectively (Dancey and Reidy 2007). ROC curves and the area under the curves (AUC) with 95% confidence intervals (CI) using HBW ≥ 293 as the criterion were generated for RL-VHS, DV-VHS, RLAD and VLAS. The AUC values of the four measurements were compared using Delong's method (DeLong *et al*., 1988, MedCalc® Statistical Software version 20.115, 2022, MedCalc Software Ltd., Ostend, Belgium). Sensitivity and specificity for RL-VHS, DV-VHS, RLAD and VLAS were determined with the Youden index in order to determine optimal cut-off values. Intra- and inter-observer variabilities were assessed for RL-VHS, DV-VHS, RLAD and VLAS by means of intraclass correlation coefficient (ICC) estimates and 95% confidence intervals based on a single rater, absolute agreement, with a two-way random (inter-observer) and mixed (intra-observer) effect. ICC values > 0.9 were regarded as excellent, 0.75 to 0.9 as good, 0.5 to 0.75 as moderate and values < 0.5 as poor (Koo and Li 2016). P values < 0.05 were regarded as statistically significant.

Results

With the exception of terminal BW, statistically significant differences were observed for all other values between the control group and the VO group (Table 1, Figure 1).

Table 1 Descriptive data of the radiographic cardiac indices and other values between control and VO groups

Variables	Groups			
	Control		VO	
	Median	IQR	Median	IQR
Terminal BW (g)	465	430-505	450	405-495
HW (g)	1.09	1.03-1.22	1.51*	1.43-1.69
HBW ([mg/g]100)	232	206-251	338*	293-375
RL-VHS	7.8	7.5-8.8	9.3*	8.7-9.9
DV-VHS	7.9	7.7-8.9	9.5*	8.9-10.2
RLAD	1.3	1.1-1.5	1.9*	1.4-2.6
VLAS	1.8	1.6-2.2	2.3*	2-3.1

Data is presented in median and interquartile ranges (IQR) unless stated otherwise
Abbreviations: VO, volume overload; BW, body weight; HW, heart weight; HBW, heart wight:body weight ratio; RL-VHS, right lateral vertebral heart size; DV-VHS, dorsoventral vertebral heart size; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size
*VO *vs*. control: $P < 0.001$

A positive correlation was determined between HW and HBW (r = 0.877, $P < 0.0001$), HW and RL-VHS (r = 0.889, $P < 0.0001$), HW and DV-VHS (r = 0.840, $P < 0.0001$), HW and RLAD (r = 0.771, $P < 0.0001$), HW and VLAS (r = 0.761, $P < 0.0001$), HBW and RL-VHS (r = 0.874, $P < 0.0001$), HBW and DV-VHS (r = 0.862, $P < 0.0001$), HBW and RLAD (r = 0.739, $P < 0.0001$), HBW and VLAS (r = 0.705, $P < 0.0001$).

Diagnostic accuracy, cut-offs and likelihood ratios for RL-VHS, DV-VHS, RLAD and VLAS for the radiographic prediction of cardiomegaly and LA enlargement with a criterion of HBW ≥ 293 in 74 rats are summarized in Table 2. The confidence interval of the ROC curves and AUC values for RL-VHS, DV-VHS, RLAD and VLAS are shown in Figure 2. The AUC values for RL-VHS, DV-VHS, RLAD and VLAS were 0.99 (95% CI 0.94 - 1.00), 0.98 (95% CI 0.94 - 1.00), 0.98 (95% CI 0.92 - 1.00) and 0.95 (95% CI 0.87 - 0.99), respectively. A cut-off value of > 8.7v using the RL-VHS yielded 100% sensitivity and 95% specificity (Youden index 0.95). A DV-VHS cut-off value of > 8.8v yielded 98.43% sensitivity and 96.63% specificity (Youden index 0.9261). In terms of RLAD, a cut-off value of > 1.6 was 97.56% sensitivity and 90.0%

specificity (Youden index 0.8756). A cut-off value of > 2.1v using VLAS yielded a sensitivity of 92.68% and specificity of 89.11% (Youden index 0.7768).

Both intra- and interobserver variabilities assessed using ICC values demonstrated good to excellent agreement for all radiographic cardiac indices (ICC > 0.75, $P < 0.05$) (Table 3).

Table 2 Receiver operating characteristic curve analyses for determining the diagnostic accuracy of the radiographic variable cut-offs for radiographic prediction of cardiomegaly and LA enlargment when the heart weight:body weight ratio (HBW) ≥ 293 was adopted as a criterion in 74 rats

Radiographic variables	AUC* (95% CI)	*z* and *P* values	Cutoff (vertebrae)	Sn (%)	Sp (%)	+LR	-LR	Youden Index J
RL-VHS	0.988±0.002 (0.93-1.00)	60,85 < 0.0001	> 8.7	100,00	95.00	20,0	n/a	0.95
DV-VHS	0.983±0.001 (0.94-1.00)	54,53 < 0.0001	> 8.8	98.43	96.63	n/a	0.13	0.9261
RLAD	0.987±0.01 (0.92-1.00)	41.97 < 0.0001	> 1.6	97.56	90.0	n/a	0.20	0.8756
VLAS	0.948±0.02 (0.87-0.99)	19.33 < 0.0001	> 2.1	92.68	89.11	6.18	0.087	0.7768

Abbreviations: AUC, area under the curve; CI, confidence interval; Sn, sensitivity; Sp, specificity; +LR, positive likelihood ratio; −LR, negative likelihood ratio; RL-VHS, right lateral vertebral heart size; DV-VHS, dorsoventral vertebral heart size; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size

*RL-VHS *vs*. DV-VHS: $P = 1.00$; RLAD *vs*. RL-VHS: $P = 0.19$; RLAD *vs*. DV-VHS: $P = 0.19$; RL-VHS *vs*. VLAS: $P = 0.04$; DV-VHS *vs*. VLAS: $P = 0.04$; RLAD *vs*. VLAS: $P = 0.11$

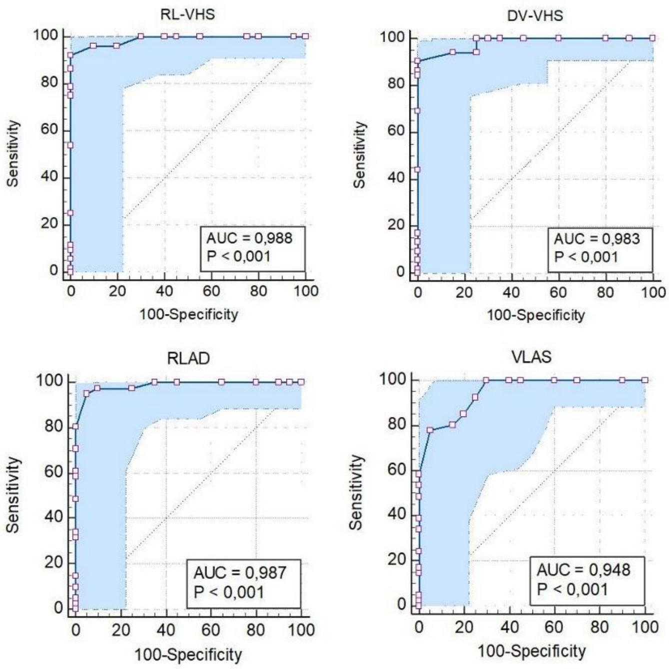

Figure 2 95% confidence interval for ROC curves of the variables of right lateral (RL) and dorsoventral (DV) vertebral heart score (VHS), radiographic left atrial dimension (RLAD), and vertebral heart size (VLAS) measured in 20 rats with heart weight : body weight (HBW) values <293, and 54 rats with HBW ≥293. The optimal cut-off points for these variables are 8.7 thoracic vertebrae (v) for RL-VHS (AUC = 0.99), 8.8v for DV-VHS (AUC = 0.98), 1.6v for RLAD (AUC = 0.99), and 2.1v for VLAS (AUC = 0.95)

Table 3 Intra- and inter-observer agreements for RL-VHS, DV-VHS, RLAD, and VLAS in rats

Radiographic Variables		Intra-observer agreement			Inter-observer agreement		
		ICC	95% CI	*P* value	ICC	95% CI	*P* value
RL-VHS	Control	0.90	0.61-0.97	< 0.01	0.83	0.30-0.96	< 0.01
	VO	0.86	0.43-0.97	< 0.01	0.84	0.36-0.96	< 0.01
DV-VHS	Control	0,91	0.62-0.98	< 0.001	0.88	0.51-0.97	< 0.01
	VO	0.84	0.36-0.96	< 0.01	0.92	0.66-0.98	< 0.001
RLAD	Control	0.93	0.73-0.98	< 0.001	0.87	0.52-0.98	< 0.01
	VO	0.90	0.81-0.98	< 0.01	0.89	0.55-0.97	< 0.01
VLAS	Control	0.95	0.81-0.98	< 0.001	0.92	0.68-0.98	< 0.001
	VO	0.88	0.52-0.97	< 0.001	0.91	0.63-0.98	< 0.001

Abbreviations: VO, volume overload; RL-VHS, right lateral vertebral heart size; DV-VHS, dorsoventral vertebral heart size; RLAD, radiographic left atrial dimension; VLAS, vertebral left atrial size

Discussion

Heart size can be evaluated objectively with VHS from thoracic radiographs and LA size from VLAS and RLAD. In addition, VHS has been shown to be a reliable index for assessing cardiac enlargement resulting from eccentric hypertrophy, particularly due to VO (Buchanan and Bucheler, 1995; Buchanan, 2000). Although VHS is a simple method for evaluation of the cardiac silhouette, the silhouette may be difficult to identify in some exotic mammals (Giannico *et al*., 2015; Garcia *et al*., 2016), including rats, due to superimposition of soft tissue opacity in the cranial mediastinum over the cranial cardiac border, which can result in inaccurate VHS values being obtained (Çetinkaya and Kaya, 2022). Dias *et al*. (2021), showed that this can be partially corrected by means of positive pressure breath hold and mechanical ventilation during radiographic exposure. We have shown that heart can be evaluated rather than heart silhouette by using lateral thoracic contrast radiography. This imaging method, which is easy and uncomplicated, provides a clear definition of all the borders of the heart and is more effective than lateral thoracic plain radiography (Çetinkaya and Kaya, 2022, Kaya et al., 2022). Radiographic cardiac indices were therefore evaluated using only contrast radiography in the present study. During VHS calculations, a slight variation was subsequently introduced by measuring the S axis from the dorsal border of the CaCV for the purpose of including the LA body at lateral thoracic radiography (Buchanan, 2000). In the present induced eccentric hypertrophy study, RL-VHS measurements were performed on the basis of that variation in order to include an increase in size due to LA enlargement in the analysis.

Cardiac hypertrophy and ventricular dilation develop rapidly (within 1-2 weeks) as a response to VO in rats with ACS. Dilatation of the LV chamber takes place through elongation of the surrounding myocytes, resulting in sarcomeric replication in series (Carabello, 2002). Cardiac hypertrophy, a progressive entity, occurs in both the left and right ventricles in rats (Abassi *et al*., 2011). This in turn leads to a progressive increase in cardiac mass (Wang *et al*., 2003). Interstitial fibrosis, cardiomyocyte hypertrophy and atrial dilatation were reported one month following ACS surgery in rats (Cao *et al*., 2018). In this context, the evaluation of eccentric cardiac hypertrophy using radiographic cardiac indices was conducted within a six-week postoperative period.

HW rises in rats with cardiac hypertrophy and a powerful correlation has been reported between HW and the echocardiographic left ventricular mass index (Cantor *et al*., 2005). HBW is commonly employed in experimental cardiac studies as a general marker for cardiac hypertrophy (Wang *et al*., 2003, Cantor *et al*., 2005, Melenovsky *et al*., 2012, Ku and Su, 2014, Cao *et al*., 2018, Aimoto *et al*., 2022). Similar to the results of the present research, HW and HBW increased significantly in the VO group compared to the control group in other research (Cantor *et al*, 2005, Carabello, 2002). Moreover, a positive strong correlation was observed between radiographic cardiac indices and both HW and HBW in this study. The lowest HBW value (292) for IQR in the VO group was therefore employed as the classifier in ROC curves and AUC analyses of radiographic variables.

Research has shown that LA size can be measured objectively in dogs and that VLAS and RLAD can be employed in the radiographic diagnosis of myxomatous mitral valve disease (Malcom *et al*., 2018; Mikawa *et al*., 2020; Stepien *et al*., 2020; Lam *et al*., 2021). VLAS and RLAD methods are positively correlated with echocardiographically-derived left atrial-to-aortic root (LA:Ao) ratio values in dogs. LA:Ao ≥ 1.6 was used as the classifier to determine the sensitivity and specificity of radiographic cardiac indices (Malcom *et al*., 2018, Sanchez Salguero *et al*., 2018, Lam *et al*., 2021). Sensitivity and specificity were 87% and 67%, respectively, for VLAS ≥ 2.5v (Malcom *et al*., 2018), 93.5% and 96.8% for RLAD ≥ 1.8v, and 76.1% and 93.5% for VHS ≥ 11.1 (Sanchez Salguero *et al*., 2018). In order to assess the value of radiographic cardiac measurements for the quantification of cardiomegaly and LA enlargement in the present study, their respective sensitivity and specificity values were estimated and cut-off values were determined. RL-VHS emerged as a more sensitive (100%) and specific (95%) tool than DV-VHS for cardiomegaly due to VO, with a cut-off value of 8.7v. RLAD exhibited higher sensitivity (97.56%) and specificity (90%) at a cut-off value of 1.6v compared to VLAS. The cut-off values obtained in this study for radiographic cardiac indices were lower than those previously reported for dogs (Malcom *et al*., 2018, Sanchez Salguero *et al*., 2018, Duler *et al*., 2021, Lam *et al*., 2021). Furthermore, the cut-off values for radiographic cardiac indices were estimated only by means of HBW, which although widely accepted as a useful value in experimental cardiac studies, does not constitute a real gold standard method for this purpose, compared to echocardiography, magnetic resonance imaging, or computed tomography. Cross-sectional imaging would constitute a useful tool for comparison in future studies involving cardiac hypertrophy assessment with VHS and LA enlargement evaluation with RLAD and VLAS in rats as the gold standard method.

Melenovsky *et al*. (2012), reported that the higher the HW in hypertrophies developing as a result of VO, the greater the risk of mortality. Additionally, atrial dilatation in rats with ACS-induced chronic VO constitutes a risk factor for atrial fibrillation (Cao *et al*., 2020, Aimoto *et al*., 2022). Accordingly, high VHS values may be considered in terms of mortality in rats with eccentric cardiac hypertrophy and elevated RLAD and VLAS values as prognostic values in terms of atrial fibrillation.

Inter-observer variability has been described as one of the factors impacting on VHS, RLAD and VLAS (Hansson *et al*., 2005; Bagardi *et al*., 2021). In the present study, good to excellent agreement was observed in intra- and inter-observer variabilities for all radiographic cardiac indices in both the control and VO groups (Table 3).

In conclusion, radiographic cardiac indices powerfully correlated with HBW in this study exhibiting high sensitivity and specificity in the evaluation of LA enlargement and cardiomegaly deriving from eccentric cardiac hypertrophy in rats.

The proposed cut-off values for predicting cardiomegaly and LA enlargement in rats are 8.7v for RL-VHS, 8.8v for DV-VHS, 1.6v for RLAD, and 2.1v for VLAS. These indices would provide both veterinarians working with domestic pets and experimental researchers with a simple, repeatable and cost-effective tool for the prediction of cardiomegaly and LA enlargement. Further research is now needed to determine the diagnostic value of these indices in monitoring disease progression in rats with eccentric cardiac hypertrophy and other diseases, such as dilated cardiomyopathy, characterized by cardiomegaly and LA enlargement.

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