PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ
ESCOLA DE CIÊNCIAS DA VIDA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

ANA PAULA SARRAFF LOPES

ASSESSMENT OF LEFT ATRIAL FUNCTION USING TISSUE MITRAL ANNULAR DISPLACEMENT IN HEALTHY AND CHRONIC MITRAL VALVE DISEASE DOGS

CURITIBA
2019

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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal, área de concentração em Clínica e Cirurgia Animal, da Escola de Ciências da Vida da Pontifícia Universidade Católica do Paraná, para obtenção do título de Doutor em Ciência Animal.

Orientador(a): Prof. Dr. Marconi Rodrigues de Farias

Coorientador(a): Prof. Dr. Marlos Gonçalves Sousa

CURITIBA

2019

Pontifícia Universidade Católica do Paraná
Programa de Pós-Graduação em Ciência Animal
Câmpus Curitiba

ATA Nº 0012 E PARECER FINAL DA DEFESA DE TESE DE DOUTORADO EM CIÊNCIA ANIMAL DA ALUNA ANA PAULA SARRAFF LOPES

Aos vinte e nove dias do mês de outubro do ano de dois mil e dezenove, às 8h30, realizou-se na sala de Tele Saúde, 1º andar do Bloco Verde, da Pontifícia Universidade Católica do Paraná, localizada no Campus de Curitiba, Rua Imaculada Conceição, nº 1155, Prado Velho – Curitiba – PR, a sessão pública de defesa de tese da doutoranda **Ana Paula Sarraff Lopes**, intitulada: **"ASSESSMENT OF LEFT ATRIAL FUNCTION USING TISSUE MITRAL ANNULAR DISPLACEMENT IN HEALTH AND CHRONIC MITRAL VALVE DISEASE DOGS"**. A doutoranda concluiu os créditos exigidos para obtenção do título de Doutor em Ciência Animal, segundo os registros constantes na secretaria do Programa. Os trabalhos foram conduzidos pelo Professor orientador e Presidente da banca, Dr. Marconi Rodrigues de Farias (PUCPR), auxiliado pelos Professores Doutores Pedro Vicente Michelotto Junior (PUCPR), Tilde Rodrigues Froes (UFPR), Rosângela de Oliveira Alves Carvalho (UFG) e Ruthnéa Aparecida Lázaro Muzzi (UFLA). Procedeu-se à exposição da tese, seguida de sua argüição pública e defesa. Encerrada a fase, os examinadores expediram o parecer final sobre a tese, que foi considerada Aprovada.

MEMBROS	ASSINATURA
Prof Dr Marconi Rodrigues de Farias - Orientador	
Prof Dr Pedro Vicente Michelotto Junior (PUCPR)	
Profa Dra Tilde Rodrigues Froes (UFPR)	
Profa Dra Rosângela de Oliveira Alves Carvalho (UFG)	
Profa Dra Ruthnéa Aparecida Lázaro Muzzi (UFLA)	

Proclamado o resultado, o Presidente da Banca Examinadora encerrou os trabalhos, e para que tudo conste, eu Caroline Nocera Bertton, confiro e assino a presente ata juntamente com os membros da Banca Examinadora.

Curitiba, 29 de outubro de 2019.

Caroline Nocera Bertton
Caroline Nocera Bertton
Secretária do Programa de Pós-Graduação em Ciência Animal

Renata Macedo
Profa. Dra. Renata Ernlund Freitas de Macedo
Coordenadora do Programa de Pós-Graduação em Ciência Animal

SUMÁRIO

"*Feliz é aquele que transfere o que sabe e aprende o que ensina*".

Cora Coralina

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À Deus, que sempre me guiou, colocando as pessoas certas no meu caminho e me dando forças para que eu chegasse ao final dessa etapa tão desejada. Agradeço por me ajudar a superar o cansaço, o desânimo e as dificuldades pelas quais passei para conseguir chegar até aqui!

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RESUMO GERAL

O átrio esquerdo (AE) tem importância na performance cardíaca por modular o enchimento do ventrículo esquerdo (VE). Uma forte correlação entre a disfunção do AE e a gravidade da cardiopatia tem sido observada em pacientes humanos com diferentes cardiopatias, fato também observado em cães, nos quais o tamanho e a função do AE têm grande correlação com prognóstico de cães com doença valvar mitral crônica (DVMC). A função do AE pode ser avaliada usando-se técnicas ecocardiográficas volumétricas, pelo *Doppler* espectral ou tecidual e pelo *speckle tracking* (ST). O deslocamento tecidual do ânulo mitral (*Tissue motion annular displacement*, TMAD) é uma técnica recentemente estudada na medicina, que utiliza o ST e avalia o movimento do anel valvar mitral durante o ciclo cardíaco, fornecendo informações sobre a função sistólica ventricular a partir do grau de deformação longitudinal do anel mitral. O objetivo do presente trabalho foi de analisar o deslocamento tecidual do ânulo mitral (TMAD) pelo *speckle tracking* para a avaliação da função longitudinal do átrio esquerdo em pacientes saudáveis e com DVMC em diferentes estágios, estabelecer valores de normalidade para essa técnica e comparar com outros métodos de avaliação da função atrial esquerda. O estudo foi prospectivo e transversal e incluiu 100 cães saudáveis e 95 com DVMC. Os pacientes foram submetidos ao exame clínico, eletrocardiográfico e ecocardiográfico convencional. A função do átrio esquerdo foi analisada pelos métodos biplanar área-comprimento, *strain* global pelo *speckle tracking*, deslocamento tecidual do ânulo mitral pelo *speckle tracking* e pela onda A' mitral pelo *doppler* tecidual. O TMAD global, indicador de função reservatório do átrio esquerdo, foi comparado às técnicas de fração de esvaziamento do AE e *strain* global do AE. O TMAD sistólico, indicador de função contrátil do átrio esquerdo, foi comparado às técnicas de fração de ejeção do AE e onda A' mitral. O TMAD global e sistólico (em mm) variou de acordo com o peso do paciente, sendo maiores conforme o peso aumentou, porém quando normalizados (por mm/m^2), foram menores nos animais mais pesados. Houve bom coeficiente de variação interobservador e intraobservador nos pacientes saudáveis para a técnica de TMAD. Nos pacientes com DVMC o TMAD global foi maior nos pacientes B1, C e D e o TMAD sistólico aumentou do controle para o B1 e diminuiu no D. O melhor método para diferenciar animais sem (B1) dos com remodelamento (B2, C e D) foi o TMAD global (mm/m^2) e para diferenciar os assintomáticos (B1 e B2) dos sintomáticos (C e D) foram o *strain* global, fração de esvaziamento e a fração de ejeção do AE. No grupo controle, o TMAD global e o sistólico (mm/m^2) apresentaram correlação forte e negativa como peso do paciente. Baseando-se nas curvas ROC (receiver operating curves), o melhor método para discriminar animais assintomáticos dos sintomáticos, bem como os sem ou com remodelamento cardíaco foi o TMAD global, com área abaixo da curva (AUC) de 0,715 e 0,756 respectivamente. O melhor ponto de corte do TMAD global para diferenciar animais assintomáticos dos sintomáticos foi de

21,07 mm/m^2, com sensibilidade de 61,1% e especificidade de 76,3% e para diferenciar animais sem ou com remodelamento cardíaco foi de 17,59 mm/m^2, com sensibilidade de 66% e especificidade de 81%. Os resultados desse estudo mostraram que o TMAD é um método simples e rápido para avaliar a função atrial esquerda e varia de acordo com o peso do animal. Nos cães com DVMC o TMAD permitiu avaliar a função global do AE e diferenciar os pacientes nas fases iniciais daquelas nas fases mais avançadas da doença, podendo ser um método diagnóstico complementar aos já existentes.

Palavras-chave: Ecocardiografia, Doença cardíaca, *Speckle tracking*, *Strain*, Insuficiência mitral.

ABSTRACT

The left atrium (LA) plays an essential role in cardiac performance by modulating left ventricular (LV) filling. A strong correlation between LA dysfunction and heart disease severity is observed in human patients. The same fact also occurs in dogs, where the size and function of the LA have a strong correlation with the prognosis of myxomatous mitral valve disease (MMVD). Left atrial function can be assessed using volumetric techniques, spectral or tissue Doppler, and speckle tracking (ST) echocardiography. Tissue motion annular displacement (TMAD) is a technique recently studied in medicine that uses the ST and evaluates mitral valve ring movement during the cardiac cycle, providing information on ventricular systolic function from the degree of longitudinal deformation of the mitral annulus. The present study aimed to assess the tissue mitral annular displacement (TMAD) by ST for the evaluation of left atrial longitudinal function in healthy patients with MMVD in different stages, to establish reference values for this technique and to compare with other echocardiographic techniques of left atrial function. This study was prospective, cross-sectional observational and included 100 healthy dogs and 95 MMVD dogs. All dogs underwent complete physical examination, electrocardiography and conventional echocardiography. Left atrial function was evaluated by biplanar area-length method, LA global strain by speckle tracking, TMAD by speckle tracking and by mitral A' by tissue doppler imaging. Global TMAD was compared to global LA strain and LA emptying fraction as measurements of reservoir function and systolic TMAD was compared to LA ejection fraction and mitral A' as measurements of contractile LA function. Global and systolic TMAD (in mm) varied according to the body weight of the animals, being greater in larger dogs, whereas, when normalized (by mm/m^2), TMAD decreased as weight increased. Intraobserver and interobserver repeatibility analyses showed good coefficients of variation in healthy dogs. In patients with DVMC left atrial global TMAD was greater in groups B2, C and D and left atrial systolic TMAD increased from control to B1 dogs and decreased in D dogs. The best method to differentiate animals without (B1) and with remodeling (B2, C and D) was LA global TMAD (mm/m^2), and asymptomatic (B1 and B2) and symptomatic animals (C and D), were LA strain, LA emptying fraction and LA ejection fraction. In the control group, the patients' weight showed strong negative correlation with global and systolic TMAD (mm/m^2). Based on ROC (receiver operating curves) analysis, the best discriminator between the asymptomatic from symptomatic and between animals with and without cardiac remodeling was LA global TMAD, with and area under the curve (AUC) of 0.715 and 0.756 respectively. The best cutting point of global TMAD to discriminate between the asymptomatic from symptomatic dogs was 21.07 mm/m^2 with sensibility of 61.1% and specificity of 76.3% and between animals with and without cardiac remodeling was 17.59 mm/m^2 with sensibility of 66.0% and specificity of 81.0%. The results of this study showed that TMAD is a fast and

straightforward method for assessing left atrial function and varies according to animal weight. In dogs with DVMC, TMAD allowed to evaluate the overall function of the LA and differentiate patients in the earlier stages from those in the more advanced stages of the disease and maybe a complementary diagnostic method to existing ones.

Keywords: Echocardiography, Cardiac disease, Speckle tracking, Strain, Mitral insufficiency.

CAPÍTULO 1

INTRODUÇÃO E CONTEXTUALIZAÇÃO

Esta tese é composta por:

- **Artigo 1**, intitulado: “Is the Tissue Mitral Annular Displacement A Reliable Technique to Assess Left Atrial Function? A Study in Healthy Dogs”, que será enviado para a revista: *Journal of Veterinary Cardiology.*
  -O trabalho foi apresentado, na forma oral, no 2019 ACVIM (*American College of Veterinary Internal Medicine) Forum,* que ocorreu de 6 a 8 de junho, em Phoenix, Arizona, EUA.
  -O resumo foi publicado no periódico *Journal of Veterinary Internal Medicine*, *vol 33, issue 4, july/august*, 2019.

- **Artigo 2**, intitulado: “Assessment of Left Atrial Function Using Tissue Mitral Annular Displacement in Dogs with Chronic Mitral Valve Disease”, que será enviado para a revista: *Journal of Veterinary Cardiology.*

CAPÍTULO 2

Artigo científico a ser submetido ao periódico: Journal of Veterinary Cardiology

Is the tissue mitral annular displacement a reliable technique to assess left atrial function? A study in healthy dogs ☆

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Abstract

Introduction/objectives: The methods to evaluate volume and deformation of the left atrium (LA) are time-consuming and difficult to use in clinical practice. The aim of this study was to demonstrate that the displacement of the mitral annulus,

obtained by speckle tracking, can be an alternative straightforward method to study left atrial function.

Animals: One hundred client-owned healthy dogs.

Materials and Methods: Prospective cross-sectional study. The dogs underwent physical examination, electrocardiography and standard echocardiography. Left atrial function was assessed by tissue mitral annular displacement (TMAD), which was compared to LA strain and biplane area-length method. Left atrial reservoir function was evaluated by LA global TMAD, global LA strain and LA emptying fraction while LA systolic TMAD and LA ejection fraction were used to assess LA systolic function.

Results: The normalized global and systolic TMAD decreased as body weight increased ($p < 0.0001$). Global TMAD showed a moderate positive correlation with LA emptying fraction ($R = 0.552$; $p < 0.0001$) and a strong positive correlation with global LA strain ($R = 0.750$; $p < 0.0001$). Systolic TMAD showed a moderate correlation with LA ejection fraction ($R = 0.514$; $p < 0.0001$). Coefficients of variation documented for the intraobserver and interobserver analyses were good for global (0.39%; 3.58%) and systolic TMAD (0.83%; 10.93%).

Conclusions: TMAD may be used as a simple yet reliable surrogate for left atrial function. Our study documented the role played by body weight on LA TMAD, which makes it necessary to normalize TMAD, but found no influence of age or heart rate.

KEY WORDS: Echocardiography; Speckle tracking; Left atrial; Strain imaging

Abbreviation Table

AP4 apical 4-chamber

AP2 apical 2-chamber

CV coefficient of variation

E_{mitral} peak velocity of early diastolic transmitral flow (m/s)

E_{mitral}:A_{mitral} Ratio of E to A

E:IVRT ratio of E to IVRT

IVRT isovolumetric relaxation time (ms)

LA left atrium

LV left ventricle

TMAD tissue mitral annular displacement

Introduction

Left atrium (LA) plays an important role in cardiac performance by modulating left ventricular filling, by its reservoir function during ventricular systole (atrial diastole), conduction during the onset of ventricular diastole, and pump during the end of ventricular diastole (atrial systole), contributing 15 to 30% of the left ventricle (LV) filling [1].

Left atrial function has been the subject of study for a long time. Atrial function can be assessed using volumetric, spectral or tissue Doppler imaging, and speckle tracking techniques. However, its peculiar shape makes the volumetric methods employed for the left ventricle difficult to use [2]. The global change in atrial volume during the cardiac cycle is simpler than that of the left ventricle, having mainly a longitudinal component, mostly due to the motion of the mitral annulus. To characterize the left atrial function using a volumetric approach, the Simpson or area-length methods can be used, however they are

laborious and time consuming to use in real clinical practice, because three volumes are needed (the maximal atrial volume, the minimal volume and the pre-A volume), corresponding to the reservoir, conduit and contractile function [3,4,5]. The most practical method, proven to be accurate and fast, allowing the evaluation of all atrial volumes in a very rapid way is the use of tridimensional imaging, that shows concordance with MRI or CT [6,7]. Nonetheless, for three-dimensional techniques it is important to have sufficient image quality to have reliable and reproducible results [6].

Strain is the fractional change in the length of a myocardial segment and represents the myocardial deformity. During the reservoir phase, the left atrium is stretched as it fills with blood from the pulmonary veins. At this stage the longitudinal strain increases, reaching a positive peak at the end of the filling of the left atrium. This phase is also influenced by the movement of the mitral annulus towards the cardiac apex resulting from contraction of the left ventricle. Therefore, the LA strain during the reservoir phase reflects not only the compliance of the LA, but also the contraction of the LV. During the conduction phase, the LA empties and shortens rapidly after opening of the mitral and the longitudinal strain decreases until a plateau, corresponding to the diastasis of the LA. The LA wall subsequently shortens in the longitudinal direction during the contraction phase of the LA, which leads to a decrease in the strain curve, reflecting LA pump function. The LA strain indices are calculated by the mean regional strains observed in the segments evaluated (global strain) [4].

Motion of the mitral annulus is associated with left ventricular and atrial function, which has led many investigators to focus on assessment of mitral annular physiology [8]. Previous studies have used M-mode echocardiography

[9] and 2-dimensional echocardiography to investigate the dynamics of the mitral annulus [10-12]. Tissue Doppler and 3-dimensional echocardiography have also been used to evaluate mitral annulus motion [13,14]. These studies have demonstrated that analysis of mitral annular movement has potential in the assessment of global and regional left ventricular systolic and diastolic function and for diagnosis of mitral valvular disease and left ventricular disorders.

Tissue Motion Annular Displacement (TMAD) is a quite recent technique, which uses speckle tracking. It is independent of the Doppler angle, and evaluates the movement of the mitral valve annulus during the cardiac cycle, bringing information of systolic function from the degree of longitudinal deformation of the mitral annulus in the apical two (AP2) and apical four chambers (AP4) images. The method consists on evaluating the displacement of the mitral annulus towards the left ventricular apex [15]. Studies have shown that it is a straightforward technique, independent of high definition images and extensive experience of the operator [16]. Research in humans have shown that tissue displacement of the mitral annulus using speckle tracking is a marker for left ventricular function, comparable to magnetic resonance imaging [17]. In a study in people, either healthy or with heart diseases, LA function was evaluated using TMAD, three-dimensional echocardiography, tissue Doppler and strain. Interestingly, TMAD was shown to be a simple, fast, independent of image quality and reliable method to evaluate left atrial function [18].

With that in mind, the aims of this study were: (1) to evaluate the longitudinal left atrial function in healthy dogs by tissue mitral annular displacement; (2) Establish normal values for this technique in healthy dogs; and

(3) Compare whether TMAD and other methods of left atrial function obtained by echocardiography.

Materials and methods

Animals

This prospective cross-sectional observational recruited 100 client-owned healthy dogs of different breeds, ages and sexes between March 2017 and October 2018 from a Veterinary Teaching Hospital and a Private Referral Hospital. All dogs were considered healthy based on medical history, complete physical examination, electrocardiography[a] and echocardiography. Exclusion criteria consisted of any clinical signs of systemic or cardiac disease. The animals were separated into quartiles according to the body weight, and each group included 25 dogs.

The study was approved by the Institutional Ethics Committee on Animal Research (protocol 01084/2017).

Echocardiography

Each animal underwent a complete standard echocardiography[b]. Dogs were examined without sedation and were only gently restrained in left and right lateral recumbency. The exams were performed following the recommendations of the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine. M-mode echocardiography measurements (left ventricular end-diastolic and end-systolic diameters, left ventricular free-wall and interventricular septal thickness in diastole and systole and fractional shortening) were obtained from the right parasternal short axis view. The left atrial-to-aorta ratio was obtained from the left parasternal short axis

view. From the left AP4 view, pulsed wave Doppler was used to record peak early (E) and late (A) diastolic mitral inflow velocities and the E:A ratio and tissue Doppler was used to measure the early diastolic (E') velocity, late diastolic velocity (A') and E':A' ratio of the septal mitral annulus. From the left apical 5-chamber view, pulsed wave Doppler between transmitral and aortic flow was used to record isovolumic relaxation time [19]. Apical 4 and 2-chamber videos were recorded for offline analyses.

Biplane Area-Length method

Using the left AP4 and AP2 views, LA area was calculated in three different points over the cardiac cycle: just before the mitral valve opening (maximum left atrium area); at the onset of the P wave on the electrocardiogram (preatria contraction area, LA P Volume), before left atrium contraction and at mitral valve closure (minimal LA area). LA area was measured with planimetry by tracing the endocardium border, excluding the confluence of the pulmonary veins and LA appendage. A straight line connecting both the hinge points of the mitral valve leaflets was taken as the border of the LV. LA length was measured from the midline of the plane of the mitral annulus to the opposite aspect of the LA. Three consecutive cardiac cycles were measured and averaged. All LA volume measurements were calculated using the biplane area-length method [20].

LA volume = (0.85 x A1 x A2) / L

where A1 and A2 represent the planimetric LA area acquired from left AP2 and AP4 views and L is the length.

LA reservoir function was assessed using the equation:

LA emptying fraction (%) = 100 x (Maximal LA volume – Minimal LA volume) / Minimal LA volume)

LA pump function was assessed using the equation:

LA ejection fraction (%) = 100 x (LA P volume - Minimal LA volume) / LA P volume).

LA emptying fraction was used as a surrogate for global LA function while LA ejection fraction was a surrogate for contractile LA function.

Longitudinal left atrial strain

Left atrial strain was obtained using two-dimensional speckle tracking. Using AP2 and AP4 images, the endocardial border of the left atrium was manually defined and epicardial surface tracing was automatically generated by the software[c], creating a region of interest that was manually adjusted to cover the full thickness of the myocardium. After processing, the LA myocardium was automatically divided into six segments, and time-longitudinal strain curves were produced for each atrial segment throughout the cardiac cycle. Also, an averaged strain of all six segmental values (global strain) was documented. The global LA strain measurement was then defined as the average of results obtained from both AP2 and AP4 views. The longitudinal global strain was used as a surrogate for the reservoir function of the LA [4] (Fig. 1).

Tissue mitral annular displacement

Mitral annular displacement was calculated offline using AP4 and AP2 images. Two points were selected at the insertions of the mitral valve leaflets and the septal and lateral parts of the AP4 view, as well as the anterior and inferior parts of the AP2 view. A third point was placed at the dorsal wall of the LA. After

setting these points, tracking was performed automatically by the equipment software.

Time-displacement curves showing the displacement, in millimeters, of a medial virtual point between the two annular points and the dorsal wall of the LA. The maximum negative and positive displacements were recorded, as well as the position during diastasis. The baseline was considered at the onset of the QRS complex. The global displacement (global TMAD) was calculated as the average of the four annular points (from AP2 and AP4 views) between the maximal negative and positive positions. In contrast, the systolic displacement (systolic TMAD) was calculated as the average of the four annular points (from AP2 and AP4 views) from diastasis and the maximal positive position [18] (Fig. 1).

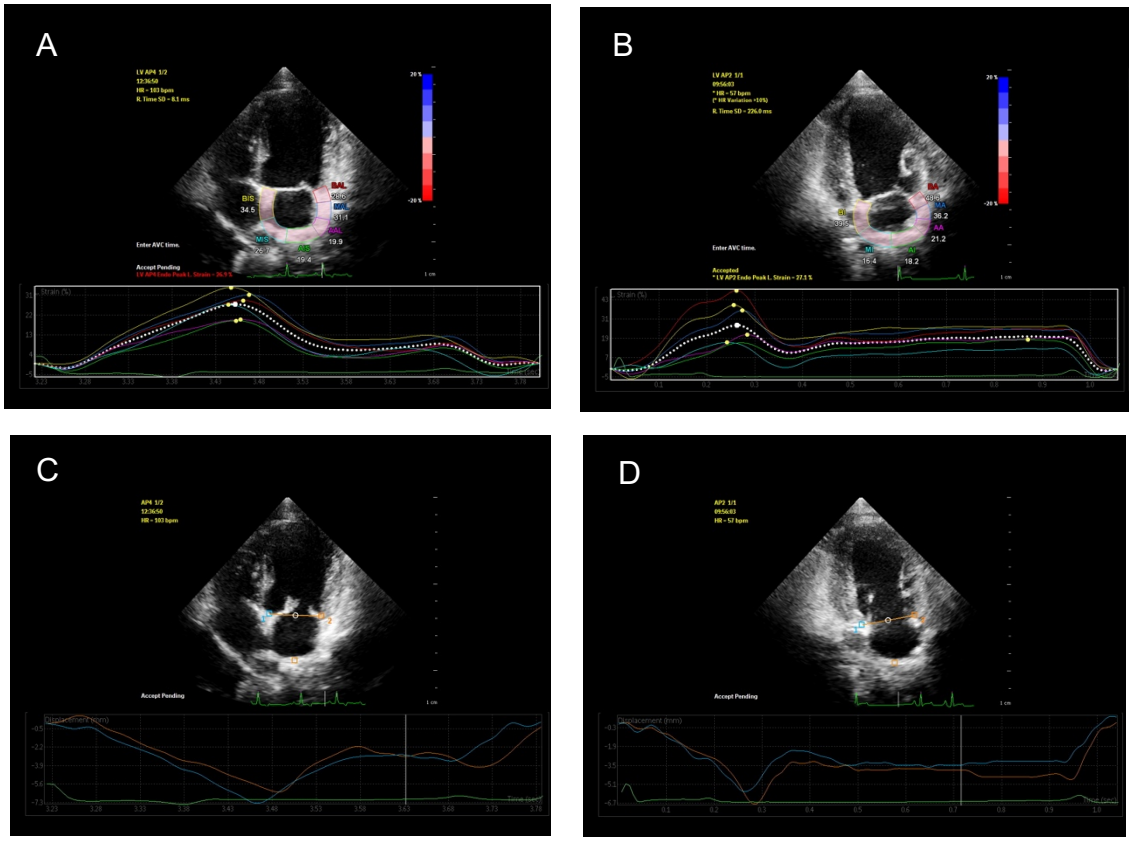


Figure 1 Left atrial strain in 4-chamber (A) and 2-chamber (B) images. The mean of both results represents the global left atrium strain. For tissue mitral annular displacement we selected two points at the insertion of the mitral valve leaflets and the dorsal wall of the left atrium in both 4-chamber (C) and 2-chamber (D). TMAD is automatically determined by the software.

Intraobserver and interobserver variability

For the repeatability study, 20 animals were reassessed by the same observer with a minimum interval time of 30 days from the first evaluation to calculate the intraobserver variability. The same animals were examined by another observer (M.W.), who was blinded to the results of the first investigation, to measure interobserver variability.

Statistical analysis

Data was examined for normality using the Shapiro-Wilk test. For normally distributed variables we used ANOVA followed by Tukey's test and the results are presented as mean and standard deviation. For non-normal variables we used Kruskal-Wallis followed by Dunn's test and the results are presented as median and interquartile range. Correlations were investigated using Pearson's or Spearman's method. Coefficients of variation were calculated to assess intraobserver and interobserver measurements. All statistical analyses were performed with IBM statistics SPSS[d], and statistical significance was set at p value < 0.05 for all analyses.

Results

A total of 100 dogs were recruited for this study. Thirty-three dogs were male (33%), and 67 (67%) female. The age varied between one and 13 years (mean 3.5; median 2.5) and the BW ranged from 1.2 to 61.6 kg (mean 15.1; median 11.098). The population included small to large-sized dogs. Among the various breeds, Beagle was the most frequently presented (n = 17); followed by German Shephard (n = 7); Yorkshire Terrier (n = 6); Shih-Tzu (n = 4); French Bulldog, Lhasa Apso, Mallinois Shephard, Miniature Schnauzer, Poodle, Pug and

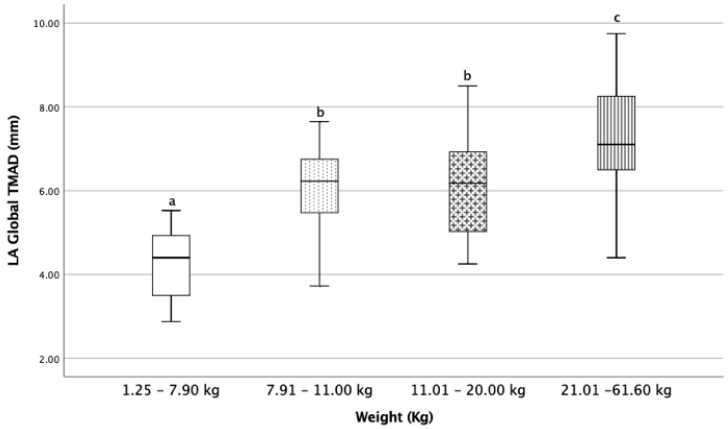
Rottweiler (n = 3); Border Collie, Golden Retriever, Labrador Retriever, Miniature Pinscher and Whippet (n = 2); American Pit Bull Terrier, Basset Hound, Bearded Collie, Cocker Spaniel, Collie, Doberman Pinscher, Great Dane, Miniature Dachshund, Saint Bernard and Shar-Pei (n = 1). Also, a total of 25 mixed-breed dogs were enrolled in this investigation.

Global and systolic TMAD (in mm) varied according to the body weight of the animals, being greater in larger dogs, whereas, global and systolic the normalized TMAD (either by mm/m^2, mm/kg or mm/LAL) decreased as weight increased (Table 1, Fig. 2).

Table 1 - Comparison of age, LA TMAD, LA Strain, LA Emptying and LA Ejection Fraction and p value in accordance with body weight.

	Body Weight (quartiles)				
	1.25-7.9 kg	7.9-11.00 kg	11.01-20.00 kg	20.01-60 kg	p
(n)	25	25	25	25	
Age (years)	2.0 (0.9-5)	2.5 (2.0-5.0)	2.0 (2.0-3.5)	3.0 (2.0-5.5)	0.4927
LA TMAD (mm)					
Global AP 2,4	4.24 (0.79)^{A}	6.05 (1.06)^{B}	6.06 (1.20)^{B}	7.16 (1.36)^{C}	<0.0001
Systolic AP 2,4	2.55 (2.41-3.27)^{A}	2.97 (2.42-3.86)^{AB}	3.40 (2.65-4.10)^{BC}	3.75 (3.20-4.58)^{C}	0.0004
LA TMAD (mm/m^2)					
Global AP 2,4	18.48 (11.43-21.30)^{A}	13.51 (12.11-15.07)^{A}	10.36 (8.69-11.85)^{B}	7.49 (5.93-8.41)^{C}	<0.0001
Systolic AP 2,4	10.47 (7.59-13.66)^{A}	6.70 (5.31-8.75)^{B}	5.93 (4.35-6.78)^{B}	3.76 (2.82-4.99)^{C}	<0.0001
LA TMAD (mm/kg)					
Global AP 2,4	1.15 (0.64-1.57)^{A}	0.64 (0.56-0.71)^{A}	0.42 (0.36-0.48)^{B}	0.24 (0.17-0.28)^{C}	<0.0001
Systolic AP 2,4	0.63 (0.42-0.95)^{A}	0.33 (0.25-0.40)^{B}	0.25 (0.18-0.29)^{B}	0.12 (0.08-0.17)^{C}	<0.0001
LA TMAD (mm/LAL)					
Global AP 2,4	0.29 (0.21-0.32)^{AB}	0.28 (0.25-0.30)^{A}	0.24 (0.23-0.26)^{B}	0.20 (0.18-0.25)^{C}	<0.0001
Systolic AP 2,4	0.17 (0.13-0.20)^{A}	0.13 (0.10-0.18)^{AB}	0.13 (0.12-0.17)^{AB}	0.11 (0.08-0.14)^{B}	0.0037
LA Strain (%)	34.47 (7.12)^{A}	33.76 (6.19)^{A}	29.08 (6.54)^{B}	21.40 (4.65)^{C}	<0.0001
LA Empt Fr (%)	63.43 (6.40)^{A}	62.27 (6.61)^{A}	60.16 (6.69)^{A}	54.97 (7.10)^{B}	<0.0001
LA Ej Fr (%)	42.14 (36.15-49.98)^{A}	38.13 (29.72-44.35)^{AB}	38.38 (33.23-40.77)^{B}	35.39 (26.64-44.73)^{B}	0.0323

(n), number of animals in quartile; LA, left atrium; TMAD, tissue motion annular displacement; AP4, apical 4-chamber; AP2, apical 2-chamber; AP 2,4, average of 2 and 4 chamber; kg, kilograms; LAL, left atrium length; Emp Fr, Emptying fraction; Ej Fr, Ejection fraction. Data are expressed as means (standard deviation) or medians (interquartile range) depending on the parameter attaining a normal distribution or not on the Shapiro-Wilk normality test. Values with different superscripted letters indicate statistically significant differences between groups.



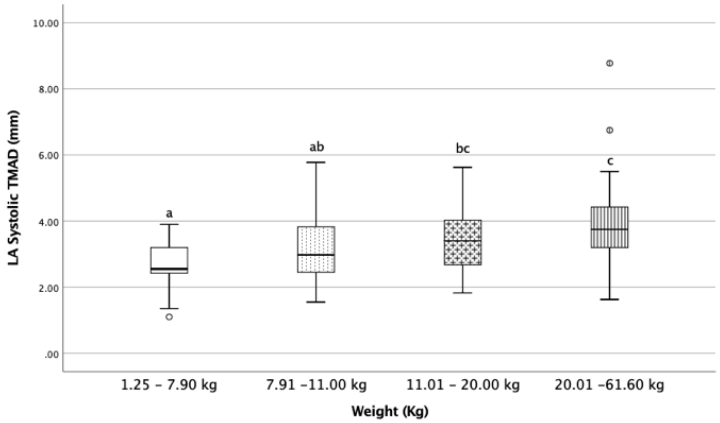


Figure 2 Box and whisker plots of left atrial global and systolic tissue mitral annular displacement (TMAD; mm) measurements obtained in dogs subdivided according to the body weight.

While LA strain varied according to the weight, being lower in dogs weighting more than 11 kg ($p < 0.0001$), LA emptying fraction was lower in dogs weighting more than 20 kg ($p < 0.0001$). A significantly higher LA ejection fraction was observed in dogs weighting less than 8 kg ($p = 0.0323$) whereas no difference was observed between the larger ones ($p > 0.05$).

Regarding sex, there was no difference ($p = 0.437$) in global TMAD between males and females, but a significantly ($p = 0.022$) higher systolic TMAD was obtained in females. Global and systolic TMAD showed correlation with neither age nor heart rate ($p > 0.05$).

Global TMAD was compared to both LA emptying fraction and global LA strain as measurements of global LA function. Global TMAD (mm/m^2, mm/kg, mm/LAL) showed a moderate positive correlation with LA emptying fraction ($R = 0.552$; $p < 0.0001$, $R = 0.547$; $p < 0.0001$, $R = 0.394$; $p < 0.0001$) and a strong positive correlation with the global LA strain ($R = 0.750$; $p < 0.0001$,$R = 0.747$; $p < 0.0001$, $R = 0.684$; $p < 0.0001$). A moderate positive correlation was obtained between global LA strain and LA emptying fraction ($R = 0.511$; $p < 0.0001$). Isovolumic relaxation time was the only diastolic parameter that correlated with

global TMAD (mm/m^2, mm/kg, and mm/LAL) (R = -0.358; $p < 0.0001$, R = -0.413; $p < 0.0001$, R = -0.261; $p = 0.008$) (Table 2).

Table 2 Correlation between either global BSA-indexed LA TMAD (mm/m2), global TMAD (mm/kg) or global TMAD (mm/LAL) with data obtained by Doppler, volumetric and deformation echocardiography modalities.

	LA Global TMAD (mm/m^2)			**LA Global TMAD (mm/kg)**			**LA Global TMAD (mm/LAL)**		
	R	95% CI	*p*	R	95% C I	*p*	R	95% CI	*p*
LA Emptying Fraction	0.552	0.413 to 0.672	**< 0.0001**	0.547	0.397 to 0.664	**< 0.0001**	0.394	0.230 to 0.536	**< 0.0001**
LA Ejection Fraction	0.363	0.177 to 0.533	**< 0.0001**	0.355	0.158 to 0.524	**< 0.0001**	0.332	0.166 to 0.485	**0.001**
LA Strain	0.750	0.636 to 0.823	**< 0.0001**	0.747	0.632 to 0.821	**< 0.0001**	0.684	0.592 to 0.769	**< 0.0001**
E_{mitral}	- 0.042	- 0.002 to 0.108	0.680	- 0.106	- 0.392 to 0.118	0.291	0.048	- 0.145 to 0.252	0.633
E_{mitral}:A_{mitral}	0.072	- 0.134 to 0.265	0.473	0.091	- 0.118 to 0.289	0.368	0.053	- 0.142 to 0.257	0.599
E_{mitral}:E'	0.032	- 0.172 to 0.237	0.747	0.064	- 0.148 to 0.261	0.523	0.044	- 0.154 to 0.237	0.660
IVRT	- 0.358	- 0.520 to - 0.163	**< 0.0001**	- 0.413	- 0.574 to – 0.235	**< 0.0001**	- 0.261	- 0.440 to – 0.067	**0.008**
E:IVRT	0.195	-0.020 to 0.382	0.051	0.183	- 0.013 to 0.385	0.067	0.170	- 0.047 to 0.357	0.090

TMAD, tissue motion annular displacement; BSA, body surface area; LA, left atrium; LAL, left atrium length; CI, confidence interval; E, peak velocity of early diastolic transmitral flow; A, peak velocity of late transmitral flow; E', peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler, IVRT, isovolumetric relaxation time; E:IVRT, ratio of E to IRVT.

In general, systolic TMAD was negatively correlated with E_{mitral}, E_{mitral}:A_{mitral} and IVRT. Regarding diastolic parameters, a weak negative correlation existed between systolic TMAD (mm/m^2) and both E_{mitral} (R = -0.244; $p = 0.014$) and E_{mitral}:A_{mitral} (R = -0.216; $p = 0.030$), while a moderate negative correlation was found with IVRT (R = -0.313; $p = 0.001$). Systolic TMAD (mm/kg) also showed a weak negative correlation with E_{mitral} (R = -0.258; $p = 0.009$) and a moderate negative correlation with IVRT (R = -0.380; $p < 0.0001$). Systolic TMAD (mm/LAL) showed a weak negative correlation with E_{mitral} (R = -0.271; $p = 0.006$) and E_{mitral}:A_{mitral} (R = -0.340; $p = 0.001$) (Table 3).

Table 3 Correlation between either systolic BSA-indexed LA TMAD (mm/m2), systolic TMAD (mm/kg) or systolic TMAD (mm/LAL) with data obtained by Doppler, volumetric and deformation echocardiography modalities.

Echocardiographic Parameters	**LA Systolic TMAD (mm/m^2)**			**LA Systolic TMAD (mm/kg)**			**LA Systolic TMAD (mm/LAL)**		
	R	95% CI	*p*	R	95% CI	*p*	R	95% CI	*p*
LA Emptying Fraction	0.407	0.229 to 0.564	**< 0.0001**	0.441	0.253 to 0.580	**< 0.0001**	0.159	- 0.048 to 0.360	0.112
LA Ejection Fraction	0.514	0.331 to 0.663	**< 0.0001**	0.480	0.289 to 0.621	**< 0.0001**	0.482	0.291 to 0.636	**< 0.0001**
LA Strain	0.515	0.351 to 0.650	**< 0.0001**	0.595	0.443 to 0.715	**< 0.0001**	0.300	0.094 to 0.479	**0.032**
E_{mitral}	- 0.244	- 0.434 to – 0.042	**0.014**	- 0.258	- 0.453 to – 0.054	**0.009**	- 0.271	- 0.457 to – 0.076	**0.006**
E_{mitral}:A_{mitral}	- 0.216	- 0.391 to – 0.007	**0.030**	- 0.125	- 0.311 to 0.064	0.214	- 0.340	- 0.508 to – 0.143	**0.001**
E_{mitral}:E'	- 0.017	- 0.208 to 0.199	0.867	0,034	- 0.159 to 0.231	0.738	- 0.013	- 0.199 to 0.167	0.894
IVRT	- 0.313	- 0.494 to – 0.117	**0.001**	- 0.380	- 0.554 to – 0.197	**< 0.0001**	- 0.141	- 0.348 to 0.065	0.159
E:IVRT	0.034	- 0.184 to 0.246	0.733	0.066	- 0.132 to 0.271	0.515	- 0.089	- 0.290 to 0.111	0.374

TMAD, tissue motion annular displacement; BSA, body surface area; LA, left atrium; LAL, left atrium length; CI, confidence interval; E, peak velocity of early diastolic transmitral flow; A, peak velocity of late transmitral flow; E', peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler, IVRT, isovolumetric relaxation time; E:IVRT, ratio of E to IRVT.

Intraobserver and interobserver repeatability analyses showed good coefficients of variation, (CV) [21]. While the intraobserver CV for global and systolic TMAD were 0.39% and 0.83%, respectively, interobserver CV were 3.58% and 10.93%, respectively.

Discussion

This investigation evaluated LA global and systolic function, comparing TMAD with LA strain and several standard echocardiographic parameters. Important to say, at least to the best of the author's knowledge, this is the first study that evaluated mitral annular displacement as a surrogate for left atrial function in dogs. Our results showed that TMAD is a feasible, reliable and simple method to assess left atrial function in a population of normal dogs.

Many studies have evaluated the deformation of the left atrium as a surrogate of left atrial function, and its prognostic importance [22-24]. Nevertheless, most methods are still highly dependent on image quality [4]. Moreover, the far-field location of the atrium, reduced signal-to-noise ratio, the thin atrial wall, and the presence of the appendage and pulmonary veins are challenges yet to overcome when applying deformation analysis of the left atrium [25].

Speckle tracking echocardiography has been studied recently in veterinary medicine to assess longitudinal atrial function in healthy dogs [24,26-28] as well as in dogs with mitral valve disease [23,28,29], but none of these studies used TMAD to evaluate left atrial function. Tissue motion annular displacement is a tracking technique, has no angle dependency and does not need a high-quality image. It is also much simpler to accomplish than two-dimensional tracking of the

atrial wall, because there is no need to trace the endocardial borders, but only a good visualization of the hyperechoic annulus, and the thickness of the LA wall also poses no problem [18]. Recent studies in man and dogs using TMAD by speckle tracking to evaluate left ventricular function have shown good correlation with ejection fraction, even when the quality of the image was not excellent [30,31]. Another paper that described TMAD to evaluate left atrial function in people showed promising results [18]. In this study, global and systolic TMAD (in mm) were higher in heavier dogs, owing to the larger heart size causing greater absolute displacement from mitral annulus toward the dorsal wall of the left atrium. In human cardiology literature, the adult heart size is relatively constant, which contrasts with the large variations of weight in adult dogs. When global and systolic TMAD were normalized (mm/m^2, mm/kg or mm/LAL), higher values were seen in smaller dogs. Similar results were previously found when evaluating mitral annulus motion [9]. In another study that evaluated left atrial ejection fraction, a negative correlation with body weight was identified in control dogs [32]. These findings highlight the need to take body weight into account when interpreting deformation indices.

Global TMAD did not change in relation to sex, whereas the systolic TMAD was higher in females. Although intriguing at first, the explanation for this finding relies on the sex distribution among the four groups. While 40.3% of the females belonged to the group of lower weight (1.2 to 7.9 kg), which present a higher indexed TMAD, 40.0% of the males were included in the group of greater weight (20.01 to 60 kg), which present smaller indexed TMAD. In people, left atrial morphology and function was demonstrated to be similar between sexes [33].

In people, there was a strong correlation between global strain and TMAD, but LA volume was measured through 3D echocardiography [18]. This difference might be ascribed to underestimation of LA volume when assessed two-dimensionally as compared to 3D echocardiography and other imaging modalities [2,34,35].

The negative correlation between TMAD (mm/m^2) and IVRT may be explained by the heavier dogs presenting a longer IVRT and a smaller TMAD. Conversely, the negative correlation between systolic TMAD (mm/m^2) and both E_{mitral} and E_{mitral}:A_{mitral} is likely attributable to dogs with smaller the E_{mitral} (lower passive filling) having greater atrial contraction (active filling), which translates into a higher systolic TMAD. Also, the reduction in E_{mitral} is accompanied by an increase in A wave, therefore reducing the E_{mitral}:A_{mitral} ratio.

The CV of TMAD (mm) were lower for intraobserver evaluation when compared to interobserver evaluation. We speculate that the CV was higher for systolic TMAD in the interobserver evaluation because of the greater difficulty in determining the exact diastasis point needed for its measurement. The high TMAD repeatability supports its reliability as a diagnostic tool, as well as its angle independency, which is an advantage over Doppler-derived techniques [1,25,36]. Also, TMAD is known to be less dependent on image quality as compared to strain analysis. TMAD only requires good visualization of the mitral annulus [30].

Some limitations of this study must be acknowledged. The equipment's speckle tracking software is designed for assessment of the left ventricle, not for the left atrium. However, several other studies have used this same adaptation to assess left atrial function [18]. Worth to mention that speckle tracking software does not use a uniform methodology. Different vendors use different techniques,

in a way that comparisons between results obtained in different machines might be inappropriate. Also, animals with higher heart rates were more difficult to determine the exact point for measuring systolic TMAD.

Conclusion

This study demonstrated that Tissue Mitral Annular Displacement may be used as a simple and reliable surrogate for left atrial function. TMAD varies according to the dog's size, which makes it necessary to consider the weight of the animal when interpreting deformation indices. Further studies of these novel parameters are warranted to clarify how they perform in dogs with cardiac diseases.

Conflicts of Interest Statement

The authors do not have any conflict of interest to disclosure

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Footnotes

☆This study was presented as an oral abstract at the 2019 ACVIM Forum in Phoenix.

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[a] TEB – Tecnologia Eletrônica Brasileira, São Paulo, Brazil.

[b] Philips Affinity 50 ultrasound system equipped with 2-4, 3-8 and 4-12MHz phased array transducers.

[c] QLAB Software – Auto Cardiac Motion Quantification (aCMQ)

[d] IBM statistics SPSS® 25.0

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CAPÍTULO 3

Artigo científico a ser submetido ao periódico: Journal of Veterinary Cardiology

Assessment of Left Atrial Function Using Tissue Mitral Annular Displacement in Dogs with Chronic Mitral Valve Disease

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Abstract

Introduction/objectives:

A strong correlation between left atrial disfunction and heart disease severity and prognosis are observed in dogs with myxomatous mitral valve disease (MMVD). The methods used to evaluate left atrial function are highly dependent on

technical factors and difficult and time consuming to use in clinical practice. The aims of this study were to study longitudinal left atrial function in dogs with chronic mitral valve disease by tissue mitral annular displacement (TMAD), correlates TMAD with other echocardiographic methods of left atrial function and compare sensibility and specificity of this new method to differentiate MMVD stages.

Animals: One hundred fifty-five client-owned dogs, 95 dogs with myxomatous mitral valve disease (MMVD) and 60 healthy control dogs.

Materials and Methods: Prospective cross-sectional study. The animals were divided into control, B1, B2, C and D groups, according to the American College of Veterinary Internal Medicine (ACVIM) consensus. All dogs underwent physical examination, electrocardiography and standard echocardiography. Left atrial reservoir function was evaluated by LA global tissue mitral annular displacement (TMAD), LA global strain and LA emptying fraction and LA systolic function by LA systolic TMAD, LA ejection fraction and mitral A' (tissue Doppler imaging).

Results: Left atrial global TMAD was greater in B2, C and D groups and LA systolic TMAD was greater in B1, B2 and C and lower in D group. Left atrial global TMAD was an excellent method to differentiate dogs with from without remodeling, whereas global strain and ejection fraction were better to discriminate the asymptomatic from the symptomatic dogs.

Conclusions: Left atrial global TMAD can be used as a complementary method in conjunction with traditional echocardiographic examination to assess global atrial function in patients with MMVD.

KEY WORDS: Echocardiography; Speckle tracking; Mitral regurgitation; Left Atrial strain; Canine

Abbreviation Table

A’ peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler (cm/s)

ACVIM American College of Veterinary Internal Medicine

BW body weight

CHF congestive heart failure

E peak velocity of early diastolic transmitral flow (m/s)

ECG electrocardiogram

LA left atrium

LA:Ao Ratio of the left atrial dimension to the aortic annulus dimension

LV left ventricle

MMVD myxomatous mitral valve disease

PH pulmonary hypertension

ROC Receiver operating curves

ST speckle tracking

TDI Tissue Doppler imaging

TMAD tissue mitral annular displacement

Introduction

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs. It is caused by progressive myxomatous degeneration of the mitral valve, leading to incomplete leaflet coaptation and valve regurgitation [1]. The prevalence of MMVD in small dogs varies from 14 to 40% depending on the breed

[2,3]. Although most cases remain asymptomatic for years, the progression of mitral regurgitation can lead to congestive heart failure (CHF) and death in some cases [4-6].

Left atrial (LA) plays an important role in cardiac performance by modulating left ventricular (LV) filling, by its reservoir function during ventricular systole (atrial diastole), by the conduit function during ventricular diastole onset, and by pump function during the end of ventricular diastole (atrial systole). During the LA reservoir phase, blood is received from the pulmonary veins during LV systole and isovolumetric relaxation. This function is influenced by LV contraction and mitral annulus displacement, right ventricle contraction through pulmonary flow, and LA compliance. In the conduit phase, blood passes passively from LA to LV during the onset of LV diastole and diastasis and is influenced by LV relaxation and initial diastolic pressure. In the pump phase, blood is actively pumped from the left atrium to the left ventricle, during left atrial contraction, at the end of the left ventricular diastole. This phase is influenced by LA contractility, LV compliance and LV end-diastolic pressure [7].

Diastolic dysfunction, increased filling pressure, LV hypertrophy, and mitral insufficiency contribute to pathological and morphological changes that occur in LA [7]. The increase in LA volume may be accompanied by progressive impairment of its function, and both may precede the development of clinical signs in the patient, which affects their prognosis [8].

The LA function has a significant impact on cardiovascular disease and has been addressed through different noninvasive methods, but several limitations, including dependence on good image quality, single-plane evaluation

and time-consuming procedures, have restricted the diffusion of clinical applicability of left atrial function evaluation [9].

Until recently, the echocardiographic study of the left atrium was performed using two-dimensional measurements, extrapolation of phasic volumes, and Doppler flow assessment of the mitral valve and pulmonary veins [10]. However, these indices depend on hemodynamic volume conditions, the geometric structure of the atrium and the difficulties with the timing of various atrial events [11]. Three-dimensional echocardiography significantly improved LA volume calculation because of the automated border detection and the acquisition of data set at different phases of the cardiac cycle [12,13]. These problems have been solved by the use of echocardiographic-based automated techniques for analysis of myocardial displacement, such as tissue Doppler imaging (TDI) and speckle tracking (ST) [14,15].

Speckle tracking is a recent echocardiographic modality which is independent of the Doppler angle, using two-dimensional images that, through an automatic system, track natural acoustic markers (speckles) of the myocardium, which are followed frame by frame during the cardiac cycle. Echocardiography by ST assesses myocardial deformity by measuring myocardial strain (percentage of deformity from its original dimension) and myocardial strain rate (myocardial deformity velocity). Longitudinal strain analysis by ST has been increasingly used in different heart diseases in human patients [16]. Although ST was originally developed to assess ventricular function, it has recently been used to assess atrial chamber function [17-19].

Tissue motion annular displacement (TMAD) is a recent technique studied in medicine, which uses the ST, is independent of the Doppler angle, and

evaluates the movement of the mitral valve annulus during the cardiac cycle. It offers an advantage over other methods, as it can be performed in most patients because it does not rely on optimal endocardial definition and the mitral annulus can often be visualized and tracked even when there is poor image quality due to inadequate endocardial visualization. It provides information on systolic ventricular function from the degree of longitudinal deformation of the mitral annulus in the two- and four-chamber apical images [20]. Studies reported an accurate assessment of global longitudinal systolic function, compared with cardiac magnetic resonance. The technique also was highly reproducible with low inter- and intra-observer variability [21].

A recent human study demonstrated that the TMAD by ST is a reliable and simple method to evaluate the longitudinal left atrial function and showed an excellent association with the deformation parameters (strain) [22].

The purposes of this study were: (1) evaluate the longitudinal left atrial function in dogs with chronic mitral valve disease by TMAD; (2) investigate whether a correlation exists between TMAD and other echocardiographic methods of left atrial function; and (3) compare the sensibility and specificity of LA TMAD to differentiate the stages of heart disease in patients.

Materials and methods

Animals

The study was approved by the Institutional Ethics Committee on Animal Research (protocol 01084/2017).

This study was prospective cross-sectional observational study, and included 155 client-owned dogs, 95 dogs with MMVD, and 60 healthy control,

regardless of breed or sex and age > 1 year old. The animals were enrolled between April 2017 and June 2019 from a Veterinary Teaching Hospital and a Private Referral Hospital.

All dogs included in this study underwent complete physical examination, electrocardiography[a] and echocardiography. The dogs were divided into groups according to the standardized by the American College of Veterinary Internal Medicine (ACVIM) consensus [23]. Healthy dogs (A group); dogs without clinical signs of MMVD, with normal LA size or echocardiographic evidence of LA enlargement (left atrial to aortic root ratio [LA/Ao] $\leq$ 1.6) [24] or ventricular enlargement (left ventricular internal diameter in diastole, normalized for body weight $\leq$ 1.7) [25] (B1 group); dogs having MMVD with LA enlargement (LA/Ao $\geq$ 1.6) and left ventricular internal diameter in diastole, normalized for body weight $\geq$ 1.7, but without clinical signs or confirmed radiographic evidence of CHF (B2 group); dogs with symptomatic MMVD, LA and LV enlargement and radiographic evidence of pulmonary edema (C group); dogs with refractory CHF (D group).

Exclusion criteria consisted of any clinical signs of systemic or cardiac disease. Dogs with atrial flutter or fibrillation were also excluded. If the animals were receiving medications as angiotensin-converting enzyme inhibitors, furosemide, spironolactone or pimobendan they were also included.

Echocardiographic Assessment

Each animal was evaluated by complete conventional echocardiography[b]. Dogs were not sedated and were gently restrained in left and right lateral recumbency with continuous electrocardiographic recording, in accordance with the recommendations of the Echocardiography Committee of the Specialty of

Cardiology of the American College of Veterinary Internal Medicine [26]. The LA/Ao was obtained from the right parasternal short axis 2D view on the first frame after closure of the aortic valve as previously described [27]. The LV diameter in diastole and in systole were measured on the M-mode echocardiogram from the right parasternal short-axis 2D view with concomitant ECG registration. M-mode values were used to derive the fractional shortening and ejection fraction. From the left apical 4-chamber view, pulsed wave Doppler was used to measure the peak early (E) and late (A) diastolic mitral inflow velocity and tissue Doppler was used to measure the early diastolic (E') and late diastolic (A') velocity of the septal mitral annulus [28,29]. Left Apical 4 and 2-chamber videos were recorded, from a standard left apical four-chamber view, for offline analysis.

Biplane Area-Length method

Parameters of LA phasic function were determined as previously reported [30]. LA area was measured with planimetry in the left AP2 and AP4 views by tracing the endocardial border, excluding the confluence of the pulmonary veins and LA appendage. LA area was measured at 3 different points in cardiac cycle: just before the mitral valve opening (maximum left atrium area), at the onset of the p-wave on the ECG (preatria contraction area, LA p-Volume) and before left atrium contraction and at mitral valve closure (minimal LA area). A straight line connecting both the hinge points of the mitral valve leaflets was taken as the border of the LV. LA length was measured from the midline of the plane of the mitral annulus to the opposite aspect of the LA. Three consecutive cardiac cycles were measured and averaged. All LA volume measurements were calculated using the biplane area-length method [31]:

LA volume = (0.85 x A1 x A2) / L

where A1 and A2 represent the planimetric LA area acquired from left apical two and four-chamber views and L is the length.

LA reservoir function was assessed using the equation:

LA emptying fraction (%): LA Ef = 100 x (Maximal LA volume – Minimal LA volume) / Minimal LA volume

LA pump function was assessed using the equation:

LA ejection fraction (%): 100 x (LA p-Volume - Minimal LA volume) / LA p-Volume.

LA emptying fraction was used to compare to global / reservoir LA function and the LA ejection fraction to compare do contractile LA function.

Longitudinal left atrial strain

LA strain was estimated using two-dimensional speckle tracking method, from the left AP2 and AP4 views, obtained with simultaneous ECG trace recording, analyzed offline, with a software[c] originally designed to conduct LV analysis [32].

The endocardial border of the LA was manually defined and epicardial surface tracing was automatically generated by the system, creating a region of interest that was manually adjusted to cover the full thickness of the myocardium. After processing, the software divides the endocardial border into 6 segments and automatically produces time-longitudinal strain curves for each atrial segment throughout the cardiac cycle and the average of all six segmental values (global strain). The average of global LA strain measurements was obtained from values from AP2 and AP4 views. We retained the longitudinal global strain (mean

maximal atrial strain during atrial diastole) as a marker of the reservoir function of the LA [33].

Tissue mitral annular displacement

Mitral annular displacement was calculated offline in both AP2 and AP4 images, using a two-dimensional strain-based tissue tracking technique. In the AP2 view, the anterior and inferior aspects of the mitral annulus and the dorsal wall of the LA were selected. In the AP4 view, the hinge points of the mitral valve leaflets with the septal and lateral aspects of the mitral annulus and dorsal wall of the LA were selected. Three regions of interest (ROI) or points were placed. ROIs were tracked during cardiac cycle in each view. The ST software automatically plots the measured mitral annular longitudinal displacement of each tracked point frame to frame, in millimeters, creating time-displacement curves, from the medial point between the two points at the annulus and the dorsal wall of the LA. The maximum negative and positive displacement points were noted, as well as the position during diastasis. The baseline is considered the onset of QRS. The global displacement (Global TMAD) was calculated by averaging the four annular points (from AP2 and AP4 views), between the maximal negative and positive positions. The systolic displacement (Systolic TMAD) was obtained by the average of the four annular points (from AP2 and AP4 views), from diastasis and maximal positive position [22] (Fig. 1).

To minimize the body weight bias between the animals, the TMAD was indexed by body surface area, according to the following formula:

$BSA = K \times (\text{body weight in grams}^{2/3}) \times 10^{-4}$

K = constant (10.1 for dogs)

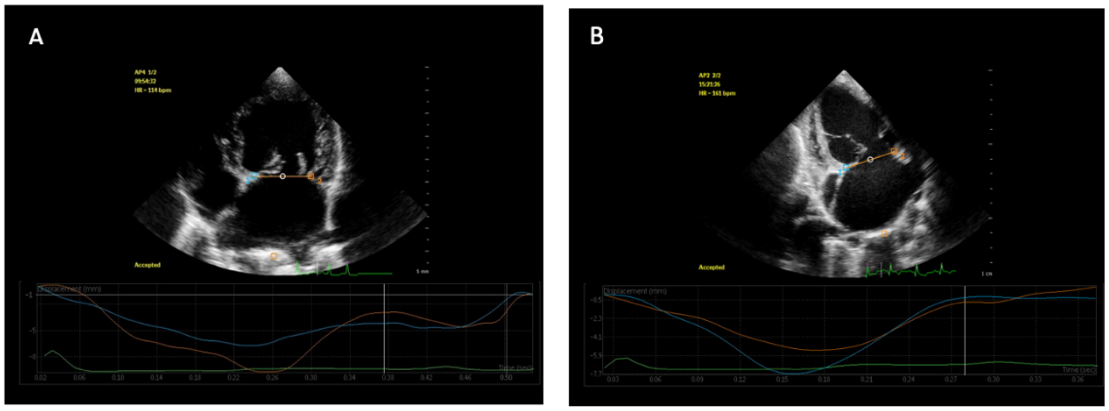

Figure 1 For tissue mitral annular displacement, two regions of interest were defined at the insertion of the mitral valve leaflets, at the septal and lateral parts for the apical-4 view (A) and at the anterior and inferior parts for apical-2 view. A third point was fixed at the dorsal wall of the left atrium. The curves are automatically determined by the equipment software.

Global TMAD was compared to global LA strain and LA emptying fraction as measurements of reservoir LA function and systolic TMAD was compared to LA ejection fraction and mitral A' as measurements of contractile LA function.

Statistical analysis

Data were examined for normality using the Shapiro-Wilk test. For normally distributed variables we used ANOVA followed by Tukey's test and the results are presented as mean (standard deviation). For asymmetrical variables we used Kruskal-Wallis followed by Dunn's test and the results are presented as median (interquartile range). To evaluate the correlations between the desired variables in all animals and between groups, either Pearson's or Spearman's correlation coefficient was used. The evaluation of the differences of the variables of interest according to sex in the control group animals was estimated by the Mann-Whitney U test and according to cardiac rhythm by the Kruskall-Wallys test. Receiver operating curve analysis was used to estimate the discriminative power of the different methods. The tests were considered significant when p value < 0.05 and all statistical analyses were performed with SPSS 21.0 statistics software[d].

Results

A total of 155 dogs were enrolled. The population included 60 dogs in stage A (healthy dogs), nineteen mixed-breed dogs and 41 from different breeds: Beagle (n = 16); Yorkshire Terrier (n = 6); Pug and Shi tzu (n = 4); Miniature Schnauzer (n = 3); English Bulldog and Miniature Poodle (n = 2), Border Collie, Lhasa Apso, Miniature Pinscher and Dachshund (n = 1). Forty two dogs were in stage B1 (44%): Miniature Poodle (n = 13); mixed-breed dog (n = 7); Miniature Schnauzer (n = 4); Miniature Pinscher and Shi tzu (n = 3); Bichon Frisé, Lhasa Apso and Dachshund (n = 2), Beagle, French Bulldog, Maltese, Pekingese, Whippet, and Yorkshire Terrier (n = 1). Seventeen dogs were in stage B2 (18%): mixed-breed dog (n = 5); Miniature Schnauzer (n = 3), Dachshund and Yorkshire Terrier (n = 2); Beagle, Brazilian Terrier, Miniature Pinscher and Shi tzu (n = 1). Thirty one dogs were in stage C (33%): mixed-breed dog (n = 9); Miniature Poodle (n = 5); Maltese and Miniature Schnauzer (n = 4); Chihuahua, Cocker Spaniel and Lhasa Apso (n = 2), Miniature Pinscher, Dachshund and Yorkshire Terrier (n = 1). Five dogs were in stage D (5%): Lhasa Apso (n = 3); Maltese and Pit Bull Terrier (n = 1). A hundred and one dogs were female (65,2%), and 54 were male (34,8%). The age varied between four months and 17 years (median 9.0; 3.0-13.0), and the body weight (BW) ranged from 1.25 to 20 kg (median 8; 5.4-10.6). The most common cardiac rhythm was sinus arrhythmia (70.32%) followed by sinus rhythm (14.84%) and sinus tachycardia (14.19%). Three dogs presented isolated ventricular premature complexes and four dogs isolated atrial premature complexes.

Left atrial global TMAD varied according to patient group, being greater in groups B2, C and D ($p < 0.001$). Left atrial systolic TMAD increased from control

to B1 dogs and decreased in D dogs ($p < 0.001$). Left atrial strain and LA emptying fraction were lower in C and D dogs ($p < 0.001$). Left atrial ejection fraction was higher in B1 and B2 and lower in C and D dogs ($p < 0.001$). A higher mitral A' wave was observed in B1, B2, C and D dogs ($p < 0.001$) (Figures 2 and 3).

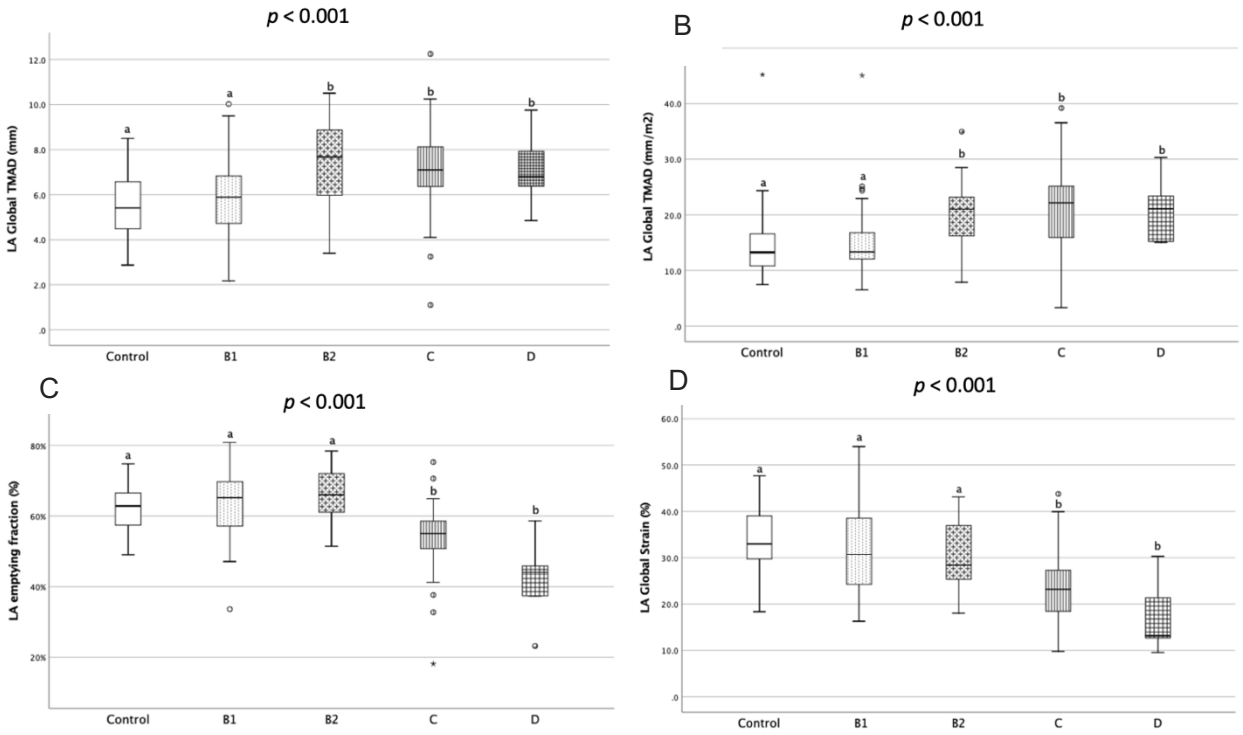

Figure 2 Box and whisker plots of left atrial global function by: A) LA global tissue mitral annular displacement (TMAD in mm); B) LA global tissue mitral annular displacement (TMAD in mm/mm^2); C) LA emptying fraction (%) and D) LA global strain (%) measurements obtained in dogs according to the groups.

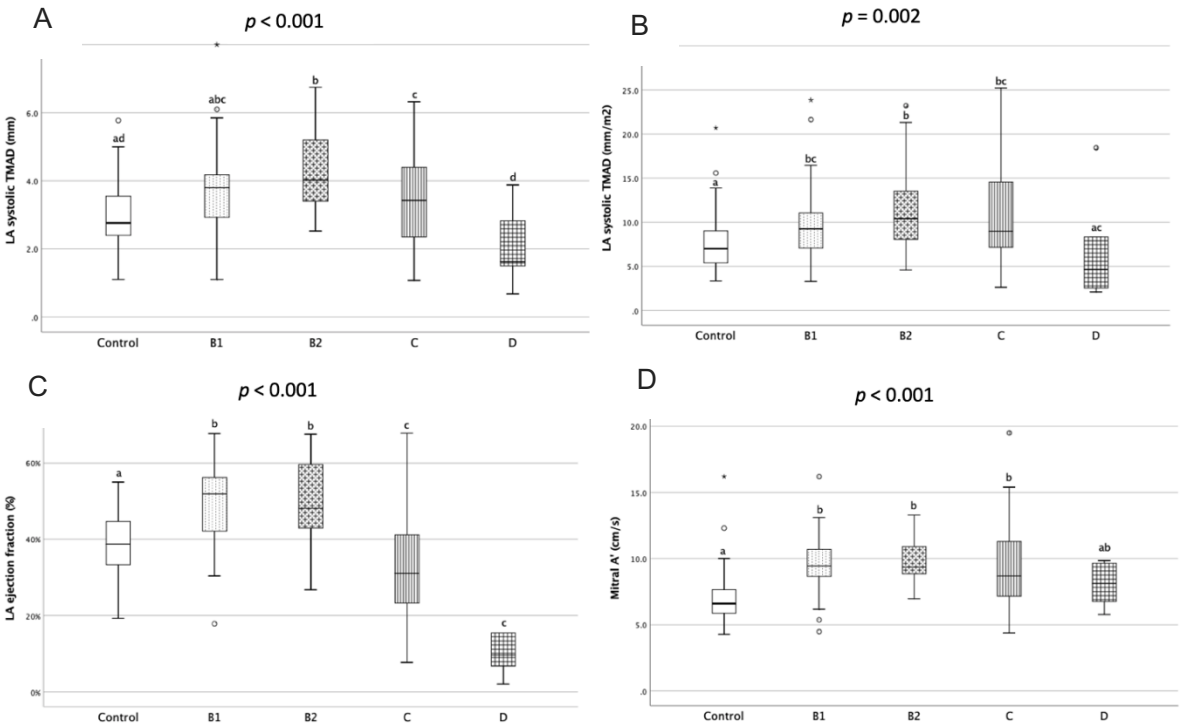

Figure 3 Box and whisker plots of left atrial systolic function by: A) LA systolic tissue mitral annular displacement (TMAD in mm); B) LA systolic tissue mitral annular displacement (TMAD in mm/mm^2); C) LA ejection fraction (%) and D) Mitral A' (cm/s) measurements obtained in dogs according to the groups.

Left atrial reservoir function was assessed by LA global TMAD, LA emptying fraction and LA global strain (Table 1).

Table 1 Comparison of LA TMAD, LA Strain, LA Emptying, LA Ejection Fraction and Mitral A' wave in accordance with groups.

	Groups					
	Control	B1	B2	C	D	*p*
(n)	60	42	17	31	5	
LA TMAD (mm)						
Global AP 2,4	5.45 (1.36)^{A}	5.87 (1.61)^{A}	7.43 (1.89)^{B}	7.14 (2.17)^{B}	7.14 (1.82)^{AB}	<0,001
Systolic AP 2,4	2.76 (1.02)^{AD}	3.80 (1.31)^{ABC}	4.02 (1.92)^{B}	3.42 (2.03)^{C}	1.62 (2.26)^{D}	<0,001
LA TMAD (mm/m²)						
Global AP 2,4	13.25 (5.68)^{A}	13.33 (4.80)^{A}	21.03 (8.94)^{B}	22.16 (9.94)^{B}	21.09 (11.73)^{B}	<0,001
Systolic AP 2,4	7.00 (3.63)^{A}	9.24 (4.23)^{BC}	10.39 (8.83)^{B}	8.97 (8.66)^{BC}	4.65 (11.07)^{AC}	0,002
LA Strain (%)	33.61 (6.72)^{A}	31.17 (8.98)^{A}	29.96 (7.75)^{A}	23.41 (7.37)^{B}	17.42 (8.41)^{B}	<0,001
LA Empt Fr (%)	62.84 (9.15)^{A}	65.19 (12.73)^{A}	65.99 (12.59)^{A}	55.05 (8.52)^{B}	44.10 (21.96)^{B}	<0,001
LA Ej Fr (%)	38.61 (8.59)^{A}	49.19 (10.38)^{B}	49.78 (12.59)^{B}	31.62 (13.69)^{C}	17.59 (20.90)^{C}	<0,001
TDI A' (cm/s)	6.60 (1.79)^{A}	9.45 (2.10)^{B}	9.53 (2.69)^{B}	8.68 (4.47)^{B}	8.12 (3.48)^{AB}	<0,001

(n), number of animals in quartile; LA, left atrium; TMAD, tissue mitral annular displacement; AP4, apical 4-chamber; AP2, apical 2-chamber; AP 2,4, average of 2 and 4 chamber; kg, kilograms; LAL, left atrium length; Emp Fr, Emptying fraction; Ej Fr, Ejection fraction, TDI A', Peak velocity of diastolic mitral annular motion as determined by tissue Doppler imaging.

Data are expressed as means (standard deviation) or medians (interquartile range) depending on the parameter attaining a normal distribution or not on the Shapiro-Wilk normality test. Values with different superscripted letters indicate statistically significant differences between groups.

In order to compare control to MMVD groups, global TMAD (mm/m^2) allowed the discrimination the animals between control and B1 group from B2, C

and D ($p < 0.001$). Left atrial strain and emptying fraction differentiated the controls only from C and D groups ($p < 0.001$) (Table 1).

To compare animals without (B1) and with remodeling (B2, C and D), LA global TMAD (mm/m^2) discriminated B1 from B2 ($p = 0.011$), C ($p < 0.01$) and D ($p = 0.037$). LA global strain and LA emptying fraction discriminated B1 from C ($p < 0.01$; $p < 0.01$) and D ($p = 0.006$; $p = 0.001$) (Table 1).

Comparing asymptomatic (B1 and B2) and symptomatic animals (C and D), LA global TMAD (mm/m^2) discriminated B1 from C ($p < 0.001$) and D ($p = 0.037$) but B2 did not show significant difference from C ($p = 0.510$) and D ($p = 0.911$) groups. LA strain and LA emptying fraction discriminated the asymptomatic from the symptomatic dogs, LA emptying fraction being better than LA strain (Table 1).

Left atrial systolic function was assessed by LA systolic TMAD, LA ejection fraction and mitral A' (Table 1).

When comparing control to MMVD dogs, systolic TMAD (mm/m^2), differentiated the control from B1 ($p = 0.014$), B2 ($p = 0.002$) and C ($p = 0.005$), but not from D animals ($p = 0.518$). LA ejection fraction discriminated controls from B1 ($p < 0.001$) and B2 ($p = 0.002$), but not from C ($p = 0.049$) and D groups ($p = 0.065$). Mitral A' discriminated controls from B1 ($p < 0.001$), B2 ($p < 0.001$) and C ($p < 0.001$), but not from D group ($p = 0.242$) (Table 1).

Comparing animals without (B1) and with remodeling (B2, C and D), LA ejection fraction discriminated B1 from C ($p < 0.001$), and D ($p < 0.001$). LA systolic TMAD (mm/m^2) and mitral A' didn't discriminate between animals with from without remodeling (Table 1).

LA systolic TMAD (mm/m^2) didn't discriminate between asymptomatic (B1 e B2) from symptomatic animals (C and D). Ejection fraction discriminated asymptomatic from symptomatic animals ($p \leq 0.001$), but Mitral A' didn't (Table 1).

Correlations of variables in MMVD group

Global TMAD (mm/m^2) was not correlated to LA global strain and was weak and positively correlated to LA emptying fraction (R = 0.183; p = 0.022).

Systolic TMAD (mm/m^2) showed a moderate positive correlation with LA ejection fraction (R = 0.479; $p < 0.001$) and with mitral A' (R = 0.321; $p < 0.001$).

Correlations of variables in control group

LA global TMAD (mm/m^2) was weak and negatively correlated to age (R = -0.277; p = 0.032) but was not correlated to sex, heart rate (HR) or cardiac rhythm. Left atrial Strain were not affected by age, sex, HR or cardiac rhythm ($p > 0.05$). The LA systolic TMAD were also not affected by age, HR or cardiac rhythm, however, it was significantly correlated with sex (p = 0.022), with females presenting higher values. The patient's weight showed strong negative correlation with global and systolic TMAD (mm/m^2) (R = -0.640; $p < 0.001$; R = -0.616; $p < 0.001$), and weak negative correlation with LA strain (R = -0.270; p = 0.037).

Global and systolic TMAD correlated significantly with several echocardiographic surrogates of diastolic function (Table 2).

Table 2 Correlation between LA global and systolic TMAD (mm/m^2) with data of diastolic function obtained by conventional echocardiography.

	LA global TMAD (mm/m^2)		LA systolic TMAD (mm/m^2)	
	R	*p*	R	*p*
LA:Ao	0.178	0.027	0.125	0.121
Mitral E	0.261	0.001	0.020	0.809
Mitral A	0.368	<0.001	0.470	<0.001
E:A	-0.017	0.839	-0.337	<0.001
IVRT	-0.233	0.003	-0.104	0.197
E:IVRT	-0.231	0.004	0.034	0.671
Mitral E'	0.084	0.301	-0.143	0.079
Mitral A'	0.234	0.004	0.321	<0.001
E':A'	-0.136	0.093	-0.371	<0.001
E:E'	0.191	0.018	0.119	0.143

LA, left atrium; TMAD, tissue mitral annular displacement; LA:Ao, ratio of the left atrial dimension to the aortic annulus dimension; Mitral E, peak velocity of early diastolic transmitral flow; Mitral A, peak velocity of late transmitral flow; E:A, Ratio of E to A; IVRT, Isovolumetric relaxation time; E:IVRT, Ratio of E to IVRT; Mitral E', Peak velocity of early diastolic mitral annular motion determined by pulsed wave Doppler; Mitral A', Peak velocity of diastolic mitral annular motion determined by pulsed wave Doppler; E'A', Ratio of E' to A'; E:E', Ratio of E to E'.

Nine dogs from C and D stages presented mild pulmonary hypertension (PH) (24.3%), seven moderate PH (18.9%) and five severe PH (13.5%). Neither global nor systolic TMAD values correlated with PH severity in the present study.

Receiver operating curves (ROC) were plotted to compare echocardiographic parameters to discriminate between asymptomatic (B1 and B2) from symptomatic animals (C and D) and animals without cardiac remodeling (B1) from the animals with cardiac remodeling (B2, C and D). Left atrial global Strain and LA emptying fraction were compared to LA global TMAD as measurements of the LA global / reservoir function and ejection fraction and mitral A' were compared to LA systolic TMAD as measurements of the LA systolic function. The results are presented in tables 3 and 4, and graphically represented in figures 4 and 5.

Table 3 Receiver operator characteristic curve analysis to differentiate asymptomatic and symptomatic dogs.

	ROC area under the curve 95% Confidence interval			
Functional parameter	Area under the curve	Lower bound	Upper bound	*p*
LA Global TMAD (mm/m^2)	0.715	0.607	0.823	<0.001
LA Global Strain (%)	0.242	0.143	0.341	<0.001
Emptying fraction (%)	0.198	0.108	0.289	<0.001
LA Systolic TMAD (mm/m^2)	0.465	0.347	0.603	0.687
Ejection fraction (%)	0.149	0.065	0.232	<0.001
TDI A' wave (cm/s)	0.391	0.263	0.520	0.079

ROC, receiver operating characteristic; LA, left atrial; TMAD, tissue mitral annular displacement; TDI, tissue doppler imaging.

Table 4 Receiver operator characteristic curve analysis to differentiate dogs without and with cardiac remodeling.

	ROC area under the curve 95% Confidence interval			
Functional parameter	Area under the curve	Lower bound	Upper bound	*p*
LA Global TMAD (mm/m^2)	0.756	0.656	0.855	<0.001
LA Global Strain (%)	0.313	0.206	0.420	0.002
Emptying fraction (%)	0.322	0.213	0.430	0.003
LA Systolic TMAD (mm/m^2)	0.537	0.420	0.654	0.543
Ejection fraction (%)	0.266	0.163	0.368	<0.001
TDI A' wave (cm/s)	0.434	0.317	0.551	0.273

ROC, receiver operating characteristic; LA, left atrial; TMAD, tissue mitral annular displacement; TDI, tissue doppler imaging.

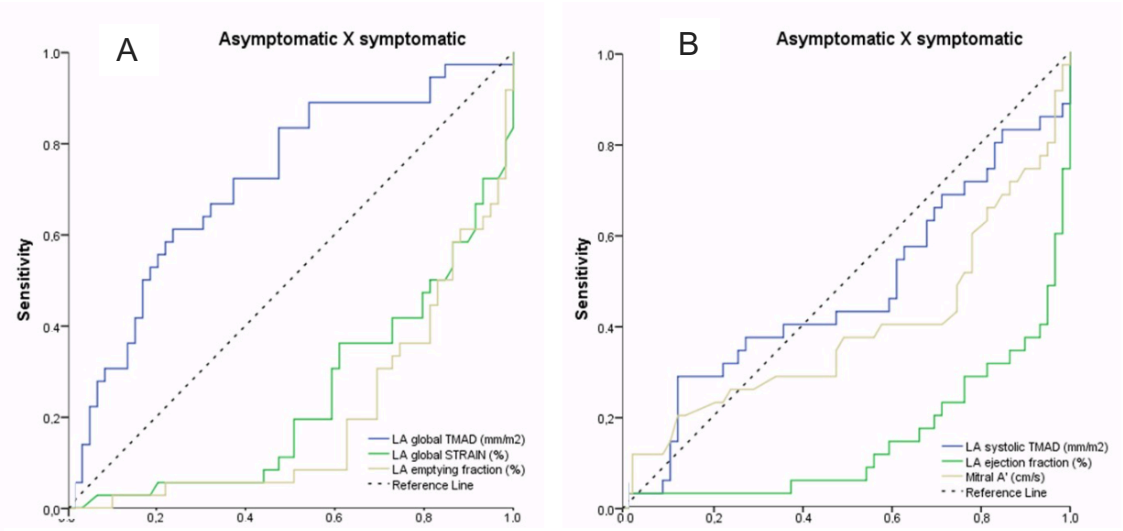

Figure 3: Receiver operator characteristic curves to distinguish asymptomatic from symptomatic animals. A) Global function parameters: LA global TMAD, LA global strain and LA emptying fraction. Left atrial Global TMAD showed larger area under the curve than LA strain and LA emptying fraction. Left atrial global TMAD showed larger area under the curve than LA strain and LA emptying fraction. B) Atrial systolic parameters: LA systolic TMAD, LA ejection fraction and A' mitral. TMAD, tissue mitral annular displacement; LA, left atrial; Mitral A', Peak velocity of diastolic mitral annular

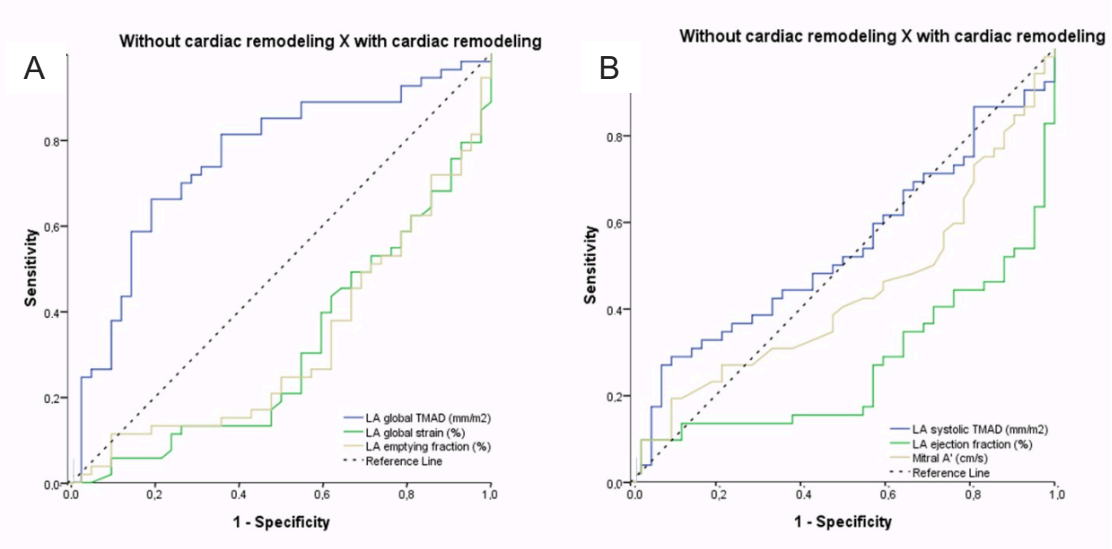

Figure 4: Receiver operator characteristic curves to distinguish animals without cardiac remodeling from with cardiac remodeling. A) Global function parameters: LA global TMAD, LA global strain and LA emptying fraction. Left atrial Global TMAD showed larger area under the curve than LA strain and LA emptying fraction. B) Atrial systolic parameters: LA systolic TMAD, LA ejection fraction and A' mitral. TMAD, tissue mitral annular displacement; LA, left atrial; Mitral A', Peak velocity of diastolic mitral annular motion determined by pulsed wave Doppler.

Based on ROC analysis, the best discriminator between the asymptomatic from symptomatic and between animals with and without cardiac remodeling was the LA Global TMAD, with an area under the curve (AUC)=0.715 and (AUC)=0.756 respectively. The other variables analyzed were not good discriminators between the groups. The best cutting point of global TMAD to discriminate between the asymptomatic from symptomatic dogs was 21.07 mm/m^2 with sensitivity of 61.1% and specificity of 76.3% and between animals with and without cardiac remodeling was 17.59 mm/m^2 with sensitivity of 66.0% and specificity of 81.0%.

Discussion

In this study, we applied the tissue mitral annular displacement, used in medicine, to healthy and MMVD dogs with good feasibility and reproducibility for the evaluation of left atrial function.

Our study showed that severe MMVD (C and D groups) is associated with a significant decrease in global strain and LA emptying fraction (reduction in reservoir function) probably due to lower left atrial compliance and higher stiffness caused by atrial remodeling in the advanced mitral disease. Ultrastructural changes of the LA myocardium, including interstitial fibrosis, myocyte hypertrophy, and chronic inflammatory changes might be an important pathophysiological feature in the reduction of compliance [11,34]. The study of Cameli (2011) [35] and Nakamura *et al.* (2017) [36] also demonstrated that LA longitudinal strain during ventricular systole (indicator of reservoir function) were lower in dogs with CHF due do MMVD than those without CHF. Although the LA enlarges in accordance with the ACVIM stage, LA strain was maintained until the onset of CHF. LA deformation was correlated with the extend of LA fibrosis in prior studies, supporting the LA strain by ST as a noninvasive tool as a surrogate marker of LA stiffness. It is probable that a more extensive LA fibrotic change results in poorer LA function and higher likelihood of causing heart failure symptoms as well as a poorer outcome [37].

Interestingly, LA global TMAD was greater in B2, C and D patients. It increased with cardiac enlargement (B2) and didn't decrease in patients with refractory congestive heart failure (D). The more significant movement from mitral annulus toward the cardiac apex during ventricular systole, was probably induced by the increased preload and ventricular systolic function in advanced MMVD, by moving more the annulus per the hyperdynamic ventricle. Our findings contrast those of Strachinaru *et al.* (2016) [22], that found decreased values of total mean annular displacement in patients with enlarged left atrium, but they present heart

diseases of several etiologies that would result in different echocardiographic parameters.

Left atrial strain and emptying fraction were useful methods to differentiate advanced stages of the disease, but global TMAD distinguished earlier stages (B2), being better to differentiate patients without or with remodeling. Therefore, the methods are complementary and cannot be substituted for each other.

Left atrial systolic TMAD increased from control to B1 dogs, and then decreased in dogs from D groups. Animals at advances phase the diastole will depend more on the initial filing. This means that during the late diastole, the annulus moved less intensely, contributing less for LV diastole, probably caused by Frank Starling exhaustion. Systolic TMAD wasn't an excellent method to evaluate LA contractile function probably due to difficulty in determining the exact point of diastasis to calculate the systolic displacement in animals with tachycardia.

Left atrium ejection fraction (active fractional area change), a measure of LA booster pump function, is one of the most common techniques used to measure LA contractile function in dogs [8,30,38]. In the present study, the LA ejection fraction was the best method to evaluate LA contractile function, showing a transient increase in patients B1 and B2 and then declining in dogs from C and D groups, performing early diagnosis as well as in the advanced stages of the disease. Similar results were found by Höllmer *et al.* (2017) [39].

The main limitation of LA ejection fraction is the inability to distinguish between the increase in LA function due to a larger volume of blood received and a real increase in LA intrinsic contractility [7]. It was significantly lower in CHF

dogs than in those without CHF according to one study. There was a significant correlation between LA longitudinal strain during atrial contraction and ejection fraction, but strain had a higher predictive power for CHF. The difference in preload dependency may have contributed to the superiority of strain over active fractional area changes in the study [36].

Many studies have reported the value of echocardiography for predicting survival time and providing prognostic indicators in dogs with MMVC [40-42]. The findings of one study indicate that LA size and function are strongly correlated with early death in dogs with MMVD. Although several echocardiographic parameters were significantly different between the groups, left atrial ejection fraction, the parameter corresponding to the booster pump function, was the most significant independent predictor of mortality in one study [8].

LA booster pump dysfunction can be more attributable to afterload mismatch than to LA intrinsic contraction abnormalities. In chronically decompensated MR, LV stiffness and end-diastolic pressure rise with LA pressure. This situation contributes to decreased transmitral flow during LA contraction (restrictive pattern). In human heart failure patients, reduced transmitral A wave velocity is recovered after reduction in LV filling pressure with optimal treatment of heart failure. This reversibility of mitral A flow suggests that LA dysfunction results from LA afterload mismatch rather than intrinsic LA abnormality [43,44].

Mitral A' velocity by TDI discriminated only controls from B1, B2, C and D, but there was not significant difference between the symptomatic and asymptomatic dogs and wasn't a reliable method for evaluation of LA systolic function. In one study, there was also no significant difference in A' between the

survivors and nonsurvivor dogs, reflecting a lower value for this prognostic predictor in dogs with MMVD [8]. In humans, several studies have demonstrated that Mitral A' velocity can be used as a rapid and accurate marker of global atrial function [22,45]. Mitral A' velocity by TDE didn't correlate with atrial function in dogs with MMVD and should not be used as a prognostic method for canine patients. Probably the increased preload (increased A') and elevation of LV filling pressure (decreased A') that occur at C and D patients, leads to a preserved A' velocity. In human researches, congestive heart failure has diverse etiology and with varied hemodynamic determinants that could influence tissue doppler parameters.

In the control group LA global TMAD (mm/m^2) was weak and negatively correlated to age. This could be caused to lower atrial compliance due to fibrosis present in older animals since the included animals ranged from 4 months to 10 years of age.

In healthy dogs the bodyweight showed a strong and negative correlation with global and systolic TMAD (mm/m^2), and weak and negative correlation with LA strain. Probably the LA function also varies according to the size and weight of the patient, as occurs with the systolic ventricular myocardial function, reportedly lower in larger dogs [46]. Therefore, we cannot exclude a possible effect of BW on the ST variables. Similarly, a study evaluating LA strain in healthy dogs found a negative correlation between strain and BW [47,48]. Dickson *et al.* (2017) [48] also found a and negative correlation between the patient weight and total LA emptying fraction and LA reservoir function. These findings sup

port the consideration of the effect of BW on TMAD, strain and volumetric indices interpretation of cardiac diseases.

In the control group, LA systolic TMAD (mm/m^2) was significantly correlated with sex, with females presenting higher values. The 45 females included in the control group, weighted from 1.25 to 19.8 kg (mean 8.29; median 8.6) and the 15 males, weighted from 2.6 to 15.5 (mean 9.83; median 10.2). Probably the females showed higher values of systolic TMAD because they present lighter weight comparing to males, since the patient weight is negative correlated to systolic TMAD.

Pulmonary hypertension severity didn't correlate to TMAD, so this method doesn't have prognostic value for PH in the present study, however the number of animals that presented PH was small.

Based on ROC analysis, the deformation (Global LA strain) and volumetric indexes (emptying fraction) were not good discriminators between asymptomatic from symptomatic and between animals with and without cardiac remodeling, and the best discriminator method between them was the LA Global TMAD, showing better positive predictive value.

When evaluating systolic LA function, no method was effective to discriminate between the asymptomatic from symptomatic and between animals with from without cardiac remodeling. Different results were obtained in people, in which the best discriminator of abnormal versus normal patients regarding global function was global LA strain (AUC=0.921), emptying fraction of the LA (AUC=0.872) and global TMAD (AUC=0.872). When evaluating systolic function, the best method was LA ejection fraction (AUC=0.915), followed by, systolic TMAD (AUC=0.843) and Mitral A' (AUC=0.807) [22].

Some limitations of this study must be considered. First, the patients were selected so as to have a good quality image, to allow the use of deformation method (strain). When the animal was tachycardic, it was more difficult to accurately determine the diastasis point for systolic TMAD calculation. When the patient was tachypneic, imaging and evaluation of mitral annulus movement was hampered by excessive pulmonary movement due to rapid breathing. Lastly is the software used, that is originally designed for the ventricle, and a specific for the atria is not already available.

Conclusion

This study shows that motion annular displacement by speckle tracking is a feasible, simple and promising tool for assessing left atrial function. Left atrial global TMAD was higher and LA systolic TMAD was lower in advanced stages of MMVC. LA global TMAD allowed the discrimination between patients with and without remodeling and can be a complementary method to be used together to existing ones to assess left atrial function in patients with MMVD.

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Conflicts of Interest Statement

The authors do not have any conflict of interest to disclosure.

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Footnotes

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[a] TEB – Tecnologia Eletrônica Brasileira, São Paulo, Brazil.

[b] Philips Affinity 50 ultrasound system equipped with 2-4, 3-8 and 4-12MHz phased array transducers.

[c] QLAB Software – Auto Cardiac Motion Quantification (aCMQ)

[d] IBM statistics SPSS® 21.0 (2012)

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CAPÍTULO 4

CONSIDERAÇÕES FINAIS

A análise dos resultados desse estudo mostrou que o deslocamento tecidual do anel mitral é uma técnica rápida, com boa repetitividade e que fornece informações adicionais na avaliação funcional do átrio esquerdo. Esse método permitiu a diferenciação entre os pacientes com DVMC sintomáticos dos assintomáticos, podendo ser incorporado na rotina ecocardiográfica principalmente quando se objetiva classificar os pacientes de acordo com a gravidade e remodelamento cardíaco. É importante destacar que o método não deve substituir os demais, mas sim ser complementar aos já existentes.

ANEXO 1 – Parecer de aprovação do CEUA

Pontifícia Universidade Católica do Paraná
Pró-Reitoria de Pesquisa e Pós-Graduação
Comissão de Ética em Pesquisa no Uso de Animais

Curitiba, 11 de maio de 2017.

PARECER DE PROTOCOLO DE PESQUISA

REGISTRO DO PROJETO: **01084/2017 – Emenda (Inclusão de Pesquisador e mudança de Técnica)**

TÍTULO DO PROJETO ORIENTADOR: *AVALIAÇÃO DA FUNÇÃO ATRIAL ESQUERDA PELO DESLOCAMENTO TECIDUAL DO ANEL MITRAL EM CÃES SAUDÁVEIS E COM DOENÇA VALVAR MITRAL.*

PESQUISADOR RESPONSÁVEL

Marconi Rodrigues de Farias

EQUIPE DE PESQUISA

Ana Paula Sarraf Lopes , Marlos Gonçalves Souza, Bruna Cristina Brüler, Giovana Laís Ruviaro Tuleski, Marcela Wolf, Stephany Buba Lucina, Vinícius Bentivóglio Costa Silva, Júlio Pereira dos Santos.

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VIGÊNCIA DO PROJETO	11/2016 a 12/2017	**QUANTIDADE DE ANIMAIS**	40 (já liberados em 2016)
ESPECIE/LINHAGEM	*Canis lupus familiaris*	**Nº SISBIO** (Somente animais de vida livre)	Não se aplica
SEXO	MF	**ATIVIDADES** (Somente animais de vida livre)	Não se aplica
IDADE / PESO	A partir de 06 anos	**ESPECIÉ – GRUPO TAXONÔMICOS** (de vida livre)	Não se aplica
ORIGEM DO ANIMAL	Hospital Veterinário PUCPR	**LOCAL (IS)** (Somente animais de vida livre)	Não se aplica

O colegiado do CEUA certifica que este protocolo que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto homem), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794/2018 e Decreto nº 6.899/2009, e com as normas editadas pelo CONCEA e foi **APROVADO** pela CEUA - PUCPR em reunião de ***11.05.2017.*** Se houver mudança do protocolo o pesquisador deve enviar um relatório á CEUA descrevendo de forma clara e sucinta, a parte do protocolo a ser modificado e as suas justificativas. Se a pesquisa, ou parte dela for realizada em outras instituições, cabe ao pesquisador não iniciar antes de receber a autorização formal para a sua realização. ***O documento que autoriza o início da pesquisa deve ser carimbado e assinado pelo responsável da instituição e deve ser mantido em poder do pesquisador responsável, podendo ser requerido por esta CEUA em qualquer tempo. Lembramos ao pesquisador que é obrigatório encaminhar qualquer alteração no protocolo de pesquisa e o relatório final a esta CEUA.***

Atenciosamente,
Prof. Dr. Sérgio Luiz Rocha
Coordenador
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ANEXO 3 – Resumo do trabalho oral apresentado no ACVIM (American College of Veterinary Internal Medicine) Forum 2019.

Speaker List

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Assessment of Atrial Function Using Tissue Mitral Annular Displacement in Healthy Dogs (Abstract C18)

ACVIM 2019

Ana Paula Sarraff Lopes[1]; Vinícius Silva[2]; Marcela Wolf[2]; Giovana Tuleski[2]; Leticia Queiroz[1]; Marconi Farias[1]; Marlos Sousa[2]

[1]Pontifical Catholic University of Paraná - PUCPR, Curitiba, PR, Brazil; [2]Federal University of Paraná - UFPR

Recently there have been advances in terms of ability to characterize and quantitate left atrium (LA) function using non-invasive imaging. The aim of this study was to demonstrate that Tissue Mitral Annular Displacement (TMAD) by two-dimensional Speckle Tracking, can be an easy and fast method do evaluate the longitudinal left atrium function.

In this prospective cross-sectional observational study, a hundred healthy dogs (1–13 y / 1–61 kg) underwent echocardiogram. Apical 4-chamber (AP4) and 2-chamber (AP2) images were obtained, which allowed the calculation of TMAD (global and systolic), Longitudinal Strain and LA volume measurements. TMAD and Strain were acquired from Speckle Tracking and LA volumes from area-length method. LA ejection and emptying fractions were calculated from the volumes obtained. Data underwent the Shapiro-Wilk test to check for a normal distribution.

Both TMAD and LA Strain varied in accordance with the size of the animals (Table-1). Our results showed that strain was higher in smaller dogs, which contrasts with the greater displacement of the mitral annulus (in mm) in heavier dogs. However, when TMAD was indexed, positive correlations were found to exist. Global TMAD (mm) varied with regard to sex ($p=0.003$), (female=5.56 (1.50); male=6.52 (1.38)), while systolic TMAD didn't ($p=0.393$). LA ejection and emptying fraction presented lower values in larger dogs ($p<0.05$). Global Strain was strongly correlated with global TMAD (indexed) and moderated with systolic TMAD. Global and systolic TMAD was moderated correlated with LA emptying and ejection fractions. There was no correlation between global TMAD and echocardiographic indices of diastolic function except for isovolumetric relaxation time (TRIV) (negative correlation), whereas systolic TMAD showed a negative correlation with some (E, E/A, TRIV).

TMAD by speckle tracking is a reliable and fast method for assessment of LA longitudinal function and is less dependent on image quality. Further studies are warranted to validate the clinical applicability of TMAD in animals with heart diseases.

SPEAKER INFORMATION
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Ana Paula Sarraff Lopes
Pontifical Catholic University of Paraná - PUCPR
Curitiba, Paraná, Brazil

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ANEXO 4 – Resumo do ACVIM Forum 2019 publicado no periódico Journal of Veterinary Internal Medicine

(PG). Post-procedural success was defined as a reduction in the transpulmonic pressure gradient by at least 50% of the pre-procedural PG or to less than 75mmHg one month post procedure.

Ten client-owned animals with severe valvular PS based on transthoracic echocardiography (TTE) were enrolled in the study. The BVP procedure was performed by standard technique with the use of a NuCLEUS-X™ BVP instead of a conventional BVP. Right-sided pressure and cardiac output measurements were made pre- and post-balloon valvuloplasty and effective valve orifice area was calculated. The NuCLEUS-X™ balloon size was selected to achieve a balloon-to-annulus ration between 1.2-1.5 and hand inflated with an iodinated contrast solution until loss of the stenotic valve waist was observed using fluoroscopic guidance. Pre- and 1-month post TTE PG measurements were recorded with continuous wave Doppler. Descriptive data is listed as mean ± standard deviation for normally distributed data and median [IQR] for non-normal data. Paired t-tests were used to determine significant differences in continuous variables with significance set at 0.05.

The median body weight was 16.6 kg ± 5.3 kg and age at BVP was 9 months [3.6-21 months]. All 10 cases achieved balloon stability centered at the pulmonic valve on the first inflation. Nine dogs had two total procedural inflations and one dog had a single inflation due to pronounced systemic hypotension following balloon deflation that resolved with balloon catheter removal. The median TTE derived pre-operative transpulmonic PG was 141 mmHg ± 41 mmHg and the 1-month post-operative PG was 83 mmHg ± 41 mmHg. Procedural success was achieved in 60% of patients and no significant complications were noted using the NuCLEUS-X™ catheter. One instance of resistance when removing the deflated catheter from the right ventricular outflow tract was encountered, but resolved following reinflation and deflation of the BVP. Nine dogs had a single NuCLEUS-X™ catheter used; one dog required upsizing with a conventional BVP catheter after the NuCLEUS-X™ inflation resulted in no apparent catheter waist. Using the angiographic pulmonic valve annulus measurement, the median balloon to annulus ratio was 1.2 ± 0.1 for the balloon waist, and 1.4 ± 0.1 for the distal portion of the NuCLEUS-X™ balloon. There was a significant difference in invasive peak-to-peak PG post-BVP compared to pre-BVP ($p = 0.028$) and no significant difference between pre- and post- pulmonic valve orifice area ($p = 0.1$).

The results of our study indicate that use of the pediatric NuCLEUS-X™ catheter is feasible for BVP in dogs with severe PS. The unique balloon shape provided catheter stability on the first inflation in all dogs. The NuCLEUS-X™ may be particularly useful in cases when stabilization of a conventional BVP catheter cannot be achieved.

C18

Assessment of Atrial Function Using Tissue Mitral Annular Displacement in Healthy Dogs

Ana Paula Sarraff Lopes – Pontifical Catholic University of Paraná - PUCPR, Curitiba, PR, Brazil; Vinícius Silva – Federal University of Paraná - UFPR; Marcela Wolf – Federal University of Paraná - UFPR; Giovana Tuleski – Federal University of Paraná - UFPR; Leticia Queiroz – Pontifical Catholic University of Paraná – PUCPR; Marconi Farias – Pontifical Catholic University of Paraná – PUCPR; Marlos Souza – Federal University of Paraná - UFPR

Recently there have been advances in terms of ability to characterize and quantitate left atrium (LA) function using non-invasive imaging. The

Table 1 - Comparison of age, LA TMAD, LA Strain, LA Emptying and LA Ejection Fraction in accordance with body weight

	Body Weight (quartiles)				
	1.25–7.90 kg	7.91–11.00 kg	11.01–20.00 kg	20.01-61.60 kg	p
(n)	25	25	25	25	
Age (years)	2.0 (0.9-5)	2.5 (2.0-5.0)	2.0 (2.0-3.5)	3.0 (2.0-5.5)	0.4927
LA TMAD (mm)					
Global AP 2,4	4.24 (0.79)^{A}	6.05 (1.06)^{B}	6.06 (1.20)^{B}	7.16 (1.36)^{C}	<0.0001
Systolic AP 2,4	2.55 (2.41-3.27)^{A}	2.97 (2.42-3.86)^{AB}	3.40 (2.65-4.10)^{BC}	3.75 (3.20-4.58)^{C}	0.0004
LA TMAD (mm/m²)					
Global AP 2,4	18.48 (11.43-21.30)^{A}	13.51 (12.11-15.07)^{A}	10.36 (8.69-11.85)^{B}	7.49 (5.93-8.41)^{C}	<0.0001
Systolic AP 2,4	10.47 (7.59-13.66)^{A}	6.70 (5.31-8.75)^{B}	5.93 (4.35-6.78)^{B}	3.76 (2.82-4.99)^{C}	<0.0001
LA TMAD (mm/kg)					
Global AP 2,4	1.15 (0.64-1.57)^{A}	0.64 (0.56-0.71)^{A}	0.42 (0.36-0.48)^{B}	0.24 (0.17-0.28)^{C}	<0.0001
Systolic AP 2,4	0.63 (0.42-0.95)^{A}	0.33 (0.25-0.40)^{B}	0.25 (0.18-0.29)^{B}	0.12 (0.08-0.17)^{C}	<0.0001
LA TMAD (mm/LAL)					
Global AP 2,4	0.29 (0.21-0.32)^{AB}	0.28 (0.25-0.30)^{A}	0.24 (0.23-0.26)^{B}	0.20 (0.18-0.25)^{C}	<0.0001
Systolic AP 2,4	0.17 (0.13-0.20)^{A}	0.13 (0.10-0.18)^{AB}	0.13 (0.12-0.17)^{AB}	0.11 (0.08-0.14)^{B}	0.0037
LA Strain (%)	34.47 (7.12)^{A}	33.76 (6.19)^{A}	29.08 (6.54)^{B}	21.40 (4.65)^{C}	<0.0001
LA Empt Fr (%)	63.43 (6.40)^{A}	62.27 (6.61)^{A}	60.16 (6.69)^{A}	54.97 (7.10)^{B}	<0.0001
LA Ej Fr (%)	42.14 (36.15-49.98)^{A}	38.13 (29.72-44.35)^{AB}	38.38 (33.23-40.77)^{B}	35.39 (26.64-44.73)^{B}	0.0323

(n), number of animals in quartile; LA, left atrium; TMAD, tissue motion annular displacement; AP4, apical 4-chamber; AP2, apical 2-chamber; AP 2,4, average of 2 and 4 chamber; kg, kilograms; LAL, left atrium length; Empt Fr, Emptying fraction; Ej Fr, Ejection fraction.
Data are expressed as means (standard deviation) or medians (interquartile range) depending on the parameter attaining a normal distribution or not on the Shapiro-Wilk normality test. Values with different superscripted letters indicate statistically significant differences between groups.

aim of this study was to demonstrate that Tissue Mitral Annular Displacement (TMAD) by two-dimensional Speckle Tracking, can be an easy and fast method do evaluate the longitudinal left atrium function.
In this prospective cross-sectional observational study, a hundred healthy dogs (1-13 y / 1-61 kg) underwent echocardiogram. Apical 4-chamber (AP4) and 2-chamber (AP2) images were obtained, which allowed the calculation of TMAD (global and systolic), Longitudinal Strain and LA volume measurements. TMAD and Strain were acquired from Speckle Tracking and LA volumes from area-length method. LA ejection and emptying fractions were calculated from the volumes obtained. Data underwent the Shapiro-Wilk test to check for a normal distribution.
Both TMAD and LA Strain varied in accordance with the size of the animals (Table-1). Our results showed that strain was higher in smaller dogs, which contrasts with the greater displacement of the mitral annulus (in mm) in heavier dogs. However, when TMAD was indexed, positive correlations were found to exist. Global TMAD (mm) varied with regard to sex ($p = 0.003$), (female = 5.56 (1.50); male = 6.52 (1.38)), while systolic TMAD didn't ($p = 0.393$). LA ejection and emptying fraction presented lower values in larger dogs ($p < 0.05$). Global Strain was strongly correlated with global TMAD (indexed) and moderated with systolic TMAD. Global and systolic TMAD was moderated correlated with LA emptying and ejection fractions. There was no correlation between global TMAD and echocardiographic indices of diastolic function except for isovolumetric relaxation time (TRIV) (negative correlation), whereas systolic TMAD showed a negative correlation with some (E, E/A, TRIV).TMAD by speckle tracking is a reliable and fast method for assessment of LA longitudinal function and is less dependent on image quality. Further studies are warranted to validate the clinical applicability of TMAD in animals with heart diseases.

C19

Related Factors for Residual Coughing in Dogs After Mitral Valve Repair

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Coughing is one of the related clinical signs of dogs with myxomatous mitral valve disease (MMVD). Previous study has reported left atrial enlargement were associated with coughing in dogs with MMVD. Mitral valve repair (MVR) leads to reduce the heart size. The objective of this study was whether the reverse remodeling after MVR affects appearance of coughing in dogs. We retrospectively reviewed cases that underwent MVR between January 2018 and November 2018. 133 dogs that were coughing pre-operatively were separated into two groups, those that continued to cough 1-month after MVR (Cough group, n = 50) and those in which the cough disappeared (non-Cough group, n = 83). We then analyzed the data concerning demographics, tracheal collapse, bronchomalacia, and pre- and 1-month post-operative vertebral heart size (VHS), vertebral left atrial size (VLAS), left atrial to aortic root rate (LA/Ao), left ventricular internal diameter in diastole normalized to bodyweight (kg) (LVIDDn). ACVIM heart failure classification was significantly different between the groups, and post-operative VHS, LA/Ao and LVIDDn was significantly smaller in the non-Cough group than in the Cough group. Multivariable analysis revealed that post-operative LA/Ao (OR : 11.831, 95%CI : 1.679 – 83.354, $P = 0.013$) was significantly related to post-operative coughing. Our study findings revealed that post-operative LA/Ao is a related factor for residual coughing in dogs after MVR. Therefore, if enough reverse remodeling of the left atrium occurs after MVR, the cough disappears.

C20

Disorganization of β-Catenin in Cats with Hypertrophic Cardiomyopathy

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β-catenin is a member of the catenin protein family, it is widely expressed in many tissues and exists in two forms, membrane associated or cytosolic. In cardiac muscle, membrane associated β-catenin localizes to adherens junction proteins such as cadherin in intercalated discs, which are critical for electrical and mechanical coupling between adjacent cardiomyocytes. Perturbation of strict segregation of cell-cell and cell-matrix contact has been observed in human hypertrophic cardiomyopathy (HCM) by detecting laterally located membrane associated β-catenin in cardiomyocytes. Stabilization of β-catenin in the cytoplasm leads to increased cytosolic β-catenin which can functions as a transcriptional regulator that promotes cardiomyocyte growth in response to hypertrophic-associated stimuli. The objective of the study was to investigate the expression and localisation of β-catenin in the left ventricle of cats with HCM.
The expression of β-catenin in the myocardium from 10 normal cats and 9 cats with HCM was investigated using Western blotting. The intensity of the bands was expressed as fold change after normalization with a housekeeping internal control, GAPDH. To further explore the expression and localization of β-catenin, hearts from 5 normal cats, 14 cats with HCM were fixed in formalin, processed, and embedded in paraffin for fluorescent immunohistochemistry. Final confirmation of HCM was based on histopathological examination by an experienced specialist veterinary pathologist. The slides were coded and triple stained with β-catenin, desmin for better visualisation of the boarder of each individual cardiomyocyte, and DAPI the nucleus dye. 10 images were acquired under 40x magnification per cat and the presence of laterally located β-catenin was noted. The signal detected in cytoplasm was scored (0-3) with the observer blinded to the disease status of the cat. The results were compared between groups using Fisher's exact test and Mann Whitney test. Spearman's test was used to detect correlation between cytosolic β-catenin and disease severity.
There was no difference in the age and sex of different groups of cats ($p > 0.05$). No difference was detected in the protein level of β-catenin between HCM and Control ($p = 0.0789$). Laterally distributed β-catenin was only observed in HCM group and the presence of this distribution was significant compared to controls ($p = 0.0048$). HCM showed more β-catenin in the cytoplasm compared to controls