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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

Relatório Final de Estágio

Mestrado Integrado em Medicina Veterinária

Diagnostic accuracy of radiographic techniques for the evaluation of left atrium enlargement in dogs with mitral valve disease

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Orientadora:

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ABSTRACT

OBJECTIVE: Myxomatous mitral valve disease (MMVD) is the most frequent acquired cardiac disease in dogs and echocardiography is the gold standard for its diagnosis. Radiographic evaluation is a valuable toll to evaluate left atrial dimensions when echocardiography is not available. The aim of the present study was to evaluate the diagnostic potential of the vertebral heart score (VHS), radiographic left atrial dimensions (RLAD) and vertebral left atrial size (VLAS) for the detection of left atrium enlargement (LAE) in dogs with MMVD.

MATERIALS AND METHODS: In this prospective study measurements of VHS, RLAD, VLAS and echocardiographic left atrium-to-aorta ratio (LA/Ao) were performed and RLAD and VLAS were correlated with LA/Ao. Concerning radiographic measurements, the influence of the lateral decubitus side was also evaluated (right lateral recumbency (RLR) vs. left lateral recumbency (LLR)). The diagnostic accuracy was evaluated for LAE prediction.

RESULTS: Differences between recumbencies were statistically different for VHS ($p<0.001$) and RLAD ($p=0.031$). A positive correlation was found between LA/Ao and RLAD ($r = 0.41$, $p=0.004$ for the RLR and $r = 0.57$, $p<0.0001$ for the LLR) and between VLAS and LA/Ao ($r = 0.47$, $p=0.0008$ for the RLR and $r = 0.50$, $p=0.0002$ for the LLR). The diagnostic accuracy of the three techniques was moderate (area under the curve [AUC] of 0.833/0.771 for VHS in the RLR/LLR, respectively; 0.824/0.772 for VLAS in the RLR/LLR, respectively; 0.745/0.788 for RLAD in the RLR/LLR, respectively), although not statistically different between techniques for the detection of LAE ($p>0.05$).

CONCLUSION: All 3 techniques are useful for the detection of LAE in canine MMVD, showing similar diagnostic potential. The optimal cut-off for RLAD showed maximum specificity, which represents a clinical advantage. The differences between recumbencies warrant standardization, and the same recumbency should be used when sequential measurements are intended.

KEYWORDS: myxomatous mitral valve disease; left atrium dimensions; VHS; RLAD; VLAS.

RESUMO

OBJECTIVO: A doença mixomatosa da válvula mitral (DMVM) é a doença cardíaca adquirida mais frequente em cães e a ecocardiografia é o exame de eleição para o seu diagnóstico. A avaliação radiográfica é uma ferramenta valiosa para avaliar as dimensões do átrio esquerdo quando a ecocardiografia não está disponível. O objetivo deste estudo foi avaliar o potencial diagnóstico do índice cardiovertebral (ICV), dimensão atrial esquerda radiográfica (DAER) e tamanho atrial esquerdo vertebral (TAEV) para a deteção de dilatação atrial esquerda (DAE) em cães com DMVM.

MATERIAIS E MÉTODOS: Neste estudo prospetivo foram realizadas medições do ICV, DAER, TAEV e rácio átrio esquerdo-aorta (AE/Ao) ecocardiográfico e o TAEV e DAER foram correlacionados com o AE/Ao. Relativamente às medições radiográficas, a influência do decúbito lateral foi ainda avaliada (decúbito lateral esquerdo (DLE) vs. decúbito lateral direito (DLD)). A precisão diagnóstica foi avaliada para previsão de DAE.

RESULTADOS: Diferenças entre decúbitos foram estatisticamente diferentes para o ICV ($p<0.001$) e DAER ($p=0.031$). Uma correlação positiva foi encontrada entre AE/Ao e DAER ($r = 0.41$, $p=0.004$ para o DLD e $r = 0.57$, $p<0.0001$ para o DLE) e entre TAEV e AE/Ao ($r = 0.47$, $p=0.0008$ para o DLD e $r = 0.50$, $p=0.0002$ para o DLE). A precisão diagnóstica das três técnicas foi moderada (área sob a curva de 0.833/0.771 para o ICV no DLD/DLE, respetivamente; 0.824/0.772 para o TAEV no DLD/DLE, respetivamente; 0.745/0.788 para o DAER no DLD/DLE, respetivamente), contudo não foi estatisticamente diferentes entre técnicas para a deteção de DAE ($p>0.05$).

CONCLUSÃO: As 3 técnicas são úteis na deteção de DAE na DMVM canina, mostrando potencial diagnóstico semelhante. O ponto de corte ótimo para o DAER mostrou especificidade máxima, sendo uma vantagem clínica. As diferenças entre decúbitos justificam que haja padronização, devendo ser usado o mesmo decúbito quando se pretendem medições sequenciais.

PALAVRAS-CHAVE: doença mixomatosa da válvula mitral; dimensão do átrio esquerdo; ICV; TAEV; DAER.

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Lastly, I thank all the people who supported me, not just during the time frame regarding my internship and research project but during my entire life, particularly my family. Specially, and above all, I thank my parents that always believed in me and have always been there for me, encouraging me and helping me through both the happy and difficult times, without them I would not have gotten to where I am today, and for that I thank them from the bottom of my heart!

Porto, July 2021
Sara Gama

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ABBREVIATIONS LIST

ACEI	Angiotensin converting enzyme inhibitors
ACVIM	American College of Veterinary Internal Medicine
AE/Ao	Rácio átrio esquerdo-aorta
AUC	Area under the curve
CHF	Congestive heart failure
CKCS	Cavalier King Charles Spaniel
CVC	Caudal vena cava
DAE	Dilatação atrial esquerda
DAER	Dimensão atrial esquerda radiográfica
DLD	Decúbito lateral direito
DLE	Decúbito lateral esquerdo
DMVM	Doença mixomatosa da válvula mitral
DV	Dorsoventral
ECG	Electrocardiography
HF	Heart failure
HR	Heart rate
HVP	Hospital Veterinário do Porto
ICBAS	Instituto de Ciências Biomédicas de Abel Salazar
ICV	Índice cardiovertebral
IQR	Interquartil range
Kg	Kilograms
LA	Left atrium
LA/Ao	Left atrium-to-aorta ratio
LAE	Left atrium enlargement
LLR	Left lateral recumbency
LMSB	Left mainstem bronchus
LSE	Left sided enlargement
LVIDd	Left ventricular internal diameter in diastole
LVIDdN	Left ventricular internal diameter in diastole normalized
MMVD	Myxomatous mitral valve disease
MR	Mitral regurgitation
M-VLAS	Modified vertebral left atrial size
NT-proBNP	N-Terminal pro-B-type Natriuretic Peptid
PE	Pulmonary oedema

PH	Pulmonary hypertension
PO	Per os
RLAD	Radiographic left atrial dimension
RLR	Right lateral recumbency
ROC	Receiver operating characteristic
RR	Respiratory rate
Se	Sensitivity
Sp	Specificity
TAEV	Tamanho atrial esquerdo vertebral
TID	Ter in die
VBU	Vertebral body units
VD	Ventrodorsal
VHS	Vertebral heart score
VLAS	Vertebral left atrial size

1. INTRODUCTION

My curricular internship was done in Hospital Veterinário do Porto (HVP). In HVP, I had the chance to experience all the fields that the hospital has to offer by doing weekly rotations in surgery, anaesthesiology, internment, cardiology and general or speciality appointments, which allowed me to experience other areas even if there was not a rotation specifically designed for those fields (for example oncology, dermatology, etc). I also had the chance to participate in emergency and critical care situations and to work night shifts. This part of my internship had the purpose of learning how to put in practise all the theoretical knowledge previously acquired and further develop it.

Simultaneously, I integrated a research project with the purpose of comparing the diagnostic accuracy of radiographic techniques for the evaluation of the left atrium (LA) dimensions in dogs. The echocardiographic and radiographic examination were performed in HVP and UPVET (ICBAS-UP) by Professor Luís Lobo and Professor Ana Patrícia Fontes de Sousa, respectively; Professor Cláudia Baptista from ICBAS-UP helped with the radiographic measurements. The parameters included were selected based on scientific evidence from veterinary literature for small animal cardiology, more precisely myxomatous mitral valve disease (MMVD), and thoracic radiology area. In this part of the internship, I had the chance to accompany the patients in their echocardiographic and radiographic examinations in HVP.

With this study, the goal was to assess the usefulness of the radiographic techniques in the clinical context, specifically when the echocardiography is not available. The relevance and contribution of this study relies on highlighting the importance of detection of cardiac remodelling in MMVD patients and the fact that radiographic examination would facilitate patient monitoring if proven similar in diagnostic accuracy to the echocardiographic examination.

2. STATE OF THE ART

2.1. Myxomatous mitral valve disease

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs (representing 75% of the canine heart diseases) [1, 2] and the most common cause of congestive heart failure (CHF) [3]. Almost one third of the cases have involvement of the tricuspid valve [3]. Disease begins with several cellular changes occurring in all the valvular structures, however the cause triggering these changes is still not clear [4, 5]. These cellular changes lead to deformation of the valve's apparatus, causing a poor coaptation of the valvular leaflets and, consequently, the appearance of regurgitation [4, 5].

The degenerative character of the disease causes further valvular deformation and regurgitation worsening, leading to volume overload, which in turn promotes further regurgitation and the development of left sided enlargement (LSE), as the mitral valve is the primarily affected [2, 5, 6]. Mitral valve insufficiency is manifested by the presence of a typical left apical systolic murmur, which can radiate towards the cardiac base and to the right side. Also, a right apical systolic murmur might be heard due to tricuspid regurgitation [1-3]. Murmur intensity has been associated with disease severity [2, 7]. Aggravation of mitral regurgitation (MR) leads to higher left ventricle and atrium pressures, potentiating the development of pulmonary venous congestion and further on leading to pulmonary oedema (PE) and progression to left-sided CHF [2]. An acute evolution associated with an acute development of PE may however occur due to rupture of chordae tendineae [8]. The chronic evolution of disease may lead to right sided CHF associated with progression of concomitant tricuspid insufficiency and/or pulmonary hypertension (PH) and, in cases of severe LA dilation, although rare, rupture of the LA and cardiac tamponade can occur [2, 8].

This valvular disease is more frequent in males and smaller dogs; however, when large dogs are affected, the course of the disease tends to be faster [2, 9]. Death by MMVD is generally due to CHF, but sudden death may also occur [3, 10]. Even so, the most common is for the manifestation of heart failure (HF) to occur only years after the onset of mitral valve murmur [1, 11] and there are even dogs that always remain asymptomatic [2, 11]. Some breeds have a higher predisposition for MMVD, like the Cavalier King Charles Spaniel (CKCS), in which 90% of the population over 10 years old is affected by the disease [1]. Although it is more frequent with aging [3], the disease tends to appear early in the CKCS breed [2, 12]. Inheritability and severity are also associated with some breeds

genetics [2, 12]. Several factors have been described as helpful for the identification of patients at risk for CHF, including increase in left-atrium-to-aortic-root ratio (LA/Ao), N-Terminal pro-B-type Natriuretic Peptid (NT-proBNP), left ventricular internal diameter in diastole (LVIDd), vertebral heart score (VHS), murmur intensity, E wave transmitral peak velocity and possibly also age and heart rate (HR) [7, 10, 13]. While the increase in heart size, measured through the VHS, tends to be gradual (already noticeable up to 1 year before), the increases of HR, respiratory rate (RR) and resting RR occur closer to CHF onset (during the few months before); however, all these have shown to have higher rates of change right before CHF onset [14]. The bigger changes were noticed in RR and resting RR (58% and 78% increases, respectively, comparing to increases of 20% in HR and of 15% in VHS) [14]. Very recently a scoring system, named MINE score, was developed for severity assessment of MMVD, through a scale of four severity classes into which patients are allocated based on the sum of individual scores from four echocardiographic parameters routinely obtained [15]. This scoring system proved to be associated with survival time and could, therefore, provide useful information regarding risk of death, even within asymptomatic patients, and prognosis [15].

In MMVD, it is common for PH to develop, especially in B2 and more advanced stages, and a worse prognosis is associated with estimated systolic pulmonary artery pressure > 55 mmHg [16] (considered a moderate PH) [6]. In a study evaluating prevalence and prognosis of PH in MMVD patients, 1/3 of B2 dogs had PH [16] and in another study B2 dogs had a lower median survival time when LA/Ao > 1.91 and LVIDd normalized for body weight (LVIDdN) > 1.90, while for B1 patients the median survival time was not affected by the degree of the LA enlargement (LAE) [17]. This suggests heterogenicity within the B2 population and different possible outcomes. Owner compliance, response to treatment, development of PH and chordae tendineae rupture secondary to MMVD [1] (poor prognosis when associated to acute episodes [3]) and existence of comorbidities may influence the survival time once CHF develops [1].

Since MMVD is an evolving condition, disease progression was divided in a staging system that allows classification of the patients according to disease presentation. The first is stage A which describes dogs with no signs of disease but that are very prone to developing it (e.g. predisposition of certain breeds) [4]. Evolution to stage B means there is already an existing alteration of the heart structure (seen in radiographic/echocardiographic examination) but without signs of HF [4]. These dogs are generally identified through detection of a left apical systolic murmur [4] (often by accident), although other signs can be detected, such as a dry and harsh cough due to left bronchial compression [2, 6], which is generally the first sign noticed by the owner [2]. This stage is divided in 2 subcategories.

In B1 belong dogs without cardiac remodelling or with some degree of cardiac remodelling that does not justify starting treatment. In stage B2 dogs the remodelling is extensive enough to cause LSE that justify initiating treatment to delay the onset of HF [4]. Appearance of signs of HF (e.g. weakness, cough, which can be productive, exercise intolerance, dyspnoea [2]), either past or current, due to MMVD marks stage C [4]. Stage D is the final stage of the disease where no response is seen to traditional treatment, therefore needing further treatment options to ease the clinical signs. Both C and D stages are divided in subcategories to separate dogs that can be managed outside the hospital and those that need to stay in the hospital due to a more acute presentation [4].

For the diagnosis, the physical examination is an important starting point. Considering the generally long asymptomatic phase, the presence of a heart murmur typical of MR can be the only sign found in early stages of the disease (B stage) [6]. When a grade V/VI murmur or higher is present a thrill can be felt in the precordial shock [2]. Stage C and D patients are already symptomatic, therefore, recognizing the clinical signs of HF in the patient's physical exam or clinical history is very important [4]. Those signs comprise tachypnoea, dyspnoea, anxiety, cough, exercise intolerance, abnormal pulmonary sounds in auscultation, syncope and, in advanced cases, poor perfusion, abdominal and venous distention and muffled cardiac sounds [2, 4, 8]. However, some of these signs can also be seen in asymptomatic patients with concomitant respiratory disease [2]. Complementary diagnostic exams are also essential. Echocardiography is the gold standard for the diagnosis and staging of MMVD [4] and thoracic radiography is vital for the detection of congestion and PE [8] and is gaining more importance for the evaluation of cardiac silhouette remodelling. Electrocardiography (ECG) allows for arrhythmia detection, with supraventricular tachycardia being the most frequent in MMVD and atrial fibrillation, when present (rare), usually reveals advanced disease with marked LAE [2]. The ECG also allows for an indirect evaluation of chamber's dilation (e.g., wider P waves suggest LAE) [2]. The determination of NT-proBNP concentration in serum can also help discern if the clinical signs presented have a cardiac basis and performance of blood and urine analysis, especially in older dogs, and blood pressure assessment is also recommended [4, 18].

Based on previous studies [19] therapeutic treatment is only recommended when disease evolves to a B2 stage. Therefore, for stages A and B1 the recommendation is based on periodical evaluations: yearly for stage A [4], with breeding schemes showing a positive impact in MMVD murmur prevalence in the offspring [20], and ≥ 1-2/year for B1 stage depending on imaging findings, with the possibility of revaluating through radiographic examination if echocardiography is not possible [4].

Stage B2 marks the beginning of life-long treatment and, as such, the criteria to classify a patient as a B2 are well defined in the 2019's American College of Veterinary Internal Medicine (ACVIM) consensus guidelines: murmur intensity ≥ III/VI, LA/Ao ratio ≥ 1.6, LVIDdN ≥ 1.7 and a breed-adjusted VHS > 10.5, although the most crucial within these criteria are the echocardiographic findings [4]. The main treatment for B2 patients is pimobendan (0.25-0.3 mg/Kg PO BID) [4, 19]. Regarding the efficacy of angiotensin converting enzyme inhibitors (ACEI) in asymptomatic MMVD patients, literature evidence is contradictory [21-24], thus its use is not consensual. A study evaluating the effects of a specific diet (for an adequate caloric and protein intake, moderate sodium restriction, etc) showed improvement in echocardiographic parameters, and, even though the influence on time to the onset of CHF and survival were not studied [25], dietary treatment is still advised [4]. The use of cough suppressants in more advanced cases may be justified. For advanced cases there is also the surgical option, which has a better outcome for B2 patients than for more advanced stages [6, 26].

For stage C, pimobendan, ACEI and furosemide are the standard protocol treatment [2, 27-29] for both acute and chronic presentations. In cases of acute presentation, oxygen supplementation, effusion relief (e.g., thoracocentesis) if deemed necessary, light sedation (e.g., butorphanol) and suitable care for dyspnoeic/sedated patients (e.g., sternal recumbency, head elevation) are also advised [4, 8]. When these actions fail, dobutamine can be also be added. Sodium nitroprusside can help in very severe cases of PE [4]. For a chronic management of stage C other recommendations are the addition of spironolactone [30-32] and use of diltiazem with/without digoxin in the presence of atrial fibrillation. The use of cough suppressants and bronchodilators in certain circumstances is not consensual [4]. Respiratory rate and HR should be monitored, especially resting RR [14]. Although in general all these options are strongly recommended, the evidence for acute treatment is weaker than for the chronic therapy (moderate evidence) [4].

For stage D, since patients are refractory to treatment, the therapy is reinforced, i.e., higher doses of furosemide or even substitution for torsemide in non-responsive patients [8], introduction of sildenafil [33] and drugs for additional afterload reduction if tolerated (e.g., dobutamine or amlodipine/hydralazine if PO), pimobendan possibly in a TID regimen (although it is an off-label use) and ACEI [4]. The use of bronchodilators is not unanimously accepted. Specifically for an acute presentation, the approach is similar to the already mentioned for stage C, with the addition of mechanical ventilation if found useful [4]. Specifically for a chronic presentation spironolactone is strongly recommended [31]. Hydrochlorothiazide could also be used as an adjunctive to the diuretic drug [8] and digoxin

for atrial fibrillation [4]. Although in general the evidence for these treatment options is weak, the recommendation is stronger for the acute measures than for the chronic [4].

Anorexia seems to be common in dogs with CHF [34] therefore body weight and appetite should be monitored and significant changes investigated. Dietary suggestions include moderate sodium restriction [2], more pronounced for stage D dogs with uncontrolled effusion, adequate calorie and protein consumption, avoidance of potassium rich foods and the necessary supplementations (e.g., magnesium, omega-3 fatty acids) [4].

2.2. Radiographic evaluation of cardiac silhouette

2.2.1. Importance of radiographic examination in MMVD

The ACVIM guidelines reinforce the importance of differentiating B1 from B2 dogs, since this means discriminating dogs that do and do not benefit from treatment. Scientific evidence support that dogs in the B2 category profit from administration of pimobendan [19]. Pimobendan was shown to be a “safe and well tolerated” drug that can delay, by approximately 15 months, the progression to stage C (CHF) and death, reducing, also, the probability of occurrence of those events to 2/3 in B2 dogs (LA/Ao ≥ 1.6, LVIDdN ≥ 1.7, and VHS > 10.5) [19]. Similar results were observed in a later study where pimobendan administration led to reduction of values of three echocardiographic parameters (LA/Ao, left ventricular internal diameter in systole and diastole normalized for body weight) associated with longer periods until CHF onset, as well as an increase in the shortening fraction, within a month of treatment [35]. For this purpose, an accurate diagnosis followed by staging is necessary. According to the ACVIM consensus guidelines of 2019, echocardiography is the gold-standard for diagnosis and staging of stage B [4]. However, the performance of thoracic radiographs is also recommended for the evaluation of the hemodynamic consequences, the comparison to future radiographs and to help distinguish if certain clinical signs like cough have a cardiac basis [4, 6]. It is also useful to detect marked cardiac changes, response to treatment [36] and to evaluate the pulmonary field, being the only broadly accessible method for a non-invasive diagnosis of PE [2]. Thoracic radiography can assist the staging process. Although definitive criteria for classifying a patient into a B2 stage through radiographic examination are lacking, if cardiomegaly is evident in radiographs (VHS ≥ 11.5 or higher than the breed-adjusted range upper limit) or there is evidence of increased rate of changes seen in radiographic parameters for enlargement, these can replace the standard echocardiographic examination according to the 2019’s ACVIM guidelines [4]. A recent parameter, the vertebral left atrial size (VLAS), is also mentioned in the guidelines for the radiographic determination of the LA dimension [4] and,

even though a cut-off for enlargement is not yet established, a value ≥ 3 likely identifies stage B2 MMVD [37, 38].

Although the gold standard for assessment of the left heart dimensions is echocardiography [4, 39], this exam (i) requires the existence of an ultrasound machine, which is not available in all veterinary care centres, (ii) requires specialized knowledge for its execution and interpretation and (iii) it is a considerably expensive exam [40]. For these reasons there has been a search for other techniques that could be useful when echocardiography is not feasible. Contrary to echocardiography, radiographic examination is much more accessible, affordable, and easily performed. Thoracic radiography has been described as a crucial component in the diagnostic protocol of MMVD, especially for HF detection, and, depending on the circumstances, many patients with MR could be managed without echocardiographic examination [2]. Therefore, different radiographic techniques have been developed in order to find radiographic measurements comparable to the LA/Ao. Enlargement of the LA reflects MMVD severity and chronicity of regurgitation, hence being considered a key indicator of higher death risk [3], thus finding radiographic techniques for the detection of LAE could be very useful.

2.2.2. Subjective radiographic evaluation of the cardiac silhouette

A radiographic evaluation of the thorax should include, at least, 2 orthogonal projections, one lateral and one ventrodorsal (VD) or dorsoventral (DV) view [41]. Certain views may be preferred according to the clinical context and the DV view is the most commonly recommended for the cardiac radiographic evaluation [42]. Nevertheless, there are some differences in the normal anatomy that should be considered when looking at different recumbencies. In left recumbency radiographs, the heart might look slightly rounder with the apex possibly being lifted from the sternum [36]. In DV views the heart tends to appear wider and with a rounder shape, possibly due to sternal compression and contact between the diaphragm and the heart, with left displacement of the apex, which can lead to erroneous perception of cardiomegaly in DV projections [43]. The magnification of the cardiac silhouette in the VD view occurs in large dogs due to a bigger distance to the imaging plates [43]. These differences in DV/VD views are more significant in medium-large dogs [36]. Other factors should also be considered when interpreting radiographs to avoid misconceptions. Overweight can decrease lung capacity giving an erroneous perception of increased lung opacity. The fat itself can contribute to increased thorax opacity with possible misdiagnosis of pulmonary disease or create a “mass effect” leading to a false impression of a mediastinal mass or cardiomegaly [36], contributing also for a bad definition of the cardiac contour [2]. The cardiac silhouette may appear larger, wider, more globular in barrel-

chested patients and conversely appear much smaller in breeds with narrow and deep thorax conformation. [2, 36]. The correct positioning of the patient is fundamental for a truthful interpretation of the thoracic radiographic image, which should include the full extent of the thoracic cavity, starting slightly cranial to the thoracic entry to a bit caudally to the last rib [36]. The thoracic limbs should be extended cranially to avoid overlap with the cranial thorax while maintaining the scapula's caudal border at the centre of the exposure field [41]. Radiographic exposure should coincide with inspiration peak, so that lung volume is optimal [18].

Cardiac diseases may also change the cardiac silhouette. In MMVD, LAE is the most frequently found radiographic alteration and one of the first to appear [8]. In lateral views the increased LA dimensions create a bulging generally in a dorsocaudal direction leading to the flattening or concave shape of the caudal cardiac border addressed as "loss of the caudal cardiac waist" [36]. The enlargement also has consequences in adjacent structures. It can cause significant elevation of the trachea at the bifurcation level and of the left mainstem bronchus (LMSB) [8], causing splitting of the mainstem bronchi [18, 41] and possible narrowing of the principal bronchi [36]. In VD/DV views, the principal bronchi can be displaced or seem to form a wider angle, addressed as "crab sign" or "bowlegged cowboy" [2]. When the enlargement is extent a summation effect will appear due to the LA's overlap on the heart [36], creating a "double wall" appearance in the radiographic image [2, 36]. If the heart silhouette is compared to a clock in VD/DV views, enlargement of the LA auricle can be seen as the appearance of a bulge localized at the 2 – 3 o'clock position [8], although this radiographic feature can also be caused by a severely enlarged LA pushing the LA auricle laterally [36].

Left ventricle enlargement can occur due to hypertrophy or dilation (although distinction is only possible with echocardiography) [36]. In concentric hypertrophy the "growth is internal", i.e. afterload increase (e.g., due to aortic stenosis) leads to cardiac wall thickening and lumen narrowing, therefore external enlargement is unlikely to be seen [36]. However, when there is an increase in the preload, due to MR for example, the eccentric hypertrophy can lead to noticeable enlargement [18]. In lateral projections, the height of the heart increases [41], elevating the intrathoracic trachea and placing it more parallel to the spine when the enlargement is severe, while in VD/DV projections, both the apex and left margin of the heart may seem rounder [36]. When dilation occurs, also due to increased preload but more likely related to HF, similar changes are seen in the lateral radiographic views or it may contribute to a global image of cardiac enlargement [36].

Due to MMVD's pathophysiology, the main changes seen in radiographs are related to "left-sided cardiomegaly". However, it is known that approximately 30% of the patients

affected by MMVD also have involvement of the tricuspid valve [3] and that PH is a common consequence of MMVD and these can cause right-sided enlargement [2, 18]. Therefore, changes in the right atrium and ventricle may also be present in MMVD patients.

Right atrium enlargement is seen in radiographs as a protuberance located in the craniodorsal border of the heart in lateral projections; however that change in cardiac silhouette is also consistent with alterations in other structures (e.g., main pulmonary artery) [36]. Straightening of the cranial cardiac border and dorsal elevation of the trachea may also be seen [8]. In the VD/DV views the protuberance is located in the 9 -11 o´clock position [18].

Similarly to the left ventricle, hypertrophy and dilation lead to the enlargement of the right ventricle and hypertrophy can be due to afterload increase (e.g., in PH); however, even if there is minimal enlargement, it is easier to detect radiographically, possibly due to the thinner ventricle wall [36]. In lateral projections an increase in sternal contact can be seen, however, chest conformation must be considered [36, 41], since breeds with deep-chest conformation have less contact between the heart and the sternum when compared to breeds with a barrel-chest conformation. A dorsal elevation of the apex can also be seen in result of hypertrophy [36]. In VD/DV projections the right ventricle seems to have a rounder shape and to extend more to the right side, resembling a “reversed letter D” [8]. The heart’s apex is usually moved to the left [18].

For the reasons explained above, when changes associated with right-sided enlargement are seen, changes due to LSE are also present. Thus, the involvement of both sides of the heart can result in appearance of cardiomegaly, which is radiographically represented as a generalized enlargement of the heart with an overall rounder shape in all projections [41].

Other signs consistent with MMVD can be seen in the pulmonary vasculature. The development of PH is due to transmission of the filling pressure in the left ventricle going backwards towards the LA and then to the pulmonary veins [2]. This is radiographically seen as the wider diameter of the pulmonary peripheral veins relatively to the respective lobar arteries since they should be approximately similar in width [41]. The pressure can be reflected even more backwards, reaching the capillaries and arteries, resulting in the appearance of PE and pulmonary artery hypertension [36]. Therefore, it is possible to find distension in both pulmonary arteries and veins in a state of HF [41]. In lateral views the enlargement should be assessed in the cranial lobes pulmonary vessels, especially in the left lateral projection due to vasculature overlap in the right recumbency. In these views the veins are ventral and the arteries are dorsal to the corresponding bronchus [36]. In the VD/DV projections the vessels should be assessed in the caudal lobes, with the artery being

located lateral to the bronchus and vein medially [36]. The DV projection is preferred since lung inflation is improved and the vessels are also more perpendicular to the x-ray beams than in the VD projection [36]. The cranial and caudal vessel's width is assessed through comparison to the forth and ninth rib, respectively [41]. The PE is seen in radiographs as an interstitial lung pattern (rare due to its short duration) and later as an alveolar pattern [2, 12, 18]. More specifically, the interstitial pattern is characterized by the loss of vascular definition and, possibly, coexisting pulmonary venous enlargement, evolving to an alveolar pattern with the presence of bronchograms, increased opacity with multifocal distribution, concealing the pulmonary vessels [2, 36]. A study has showed that MMVD patients may have either a symmetric or asymmetric distribution of PE, although the first was more common (30 vs 21 cases, respectively), and that PE pattern of distribution appears to be linked to the direction of the regurgitation [44].

It is important to note that even though a radiographic examination can be informative, serial evaluation of radiographs is much more valuable for correct interpretation, especially for the purpose of detecting cardiac enlargement [18].

2.2.3. Radiographic techniques for left atrium enlargement detection

Apart from the subjective evaluation, general lines for the quantification of the cardiac size suggest that a normal canine heart should have a width correspondent to 2.5 – 3.5 intercostal spaces and occupy nearly around 65 – 70% of the thorax in height in lateral radiographic projections [41]. For the DV view it should occupy no more than 2/3 of the thoracic width at the level of its widest portion [18]. However, radiographic techniques for a more precise quantitative evaluation of the cardiac silhouette have been described. The most common in present days is the VHS, a technique first introduced in 1995 by Buchanan & Bücheler, consisting of the sum of a long and short axis in vertebral body units (VBU) [39]. Each axis is measured separately, with the long corresponding to the length from the ventral edge of the LMSB to the farthest ventral point of the apex and, the short to the widest diameter in the central region perpendicular to the long axis [39]. Each length is then allocated parallel to the spine, from the cranial border of the T4, to be converted to VBU, to the nearest 0,1 VBU and afterwards summed [39]. Later, a variation in the measurement method was introduced in cases where significant LAE was seen, measuring the long axis caudally to the carina, from the left bronchus ventral edge, and the short axis where the heart's caudal limit meets the dorsal edge of the caudal vena cava (CVC), so that the axes length reflected the LAE [45]. Therefore, VHS expresses heart size in VBU (better correlated than sternebrae [39]). The mean value obtained for VHS in normal dogs was 9.7 ± 0.5 VBU and 10.5 VBU was the value recommended as the upper limit for a normal size

heart [39]. However, this quantitative method has large variation considering the big interval range for normal VHS values (8,7 – 10,7) and further variation caused by the respiratory and cardiac cycles that can be up to 1.0 VBU [36, 46]. Studies have shown different ranges and upper limits for normal dogs of certain breeds [47-51] and have also reported that many dogs have intermediate VHS values, i.e., over the originally suggested upper limit (10.5) but not high enough for VHS alone to be capable of accurately detecting whether there is LSE; therefore, breed adjusted ranges for VHS should be used whenever possible [52, 53].

Vertebral left atrial size, first described in 2018 by Malcolm and collaborators, is a technique used to measure the LA in order to detect LAE [40]. It represents the distance between the middle of the most ventral border of the carina and the point where the caudal border of the heart meets the dorsal edge of the CVC [40]. The line formed is then replicated parallel to the vertebral column, beginning on the cranial border of the T4, with the length represented in VBU, similarly to VHS [40]. A value for VLAS of 2.3 VBU was suggested in the original study as the cut-off for determining LAE [40]. Studies that followed also reported cut-offs for LAE detection (LA/Ao ≥ 1.6) or for detection of echocardiographic parameters for stage B2 (LA/Ao ≥ 1.6 + LVIDdN ≥ 1.7) and, interestingly, the values reported are similar, falling within a small interval of 2.2 – 2.5 [37, 38, 54-56], which includes the first reported value of 2.3. A cut-off of 2.4 VBU for the differentiation between mild enlargement (LA/Ao ≤ 1.9) and moderate/severe (LA/Ao > 1.9) has also been suggested [56]. A study reported a normal range going up to 2.2 VBU [57], which also aligns with the originally proposed cut-off value. A study attempting to evaluate breed effect in VLAS showed inferior values (mean 1.8 ± 0.2) when compared to Malcolm et al. (2018) (2.07 ± 0.25 when converted to mean ± standard deviation), possibly raising the importance to investigate the need for breed-adjusted ranges for this radiographic technique [58].

According to Lam et al. (2021), the way that the enlargement alters LA shape and size is variable, making VLAS possibly insufficient to detect LAE depending on how the enlargement occurs [59]. Therefore, a variant for the VLAS measurement was recently described and called modified VLAS (M-VLAS), where a second line is added to the original VLAS method, connecting the first line to the most distal LA border in a way that the 2 lines are perpendicular and M-VLAS then corresponds to the sum of the length of both lines in VBU [59]. In this study there were 2 cases in 21 where LAE was identified by M-VLAS (≥ 3.4 VBU) and not by VLAS (≥ 2.3 VBU), while none was missed by M-VLAS [59].

Just like VLAS, radiographic left atrial dimensions (RLAD) is also a recently proposed technique for the assessment of LA size and enlargement. The process of RLAD quantification could be seen as a “continuation” of the VHS measurement since the computer program is then used to form a line from the intersection of the VHS axes,

bisecting the angle, to the dorsal edge of the LA [60]. However, a clear distinction of the LA margins is not always seen, making RLAD quantification harder to accomplish, especially when LAE is only mild, due to LA limits overlapping with adjacent structures. In those cases, the reference point suggested is “the most dorsal aspect of soft tissue opacity” [60] seen caudally to the carina. This method therefore requires measuring VHS first, but with the variation for the short-axis introduced by Buchanan et al. (2000) [45]. The line formed for RLAD is then replicated along the vertebral column, starting at the cranial border of T4, and its length converted to VBU, similarly to VHS [60]. The optimal cut-off first described for RLAD was 1.8 VBU and other studies reported the same [56] or similar cut-offs (1.9 VBU [54]). A cut-off of 2.0 VBU has been suggested by a study for the differentiation between mild (LA/Ao ≤ 1.9) and moderate/severe (LA/Ao > 1.9) LA enlargement [56]. Due to the potential difficulty in identifying the limits of the LA, new techniques, like Bronchus-to-spine and RLAD-Spine, have been proposed to try to overcome this limitation. The first technique begins at the same point as the long axis of VHS, but instead of tracing a line to the apex, the line goes up straight until it touches the ventral border of the vertebrae immediately above [61]. The second technique consists in creating a line connecting the point where RLAD ends (i.e., at the LA border) and the ventral edge of the thoracic vertebrae over the LA, in a way that the line is perpendicular to the vertebrae, therefore RLAD also must be measured [61]. The length of the lines created is then converted to VBU. However, the authors of the study still prefer RLAD if there is a good observation of the LA limits [61].

Considering how various factors can impact the cardiac silhouette’s dimensions (thorax conformation, respiration cycle, current treatments like diuretics, cardiac pathology, etc) differentiating between mild or no enlargement is hard [41]. Therefore, relying only on a subjective assessment of LAE from radiographs could be limiting [62], which is why quantitative methods of assessing LA size are being studied, including the present study. It is known that there are differences in the thoracic image depending on the recumbency in which the dog is placed [36], however the recumbency’s effect on the diagnostic accuracy of LAE detection is still not very established, hence one of the contribution of the present study.

3. OBJECTIVES

The gold standard to evaluate the LA dimensions is the LA/Ao ratio obtained by echocardiography. Given the frequency of MMVD in dogs (within cardiac diseases), a quantitative radiographic evaluation of LA size can be a very useful resource, especially when performance of echocardiography is not available. For that reason, various studies have been conducted to identify new radiographic techniques, such as RLAD and VLAS. Although these techniques have showed good results, VHS remains the radiographic technique most frequently used.

Therefore, the objective of this prospective study was to focus on evaluating the LA dimensions, by confirming the usefulness of these radiographic techniques (RLAD and VLAS) comparatively to VHS. The specific objectives for this study were to:

- Evaluate the influence of recumbency (right or left) on the values obtained for VHS, RLAD and VLAS;
- Correlate RLAD and VLAS values obtained from right recumbency radiographs with echocardiography LA/Ao ratio values;
- Correlate RLAD and VLAS values obtained from left recumbency radiographs with echocardiography LA/Ao ratio values;
- Determine cut-off values and diagnostic accuracy for VHS, RLAD and VLAS;
- Determine if RLAD and VLAS can differentiate ACVIM stages.

The objectives also comprised the development of skills and the acquisition of knowledge necessary to carry out the study, specifically related to the echocardiographic and radiographic techniques, statistical analysis and interpretation of the results and its discussion in light of existing literature.

4. MATERIALS AND METHODS

4.1. Canine population selection

For this prospective study dogs presented to HVP and UPVET (ICBAS) were recruited from June of 2019 to March of 2021 with owner's consent. Dogs were included if they underwent a complete cardiovascular examination comprising echocardiographic, ECG and radiographic examination. The radiographs, consisting in 3 projections (VD and both lateral recumbencies), had to be performed within a maximum of 24 hours interval from the echocardiographic examination, which was the gold standard method used for LA evaluation. Relevant patient information was also retrieved (data including age, sex, weight, breed, physical exam findings).

Besides all the patient information and performance of the necessary exams, dogs needed to meet certain criteria to be included. The study included only non-sedated animals with at least a year of age, independently of sex and breed, as well as animals with cardiac disease under treatment. Dogs were excluded if the radiographic evaluation showed evidence of mispositioning of the patient, skeletal alterations like vertebral anomalies, presence of PE and of any kind of structure blocking a clear view of the thoracic anatomy, hindering the possibility of performing the measurements. The presence of severe tricuspid regurgitation, severe pulmonary arterial hypertension (> 75 mmHg [6, 63], although recent guidelines for PH do not defend the use of mild, moderate and severe classification for PH [64]), right heart enlargement and ECG arrhythmias in the echocardiographic examination also led to exclusion.

Only dogs affected by MMVD were recruited and were afterwards divided in 2 groups: (1) dogs without LAE (normal LA group) and (2) dogs with LAE, based on a cut-off value of 1.6 (LAE group).

4.2 Echocardiographic examination

The echocardiographic parameters used for the diagnosis of MMVD were the presence of valvular changes (irregularity, thickening, prolapse of the valve's leaflets) in bidimensional mode (B-mode) and presence of a jet flow consistent with MR seen with colour Doppler [2, 54].

All dogs received a complete echocardiographic examination (M-Mode, B-Mode and Doppler) with measurement repetition through 3 to 5 cycles to obtain an average value [2]. The LA/Ao ratio was the parameter used to measure the LA dimensions and a cut-off of 1.6 was selected to determine LAE [2]. This parameter was obtained from a right parasternal

short-axis view with B-mode in the first frame of early diastole (for aortic leaflets visualization) [2], as shown in figure 1. For the B2 classification the ACVIM guidelines staging system was used and, therefore, the LVIDd was also measured and then normalized (LVIDdN). The LVIDd was obtained from a right parasternal short-axis view with M-mode, measuring from “leading edge” to “leading edge” in diastole at the start of QRS [2]. Normalization to body weight (BW) was obtained through the formula: LVIDdN = LVIDd/[(BW)$^{0.294}$] [2]. The echocardiographic exams were performed by 2 veterinarians with relevant experience in cardiology (one in each recruiting centre) to whom the findings of VHS, VLAS and RLAD measurements were maintained unknown. Based on the echocardiographic results the dogs were then staged according to the ACVIM guidelines. Dogs were also allocated into 2 groups according to the cut-off of 1.6 for the LA/Ao ratio, as mentioned above; therefore, dogs with a LA/Ao ratio ≥ 1.6 were allocated in the LAE group and thus considered to have enlargement, and dogs with an inferior value were allocated in the normal LA group (without LAE). The degree of enlargement was further subdivided into 2 categories: mild (LA/Ao values from 1.6 – ≤ 1.9) and moderate to severe (LA/Ao values > 1.9) [56].

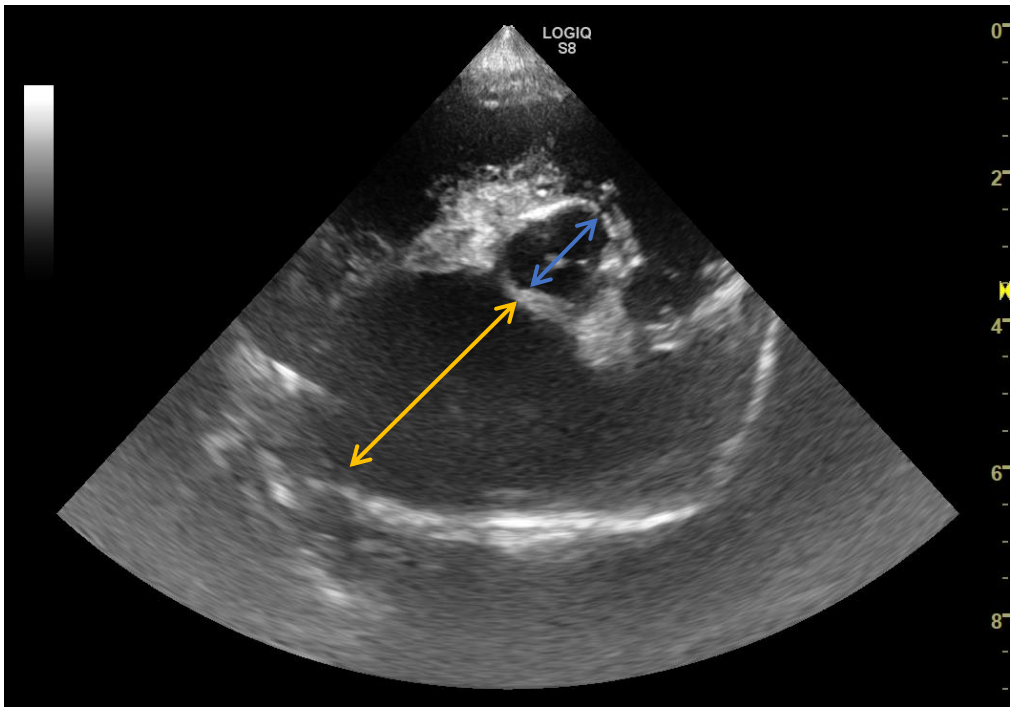

Figure 1. Exemplificative measurement of LA/Ao ratio. The blue double-headed arrow represents the measurement of aorta, and the yellow double-headed arrow represents the measurement of the left atrium.

4.3 Radiographic examination

For every dog included in this study 3 radiographic projections were taken, the VD and both lateral views (right and left recumbency) using a computed radiography system (SEDECAL standard vet stationary x-ray; Computed radiography system FCR Prima T2, Fujifilm Corporation, Tokyo, Japan in HVP and Philips Optimus 50, Philips Portuguesa S.A., Porto Salvo, Portugal; Computed radiography system Fujifilm FCR Prima, Fujifilm Europe GmbH, Vila Nova de Gaia, Portugal in UPVET). All radiographs were validated as good enough to allow correct and accurate measurements. The measurements of T4, VHS, RLAD and VLAS were made in both lateral views by an experienced imaging veterinary and repeated, at different times, by the same operator in order to obtain a total of 3 values for each parameter in each recumbency, using a computer DICOM viewer software (RadiAnt DICOM Viewer 2020.2 (64-bit), trial version, Medixant, Poznan, Poland). A mean value was then calculated from the 3 values obtained.

The measurement procedure was similar to Bagardi et al. (2021) [54] method, which consisted in measuring VHS with a variation for the short axis [54, 60] and RLAD and VLAS according to their original descriptions [40, 60]. The T4 long axis was also measured including the caudal intervertebral disc [54]. For the VHS, a long and short axis were measured. The long axis was considered from the ventral border of the LMSB (carina) to the farthest point of the cardiac apex [39]. The short axis was measured using a slight variation from the original method described by Buchanan & Bücheler et al. (1995), using the dorsal border of the CVC as a reference point, so that the LA body was included [54], instead of measuring at the wider part of the heart [39]. The short axis was therefore measured at that level using the same computer software to ensure a 90º angle between the axes. Then, the number of thoracic vertebrae (to the nearest 0.1 VBU) that correspond to the length of the lines made for the long and short axes were counted after reallocating them over and parallel to the spine [39], as shown in figure 2. For a correct count, the beginning of both lines was aligned with the cranial border of T4 and 1 unit was composed by the vertebral body and the corresponding caudal intervertebral disc [54]. The VHS then corresponded to the sum of both axes in VBU [39]. For the measurement of RLAD the computer software was then used to form a line from the intersection of the VHS axes, bisecting the angle, to the dorsal edge of the LA [60], as shown in figure 3. For the VLAS the carina was used as a reference point, more precisely, its most ventral limit, forming a line connecting it to the caudal limit of the LA at the level where the LA meets the dorsal edge of the CVC [40], as shown in figure 4. The length of the lines drawn for both RLAD and VLAS were converted to VBU, to the nearest 0.1 VBU, just like VHS [40, 60].

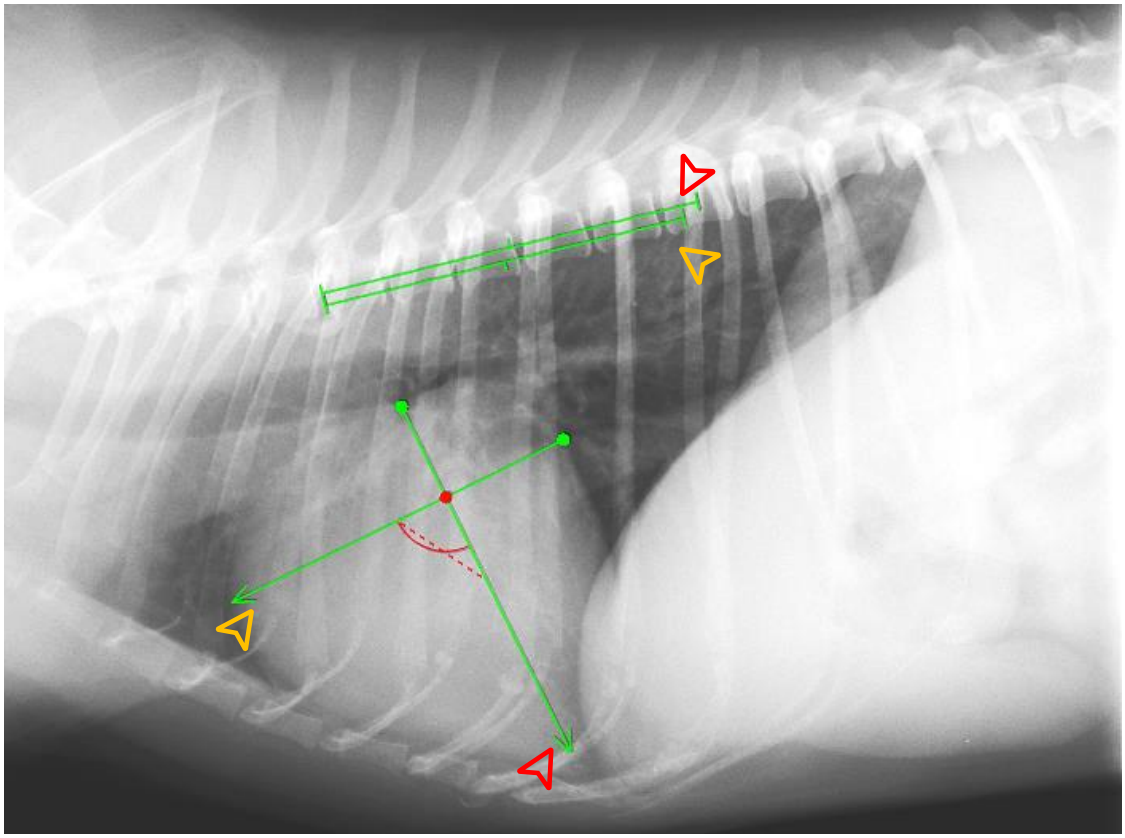

Figure 2. Exemplificative measurement of VHS on a left lateral radiograph of a dog with MMVD, with the long axis identified with a red arrowhead and short axis identified with a yellow arrowhead.

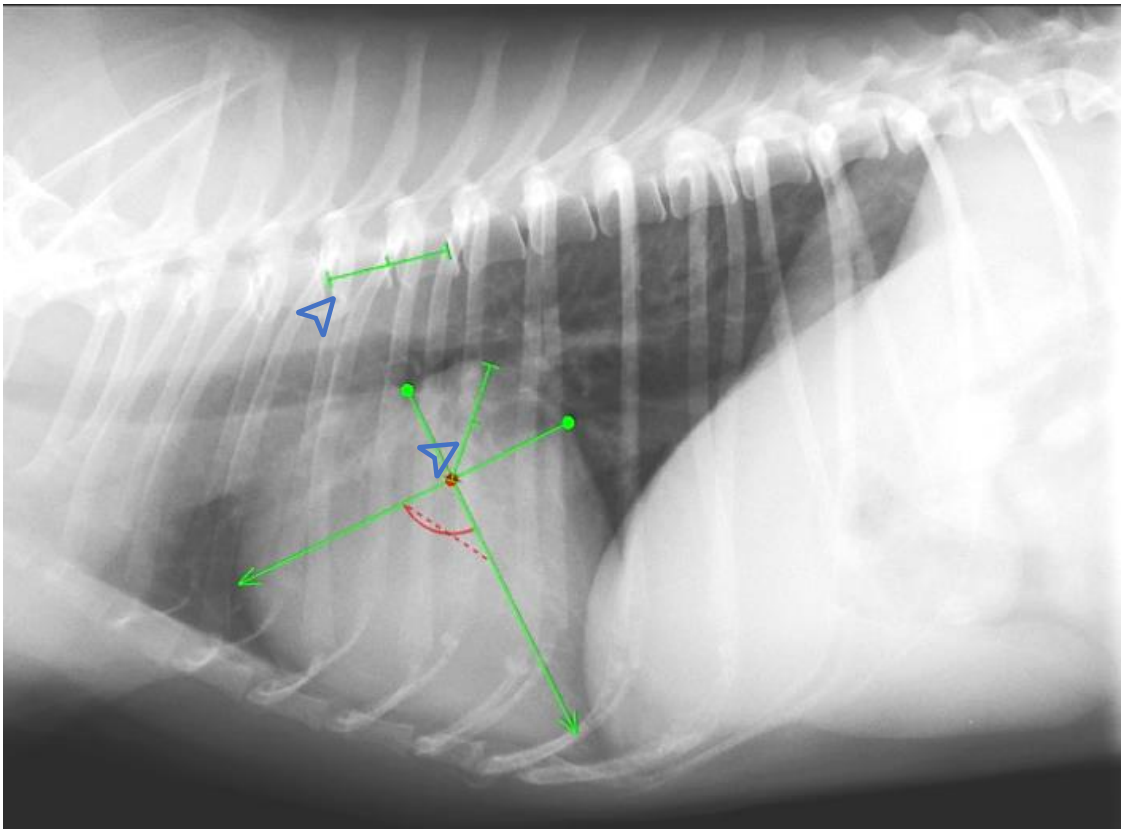

Figure 3. Exemplificative measurement of RLAD on a left lateral radiograph of a dog with MMVD identified with a blue arrowhead.

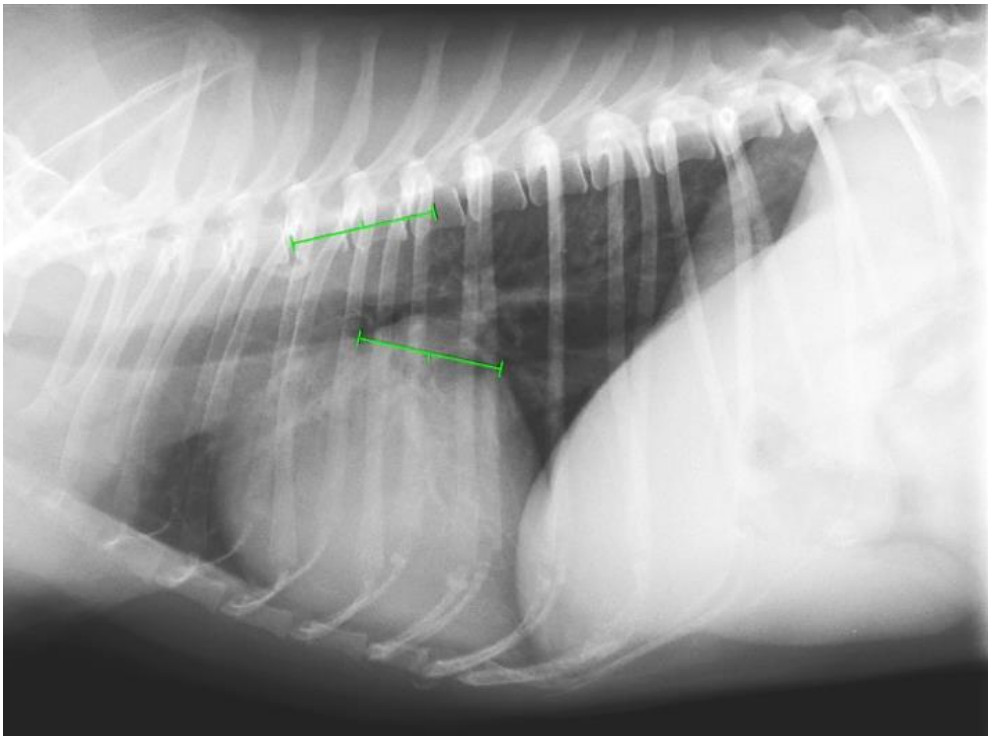

Figure 4. Exemplificative measurement of VLAS on a left lateral radiograph of a dog with MMVD.

4.4 Statistical analysis

Statistical analysis was performed with commercial statistical software (IBM SPSS Statistics 27.0; IBM Corporation Armonk, New York, USA and GraphPad Prism 9.1.2; GraphPad Software Inc, San Diego, CA, USA). The distribution of data was assessed for normality by the Shapiro-Wilk test. Descriptive statistics were calculated and, as well as the echocardiographic and radiographic variables, represented as median (interquartile range [IQR]). Descriptive data, echocardiographic and radiographic parameters were compared among the ACVIM stages (B1, B2, C+D) using one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test and among LA size groups (normal LA and LAE groups) using Student's unpaired t test or Mann-Whitney test. Pearson or Spearman's correlation was used to determine the correlation between VLAS, RLAD and LA/Ao. The diagnostic accuracy of VLAS, RLAD and VHS for the detection of LAE (LA/Ao ≥ 1.6) was assessed by means of the area under the receiver operating characteristic (ROC) curves. The Youden index (sensitivity - [1- specificity]) was used to determine the cut-off values for VHS, VLAS and RLAD that best distinguished patients according to LA size (LA/Ao ≥ 1.6) and that had maximum specificity in both recumbencies. Comparison between ROC curves was made through a non-parametric test. The measurements of VHS, VLAS and RLAD obtained from the right recumbency were compared to the measurements obtained from the left recumbency by means of Student's paired t test or Wilcoxon signed-rank test as appropriate. Values of $p<0.05$ were considered statistically significant for all analysis.

5. RESULTS

5.1. Sample characterization – dog population

From June 2019 to March 2021, 53 dogs were recruited for this study, from which 2 had to be excluded due to the presence of severe PH and PE, which were exclusion criteria. Therefore, the final canine population included 51 dogs affected by MMVD, more precisely, 31 dogs in stage B1, 11 in stage B2 and 9 in stage C+D. This population consisted of 31 males and 20 females, with a median age of 12 years (ranging from 3.5 to 17 years) and a median body weight of 8.55 Kg (ranging from 2.13 to 37.15 Kg). Thirteen dogs weighted < 5 Kg, 17 weighted between 5-9.99 Kg, 19 weighted between 10-19.99 Kg and 2 weighted ≥ 20 Kg. A summary of the descriptive data in 2 different stratification systems (ACVIM stages and LA size) is provided in table 1.

The majority of dogs were mixed breed (*n*=24), followed by Miniature Pinscher (*n*=5), Cocker Spaniel (*n*=4), Beagle (*n*=3), Maltese Bichon (*n*=2), Poodle (*n*=2), Yorkshire Terrier (*n*=2), Miniature Schnauzer (*n*=2), German Spitz (*n*=1), CKCS (*n*=1), West Highland White Terrier (*n*=1), Labrador Retriever (*n*=1), Portuguese Podengo (*n*=1), Chihuahua (*n*=1), German Shepperd (*n*=1). Stage B1 included 12 mixed breeds, 3 Beagles, 3 Miniature Pinscher, 3 Cocker Spaniels, 2 Miniature Schnauzer and 1 of the following breeds: Maltese Bichon, Labrador Retriever, West Highland White Terrier, Portuguese Podengo, Chihuahua, German Shepperd, Poodle and Yorkshire Terrier. The stage B2 included 8 mixed breeds and 1 of the following breeds: CKCS, Cocker Spaniel and German Spitz. Stage C+D included 4 mixed breeds, 2 Miniature Pinscher and 1 of the following breeds: Maltese Bichon, Poodle, Yorkshire Terrier.

There were no significant differences in body weight (p=0.49) and age (p=0.44) between the normal LA group and the LAE group (median age of 12 years for both groups and a median body weight of 8.65 Kg and 8.08 Kg, respectively). There were also no significant differences for those parameters when considering the ACVIM staging system (p>0.05) (table 1). Concerning sex, a clear tendency for a predominance of male patients in more advanced stages (B1 vs B2/C+D) when using the ACVIM staging system, and in the LAE group vs the normal LA group when using the LA size system can be seen (table 1).

5.2. Echocardiographic and radiographic data

The identification of the necessary reference points for the measurement of VHS, RLAD and VLAS was not possible in the right lateral recumbency (RLR) in 3 dogs (1 B1/

normal LA group, 1 C and 1 D/ LAE group) and in the left lateral recumbency (LLR) in 2 dogs (1 B2 and 1C/ LAE group) due to lack of detail and impossibility to visualize the CVC borders. Therefore, for the comparison between recumbencies, the canine population comprised only dogs where measurements were obtained from both RLR and LLR (n =46) and for the comparison to the gold standard (LA/Ao obtained by echocardiography) only the lateral projection in which the measurements were possible was used (48 for the RLR and 49 for the LLR) (table 1).

Regarding the echocardiographic data, of the 31 B1 dogs, 27 had normal LA dimensions and 4 had mild enlargement. Within B2 dogs, 5/11 had mild enlargement and 6/11 moderate to severe; within the C+D category, 2/9 and 7/9 dogs had mild and moderate to severe enlargement, respectively. Further results of echocardiographic and radiographic examinations are summarized in table 1.

Table 1. Summary of the descriptive, echocardiographic and radiographic data of the 51 dogs population in two different stratification systems (ACVIM stages and LA size).

Variable	ACVIM staging			LA size	
	Stage B1	Stage B2	Stage C + D	Normal LA	LAE
Nº of dogs	31	11	9	27	24
Nº of males	15	9	7	13	18
Nº of females	16	2	2	14	6
Age (years)	12 (10 - 14)	13 (9 - 15)	11 (9 - 14)	12 (9 - 14)	12 (9.25 - 14)
Weight (Kg)	9.10 (5.70 – 13.70)	8.55 (5.00 – 12.10)	4.75 (3.10 – 11.20)	8.65 (5.70 – 12.65)	8.08 (4.28 – 12.55)
LA/Ao	1.43 (1.25 – 1.52)	1.93 (1.69 – 2.06) [aaaa]	2.14 (1.89 – 2.48) [aaaa, b]	1.35 (1.24 – 1.51)	1.93 (1.68 – 2.13) [cccc]
LVIDdN	1.47 (1.29 – 1.61)	1.89 (1.70 – 2.11) [aaaa]	2.26 (1.91 – 2.41) [aaaa, b]	1.48 (1.29 – 1.62)	1.89 (1.54 – 2.24)
VHS-RLR (VBU)[dddd]	10.65 (10.15 – 10.98)	11.40 (10.57 – 13.67)	12.17 (10.53 – 13.67)	10.57 (10.05 – 10.90)	11.32 (10.74 – 12.28) [ccc]
VHS-LLR (VBU)	10.43 (9.97 – 10.80)	11.27 (10.68 – 11.59)	12.15 (10.17 – 13.07)	10.43 (9.90 – 10.80)	11.15 (10.61 – 12.11) [ccc]
VLAS-RLR (VBU)	2.16 (2.04 – 2.34)	2.73 (2.10 – 2.85) [aa]	2.83 (2.23 – 3.00) [aa]	2.14 (2.04 – 2.27)	2.52 (2.18 – 2.86) [cccc]
VLAS-LLR (VBU)	2.27 (2.00 – 2.63)	2.75 (2.24 – 3.06) [a]	2.72 (2.12 – 3.05)	2.23 (1.90 – 2.50)	2.72 (2.27 – 3.01) [ccc]
RLAD-RLR (VBU)[d]	1.99 (1.82 – 2.17)	2.70 (1.87 – 2.97) [aa]	2.63 (1.87 – 3.27) [aa]	1.99 (1.79 – 2.14)	2.55 (1.87 – 2.97) [ccc]
RLAD-LLR (VBU)	2.13 (1.90 – 2.33)	2.60 (2.17 – 2.93) [aa]	2.70 (2.13 – 3.14) [a]	2.13 (1.85 – 2.27)	2.49 (2.18 – 2.93) [ccc]

Values represent the median (IQR).

ACVIM: American College of Veterinary Internal Medicine; LA: left atrium; LAE: left atrium enlargement; LA/Ao: left atrium-to-aorta ratio; LVIDdN: left ventricular internal diameter in diastole normalized for body weight; VLAS: vertebral left atrial size; RLAD: radiographic left atrial dimension; VHS: vertebral heart score; -RLR: right lateral recumbency; -LLR: left lateral recumbency; VBU: vertebral body units; mm: millimetres; Kg: kilograms; Nº: number.

[aaaa] p<0.0001 vs. stage B1; [aa] p<0.01 vs. stage B1; [a] p<0.05 vs. stage B1; [b] p<0.05 vs. stage B2;

[cccc] p<0.0001 vs. Normal LA group; [ccc] p<0.001 vs. Normal LA group;

[dddd] p< 0.0001 vs. LLR; [d] p<0.05.

The LA/Ao and LVIDdN were significantly higher in stages B2 and C+D as compared to stage B1 ($p<0.0001$) and in stage C+D as compared to stage B2 ($p<0.05$) (table 1 and figure 5). When comparing according to the LA size system, the LA/Ao was also significantly higher in the LAE group than in the normal LA group ($p<0.0001$; table 1).

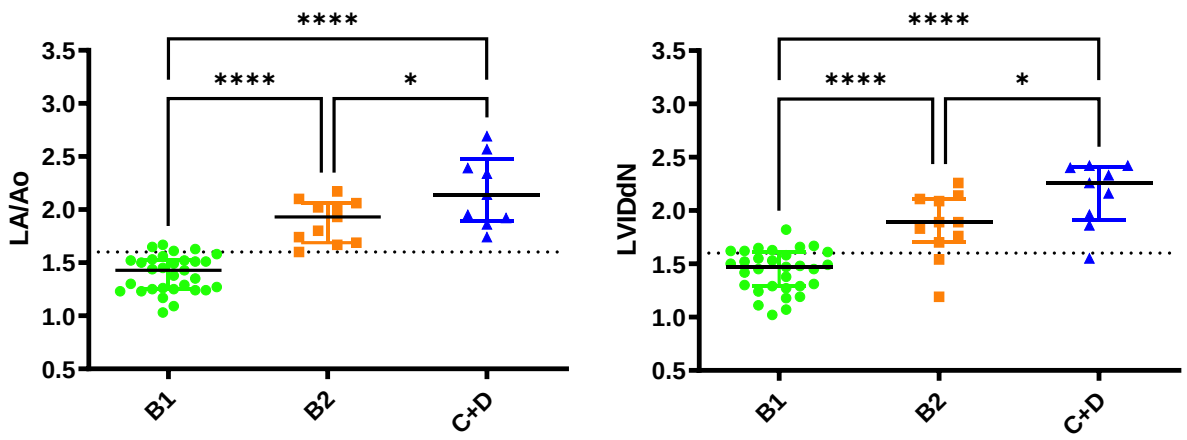

Figure 5. Scatterplots of the LA/Ao and LVIDdN according to the American College of Veterinary Internal Medicine (ACVIM) staging system. The black bars represent the median and the coloured bars represent the interquartile range. LA/Ao: left atrium-to-aorta ratio; LVIDdN: left ventricular internal diameter in diastole normalized for body weight. **** $p<0.0001$; * $p<0.05$.

Regarding the radiographic variables, for the ACVIM staging stratification system, significantly higher values were found for stage B2 in comparison to stage B1 for both RLAD and VLAS in both recumbencies ($p<0.01$ for RLAD-RLR, RLAD LLR and VLAS RLR and $p<0.05$ for VLAS LLR) and significantly higher values for stage C+D in comparison to B1 were found in RLAD RLR ($p<0.01$), RLAD LLR ($p<0.05$) and VLAS RLR ($p<0.01$) (table 1). For the LA size system, all 3 radiographic techniques (VHS, RLAD and VLAS) showed significantly higher values in the LAE group than in the normal LA group for both recumbencies ($p<0.001$ for VLAS-LLR, VHS and RLAD for both recumbencies and $p<0.0001$ for VLAS RLR) (table 1). More than 40% of the dogs in the normal LA group had a VHS value > 10.5 in both recumbencies (upper limit of the normal range originally proposed [39]).

A significant positive linear correlation was found between RLAD and LA/Ao in both recumbencies ($r = 0.41$, $p=0.004$ for the RLR and $r = 0.57$, $p<0.0001$ for the LLR) and between VLAS and LA/Ao in both recumbencies ($r = 0.47$, $p = 0.0008$ for the RLR and $r = 0.50$, $p = 0.0002$ for the LLR) (figure 6).

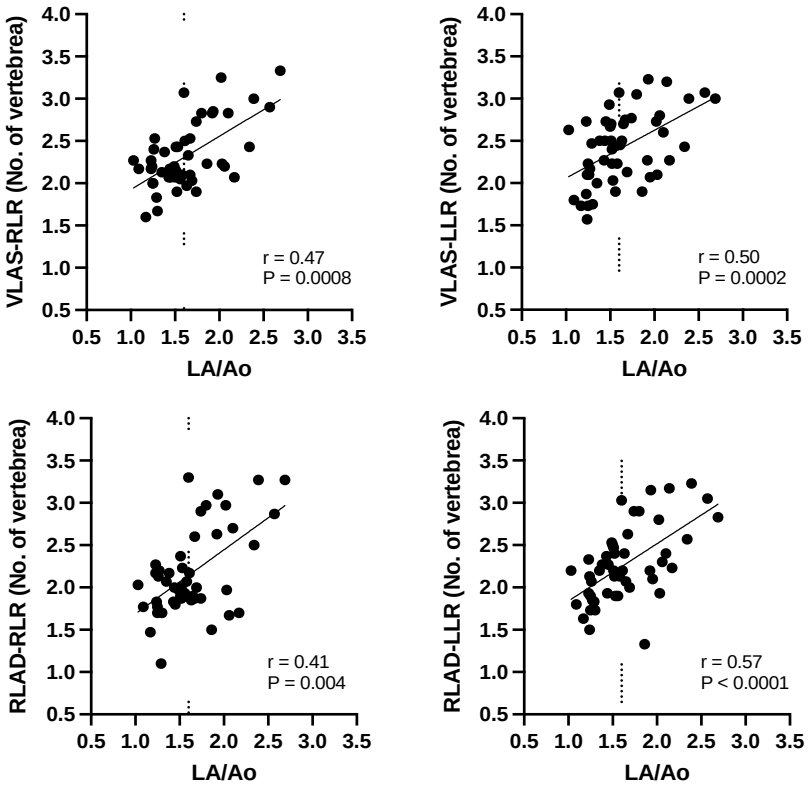

Figure 6. Positive linear correlations between the 2 radiographic techniques studied (RLAD and VLAS) and the gold standard for determination of left atrial size in echocardiography (LA/Ao). LA/Ao: left atrium-to-aorta ratio; VLAS: vertebral left atrial size; RLAD: radiographic left atrial dimension; -RLR: right lateral recumbency; -LLR: left lateral recumbency; No: number.

The results of the ROC curves and optimal cut-off values for VHS, VLAS and RLAD, for both recumbencies, for the purpose of detecting LAE (LA/Ao ≥ 1.6) are presented in table 2. For all radiographic techniques, and in both recumbencies, the diagnostic accuracy was moderate (area under the curve [AUC] values between 0.7 and 0.9), with the VHS and VLAS, both from the RLR, showing the highest AUC values (0.833 and 0.824, respectively; $p<0.0001$; table 2 and figure 7). However, there were no significant differences between the AUC curves of the 3 radiographic techniques ($p>0.05$). The cut-off values that best distinguished between LA size groups for RLAD (2.43/2.55 for RLR/LLR, respectively) showed maximum specificity for both recumbencies (table 2). For VHS and VLAS the cut-offs with maximum specificity were also calculated and were slightly higher than the optimal cut-offs, however, the observed sensitivity decreased considerably, especially for VHS (from 65% to 35% in the RLR and from 50% to 20% in the LLR) (table 2).

The comparison between recumbencies showed significant differences for VHS (median 10.82, IQR 10.31 – 11.27 for RLR; median 10.72, IQR 10.06 – 11.13 for LLR; $p<0.001$) and RLAD (median 2.02, IQR 1.83 – 2.53 for RLR; median 2.20, IQR 1.93 – 2.51

for LLR; p = 0.031). For VLAS the differences were not significant between recumbencies (median 2.22, IQR 2.07 – 2.53 for RLR; median 2.46, IQR 2.10 – 2.73 for LLR; p = 0.069).

Table 2. Summary of ROC curves results and diagnostic cut-offs for the 3 radiographic techniques for detection of LAE in both recumbencies.

ROC curves	AUC (95% CI)	P value	Cut-off (VBU)	Se (%)	Sp (%)	Cut-off type
VHS-RLR	0.833 (0.711 – 0.955)	p<0.0001	≥ 11.22	65	96,2	
			≥ 12.05	35	100	Maximum Sp
VHS-LLR	0.771 (0.630 – 0.912)	p<0.0001	≥ 11.17	50	96,2	
			≥ 12.17	20	100	Maximum Sp
VLAS-RLR	0.824 (0.692 – 0.956)	p<0.0001	≥ 2.47	60	96,2	
			≥ 2.63	50	100	Maximum Sp
VLAS-LLR	0.772 (0.635 – 0.910)	p<0.0001	≥ 2.74	45	96,2	
			≥ 2.97	30	100	Maximum Sp
RLAD-RLR	0.745 (0.581 – 0.909)	p = 0.003	≥ 2.43	60	100	Maximum Sp
RLAD-LLR	0.788 (0.649 – 0.928)	p<0.0001	≥ 2.55	50	100	Maximum Sp

ROC curves: Receiver Operating Characteristic curves; AUC: area under the curve; CI: confidence interval; VBU: vertebral body units; Se: sensitivity, Sp: specificity; VLAS: vertebral left atrial size; RLAD: radiographic left atrial dimension; VHS: vertebral heart score; -RLR: right lateral recumbency; -LLR: left lateral recumbency.

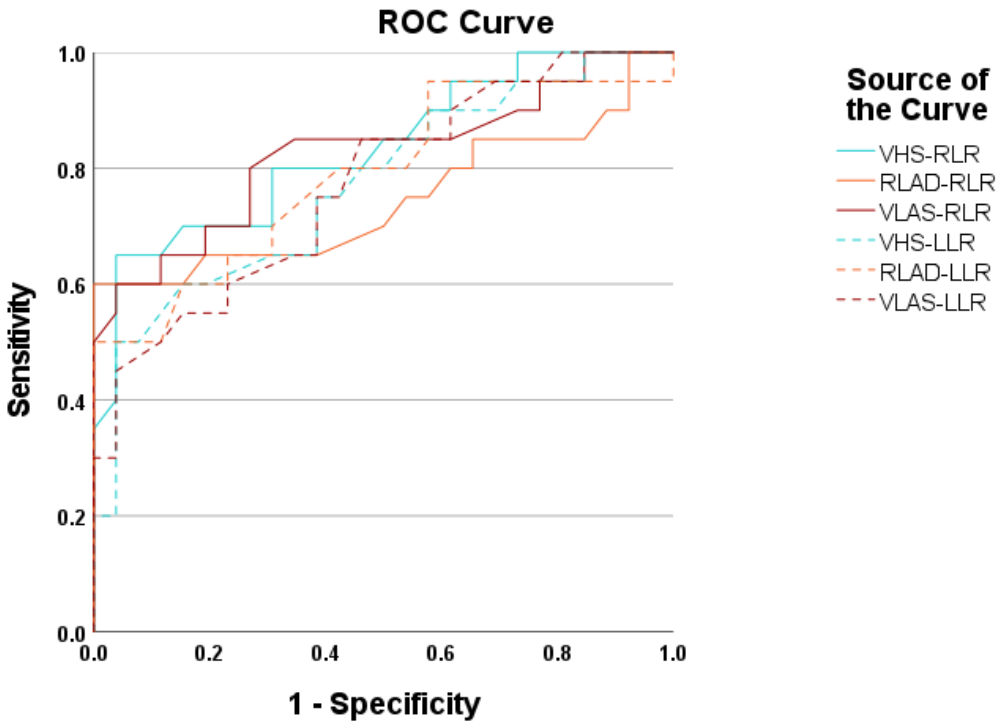

Figure 7. Comparison of the ROC curves of VLAS and RLAD for the detection of LAE. ROC curves: Receiver Operating Characteristic curves; VLAS: vertebral left atrial size; RLAD: radiographic left atrial dimension; VHS: vertebral heart score; -RLR: right lateral recumbency; -LLR: left lateral recumbency; VBU: vertebral body units.

6. DISCUSSION

The aim of our study was to evaluate radiographic techniques, VHS, VLAS and RLAD, regarding their correlation to the gold standard (LA/Ao), their diagnostic accuracy in the detection of LAE and the influence of recumbency. Although the results showed a weaker correlation between VLAS and RLAD with LA/Ao than expected (r values between 0.47 – 0.57), literature results show considerable variation, with studies reporting effectively high correlation values (e.g., r = 0.816/0.855 for VLAS and RLAD with LA/Ao, respectively [56]) and others reporting considerably lower values (e.g., r = 0.63/0.59 for VLAS and RLAD with LA/Ao, respectively [54] and r = 0.48 for VLAS with LA/Ao [52]). Still, both techniques, as well as VHS, were able to distinguish the normal LA vs LAE groups ($p<0.001$). Only in 10% of the cases were RLAD and VLAS not possible to be measured in one of the recumbencies, which shows a great applicability. However, in the present study, the exclusion of patients with certain criteria (e.g., PE) and the fact that the radiographic measurements were performed by a veterinary with high level of experience in imagiology may justify why RLAD and VLAS measurements were obtained from the big majority of the initial canine population. Those factors may also justify why both parameters were obtained from the same patients, when a higher feasibility for VLAS than RLAD could be expected, considering its easier execution, as previously reported [56]. This could also suggest that this high percentage of execution may not apply to every clinical context.

Around 42% of the dogs in the normal LA group had VHS > 10.5, which is the originally proposed upper limit of the normal range for VHS [39]. This finding agrees with previous studies that reported a considerable number of cases with “intermediate” VHS values (10.6-10.8 to 11.7) for which an accurate detection of LSE was not possible with just VHS [52, 53]. This suggests that VHS is not the most adequate radiographic technique to identify LAE and, although the radiographic examination is not intended to be used as a sole method for the identification of LAE, it appears that VHS may not provide useful additional information in a significant number of cases. This was not unexpected, since the design of VHS is better suited to identify cardiomegaly and not enlargement of a specific chamber. For the purpose of identifying specifically LAE, the design of radiographic techniques such as VLAS and RLAD seem more suited. It is true that the results might have been more favourable if VHS had been adjusted to breed-specific normal ranges, however it is also true that breed-adjusted ranges are not available for all breeds and for mixed breed dogs it is not possible to create adjusted ranges. A recent study [58] reported lower VLAS values for healthy Chihuahuas than the original study for VLAS [40] (mean 1.8 ± 0.2 vs mean 2.07 ± 0.25, respectively. Breed effect could be present and therefore elements of

other breeds could have contributed for a higher VLAS since the original study only had 3 elements of this breed in the control group. Still, the control group of the original study was composed by a small population (n=15), thus, further studies with larger populations are necessary to elucidate this possible effect of breed on VLAS [58].

The diagnostic accuracy for the detection of LAE was not significantly different between the 3 techniques, contrasting with a study which showed higher diagnostic accuracy for RLAD than VLAS [56], but agreeing with others [54, 59]. Comparison was limited since some studies only evaluate one radiographic technique [38, 40] and others that do involve at least two were not clear to the author of this present work if significance between AUC values was tested between techniques [37, 60]. Similar diagnostic accuracies also contradict with the previous argument that VHS may not be the most adequate for LAE detection. However, in the present study a variation of the VHS method was used to include the LA body (measurement of the short axis at the level of the CVC), which may have contributed to the comparable results between the VHS and the two techniques designed specifically for LAE detection. In fact, the two studies which showed similar diagnostic accuracy between techniques apply this variation in all measurements [54] or at least in cases where LAE was marked [45, 59]. The cut-offs suggested by those same studies were also similar to the ones found in the present study for VHS (11 VBU [54], 11.1 VBU [59] vs the results found in the present study, 11.22 and 11.17 VBU for RLR and LLR, respectively) and to the study originally describing RLAD which also uses the same method for VHS measurement (11.1 VBU [60]). The study which showed differences between techniques [56] and measured VHS according to the original method [39] reported a cut-off of 10.7 VBU, which diverts a little, however this cut-off was for detecting echocardiographic criteria for stage B2, which is different than detecting LAE and, therefore, could also justify the difference. The sensitivity (Se) for VHS found in the present study was considerably lower than in other studies (65% and 50% for RLR and LLR, respectively vs 76 - 78% [54, 59, 60]), while specificity (Sp) was more similar (96.2% in our study vs 93.5% [60], 96.6% [59], although lower for one study, 74% [54]). A higher proportion of patients with more severe disease could help justify the higher sensitivities found in those studies [60].

The best cut-off for distinguishing dogs with normal LA and with LAE was 2.47 VBU (60% Se, 96.2% Sp) and 2.74 VBU (45% Se, 96.2% Sp) for VLAS in the RLR and LLR, respectively. The study originally reporting VLAS [40] suggested a cut-off value of 2.3 VBU for prediction of LAE, however the clinically relevant cut-off value, using only the LA/Ao in short axis as criteria for enlargement, as was done in this study, was 2.5 (67% Se, 84% Sp), which is particularly closer to the results obtained in this study for the RLR. Two other studies reported also a cut-off of 2.5 VBU, one obtained from a population of stage B dogs

(Se 62%, Sp 87%) [38] and another determined for the distinction of B1 and B2 dogs (Se 70%, Sp 84%) [37]. The particularities in the design of these 2 studies condition the comparison, however it is interesting how the results are similar to the previously mentioned study despite those differences. Other studies reported cut-offs for LAE detection of 2.2 VBU (90% Se, 80% Sp) [56], which is lower than the cut-offs obtained in this study, particularly for the LLR, and 2.43 VBU (66% Se, 88% Sp) [54], which is also closer to the RLR value. The results show a higher similarity of the cut-off values and sensitivity found in literature with the ones found in the present study for the RLR, although one of the studies reports a significantly higher sensitivity (90% [56]). Specificity, however, was the same for both recumbencies and considerably higher in the present study (96.2%). The best cut-off for distinguishing dogs with normal LA and with LAE was 2.43 and 2.55 VBU for RLAD in the RLR and LLR, respectively, which largely diverts from the originally proposed value of 1.8 [60] and, consequently, from other studies which found the same [56] or similar values (1.9) [54]. The sensitivity also significantly diverted from the literature (60% and 50% for the RLR and LLR in the present study, respectively, vs 93.5% [60], 90% [56], although lower in one study, 71% [54]). Also, regarding specificity, in the author's knowledge, this is the first study where the cut-off value that best discriminates between normal and enlarged LA is also the value with maximum specificity (i.e., least false positives). Differences in the population could justify these discrepancies. In our study, the number of animals presenting a mild enlargement and a moderate-severe enlargement is similar (11 vs 13, respectively), however for other studies that might not be the case. In one study the proportion was 20 to 51 [56] and in another 80% of the LAE group had LA/Ao>2 [60], while in ours that percentage was 42. Therefore, in studies where there is a considerably higher number of dogs with more advanced disease, the radiographic techniques may be more accurate, especially in the case of RLAD that is a more complex technique involving several measurements and difficult landmarks which can contribute to different results. The same studies mentioned above also reported higher correlations between RLAD and LA/Ao (e.g., 0.855 [56], 0.84 [60] vs 0.41/0.57 for RLR/LLR in the present study) and VLAS and LA/Ao (0.816 [56] vs 0.47/0.50 for RLR/LLR) and a study in which only asymptomatic dogs were included showed a correlation between VLAS and LA/Ao similar to the present study (0.48 vs 0.47/0.50 for RLR/LLR) [52]. Thus, these differences in the population could also explain the superior correlations found in the literature.

In the present study VLAS ≥ 2.63 for the RLR and ≥ 2.97 for the LLR, RLAD ≥ 2.43 for RLR and ≥ 2.55 for the LLR and VHS ≥ 12.05 for the RLR and ≥ 12.17 for the LLR differentiated dogs with normal LA and enlarged LA with 100% specificity. These results may indicate that these values, for the respective variables, likely identify LAE if the

echocardiographic examination is not available. If we cross this information with the fact that those cut-offs are also the optimal cut-offs for the detection of LAE in RLAD's case, it appears that our results suggest an additional advantage in using RLAD for measuring the LA size, as those values become even more useful in a clinical context. However, it can be very challenging to determine the margins of the LA in cases where LAE is mild due to the superimposition of adjacent anatomical structures. In Vezzosi et al. (2021), although a higher feasibility was seen for VLAS than RLAD exactly due to the higher difficulty of locating the landmarks, RLAD demonstrated higher diagnostic potential, possibly related to the fact that RLAD better represents the direction in which the LA tends to enlarge, as suggested by the authors [56]. The possible inability of VLAS to detect enlargement depending on how the LA shape changes was also commented by a study comparing VLAS and a modified VLAS technique [59]. Therefore, the selection of one of the techniques requires the evaluation of all these factors.

In the present study significant differences were found between measurements obtained from right and left recumbency for VHS and RLAD. For VHS, previous studies are contradictory regarding the recumbency influence, with studies reporting no differences [37, 39, 58] and others showing significantly higher values for the RLR [48, 50, 65] as occurs in the present study. A study suggested that this effect could be related to the divergency of the x-ray beams and the fact that the heart and the cassette are farther apart in the RLR [65]. Although the difference is not very marked (median of 10.57 vs 10.43 for the RLR and LLR, respectively for the normal LA group and 11.32 vs 11.15 for the RLR and LLR, respectively for the LAE group), it becomes clinically significant when the patient in question presents a VHS value over the upper limit for the normal range in one recumbency but lower than that limit for the other recumbency, as mentioned in Greco et al. (2008) [65]. This effect is seen in the present study, considering that the median VHS in the normal LA group for the RLR is > 10.5, the upper limit originally suggested [39], but < 10.5 for the LLR. Adjustment of VHS values to breed-specific normal ranges probably will not compensate this difference, unless different ranges are created for each recumbency. To the author's knowledge, this is the first study evaluating the effect of recumbency on RLAD, therefore, comparison was not possible. However, considering that VHS is necessary for RLAD measurement, theoretically, it is rational to hypothesize that the factors influencing VHS also influence RLAD, although RLAD was higher for the RLR in the group with LAE, but lower in the normal LA group. For VLAS no differences were found, which agrees with previous studies [37, 40]. Still, in a study the results regarding VLAS in both recumbencies led the authors to advise that values obtained from different recumbencies should not be swapped when comparisons between different patients or between serial measurements

are being made [40]. This suggests that there could be a recumbency effect, possibly disguised by the type of population and protocol design of the studies evaluating that effect.

Apart from breed, the VHS can be influenced by the respiratory and cardiac cycles and by the operator regarding the landmarks placement [46, 66]. The VHS has been shown to be significantly decreased in dogs affected by Addison's disease [67] and in which manual lung inflation was performed due to general anaesthesia [68]. Since RLAD is dependent on VHS, these factors may also influence RLAD. The cardiac cycle may not affect VLAS [59, 69]. Other factors that most likely affect all radiographic techniques include anatomical abnormalities (e.g., vertebral anomalies), imperfect positioning, especially in cases where sedation is not used and even the slightest movement may cause rotation, obliquity and prevent exposure synchronicity with peak inspiration and cause discrepancies in measurements.

A more exact and broader comparison between studies within this study's subject revealed to be difficult due to the different designs and protocols used between studies, which in some cases resulted in minimal impact and in others may significantly invalidate comparison in the author's opinion. Some studies allow longer time intervals between the radiographic and echocardiographic examinations [55-57], possibility of administering medication in this period [40, 55], others show differences in the measurement process (e.g., nearing the VBUs to 0.25 instead of 0.1 [37] or using different reference points, like in VHS), some resort to the use of anaesthetic drugs [40, 52], even inclusion/ exclusion criteria differ in some cases (e.g., weight < 20 Kg) and definition of different values to define LAE (e.g., defining LAE in short axis as LA/Ao>1.68 [55]). In Malcolm et al. (2018) a small number of dogs received medication between both exams and exclusion from the analysis only resulted in little improvement of the correlations between the echocardiographic and radiographic variables (e.g., r increased from 0.7 to 0.73 between LA/Ao in short axis and VLAS) [40]. However, this leads us to hypothesize how much bigger would the differences be if a larger amount of the population had also been medicated in-between exams as studies have shown differences in the echocardiographic and radiographic parameters with alteration of hydration status [70] and short-term medication (pimobendan more precisely) [71]. Other studies also regarding these same techniques calculated cut-off values but for the purpose of differentiating B1 and B2 stages (e.g., [37]), therefore, comparisons are limited since our results are primarily for the differentiation of normal and enlarged LA, which is not entirely equal since patients with LAE could still be staged as B1.

However, the current study had some limitations. First, the gold standard used to assess LA size was the LA/Ao, defining LAE if ≥ 1.6, when a probably more accurate gold standard would measure LA volume. However, the LA/Ao was still the chosen gold standard

since it is in fact the implemented practice in MMVD, several studies concerning this disease and radiographic techniques for LA size also use it as a gold standard (e.g., [54, 56, 60]) and it is a parameter established for decision making regarding treatment [4, 19]. Second, intra- and interobserver variability were not assessed in the present study; nevertheless, the echocardiographic examinations were performed by 2 highly experienced veterinary cardiologists and the radiographic measurements were performed by a highly experienced operator specialized in imagiology. The fact that the radiographic measurements were performed by a single operator may introduce an error bias, however, the error possibly introduced has a more systematic nature, which allows for consistency. Third, only patients with good quality radiographs were included in this study and all radiographic measurements were performed by an imagiology specialist, therefore, results may differ in the clinical context where radiographs without the ideal conditions would still be analysed (e.g., radiographs showing rotation, suboptimal positioning, vertebral defects, increased lung opacity, etc) and where the clinicians have variable expertise for radiographic interpretation. As someone with very little experience, the difficulties I faced when performing these radiographic measurements were significant, a lot of radiographs leave room for different interpretations, mostly regarding the limits of the anatomical structures, especially with RLAD, which is the one with the most difficult landmarks to pinpoint the exact location out of the 3 techniques. Fourth, our study did not have a control group. However, previous studies are contradictory regarding the influence of sex on VHS, with some studies reporting no influence [39, 65], one reporting higher values for males [47] and one reporting higher values for females in only one of the 8 breeds included [51]. Similarly the correlation of VHS with body condition score/weight is not consensual (in 2 studies no correlation was found [48, 58], in another study the correlation was very weak and differences between a low and high body condition were only found in one of the breeds studied [51] and another study reported significantly higher VHS values in body scores ≥ 6 for the studied breed [49]). A study on healthy dogs showed no influence of sex and body weight on VLAS [57]. Therefore, even with a “matched” control group the result interpretation would be similar. Fifth, the size of the population was reasonable and comparable to other studies, however studies involving larger populations may be needed in order to affirm the results and allow their generalization to the canine population affected by MMVD. Sixth, the cases were recruited in 2 veterinary care facilities, therefore, differences in the radiology equipment used and exposure factors may affect the results. Lastly, the breed effect was not assessed and, studies have shown its influence on VHS [47-51], however, to the author’s knowledge, only one study has evaluated that effect on VLAS for one breed [58], therefore, further studies are needed to understand how the breed could impact these techniques.

7. CONCLUSION

The results show that VHS, VLAS and RLAD can be useful in the clinical context. The two recent radiographic techniques (RLAD and VLAS) positively correlated with the echocardiographic gold standard and differentiated between stage B1, B2, C + D patients (with the exception that VLAS-LLR only differentiated stage B1 from B2) and all techniques differentiated patients with normal and enlarged left atrium. All 3 techniques also showed a moderate accuracy for the detection of LAE in a heterogenous canine population affected by all stages of MMVD and, therefore, may be valuable for the monitorization of subclinical patients with MMVD, particularly if the echocardiographic examination is not possible. This study did not show differences in the diagnostic accuracy between the 3 techniques; therefore, one cannot be recommended over the other based on their diagnostic potential. Advantages of each technique should be weighted for the purpose of choosing one over the other, however, the fact that the optimal cut-offs obtained for RLAD for both recumbencies showed maximum specificity is a clinically useful advantage, especially if echocardiography is not feasible. Recumbency affected measurements in VHS and RLAD. The VHS in the RLR was significantly higher than in the LLR in both groups of the LA size, while RLAD was significantly higher on the RLR in the normal LA group, but higher on the LLR in the LAE group. To the author's knowledge this is the first study showing differences in RLAD measurements between the left and right recumbencies and further studies on larger populations should be performed to assess this finding. Nevertheless, this finding, together with the differences also showed for VHS, at the very least suggest that the same recumbency should always be used, especially when there is an intention of comparing serial measurements from radiographs through time.

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