Anatomic Regurgitant Orifice Area in Dogs with Myxomatous Mitral Valve Disease

Determination of the feasibility and repeatability of AROA measurements using real-time 3D transthoracic echocardiography

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October 2015

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Abstract

Background: accurate assessment of severity of mitral regurgitation (MR) has important implications in the management of dogs with myxomatous mitral valve disease (MMVD). Planimetry of anatomic regurgitant orifice area (AROA) is a feasible and accurate method to quantify MR in humans.
Objectives: to determine feasibility and repeatability of AROA measurements using real-time 3D transthoracic echocardiography (RT3DE) in dogs affected by MMVD, and to determine whether if AROA obtained using RT3DE relates to the severity of MMVD assessed both with an echocardiographic scoring system (MRSS) and the American College of Veterinary Medicine (ACVIM) staging.
Animals: 81 privately owned dogs diagnosed with MMVD.
Methods: Following a standard two-dimensional and Doppler echocardiographic examination, RT3DE datasets of the mitral valve were acquired and used for measuring AROA with dedicated software. Dogs were classified as mild, moderate or severe according to the MRSS score, and as Stage B1, B2 and C accordingly to ACVIM staging. Inter- and intra-operator variability has been tested and differences in AROA between dogs in different MRSS and ACVIM classes were investigated.
Results: AROA was measurable in 60 (74.1%) dogs. The inter- and intra-operator coefficients of variation were 26% and 21%, respectively. AROA was significantly greater in dogs with a severe MRSS compared to dogs with mild MRSS (p=0.04). There were no differences in AROA between dogs with mild versus moderate, and moderate versus severe MRSS (p=0.84 and p=0.28 respectively). There was no difference between AROA of dogs in different ACVIM clinical stages.
Conclusions and Clinical Importance: obtaining AROA using RT3DE is feasible and is promising as a non-invasive additional technique to stratify MR severity in dogs with MMVD. Diagnostic and prognostic utility of AROA deserve further investigation.

Achtergrond: accurate toetsing van de ernst van mitraalklep regurgitatie (MR) heeft belangrijke implicaties in de management van honden met myxomateuze mitraalklep degeneratie (MMVD). Planimetrie van de *anatomic regurgitant orifice area* (AROA) is een haalbare en accurate methode om MR te kwantificeren in mensen.
Doel: het bepalen van de haalbaarheid en herhaalbaarheid van het maken van AROA metingen door *real-time 3D transthoracic echocardiography* (RT3DE) te gebruiken in honden met MMVD, en om te bepalen of AROA gemeten met RT3DE gerelateerd kan worden aan de ernst van MMVD wanneer deze wordt gescoord met een echocardiografisch scoringssysteem (MRSS) en het *American College of Veterinary Medicine* (ACVIM) stageringssysteem.
Dieren: 81 privé gehouden honden gediagnosticeerd met MMVD.
Methode: RT3DE datasets van de mitraalklep zijn verkregen en gebruikt om de AROA te meten met behulp van speciale software. Honden werden geclassificeerd als mild, matig en ernstig door middel van het gebruik van de echografische MRSS en als klasse B1, B2 en C volgens de ACVIM classificatie. *Inter- en intra-operator* variabiliteit is getest en de verschillen in AROA tussen honden in de verschillende MRSS en ACVIM klassen zijn onderzocht.
Resultaten: AROA was meetbaar in 60 (74,1%) honden. De inter- en intra-operator variatiecoëfficiënten waren respectievelijk 26% en 21%. AROA was significant groter in honden met ernstige MRSS vergeleken met honden met een milde MRSS (p=0,04). Er waren geen verschillen in AROA tussen honden met milde of matige, en matige of ernstige MRSS (p=0,84 en p=0,28 respectievelijk). Er was geen verschil tussen AROA in de verschillende ACVIM klassen.
Conclusie en klinisch belang: het verkrijgen van AROA door RT3DE te gebruiken is haalbaar en is veelbelovend als een non-invasieve extra techniek om de ernst van MR te bepalen in honden met MMVD. Diagnostische en prognostische bruikbaarheid van AROA vereist meer onderzoek.

Introduction

Abbreviations

2DE	2D echocardiography
3DE	3D echocardiography
ACEi	Angiotensin Converting Enzyme inhibitor
ACVIM	American College of Veterinary Internal Medicine
AROA	Anatomic regurgitant orifice area
BW	Body weight
CHF	Congestive heart failure
CMRI	Cardiac Magnetic Resonance Imaging
CV	Coefficient of variation
Emax	Peak velocity of mitral inflow
EROA	Effective regurgitant orifice area
LA	Left atrial
LA:Ao	Left atrial to aortic root ratio
LVIDd	Left ventricular internal dimension in diastole
MMVD	Myxomatous mitral valve disease
MR	Mitral regurgitation
MRSS	Mitral regurgitation severity score
MV	Mitral valve
PISA	Proximal isovelocity surface area
RT3DE	Real-time 3D transthoracic echocardiography
Rvol	Regurgitant volume
VCW	Vena contracta width

Background on MMVD

Myxomatous mitral valve disease (MMVD) is a chronic and progressive condition affecting valve anatomy and function due to degeneration of the mitral valve and chordae tendinae. Mitral valve degeneration causes mitral insufficiency, causing regurgitation of blood from the left ventricle into the left atrium, which can eventually lead to left-sided congestive heart failure (CHF). MMVD is the most common acquired cardiac disease in dogs, and the most common cause of congestive heart failure (CHF) in this species. MMVD also exists in humans.

Epidemiology and etiology

Although MMVD is the most common cause of CHF in dogs, it is a relatively benign condition. A long preclinical period is often seen and many dogs affected do not progress to CHF.[1] MMVD is more often diagnosed in small-breed dogs, but it can also occur in large-breed dogs.[2] Male dogs are affected somewhat more often than females. Age and breed correlate with the prevalence of the disease, with a prevalence of more than 90% in Cavalier King Charles spaniels older than 10 years. Besides the Cavalier King Charles spaniel, the Dachshund and Miniature and Toy Poodles is also predisposed.[3–6]

Concomitant with MMVD, myxomatous degeneration of other valves could be present. The incidence of valve involvement is estimated based on several studies. In around 60% of dogs with valve degeneration, only the mitral valve is affected. Around 20-30% of affected dogs have mitral and tricuspid valve degeneration and less than 10% has

tricuspid valve disease alone.[7–9] The aortic and pulmonic valves are less commonly affected.[10] Genetic factors play a large role in the etiology of the disease, but the exact mechanism is unknown. Most likely the disease has a polygenic inheritance in which multiple genes influence the trait and a certain threshold has to be reached before MMVD develops.[11, 12]

Pathofysiology

Pathology of affected mitral valve leaflets shows a wide spectrum of lesion severity, such as mild to severe nodular thickening of the leaflets, elongation of the leaflets and lengthening or rupture of the chordae tendinae (fig. 1).[13] Those lesions preclude a good leaflet apposition. The mitral annulus, a saddle shaped ring which contracts and reduces surface during systole to help complete closure of the leaflets, can dilate due to MMVD. This too can attribute to poor leaflet apposition.[14] The exact mechanism of the mitral valve degeneration is unknown, but includes weakening of the connective tissue of the leaflets. Histopathology suggest accumulation of proteoglycans and glycosaminoglycan within the connective tissue matrix of the valve plays a role in the development of the disease.[10, 15]

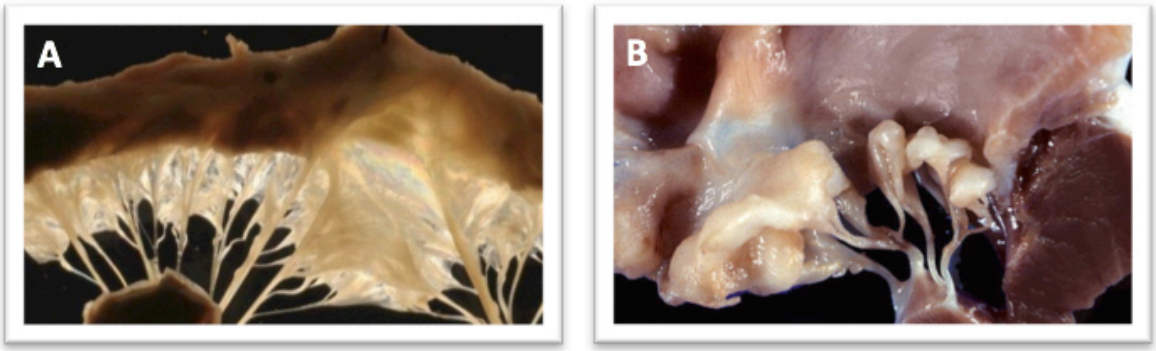

Fig. 1. Pathomorphology of mitral valve leaflets. A normal mitral valve *(A)* and a myxomatous mitral valve *(B)*. (Source Borgarelli M, Buchanan JW. Historical review, epidemiology and natural history of degenerative mitral valve disease. J Vet Cardiol 2012;14:93–101.)

Clinical signs

The first clinical sign of mitral regurgitation (MR) due to MMVD is the detection of a soft systolic left apical heart murmur. Early stages of the disease usually do not show any other clinical signs and there are usually no changes in the dog's activity level or behavior for a long period of time. Some studies show an increase in murmur intensity as the disease progresses.[16]

As valvular regurgitation progresses, cardiac work increases. Due to the volume overload, the atrium will enlarge to compensate for the filling pressure. The left ventricle will develop eccentric hypertrophy in order to manage the larger than normal stroke volume and maintain a normal cardiac output. When the ventricular myocardium is no longer able to contract adequately to compensate for the volume overload, cardiac output decreases and end-diastolic volume increases, which leads to pulmonary congestion and fluid accumulation. Development of pulmonary edema causes the first clinical signs of CHF.[6, 17]

A ruptured chorda tendinae due to MMVD can cause a sudden volume and pressure overload of left atrium and ventricle. This may result in acute onset of pulmonary congestion.[6]

Dogs with congestive heart failure show signs of reduced exercise ability, including increased heart rate, breathing rate and/or effort, panting, lethargy and cough. Ascites or sudden weakness can also occur in dogs with CHF.[10]

Diagnosis

Echocardiography is required to exclude the presence of other cardiovascular diseases leading to mitral regurgitation and to confirm and assess severity of MMVD. The right parasternal long-axis 4-chamber view is the standard view to assess the canine mitral valve.[14] Prolapse and/or thickening of one or both mitral valve leaflets are echocardiographic characteristics of MMVD.[2] A significant regurgitant jet is often present and can be detected using Doppler echocardiography. Echocardiography also plays an important role in assessing severity of the disease. Left atrial enlargement seems the most reliable prognostic parameter. Left atrial enlargement, assessed with the left atrium/aortic root ratio, increases risk of death from cardiac disease.[1, 18] Quantifying MMVD severity by determining the severity of regurgitation seems promising, but is difficult in veterinary medicine.

Thoracic radiography is recommended for dogs with MMVD to assess cardiomegaly, to obtain a baseline when the patient is asymptomatic, monitor progression of the disease and lastly to diagnose pulmonary edema as a result of congestive left sided heart failure.[6] Concomitant respiratory diseases that may be the cause of clinical signs, like cough, could also be diagnosed with the help of thoracic radiographs.[2]

Systematic investigation of the relationship between structural mitral valve abnormalities and clinical assessment of disease severity is hindered by the lack of objective and quantitative echocardiographic criteria. Recently, the prognostic relevance of abnormal mitral valve anatomy seen in dogs with MMVD has been , but there is still a lot to learn.[14, 17, 19, 20]

Classification

Clinical signs and degree of cardiac remodeling, assessed by echocardiography of thoracic radiography, is used to classify the severity of MMVD. The ACVIM consensus statement provides a classification system that is currently widely used to stage MMVD in dogs (table 1). The goal of classification is to link severity of signs to appropriate treatment at each stage of the disease.[6] Dyspnea or tachypnea as a consequence of pulmonary edema is the first clinical sign of CHF.

	Definition
Stage A	Dogs at risk for developing MMVD that have no identifiable cardiac structural disorder (i.e., Cavalier King Charles spaniel, dachshunds)
Stage B1	Dogs with MMVD that have never developed clinical signs and have no radiographic or echocardiographic evidence of cardiac remodeling
Stage B2	Dogs with MMVD that have never developed clinical signs but have radiographic or echocardiographic evidence of cardiac remodeling (i.e., left-sided heart enlargement)
Stage C	Dogs with MMVD and past or current clinical signs of heart failure associated with structural heart remodeling (dogs presenting heart failure for the first time may present severe clinical signs and may require hospitalization)
Stage D	Dogs with end-stage MMVD and heart failure that is refractory to standard therapy (i.e., furosemide, ACE-I, pimobendan)

Table 1. Classification system for dogs affected by MMVD based on clinical signs and radiographic or echocardiographic assessment (from Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Haggstrom J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R. Guidelines for the Diagnosis and Treatment of Canine Chronic Valvular Heart Disease. J Vet Intern Med 2009;23:1142–1150)

Treatment

The ACVIM consensus statement provides therapy guidelines for the different stages of MMVD. Those guidelines are expert opinions, but are formulated with supporting evidence. Dogs in Stage A or Stage B1 do not require any dietary or drug therapy. An annual re-evaluation is recommended for the B1 Stage.

There is no consensus about the advised therapy for dogs in Stage B2 of the disease. If treated at all, the most common drug therapy used for this stage is the use of an *Angiotensin converting enzyme inhibitor* (ACEi). An ACEi, like enalapril or benazepril, will cause vasodilatation and will lower blood pressure and therefore could lower the afterload of the heart. An ACEi will also lower production of aldosterone, causing an increase in fluid excretion.[21] This therapy is not convincingly beneficial for asymptomatic dogs with cardiac remodeling. There is even less evidence for beneficial effects of the use of other drugs than ACEi in this stage, and therefore no drug therapy is advised for this stage.[2, 6]

Dogs in Stage C might have past or current clinical signs of heart failure. Treatment depends on current situation. Acute therapy of Stage C dogs includes furosemide, dosing based on the severity of clinical signs and response to initial therapy. Pimobendan is mainly useful in a chronic situation. Although there is no strong clinical trial evidence, it is also recommended in acute phases by experts, based on hemodynamic and experimental evidence.[6, 22–25] Pimobendan has a positive inotropic effect, it increases cardiac contractility, increases cardiac output and lowers the hearts pre- and afterload.[21] There is no consensus about the efficacy of ACEi, in addition to furosemide and pimobendan in the acute situation, because there is no clear evidence of its additional effect. However, there is evidence ACEi gives improvement in the acute situation compared with the administration of furosemide alone.[6] Oxygen supplementation should be given if needed.

Furosemide is also indicated in the chronic therapy of Stage C, dosage to effect (1-2 mg/kg PO q12h to 4-6 mg/kg PO q8h), at the lowest dose possible to keep patients free

from clinical signs. An ACEi (0.25 -0.5 mg/kg PO q12h) or pimobendan (0.25-0.3 mg/kg PO q12h) could be given. Both pimobendan and ACEi significantly improve survival time when given besides furosemide in the chronic therapy of MMDV.[18, 26, 27] It is not known whether if a triple therapy with furosemide, pimobendan and ACEi is superior to therapy consisting of furosemide and ACEi or furosemide and pimobendan alone. However, the combination of all these drugs is widely used and no negative effects have been reported yet. There is no consensus about the use of spironolactone, digoxin or a β blocker at this stage. Those therefore are not further addressed. Weight should be monitored and weight loss should be prevented.[6]

For patients in Stage D of MMVD, conventional treatment no longer has the desired effect. In acute situations IV furosemide can be administered, besides oxygen supplementation. Extra vasodilators (amlodipine, hydrazaline or sodium nitroprusside) can be started besides pimobendan and ACEi, to further reduce afterload. Increasing furosemide dosage can be necessary in chronic Stage D therapy. Spironolactone, if not already started, is indicated in this stage too.[6]

It should be clear there is no standard therapy for a patient in a certain clinical stage of the disease and the ACVIM recommendations based on staging are only guidelines. Every patient needs his own therapeutic plan based on individual findings and follow-ups.

Background on Transthoracic Echocardiography

From 2D to 3D

The most common type of echocardiogram used in dogs is the 2D transthoracic echocardiography, which is a still or moving image of the internal parts of the heart, using ultrasound. An ultrasonic transducer, or probe, is placed on the chest of the patient to get various views of the heart. It is a non-invasive way to assess the health status of the heart. Patients generally do not need any form of sedation to undergo echocardiography.[28]

Transesophageal echocardiography in dogs is possible using a special transducer mounted on a modified endoscope, enabling acquisition of high-resolution images, superior to transthoracic echocardiography, because of the proximity to the heart. This form of echocardiography requires special gear, special training and is invasive for the patients as it requires general anesthesia. Besides that, there is the risk of complications as damage to the oropharynx, esophagus, or transmission of infectious diseases. Because of those limitations, transesophageal echocardiography in dogs is mainly used in surgical procedures and less as a diagnostic tool.[29]

Although this 2D echocardiography (2DE) is a really useful technology for imaging the heart, it has some limitations. Quantitative analysis of the heart using this technique is based on geometric assumptions about the shape of structures of the heart, spatial interpolation among available views and calculations using fixed mathematical formulas irrespective of the actual shape of the cardiac structure of interest. Some 2D techniques, for example left ventricular mass calculations, have limited reproducibility and accuracy. Looking at the complex mitral valve annulus, 2DE is not sufficient in displaying valve morphology and geometry, in human and in dogs.[20, 30]

Thanks to advancements in computer and transducer technology, a lot of those limitations are now bypassed by the use of 3D transthoracic echocardiography. Instead of only horizontal and vertical dimensions, 3D echocardiography (3DE) adds depth as third dimension, which allows visualization from multiple perspectives (fig.2). This technique enables an easier, more reproducible and accurate interpretation of the cardiac anatomy.[30]

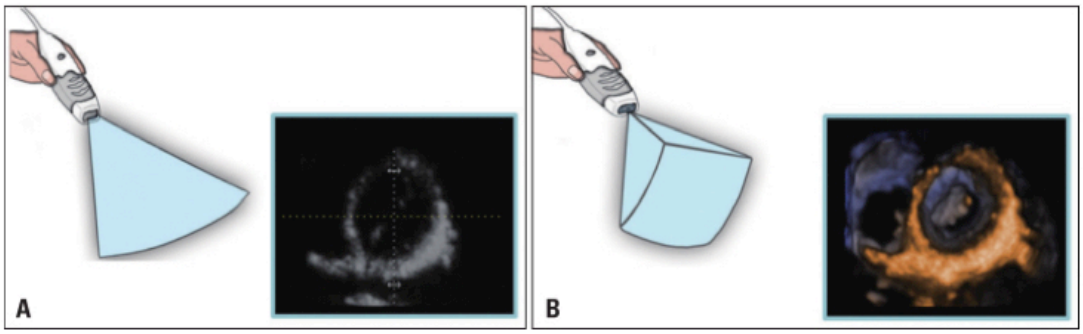

Fig. 2. Schematic representation of two-dimensional (i.e. tomographic; *A*) and single-beat three-dimensional (i.e. volumetric; *B*) of the left ventricular short-axis at mitral valve level. Volumetric rendering displays many more details and allow better appreciation of spatial relationship among cardiac structures. (from Badano LP, Boccalini F, Muraru D, Bianco LD, Peluso D, Bellu R, Zoppellaro G, Iliceto S. Current clinical applications of transthoracic three-dimensional echocardiography. J Cardiovasc Ultrasound 2012;20:1–22.)

Real-time 3D transthoracic echocardiography (RT3DE) is a 3D data acquisition method in which, beat after beat, a thin sector of a pyramidal 3D volume data set is obtained and visualized live, as in a 2DE. Fast acquisition is possible and there is no need of a reference system, electrocardiogram and respiratory gating. Unfortunately it still suffers of relatively poor spatial and temporal resolution. Multi-beat acquisition has a higher resolution but is more prone to artifacts.[30]

In humans, 3DE is used to obtain accurate and reproducible chamber volume measurements and to make realistic en face views of heart valves.[30–32] Moreover, this technique is recently gaining more popularity, and is proving its use in veterinary medicine.[17, 20, 33]

Anatomic regurgitant orifice area

The dimension of the orifice though which MR occurs, also called the anatomic regurgitant orifice area (AROA), is one of the factors affecting MR severity.[20, 31] The gold standard to assess AROA is Cardiac Magnetic Resonance Imaging (CMRI), but this technique has several limitations in veterinary medicine. Limitations include requirement for general anesthesia, time and specific personnel, which result in elevated cost. Obtaining information about the size of the orifice using 2D echocardiography and Doppler techniques is time-consuming, and has been demonstrated to lack accuracy.[20]

Planimetry of the anatomic regurgitant orifice area (AROA) is measured in humans using transesophageal 3DE.[34] Measuring AROA in human is a feasible and accurate way to quantify MR severity.[35] Moreover, compared to other conventional techniques, as measuring effective regurgitant orifice area (EROA) by proximal convergence zone method or proximal isovelocity surface area (PISA), vena contracta width (VCW) with

2D color Doppler echocardiography, or measuring mitral valve regurgitant volume with CMRI, it is a relatively fast, non-invasive and again accurate technique.[31, 32, 36] Currently, no studies are published comparing conventional techniques to quantify MR severity with transthoracic 3DE techniques in dogs. Two-dimensional echocardiography, in combination with Doppler techniques, is still the method of choice for non-invasive evaluation of MR in both humans and dogs.[19, 20, 36, 37]

Aim of the study

Current classification of severity of MMVD in dogs is based on both clinical signs and identification of cardiac remodeling by radiography and echocardiography.[6]

Quantification of mitral regurgitation (MR) severity is a valuable way to assess MMVD severity in humans.[38, 35] Several studies suggest severity of MR could be used as an independent predictor of survival in dogs with MMVD.[1, 14, 17, 19, 39] However, with the usual echocardiographic techniques there is no feasible and repeatable method to accurately classify MMVD severity based on MR severity.

In humans, planimetry of the anatomic regurgitant orifice area (AROA) using transesophageal three dimensional echocardiography proved to be a feasible and accurate way to assess MR.[31, 32] Moreover, analysis of the AROA has demonstrated several advantages compared to other conventional techniques, as it is a relatively fast, non-invasive and accurate technique.[31, 36, 37] There are no studies on the use of AROA obtained with 3DE to assess MR in dogs yet.

The aims of this study were: 1) to determine feasibility and repeatability of AROA measurements using real-time 3D transthoracic echocardiography (RT3DE) in dogs affected by MMVD; 2) to determine whether if AROA obtained using RT3DE relates to the severity of MMVD assessed by a mitral regurgitation severity score (MRSS) and the American College of Veterinary Medicine (ACVIM) clinical staging.

Materials and Methods

Study design

The study was a retrospective, observational study.

Animals

The study group comprised client-owned dogs referred to the cardiology service of the Veterinary Teaching Hospital of the Virginia-Maryland College of Veterinary Medicine (VMRVM) for evaluation of MMVD. In all animals, standard M-mode, 2D, and Doppler blood flow measurements, performed with continuous ECG monitoring in right and left lateral recumbency using an ultrasound unit equipped with 1.5–10 MHz phased array transducers were available. Three-dimensional datasets of the MV, acquired using a RT3DE capable ultrasound unit[a] equipped with a matrix array transducer[b] were also available. Thoracic radiographs were obtained if clinically indicated.

Only patients with presence of MR were included in this study. Exclusion criteria were the use of sedative drugs during echocardiographic examination and the presence of other acquired or congenital cardiovascular disorders other than MMVD, which could directly or indirectly affect the mitral valve or its function.

Diagnosis and classification of MMVD

Myxomatous mitral valve disease was diagnosed based on the echocardiographic identification of mitral valve thickening and/or prolapse typically in combination with the presence of MR identified by color Doppler.

All patients were classified according to the ACVIM staging guidelines.[6] *Left-sided heart enlargement* (Stage B2), was defined based on the following radiographic and/or echocardiographic findings: vertebral heart score >10.5, echocardiographic evidence of LA enlargement (LAvol indexed to body weight (BW) >1.1 ml/kg and/or LA:Ao >1.5),[40] increased left ventricular end-diastolic dimensions (compared to expected dimension).[41]

For each patient, a MR severity score (MRSS) was also calculated using a previously reported method,[40] integrated with the left atrial (LA) dimensions, to assess overall MR severity. The MRSS is built on the following established echocardiographic parameters: Color Doppler regurgitant jet area,[42] the peak velocity of the mitral inflow (Emax),[19, 43, 39] the left ventricular internal dimension in diastole (LVIDd),[43, 44] leaflet anatomy,[19, 45] density of the continuous wave Doppler MR signal[37] and LA enlargement using LA volume (LAvol/BW) and/or the left atrial aortic root ratio (LA:Ao), prioritizing LAvol/BW.[1, 40, 39] Individual scores assigned for each of the parameters were summed to obtain the MRSS and total scores assigned patients to their MR severity class (mild, moderate or severe) (table 2). Due to our inclusion criteria MRSS ranges from 6 to 17.

[a] Artida, Toshiba Medical Systems, Tokyo, Japan

[b] PST-25SX matrix-array transducer, Toshiba Medical Systems, Tokyo, Japan

Echocardiographic Parameter	Normal	Mild	Moderate	Severe
Color Doppler regurgitant jet area	None, or trivial (0)	<20% of LA area (1)	20-40% of LA area (2)	>40% of LA area (3)
Mitral inflow	Emax <1.2 m/s (1)	Emax <1.2 m/s (1)	Emax >1.2 m/s (dec. time >80 ms) (2)	Emax >1.2 m/s (dec. time <80 ms) (3)
Left ventricular internal dimension in diastole	Normal (1)	Normal (1)	Enlarged (<20% over upper reference limit for BW) (2)	Enlarged (>20% over upper reference limit for BW) (3)
Leaflet anatomy	Normal (1)	Thickening/mitral valve prolapse (2)	Thickening/mitral valve prolapse (2)	Flail leaflet (3)
CW Doppler MR jet density	None (0)	Incomplete/faint (1)	Dense (2)	Dense (2)
LA enlargement: LA:Ao and LA vol/BW	LA:Ao <1.5 and LAvol/BW <1.1 ml/Kg (0)	1.5< LA:Ao <1.7, or 1.1< LAvol/BW <1.3 ml/Kg (1)	1.7< LA:Ao <2, or 1.3< LAvol/BW <1.5 ml/Kg (2)	LA:Ao > 2.0, or LAvol/BW >1.5 ml/Kg (3)
Score ranges	**3**	**4-7**	**8-12**	**13-17**

Table 2. Classification system for mitral regurgitation severity for dogs with MMVD, based on echocardiographic parameters. Numbers in parenthesis represent the assigned scores for each parameter. (Partially from Wesselowski S, Borgarelli M, Bello NM, Abbott J. Discrepancies in Identification of Left Atrial Enlargement Using Left Atrial Volume versus Left Atrial-to-Aortic Root Ratio in Dogs. J Vet Intern Med 2014;28:1527–1533.)

Real-time 3D transthoracic echocardiography

In all dogs, AROA was measured from RT3DE datasets, using commercial dedicated software.[c] The datasets optimized for acquisition of the mitral valve were analyzed, starting with a reference image of decent quality and without stitching artifacts crossing the MV. A mid systolic frame was selected for the analysis. Two orthogonal long-axis planes and one short axis plane were simultaneously visualized. The two long-axis planes were moved parallel to the assumed location of the regurgitant orifice, perpendicular to the MV leaflets. From this view the short axis plane was aligned so that it crosses the center of the regurgitant orifice, as visualized from the atrial side (surgical view). The AROA was manually tracked as the area (in cm^2) of the orifice in the MV in this view (fig.3).[32]

One operator (Müller) conducted the measurements in all dogs. The number of datasets in which it was possible to measure AROA was recorded and the feasibility of this technique were expressed as the percentage of datasets in which AROA could be obtained. In addition, AROA was measured from 9 randomly selected patients twice by the same operator and also twice by a second operator (Menciotti) on the same frame. These data were used to assess intra-operator repeatability and inter-operator reproducibility of the AROA.

[c] 4D Cardio-View 3.0 TomTec Imaging Systems, Unterschleisssheim, Germany

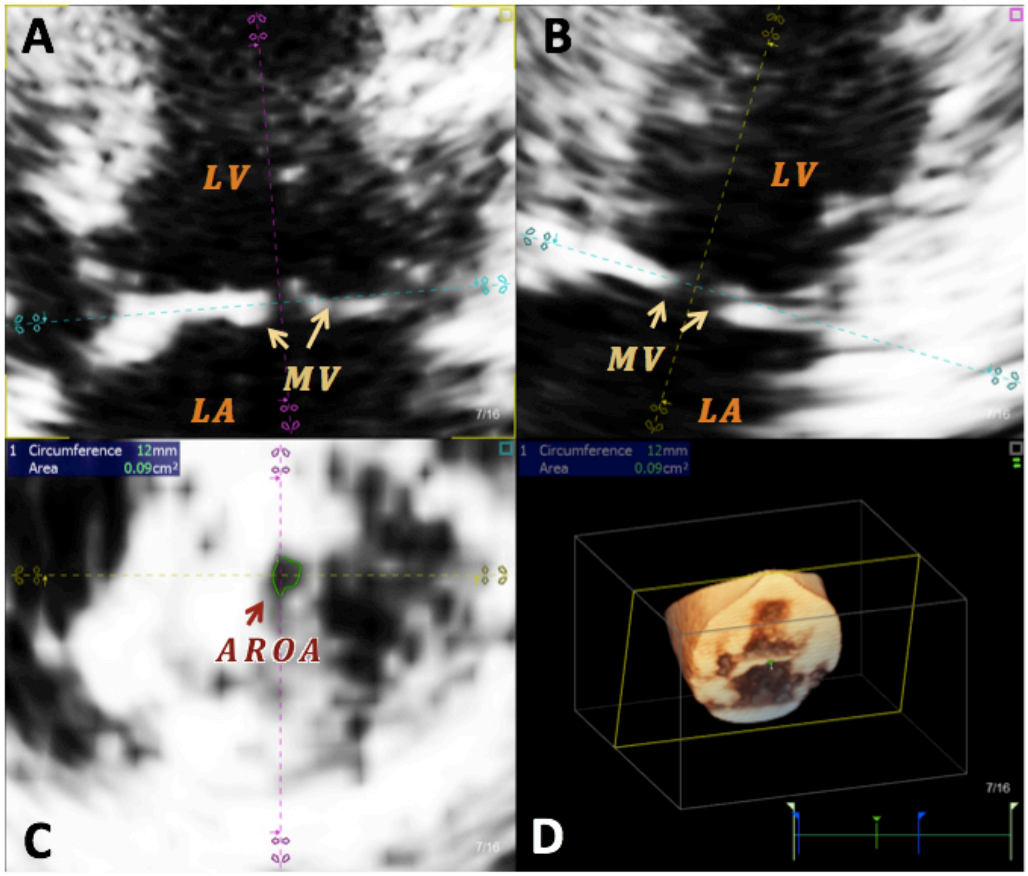

Fig. 3. Obtaining AROA from RT3DE data set. In a mid-systolic frame, two orthogonal long-axis planes, parallel to the supposed regurgitation orifice and perpendicular to the MV leaflets are visualized (A and B). The short axis plane is aligned so that it crosses the center of the regurgitant orifice so that planimetry of the regurgitant orifice area could be performed (C). The lower right image shows a 3D overview (D).

Statistical Analysis

Intra- and inter-operator repeatability of AROA measurements was calculated as % coefficient of variation (%CV). More, a variance component analysis was performed, using a nested random effect model. The effects of dog, operator (inter-operator), and repeated measure on the same image (intra-operator) were evaluated and shown as % of total variance. The distributions of age, BW and AROA were tested using the Shapiro-Wilk test. Normally distributed data are presented as mean (±SD), while non-normally distributed data are presented as median (range).

The Wilcoxon signed rank test was used for testing equality of medians between dogs with and without feasible AROA.

Differences in AROA between dogs in different MRSS classes, as well as in different ACVIM clinical stages were evaluated using a Kruskal-Wallis test for non-normally distributed data. When this test disclosed statistically significant differences, post-hoc evaluation with Dunn Test was performed to determine contingent differences between individual groups. A level of $p<0.05$ was considered significant for all the statistics tests.

Results

Animals

Eighty-one patients were enrolled in the study, consisting of 46 (57%) male and 35 (43%) female dogs with a mean age of 10.05 years (SD ± 2.63) and median BW of 9.8 kg (4.1-31.8). In 21 patients the AROA could not be obtained because of poor quality or presence of stitches artifacts in the RT3DE examinations.

The remaining 60 dogs in which AROA was measurable consisted of 32 males and 28 females with a mean age of 10.25 ± 2.61 years and median BW of 9.6 kg (4.1 – 31.8). Twenty-five breeds were represented. 11 dogs were of mixed breed origin. The most common breed was Cavalier King Charles spaniel (n=11), followed by Shih Tzu (n=4), Beagle (n=3), Dachshund (n=3) and Lhasa Apso (n=3). Twenty-two dogs were classified as ACVIM Stage B1, 29 as Stage B2, and 9 were classified as Stage C. Of the 60 patients, 15 dogs were classified as mild MRSS, 28 dogs as moderate MRSS and 17 as severe MRSS (fig.4).

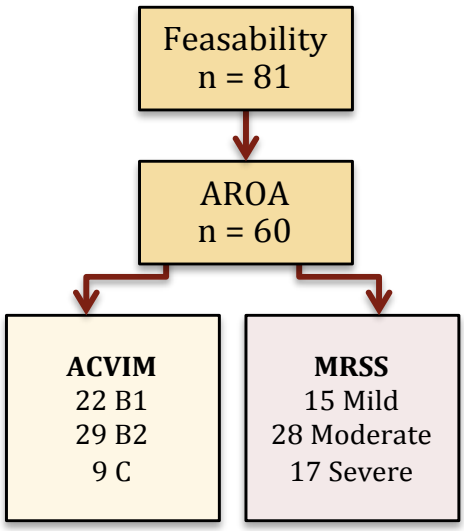

Fig. 4. Schematic representation of patient population

Feasibility

The feasibility of the technique is 74.07% (fig. 5). Dogs in which it was feasible to measure AROA had a lower BW than dogs in which AROA was not measurable (p=0.03). Between dogs with or without planimetry of AROA, there was no difference in sex (p=0.28) or age (p=0.22).

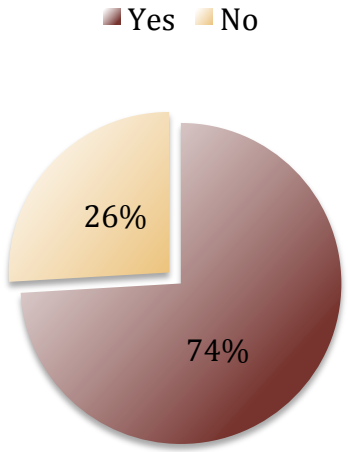

Fig. 5. Technique feasibility

The inter- and intra-operator coefficients of variation of measuring AROA were 26% and 21%, respectively. Variance component analysis showed most variation in outcome was due to operator variability (41.1%), followed by variability in measure (31.6%) and patient variability (27.3%) (fig.6).

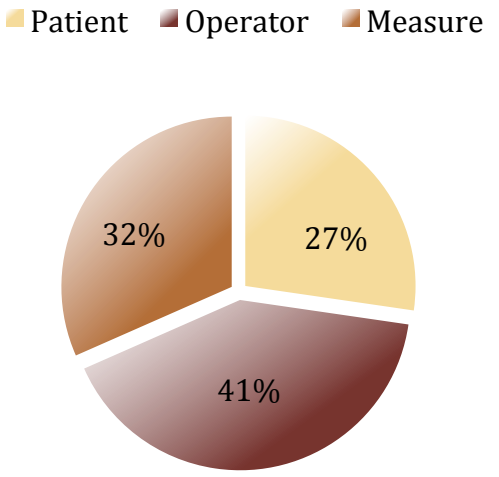

Fig. 6. Components of variation

AROA versus ACVIM staging

In patients in Stage B1, AROA had a median of 0.03cm^2 (0.01-0.09). AROA in Stage B2 had a median of 0.05cm^2 (0.02 to 0.11). Stage C AROA, median 0.06cm^2 (0.02 to 0.11) was comparable with Stage B2 (fig.7). There was no difference between AROA of dogs in different ACVIM stages. Stage B1 compared with C (p=0.31), Stage B1 compared with B2 (p=0.39) and Stage B2 with Stage C (p=1.0) did not show any significant results.

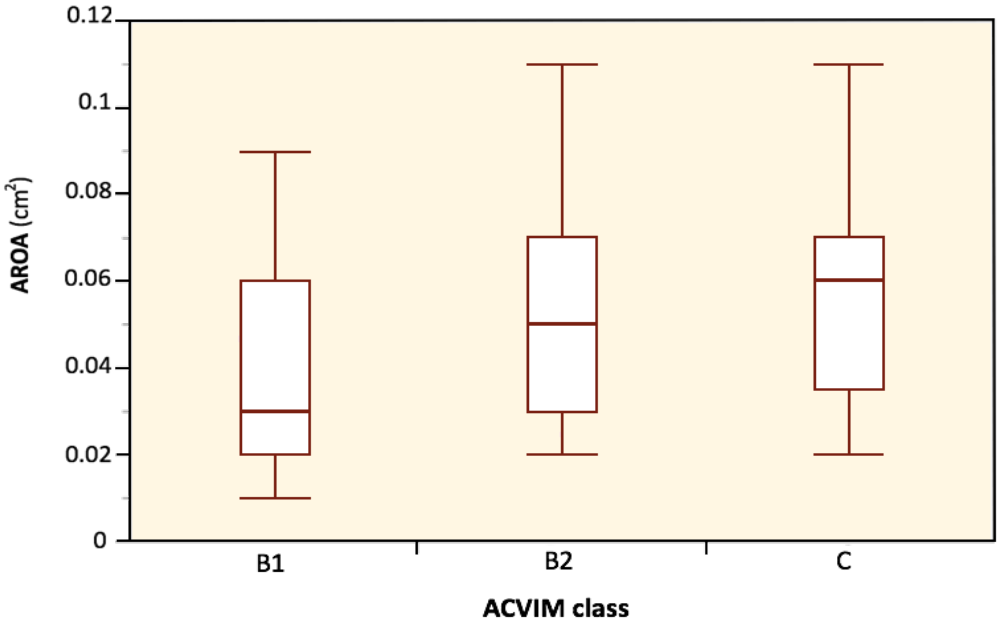

Fig. 7. Box-and-Whiskers plot of AROA in different ACVIM clinical stages.

AROA versus MRSS system

A statistically significant difference was found between AROA and different MRSS classes. AROA in patients with a severe MRSS was significantly greater (p=0.04) with a median of 0.06cm^2 (0.02-0.11), compared to the ones with mild MRSS with a median of 0.03cm^2 (0.01-0.09). Patients with a moderate MRSS had a median AROA of 0.04 (0.01-0.09) There was no significant difference in AROA between dogs in mild and moderate MRSS class (p=0.84) or moderate and severe MRSS (p=0.28)(fig. 8).

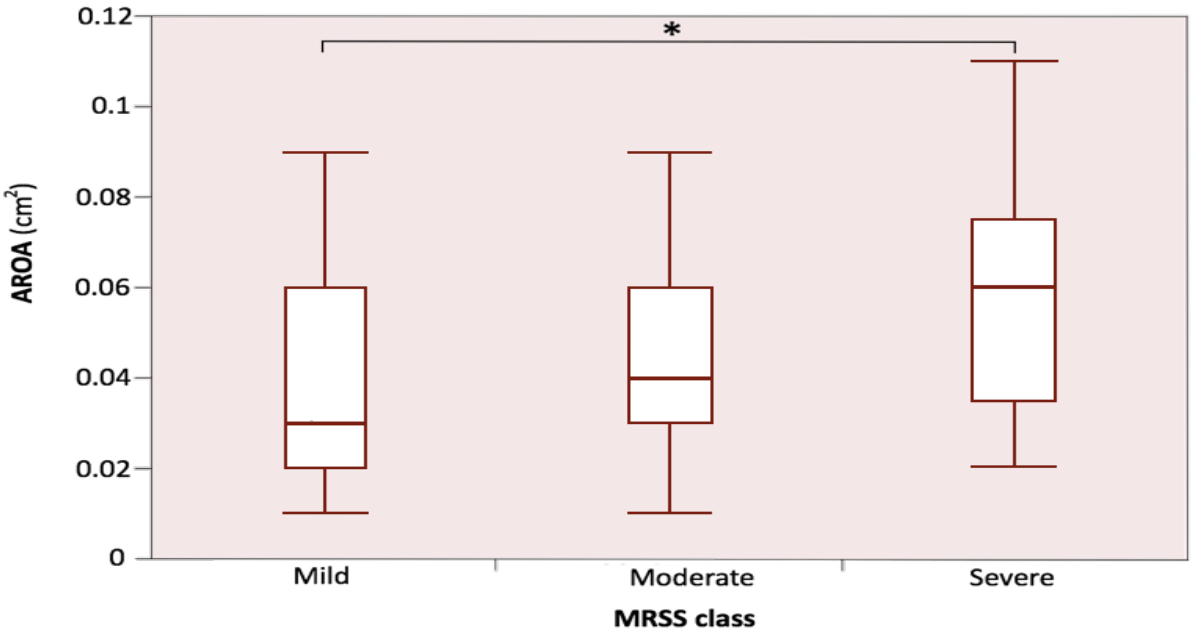

Fig. 8. Box-and-Whiskers plot of AROA in different MRSS classes. The asterisk indicates a significant difference.

Discussion

This study looked at the feasibility and repeatability of planimetry of AROA in dogs with MMVD. It also compared the size of AROA with two classification systems for MMVD severity. AROA has not been studied before in dogs. This study is the first exploratory research on this field.

Feasibility and repeatability

The first aim of the study was to determine feasibility and repeatability of AROA measurements using (RT3DE) in dogs affected by MMVD. Based on studies in human medicine, it is assumed RT3DE is an accurate way to measure AROA.[31, 35] The validity of measuring AROA with RT3DE was not tested, as AROA is not compared to the CMRI gold standard of AROA. This has not been done in veterinary medicine yet.

In 74% of the included RT3DE datasets it was feasible to obtain AROA. The RT3DE datasets of the 21 patients in which AROA could not be measured did not have a sufficiently high resolution or were not reliable due to stitching artifacts. The feasibility of measuring AROA in human studies is generally higher.[32, 34, 36] Lange (2002) found a feasibility of 95% when measured from the atrial side and 89% when measured from the ventricle.[36] This higher feasibility is likely due to the fact that those studies used transesophageal echocardiography, which increases resolution and therefore the quality of the datasets. A significant difference ($p=0.03$) was found in BW between dogs in which it was feasible to measure AROA, compared to those in which AROA could not be obtained. In patients in whom it was feasible to measure AROA, BW was lower. This could be explained by the fact the same matrix-array transducer is used in all patients, which provides a better resolution and/or less stitching artifacts in smaller patients with a less deep thorax.

Repeatability of measuring AROA was assessed by determining inter- and intra-operator %CV. The CV percentage is used to assess the repeatability and precision of the technique used to measure AROA, and to evaluate which component contributes most to the variability in the measurements.

The 26% inter-operator and 21% intra-operator CV shows that the measurement of AROA is not very precise and there is room for improvement. Variation in outcome was due to three components, namely operator variability (41.1%), variability in measurements (31.6%) and patient variability (27.3%). Repeatability data were obtained by repeated measurements of the same image loop of a dataset. This made it possible for the same operator or a different operator to obtain similar measurements acquired at a different time. Furthermore, visual quality was used to decide which image plane was used for measuring AROA. Standard image planes that included full visualization of the left atrium, left ventricle and mitral valve or image planes with a narrowed sector of the left heart, focused on the mitral valve, could be used. Images focusing on the mitral valve could have a better resolution and repeatability compared to the full image of the left side of the heart. Individual operator preferences on the level of zooming, adjusting brightness and contrast on the planes and the accuracy of planimetry of AROA can also cause variation in AROA. Variability in measure occurs due to differences in adjusting planes in each individual measurement. Standardizing

techniques of measuring AROA and provide more training to operators could improve CV. Individual patients do vary in BW, size, fat percentage and cooperation during echocardiography, which causes variation in resolution. Also, according to the size of the patient, the size of the heart and the mitral valve leaflets change, which also can be responsible for the variation.

AROA versus MRSS and ACVIM staging

The second goal of the study was to determine whether if AROA obtained using RT3DE relates to the severity of MMVD assessed by a MMVD echocardiographic score (MRSS) and the American College of Veterinary Medicine (ACVIM) clinical staging.

In humans, quantification of MR severity is used to assess MMVD severity and predicting risk. Also decisions regarding surgery are based on MR severity. The degree of MR, defined by the regurgitant volume (RVol) and EROA, is a major determinant of survival after diagnosis.[38, 46] Patients with an effective regurgitant orifice of at least 40 mm^2 have considerable rates of complications and death and represent a high-risk group, even though the presentation seems benign.[46] This results to the recommendation of mitral valve surgery for asymptomatic patients with severe MR.[47] Therefore it is important to differentiate nonsevere from severe MR.

Classification of MR severity in humans is based on several qualitative and quantitative parameters, assessed with 2DE. Together the parameters provide a grading system, resulting in a mild, moderate or severe grade of MR severity. Structural parameters as LA size, LV size and mitral leaflet anatomy are present. Color Doppler regurgitant jet area, mitral inflow, jet density, jet contour and pulmonary vein flow represent the Doppler parameters. Quantitative parameters as RVol and EROA, but also VCW and regurgitant fraction are included to help sub-classify the moderate regurgitation group into mild-to-moderate and moderate-to-severe grades.[37] The quantitative parameters can be accurate and reproducible in single centers, but those techniques seem to be modestly reliable in distinction of severe versus nonsevere MR.[48] Advances in 3DE, but also CMRI provided new tools for MR quantification, and all quantitative parameters for MR can be assessed using those more accurate techniques. They also make it possible to measure AROA and regurgitant volume (RV) derived from AROA. Although 3DE is preferable to 2DE, CMRI is the gold standard for all parameters.[35] RV derived from AROA resulted in better estimation of RV than RV derived from EROA using PISA, compared to RV obtained by CMR, resulting in an advantage of AROA over EROA.[31]

In veterinary medicine, the ACVIM classification system is widely used to make a distinction between 4 basic stages of heart disease and heart failure due to MMVD in dogs, using clinical parameters and cardiac remodeling.[6] Although this classification is useful in linking appropriate treatment recommendations to the clinical stages, it does not include parameters on MR severity and has limited prognostic value.

Although there is little conclusive evidence MR severity can be used as a prognostic factor in veterinary medicine, as it is in humans, there are various studies that support this hypothesis. Left atrial enlargement, LA:Ao ratio >1.4, is a predictor of survival on cardiac-related deaths,[1, 39] and because it reflects the degree of severity and chronic nature of mitral valve regurgitation, it is a usable parameter for MR severity. LAvol/BW

is even more accurate compared to LA:Ao ratio.[40] Also the structural parameter mitral leaflet anatomy, represented as severity of mitral prolapse and presence of a flail leaflet, predict increased mortality in dogs with MMVD.[19] Emax >1.2 is a valuable predictor of progression to a more advanced (AVCIM) stage of MMVD.[19, 39] All of those parameters on MR are thus also related to MMVD severity. There is no evidence of a prognostic value from the other Doppler parameters used in this study, regurgitant jet area, LVIDd and density of the continuous wave, but they are validated for scoring MR severity.[42, 37, 43] Also VCW and EROA could have been used to quantify regurgitation.[20, 49] Currently, no gold standard exists for quantification of MR severity. Therefore a combined assessment with 2DE and Doppler echocardiography is still the method of choice for noninvasive evaluation of MR severity.[37, 49]

The MRSS system, combining several established echocardiographic parameters, has been used in this study to provide additional descriptive data related to the severity of MMVD. The system has been used in veterinary medicine before,[40] but is also based on previously published recommendations for echocardiographic evaluation of the valvular regurgitation severity in humans.[37] It is, however, simplified so that selected parameters could be easily evaluated in every patient. It has been used to compare AROA with more than one parameter to obtain a better representation of MR severity and of MMVD severity than a single parameter, such as left atrial enlargement, would give alone.

No difference between AROA and different ACVIM stages was found. Because the ACVIM classification does not take MR severity into account, a dog in Stage B2 without clinical signs of CHF could have more severe MR compared to a dog in Stage C. Although related, MR severity and MMVD severity are not proportional to development of clinical signs. Therefore it was not expected AROA would be related to ACVIM staging.

A significant difference between AROA and MRSS classes mild and severe was found. AROA was smaller in patients with a mild MRSS compared to patients with a severe MRSS. It was expected that a bigger AROA would result in a more severe MR and therefore more severe MMVD. Although the results show that this expectation is true, we were not able to show a significant difference when comparing the other groups.

Limitations of the study

An important limitation of this study is that, although based on established echocardiographic parameters, the MRSS system has not been clinically validated in veterinary patients. It is used as a representation of MMVD severity, while there is no conclusive evidence it may function as such. Combining parameters in this system can give a better idea of MR severity, but it also includes shortcomings from every single parameter in one system. Moreover, measuring AROA with RT3DE is not validated yet in dogs by comparing it to AROA measurements with CMRI. Unfortunately this is not possible, since measuring AROA with CMRI will only be assessed when there is enough evidence for its clinical relevance.

Although there is no reference data available in literature for %CV in this type of research, it shows there is a need for improvement on method of measurement. Variability in patients cannot be improved, but homogeneous training for the different operators could improve inter- and intra-operator variability.

Another limitation is that we cannot rule out unconscious bias due to the fact this study is not blinded. Disease ACVIM stage and MRSS score could be blinded, but while measuring AROA it is not possible to avoid certain expectations on disease severity based on subjective echocardiographic findings.

Lastly, there is an imbalance in patient groups. There are only 9 patients in Stage C, against 22 and 29 in B1 and B2 respectively. This might have limited our ability to detect differences between those groups. The patients in the different MRSS classes are more equal distributed amongst the classes, but each individual class is relatively small.

Conclusion and clinical relevance

Aims of this study were to determine feasibility and repeatability of AROA measurements using RT3DE in dogs affected by MMVD and to determine whether if AROA obtained using RT3DE relates to the severity of MMVD assessed by a MRSS and ACVIM clinical staging.

This study shows that it is feasible to obtain AROA in dogs with MMVD using RT3DE, although methods need to be better standardized in order to lower inter- and intra-operator variability and improve repeatability. The technique is non-invasive as it is fast and requires the same actions taken by performing 2D echocardiography exams.

A single measurement of AROA on a patient with MMVD cannot be used to distinguish between mild, moderate or severe MR and does not give information on the severity of the disease. On the other hand, AROA is overall bigger in patients with severe MR than in patients with mild MR. This study shows that it is worthwhile to further investigate the possible diagnostic and prognostic use of AROA measurement in dogs with MMVD.

Further standardizing measurements and training of operators on measuring AROA would be the first next step in order to confirm repeatability. If variability can be lowered, it will be useful to compare AROA with a single parameter such as left atrial enlargement, which has the most robust evidence of being a prognostic factor in MMVD. Comparing AROA with other quantitative parameters such as VCW or EROA could also be considered, based on similar studies in human medicine. Lastly, a longitudinal prospective study would make it possible to follow-up MR severity and the changes in AROA over time, giving more information on the prognostic value of this measurement.

Acknowledgements

I would like to thank Dr. M. Borgarelli (Michele) and Dr. G. Menchiotti (Giulio) and the rest of the cardiology section from the Virginia-Maryland College of Veterinary Medicine from the Virginia Tech University in Blacksburg, Virginia for their help and guidance during my research program.

I would also like to thank dr. M. Dirven who was willing to be my mentor from afar, while I was doing the practical work of the research somewhere else. He provided me with really helpful feedback on my work.

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