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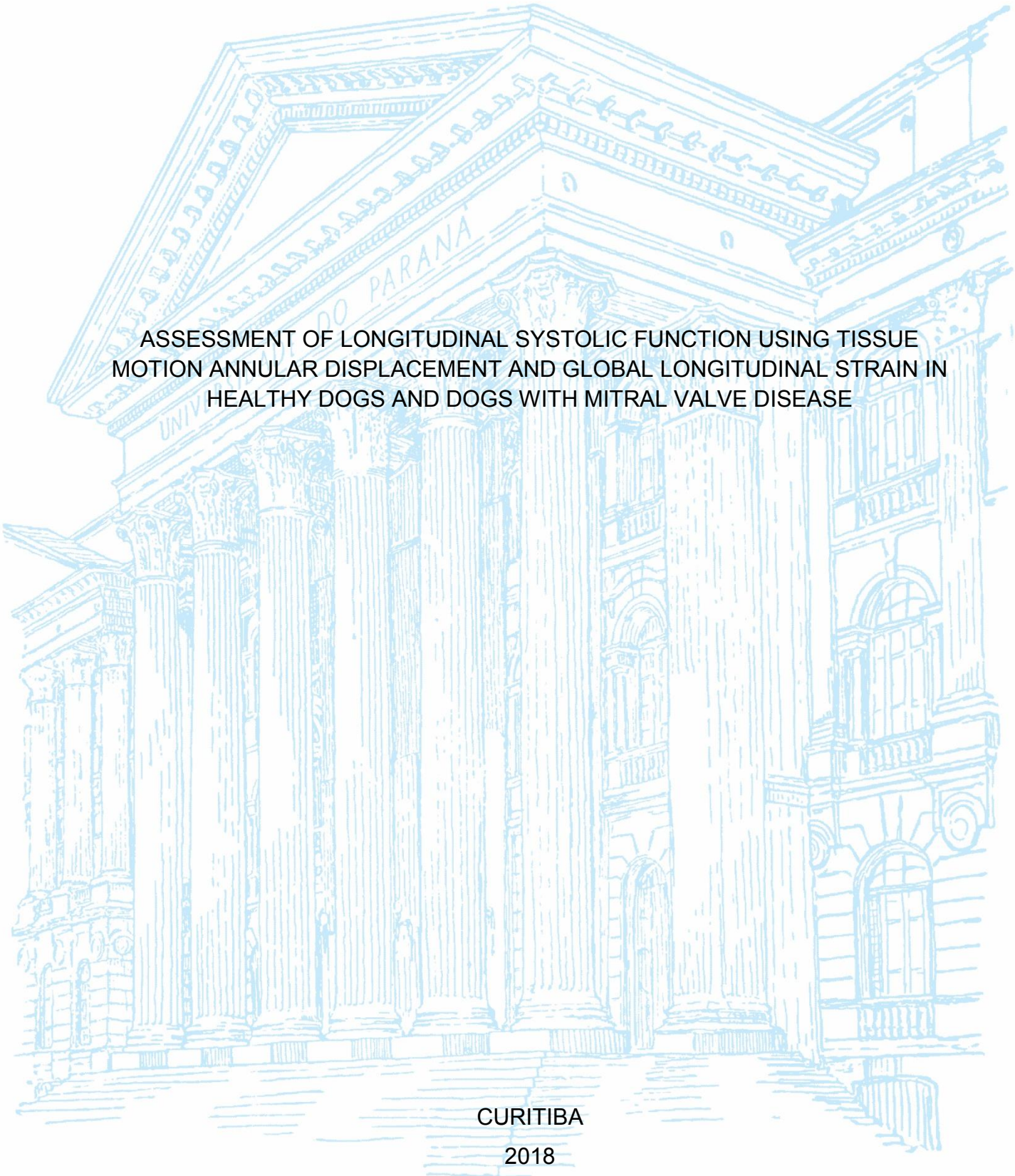

ASSESSMENT OF LONGITUDINAL SYSTOLIC FUNCTION USING TISSUE MOTION ANNULAR DISPLACEMENT AND GLOBAL LONGITUDINAL STRAIN IN HEALTHY DOGS AND DOGS WITH MITRAL VALVE DISEASE

CURITIBA

2018

MARCELA WOLF

AVALIAÇÃO DA FUNÇÃO SISTÓLICA LONGITUDINAL POR MEIO DO DESLOCAMENTO TECIDUAL DO ANEL MITRAL E *STRAIN* LONGITUDINAL GLOBAL EM CÃES SAUDÁVEIS E EM CÃES COM DOENÇA VALVAR MITRAL

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, do Setor de Ciências Agrárias, da Universidade Federal do Paraná, como requisito parcial para a obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. Dr. Marlos Gonçalves Sousa

CURITIBA

2018

FICHA CATALOGRÁFICA ELABORADA PELO SISTEMA DE BIBLIOTECAS/UFPR -
BIBLIOTECA DE CIÊNCIAS AGRÁRIAS, DOUGLAS ALEX JANKOSKI CRB 9/1167
COM OS DADOS FORNECIDOS PELO(A) AUTOR(A)

W853a

Wolf, Marcela
Assessment of longitudinal systolic function using tissue motion annular displacement and global longitudinal strain in healthy dogs and dogs with mitral valve disease / Marcela Wolf. - Curitiba, 2018.
86 f.: il., grafs., tabs.

Dissertação (Mestrado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.
Orientador: Marlos Gonçalves Sousa

1. Cães - Doenças - Diagnóstico por imagem. 2. Coração - Ventrículo esquerdo. 3. Cardiologia veterinária. I. Sousa, Marlos Gonçalves. II. Título. III. Universidade Federal do Paraná.

CDU 619.6:636.7

MINISTÉRIO DA EDUCAÇÃO
SETOR SETOR DE CIENCIAS AGRARIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS VETERINÁRIAS

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **MARCELA WOLF** intitulada: **ASSESSMENT OF LONGITUDINAL SYSTOLIC FUNCTION USING TISSUE MOTION ANNULAR DISPLACEMENT AND GLOBAL LONGITUDINAL STRAIN IN HEALTHY DOGS AND DOGS WITH MITRAL VALVE DISEASE**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
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CURITIBA, 19 de Março de 2018.

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AGRADECIMENTOS

Primeiramente, meu agradecimento a Deus, por me permitir viver tudo isso e sempre guiar meus passos, me iluminar e proteger.

Aos meus pais Tadeu e Conceição e ao meu irmão Thiago, por serem meu porto seguro, por todo o amor, apoio, suporte e compreensão. Obrigada por sempre estarem tão perto, mesmo estando tão longe.

Ao meu amor Carlos, por todo o amor, apoio, paciência e compreensão. Obrigada por sempre me incentivar.

Ao professor Marlos, pela orientação, oportunidade e acolhimento fraternal. Agradeço pela amizade, pelos conhecimentos repassados e por ser esse educador e ser humano extraordinário e inspirador. Meu eterno agradecimento e admiração.

À equipe do Laboratório de Cardiologia Comparada da UFPR, pela parceria, acolhimento, pela ajuda no desenvolvimento desse trabalho, pelos ensinamentos, pela paciência e por tornarem tudo mais leve e feliz. É um imenso prazer fazer parte dessa equipe.

A toda equipe do Hospital Veterinário da UFPR (residentes, pós-graduandos, funcionários, alunos e professores) e a todos os amigos feitos durante o mestrado, por todo o conhecimento compartilhado, companheirismo, colaboração e afeto.

Ao programa de Pós-graduação em Ciências Veterinárias e a todos os professores, pela oportunidade, pelos conhecimentos e tamanha contribuição nessa etapa de minha vida.

A todos os animais e seus tutores, pela confiança, disposição e por me permitirem crescer e amadurecer tanto ao realizar esse trabalho. À equipe do 20º Batalhão de Infantaria Blindado de Curitiba, em especial à Dra. Cristina Rauen Ribas, pela disposição e contribuição com esse estudo.

À Coordenação de Aperfeiçoamento Pessoal de Nível Superior, pelo auxílio financeiro com a bolsa de estudos.

A todos, meu sincero e emocionado muito obrigada.

“*If you can dream it, you can do it.”*

- Walt Disney

RESUMO

Nessa dissertação foi avaliada a função sistólica longitudinal do ventrículo esquerdo em cães saudáveis e em cães com doença valvar mitral por meio do deslocamento tecidual do anel mitral (TMAD) e *Strain* Longitudinal Global (GLS). O TMAD é uma técnica de ecocardiografia baseada em *Speckle Tracking* que avalia a competência das fibras miocárdicas longitudinais por meio do deslocamento do anulo mitral em direção ao ápice durante a sístole ventricular. Para tanto, esse trabalho foi subdividido em introdução e dois capítulos. O primeiro capítulo investigou a utilização do TMAD como método diagnóstico da função longitudinal em cães saudáveis. Para isso, foram avaliados 153 cães de diversas raças, pesos e idades. Os resultados mostraram que o TMAD é uma excelente técnica para avaliação da função longitudinal em cães, com correlação com diversas variáveis de função sistólica obtidas pela ecocardiografia convencional e com o GLS, além de ser rapidamente executado e com baixa variabilidade intra e interobservador. No segundo capítulo, foram recrutados 126 cães em diversos estágios da doença valvar mitral e 46 cães saudáveis como grupo controle, a fim de comparar e avaliar o comportamento da função sistólica longitudinal durante a evolução da doença para quadros de insuficiência cardíaca. Os resultados mostraram que não houve disfunção sistólica longitudinal evidente com a progressão da doença valvar mitral, visto que os valores de TMAD foram maiores nos estágios B2 e no grupo sintomático que no grupo controle e no estágio B1 da referida afecção cardíaca. O TMAD e o GLS apresentaram boa repetibilidade e se correlacionaram com diversos parâmetros ecocardiográficos, como AE/Ao, diâmetro interno do ventrículo esquerdo em sístole e diástole, onda E mitral e relação E/TRIV, sugerindo que essas técnicas são influenciadas por pré e pós carga. O TMAD é uma técnica mais rápida para ser realizada e pode ser uma alternativa ao GLS para avaliar a função sistólica longitudinal em cães saudáveis e em cães com doença valvar mitral.

Palavras-chave: *Speckle tracking*; ventrículo esquerdo; contratilidade longitudinal; ecocardiografia; endocardiose.

ABSTRACT

In this dissertation tissue motion annular displacement (TMAD) and global longitudinal strain (GLS) were used to assess the left ventricular longitudinal systolic function in healthy dogs and in dogs with mitral valve disease. TMAD is an echocardiography technique based on Speckle Tracking that evaluates the competence of the longitudinal myocardial fibers by displacement of the mitral annulus toward the apex during ventricular systole. This dissertation is subdivided in introduction and two chapters. The first chapter investigated the use of TMAD as a diagnostic method for evaluation of longitudinal function in healthy dogs. For this, 153 dogs of different breeds, varying body weights and ages were recruited. TMAD was demonstrated to be an excellent technique for the evaluation of longitudinal function in dogs. It correlated with GLS and with several surrogates of systolic function obtained by conventional echocardiography. Also, it was quickly obtained and had a low intra- and inter-observer variability. In the second chapter, 126 dogs at various stages of mitral valve disease and 46 healthy dogs as a control group were enrolled in order to compare and evaluate the behavior of longitudinal systolic function along the progression of mitral valve disease to a heart failure status. The results showed no evident longitudinal systolic dysfunction as the disease progressed. TMAD was higher in stage B2 and in the symptomatic dogs as compared to stage B1 and control groups. TMAD and GLS had a good repeatability and correlated with several echocardiographic parameters, such as LA/Ao, left ventricular internal diameter in systole and diastole, mitral E wave and E/IVRT, suggesting that changes in preload and afterload play a role in such parameters. TMAD is a faster technique to perform and may be an alternative to GLS to assess longitudinal systolic function in healthy dogs and in dogs with mitral valve disease.

Key words: Speckle tracking; left ventricle; longitudinal contractility; echocardiography; endocardiosis.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS

AP4 -	Apical 4-chamber
AP2 -	Apical 2-chamber
ACVIM -	American College of Veterinary Internal Medicine
BSA -	Body surface area
EF -	Ejection fraction
FS -	Fractional shortening
GLS -	Global longitudinal strain
HF -	Heart failure
ISACHC -	International Small Animal Cardiac Health Council
IVRT -	Isovolumic relaxation time
LA:Ao -	Left atrial to aortic ratio
LSt -	Longitudinal strain
LV -	Left ventricle
MAM -	Mitral annulus motion
MVD -	Mitral valve disease
MAPSE -	Mitral annular plane systolic excursion
ROI -	Region of interest
SBP -	Systolic blood pressure
STE -	Speckle tracking echocardiography
TMAD -	Tissue motion annular displacement

SUMÁRIO

INTRODUCTION

Left ventricular systolic function is one of the main parameters evaluated by conventional echocardiography (Atkins et al., 1992). The heart is composed by myocardial fibers oriented longitudinally and circumferentially and the joint efficiency of these fibers is one of the main intrinsic factors to determine systolic function (Sengupta et al., 2006).

The contraction of the longitudinal fibers promotes the shortening of the heart in the longitudinal direction (apex-base), while the circumferential fibers in the transverse direction (Sengupta et al., 2006). Routinely, left ventricular function is assessed in conventional transthoracic echocardiography by measures such as shortening fraction and ejection fraction, obtained in the short axis by M-mode (Bonagura and Schouber, 2009). However, by the fact to obtain these parameters in the short axis are evaluated essentially the circumferential fibers and consequently the longitudinal fibers are poorly explored in routine examination (Jones et al., 1990). Another important factor is that these parameters are highly affected by load changes and poorly correlated with invasive methods of volume assessment, especially in animals with heart disease (Atkins 1992; Bonagura and Schober, 2009).

Studies have shown that longitudinal myocardial fibers may be previously affected to circumferential fibers in the development of systolic dysfunction (Mizuguchi et al., 2008; Chetboul and Tissier, 2012). In addition, the circumferential fibers can increase their contractile competence and compensate the longitudinal deficit, keeping the volumetric indexes within normality (Mizuguchi et al., 2008). This demonstrates the importance of long axis evaluation, which can provide an early

diagnosis of systolic dysfunction (Jones et al., 1990, Suzuki et al., 2012, Fente et al., 2017).

Mitral valve disease (MVD), also known as endocardiosis or canine degenerative myxomatous mitral valve disease, is the main acquired heart disease of dogs, mainly affecting small to medium-sized animals with etiology not yet fully understood (Atkins et al., 2009). Studies have suggested that this may be a disease with polygenic inheritance (Meurs et al, 2010), resulting in a continuous deposition of glycosaminoglycans in valvular leaflets and chordae tendineae (Freed et al., 2002).

The American College of Veterinary Internal Medicine (ACVIM) classified MVD in five stages, stage A being the healthy animals (without cardiac murmur) but with high risk for the development of the disease. Asymptomatic animals are classified in stage B1 when abstinent from cardiac remodeling and B2 when present a cardiac enlargement diagnosed by echocardiography or radiography. In contrast, animals with clinical signs of heart failure responding to conventional medication are classified in stage C. Finally, stage D consists of animals in the terminal phase of the disease and refractory to therapy (Atkins et al., 2009).

Although it is a valve disease, MVD may culminate in myocardial changes, such as fibrosis, and consequently compromise systolic function (Falk et al., 2006; Borgarelli et al., 2007). Myocardial dysfunction in MVD appears to be already present in the initial phase of the disease (Urabe et al., 1992). However, some studies show that the contractile deficit occurs mainly in the more advanced stages, when there is the development of congestive heart failure (Borgarelli et al., 2007; Chetboul and Tissier, 2012). A study of 74 dogs with mitral disease showed that in the later stages of the disease, the animals presented lower values of longitudinal myocardial deformation than healthy animals, whereas there was no difference in the radial

deformation between these two groups. This shows that longitudinal function may be affected primarily in MVD (Chetboul and Tissier, 2012).

The contraction of the longitudinal myocardial fibers promotes a displacement of the mitral annulus towards the apex during systole. The amplitude of this displacement can be assessed by M-mode on conventional echocardiography by mitral annulus motion (MAM), also called mitral annular plane systolic excursion (MAPSE). This technique has already been used to determine longitudinal function in healthy dogs and in dogs with MVD (Schober e Fuentes, 2009; Sousa et al., 2016). Recent human studies have evaluated this displacement of the mitral annulus by a Speckle tracking echocardiography (STE) method, denominated Tissue Motion Annular Displascement (TMAD). The TMAD is a technique performed offline in apical images previously obtained, in which the operator determines 3 regions of interest (ROIs), being: 2 of them in the mitral annulus and one in the epicardial region of the left ventricle. After the definition of the ROIs, the software automatically provides the displacement of the ROIs of the mitral annulus towards the ROI at the apex of the left ventricle, being an effective method of evaluation of the longitudinal function (Buss et al., 2012; Suzuki et al., 2012), which for the knowledge of the authors has never been evaluated in dogs.

Others echocardiographic techniques of STE, as Strain and Strain rate also allow the evaluation of longitudinal function, because they provide the percentage of myocardial deformation (Strain) and the velocity of this deformation (Strain rate), which have already been studied in healthy dogs (Chetboul et al., 2006; Wess et al., 2011; Westrup and McEvoy, 2013) and in dogs with MVD (Zois et al., 2012; Suzuki et al., 2013 , Mantovani et al., 2015).

This dissertation is subdivided into two chapters, in which the first demonstrates a longitudinal systolic function study using TMAD and Global Longitudinal Strain (GLS) in 153 healthy dogs. From the authors' knowledge, TMAD had never been studied in dogs, so this first chapter aims to evaluate the use of TMAD in a heterogeneous group of healthy dogs and to correlate it with conventional echocardiography and GLS, which is an effective method to evaluate longitudinal function in dogs (Chetboul et al., 2006; Wess et al., 2011; Westrup and McEvoy, 2013).

With satisfactory results of the use of TMAD as diagnostic method in healthy dogs discussed in chapter 1, we developed a second study (Chapter 2) to evaluate TMAD in dogs with MVD. For this, 126 dogs with mitral valve disease in several stages were submitted to conventional and STE (GLS and TMAD) in order to evaluate the behavior of longitudinal systolic function and the use of TMAD as a diagnostic method in the course of this heart disease.

REFERENCES

ATKINS, C.; BONAGURA, J; ETTINGER, S.; FOX, P.; GORDON, S.; HÄGGSTRÖM, J.; HAMLIN, R.; KEENE, B.; LUIS-FUENTES, V.; STEPIEN, R. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. **Journal of Veterinary Internal Medicine**, v.23, n.6, p.1142-1150, 2009.

ATKINS, C.E.; CURTIS, M.B.; MCGUIRK, S.M.; KITTLESON, M.D.; SATO, T.; SNYDER, P.S. The Use of M-Mode Echocardiography in Determining Cardiac output in dogs with normal, low, and high output states: comparison to thermodilution method. **Veterinary Radiology and Ultrasound**, v.33, n.5, p.297-304, 1992.

BONAGURA, J.D.; SCHOBER, K.E. Can ventricular function be assessed by echocardiography in chronic canine mitral valve disease? **Journal of Small Animal Practice**, v.50, p.12-24, 2009.

BORGARELLI, M.; TARDUCCI, A.; ZANNATA, R.; HÄGGSTRÖM, J. Decreased Systolic Function and Inadequate Hypertrophy in Large and Small Breed Dogs with

Chronic Mitral Valve Insufficiency. **Journal of Veterinary Internal Medicine**, v.21, p.61-67, 2007.

BUSS, S.J.; MERELES, D.; EMAMI, M.; KOROSOGLOU, G.; RIFFEL, J.H.; BERTEL, D.; SCHONLAND, S.O.; HEGENBART, U.; KATUS, H.A.; HARDT, S.E. Rapid assessment of longitudinal systolic left ventricular function using speckle tracking of the mitral annulus. **Clinical Research in Cardiology**, v.101, p.273-280, 2012.

CHETBOUL, V.; SAMPEDRANO, C.C.; GOUNI, V.; NICOLLE, A.P.; POUCHELON, J.L.; TISSIER, R. Ultrasonographic assessment of regional radial and longitudinal systolic function in healthy awake dogs. **Journal of Veterinary Internal Medicine**, v.20, p.885-893, 2006.

CHETBOUL, V.; TISSIER, R. Echocardiographic assessment of canine degenerative mitral valve disease. **Journal of Veterinary Cardiology**, v.14, p.127-148, 2012.

FALK, T.; JONSSON, L.; OLSEN, L.H.; PEDERSEN, H.D. Arteriosclerotic changes in the myocardium, lung, and kidney in dogs with chronic congestive heart failure and myxomatous mitral valve disease. **Cardiovascular Pathology**, v.15, p.185-193, 2006.

FENTE, G.J.; GARG, P.; FOLEY, J.R.J.; DOBSON, L.E.; MUSA, T.A.; ERHAYIEM, B.; GREENWOOD, J.P.; PLEIN, S.; SWOBODA, P.P. The utility of global longitudinal strain in the identification of prior myocardial infarction in patients with preserved left ventricular ejection fraction. **The International Journal of Cardiovascular Imaging**, p.7-9, 2017.

FREED, L.A.; BENJAMIN, E.J.; LEVY, D.; LARSON, M.G.; EVANS, J.C.; FULLER, D.L.; LEHMAN, B.; LEVINE, R.A. Mitral valve prolapse in the general population. **Journal of the American College of Cardiology**, v.40, p.1298-1304, 2002.

JONES, C.J.H.; RAPOSO, L.; GIBSON, D.G. Functional importance of the long axis dynamics of the human left ventricle. **British Heart Journal**, v.63, p.215-220, 1990.

MANTOVANI, M.M.; MUZZI, R.A.L.; PEREIRA, G.G.; YAMATO, R.J.; SILVA, A.C.; REIS, G.F.; MUZZI, L.A.L.; GUIMARÃES, E.C. Systolic cardiac function assessment by feature tracking echocardiography in dogs with myxomatous mitral valve disease. **Journal of Small Animal Practice**, p.1-10, 2015.

MEURS, K.M. Genetics of the cardiac disease in the small animal patient. **Veterinary Clinics of North America: Small Animal Practice**, v.40, p.701-715, 2010.

MIZUGUCHI, Y.; OISHI, Y.; MIYOSHI, H.; IUCHI, A.; NAGASE, N.; OKI, T. The functional role of longitudinal, circumferential and radial myocardial deformation for regulating the early impairment of left ventricular contraction and relaxation in patients with cardiovascular risk factors: a study with two-dimensional strain imaging. **Journal of the American Society of Echocardiography**, v.21, n.10, p.1138-1144, 2008.

SCHOBER, K.E.; FUENTES, V.L. Mitral annulus motion as determined by M-mode echocardiography in normal dogs and dogs with cardiac disease. **Veterinary Radiology and Ultrasound**, v.42, n.1, p.52-61, 2001.

SENGUPTA, P.P.; KORINEK, J.; BELOHLAVEK, M.; NARULA, J.; VANNAN, M.A.; JAHANGIR, A.; KHANDHERIA, B.K. Left Ventricular Structure and Function - Basic Science for Cardiac Imaging. **Journal of the American College of Cardiology,** v.49, p.1988-2001, 2006.

SOUSA, M.G.; GAVA, F.N.; FILHO, J.C.S.; SILVA, S.N.S.; CAMACHO, R.R.; CARARETO, R.; WOLF, M.; CAMACHO, A.A. Long-axis fractional shortening and mitral annulus motion in dogs. **Semina: Ciências Agrárias**, v.37, n.5, p.3115-3124, 2016.

SUZUKI, K.; AKASHI, Y.J.; MIKUKOSHI, K.; KOU, S.; TAKAI, M.; IZUMO, M.; HAYASHI, A.; OHTAKI, E.; NOBUOKA, S.; MIYAKE, F. Relationship between left ventricular ejection fraction and mitral annular displacement by speckle tracking echocardiography in patients with different heart diseases. **Journal of Cardiology**, v.60, p.55-60, 2012.

SUZUKI, R.; MATSUMOTO, H.; TESHIMA, T.; KOYAMA, H. Clinical assessment of systolic myocardial deformations in dogs with chronic mitral valve insufficiency using two-dimensional speckle-tracking echocardiography. **Journal of Veterinary Cardiology**, v.15, p.41-49, 2013.

URABE, Y.; MANN, D.L.; KENT, R.L.; NAKANO, K.; TOMANEK, R.J.; CARABELLO, B.A.; COOPER, G. Cellular and ventricular contractile dysfunction in experimental canine mitral regurgitation. **Circulation Research**, v.70, p.131-147, 1992.

WESS, G.; KELLER, L.J.M.; KLAUSNITZER, M,; KILLICH, M.; HARTMANN, K. Comparasion of longitudinal myocardial tissue velocity strain, and strain rate measured by two-dimensional speckle tracking and by color tissue doppler imaging in healthy dogs. **Journal of Veterinary Cardiology**, v.13, p.31-43, 2011.

WESTRUP, U.; MCEVOY, F.J.. Speckle tracking echocardiography in mature Irish Wolfhound dogs: technical feasibility, measurement error and reference intervals. **Acta Veterinaria Scandinavica**, v.55, n.41, p.1-11, 2013.

ZOIS, N.E.; TIDHOLM, A.; NAGGA, K.M.; MOESGAARD, S.G.; RASMUSSEN, C.E.; HÄGGSTRÖM, J. PEDERSEN, H.D.; ABLAD, B.; NILSEN, H.Y.; OLSEN, L.H. Radial and longitudinal strain and strain rate assessed by speckle-tracking echocardiography in dogs with myxomatous mitral valve disease. **Journal of Veterinary Internal Medicine**, v.26, p.1309-1319, 2012.

CHAPTER 1 - Assessment of Longitudinal Systolic Function Using Tissue Motion Annular Displacement in Healthy Dogs

Acknowledgments

The authors are grateful for the financial support provided by Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Running head: Tissue Motion Annular Displacement in Healthy Dogs

Abstract

Introduction: Left ventricular systolic function is one of the main parameters studied in echocardiography. Longitudinal systolic function, however, is less commonly evaluated in routine examinations but may provide early information on systolic dysfunction. The movement of the mitral annulus toward the apex during systole has already been determined as a method for evaluation of longitudinal systolic function in dogs, but the study of this movement by Speckle tracking with the TMAD technique has not yet been evaluated.

Animals: One hundred fifty-three client-owned healthy dogs.

Methods: Cross-sectional study. One hundred fifty-three client-owned healthy dogs underwent physical examination, electrocardiography, blood pressure measurement, as well as a standard and speckle tracking echocardiography. Systolic function was evaluated by Global Longitudinal Strain (GLS) and TMAD. These parameters were compared with the standard echocardiographic data.

Results: A correlation was found between GLS, TMAD and body weight. Tissue motion annular displacement and GLS were significantly correlated (P<0.001) with other surrogates of systolic function, including ejection fraction and shortening fraction. There were no significant differences in TMAD between sexes. The coefficient of variation of the intra-observer evaluation in the global TMAD (*CV* 4.44) was slightly higher than the GLS (*CV* 3.74). TMAD was not influenced by cardiac rhythm and could be acquired more rapidly than GLS.

Conclusions - Tissue motion annular displacement is a rapid and reproducible method for the assessment of left ventricle longitudinal function in healthy dogs. However, more studies are needed to validate the real clinical applicability of TMAD in animals with heart diseases.

Key words: echocardiography; Speckle tracking; longitudinal contractility; strain imaging; left ventricle.

List of Abbreviations

AP4	Apical 4-chamber
AP2	Apical 2-chamber
BSA	Body surface area
BW	Body weight
EF	Ejection fraction
FS	Fractional shortening
GLS	Global longitudinal strain
HR	Heart rate
LSt	Longitudinal strain

MAM	Mitral annulus motion
ROI	Region of interest
SBP	Systolic blood pressure
TMAD	Tissue Motion Annular Displacement

INTRODUCTION

Left ventricular systolic function may be evaluated by measurement of several parameters in conventional echocardiography [1]. However, measurements such as the fractional shortening (FS) and ejection fraction (EF) obtained by M-mode specifically evaluate the circumferential myocardial fibers. It is thought that ventricular contraction is mainly driven by the circumferential myocardial fibers [2,3], but longitudinal fibers also play a relevant role in systole [4,5]. Interestingly, Jones *et al* [6] reported that the longitudinal myocardial fibers are the first to be impaired in systolic dysfunction. In dogs, standard echocardiography provides information on systolic function using parameters that are known to be affected by volume overload, as well as being highly influenced by the operator's experience and image quality [7,8]. Thus evaluation of longitudinal fibers may assist in echocardiographic assessment of systolic function.

In addition to the conventional assessment of systolic function by standard echocardiography, several echocardiographic techniques are used to evaluate the longitudinal systolic function in dogs. The difference of the mitral annular displacement in systole and diastole obtained by M-mode in the septal and lateral parts of the mitral annulus corresponds to the mitral annulus motion (MAM), a technique that provides information on long axis' systolic function [9]. Also, the longitudinal shortening fraction is another method of evaluation of the longitudinal

contraction which can be acquired by the ratio between MAM and left ventricular internal dimension at end -diastole obtained from the apical 4-chamber view [10]. Other techniques obtained by speckle tracking, such as longitudinal strain and strain rate, have also been investigated in dogs and evaluate the percentage of myocardial deformation and the velocity of such deformation [11-14].

Recent studies in man have shown that Tissue Motion Annular Displacement (TMAD) is a rapid technique that is less dependent on high definition imaging and significant operator experience when compared to measurement of ejection fraction [15,16]. Moreover, TMAD provides information on systolic function encompassing longitudinal fibers by the degree of displacement of the annulus towards the apex. Tissue Motion Annular Displacement of the mitral valve is obtained from the definition of 3 regions of interest (ROIs): two in the mitral annulus and one at the apex of the left ventricle and provides information of longitudinal systolic function based on the distance of the excursion of the points of the mitral annulus toward the cardiac apex during systole [15,17].

At least in people, TMAD is believed to allow the early diagnosis of systolic dysfunction because individuals with early systolic impairment may have a preserved ejection fraction when evaluated with conventional echocardiography although the TMAD may already be reduced [15]. Because a compensatory increase in circumferential shortening can maintain normal systolic indices despite the decreased longitudinal systolic function, a multidirectional myocardial evaluation might be rewarding for diagnostic purposes [15,18-19]. Interestingly, although other techniques that evaluate the distance of mitral annular displacement during systole have already been studied, to the author's knowledge the Speckle Tracking TMAD technique has never been investigated in dogs.

The purposes of this study were three-fold: (1) to evaluate the longitudinal systolic function in healthy dogs by TMAD; (2) to investigate whether a correlation exists between TMAD and either the Global Longitudinal Strain (GLS) or other systolic function parameters derived from conventional echocardiography; and (3) to determine if TMAD is influenced by age, sex, body weight, heart rate or cardiac rhythm.

MATERIALS AND METHODS

Animals

The cross-sectional observational study included 153 client-owned healthy dogs of various breeds and ages enrolled prospectively between October 2016 and April 2017 at the cardiology section of a veterinary teaching facility. All dogs underwent a thorough physical examination, blood pressure assessment, electrocardiography and echocardiography. Systolic blood pressure (SBP) was measured indirectly in all dogs by trained observers (MW, SBL) using the Doppler technique, as described elsewhere [20]. Several measurements were taken over 5 to 10 minutes to obtain an average of 5 values of stable measurements. Also, a 3-minute computer-based electrocardiogram[a] was recorded immediately prior to echocardiography in all animals.

Hypertensive animals (defined as systolic arterial blood pressure >150 mmHg), as well as dogs with non-sinus arrhythmias, acquired or congenital heart diseases, and cardiac neoplasms were excluded from this investigation. All procedures were previously approved by the Institutional Animal Use Committee (protocol 072-2016), and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Echocardiography

The echocardiographic examination[b] was performed in all animals without sedation, with the dogs positioned in left and right lateral recumbency in accordance with the recommendations of the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine [21]. All echocardiographic examinations and measurements were performed by the same operator (MW), and included FS and EF (calculated by the Teichholz formula) obtained by M-mode from right parasternal short axis images. Two-dimensional short axis images obtained from the right parasternal window were used to calculate the left atrium-to-aorta ratio (LA:Ao). From the left apical 5-chamber view, pulsed wave Doppler between the transmitral flow and the aortic flow was used to record the isovolumic relaxation time. Left apical 4-chamber view was used to record peak early (E) and late (A) diastolic mitral inflow velocities and the E/A ratio. Tissue Doppler was used to measure the mitral annular early diastolic (E´), late diastolic (A´) velocity, and E´/A´ ratio. Also, the aortic valve closure time (R to AV closure) was documented from the apical 5-chamber image (left ventricular outflow), and corresponded to the time from the beginning of the QRS complex to the end of the aortic valve spectra, which was obtained with the pulsed Doppler gate positioned distal to the aortic valves. That time, in milliseconds, was required for obtaining both the peak systolic Longitudinal Strain (LSt) and TMAD.

Longitudinal Strain

During transthoracic echocardiographic examination, two-dimensional apical 4- (AP4) and 2-chamber (AP2) images (Fig 1) were obtained for at least 5 cardiac cycles from the left parasternal window. The echocardiographic equipment software[c] automatically detected the left ventricular myocardium to be screened. Manual

corrections were made only a few times when the automatic tracking of the software was obviously incorrect. The LSt was determined in the apical 4- and 2-chamber views as a percentage (%) of left ventricle myocardial deformation in a heartbeat. The GLS was calculated as follows:

$$GLS = (LSt\ AP4 + LSt\ AP2)/2$$

Tissue Motion Annular Displacement

The TMAD was calculated in both the 4- and 2-chamber apical echocardiographic images (Fig 1) obtained from the left parasternal window using a two-dimensional strain-based tissue tracking technique. Two ROIs were defined: at the origin of the mitral valve leaflets at the septal and lateral parts for the apical 4-chamber view or at the anterior and inferior parts of the mitral annulus for the apical 2-chamber view. The third ROI was set at the epicardial region of the apex of the left ventricle. After setting the ROIs, tracking was performed automatically by the equipment software.

The degree of motion was automatically calculated as the base-to-apex displacement of both annulus (in mm), the midpoint, that is determined virtually between the two ROIs of the mitral annulus (in mm), and a percentage value (%) of the midpoint in relation to the total length of the left ventricle. The global TMAD was calculated in four different ways: global TMAD (mm) is the mean of the midpoint value, global TMAD (%) is the mean of the percentage value of midpoint in the AP4 and AP2 chambers view, TMAD (mm/m²) is global TMAD (mm) index to body surface area (BSA) and the cube root BW-indexed TMAD (mm/kg) as previously described [22]. Indexing by body surface area was performed according to the following formula:

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

K = constant (10.1 for dogs)

Intra- and interobserver variability and time requirements

For the repeatability study, 20 animals were randomly reassessed of previously obtained images with a minimum interval of 30 days from the first evaluation by the same observer to calculate the intra-observer variability. The same studies were examined by a co-investigator (MGS), who was blinded to the results of the first investigation, in order to measure interobserver variability. The required time for offline analyses (without image acquisition time) for both strain imaging and TMAD was documented from another 20 studies.

Statistical Analysis

The normality of the data was investigated using the Shapiro-Wilk test. Since the data was non-parametric, the results are presented as the median (interquartile range). For TMAD and LSt analyses, dogs were divided into body weight quartiles, which were compared with the Kruskal-Wallis test, followed by the *post-hoc* Dunns test. The same test was used to investigate differences in TMAD in accordance with cardiac rhythm, while the comparison between results obtained for males and females was obtained with the Mann-Whitney test. Mann-Whitney test was also used to compare the time (in seconds) for off-line analyses of GLS and TMAD. Spearman´s test was used to investigate correlations with ages, SBP, and heart rate (HR) obtained from ECG recordings immediately prior to the echocardiographic examination, as well as to correlate TMAD, LSt and the regular echocardiographic data. Also, coefficients of variation were calculated to assess intra- and inter-observer measurements. All statistical analyses were performed with either the

software Graphpad Prism®[d] or Microsoft Excel. A *P* value <0.05 was considered significant.

RESULTS

A total of 153 dogs were recruited for this study. The population included small- to large-sized dogs, including Australian cattle dog, Chowchow, Cocker, Boxer, Golden retriever, Siberian husky, Great dane, Collie, Chihuahua, Maltese, Pekingese, Samoyed (n= 1 each), Beagle (n=20), Border collie (n=2), French bulldog (n=8), Dachshund (n=4), Doberman Pinscher (n=2), Jack Russell terrier (n=2), Labrador retriever (n=6), Lhasa apso (n=9), German Shepherd (n=7), Malinois Shepherd (n=2), Miniature Pinscher (n=2), American pit bull terrier (n=2), Poodle (n=4), Pug (n=2), Rottweiler (n=3), Schnauzer (n=8), Shih tzu (n=2), Whippet (n=3), Yorkshire (n=6) and crossbreed dogs (n=47). Fifty-five dogs (35.9%) were male, and 98 (64.1%) were female. The animals were between 4 and 192 months of age (mean 54.3; median 36), while body weight ranged from 1.7 to 50 kilograms (mean 15.4; median 11.6). The most observed cardiac rhythm was sinus arrhythmia (52.3%) followed by sinus rhythm (41.2%) and sinus tachycardia (6.5%).

Both TMAD and LSt varied in accordance with the size of the animals. Differences were documented when body weight (BW) quartiles were compared and the proposal of the values for each quartile is represented on table 1. Our results show a negative correlation between BW and GLS, which contrasts with the greater displacement of the mitral annulus (in mm) in heavier dogs (Table 1, Fig. 2). However, when the LSt was compared with either the indexed TMAD or the TMAD%, positive correlations were found to exist (Fig. 3).

There were no correlation between the BSA-indexed TMAD (mm/m²) with regard to sex, SBP, and HR. However, the cube root BW-indexed TMAD (mm/kg) was significantly correlated with HR (R: -0.173; *P*: 0.033) while GLS was not (R: -0.0426 *P*: 0.6009). Global TMAD (%), BSA-TMAD (mm/m²) and the cube root BW-TMAD correlated significantly with several echocardiographic surrogates of volume, contractility and diastolic function, while the GLS correlated with FS, EF and isovolumetric relaxation time (Table 2).

Global longitudinal strain was influenced by cardiac rhythm (*P*: 0.02) whereas global TMAD was not (*P*: 0.37). Animals with sinus arrhythmia (median GLS 23.3 (20.8-25.3)) had higher GLS values than animals with sinus tachycardia (20.7 (19.4-23.2)) and sinus rhythm (21.3 (18.5-25)). Also, TMAD was shown to be a straightforward technique as compared to LSt. When the time needed to perform the offline analyses of either technique was compared, the median time for TMAD was less than the required time for strain analyses in both AP4 (TMAD:19.7s; LSt:43.5s) and AP2 (TMAD:18.9s; LST:40.1s) images.

Inter- and intra-observer repeatability analyses showed good coefficients. TMAD demonstrated either a reliability in both intra- (*CV* 4.44) and inter-observer (*CV* 4.12) analyses. The coefficients of variation of the TMAD were slightly higher than the LSt in the inter- and intra-observer evaluation, except for the comparison of the inter-observer, in which the GLS (*CV* 5.2) was higher than the TMAD (*CV* 4.12).

DISCUSSION

In this study, TMAD was compared with conventional echocardiography and GLS for the assessment of left ventricle longitudinal systolic function. Conventional echocardiography essentially evaluates radial shortening primarily involving the

circumferential myocardial fibers. Echocardiographic parameters obtained by M-mode are commonly used to provide cardiac function information, but the data obtained by this technique show little correlation when compared to invasive methods, especially in dogs with heart disease [1]. In contrast, in conventional echocardiography, the longitudinal myocardial fibers are poorly evaluated [6].

The contraction of the longitudinal fibers promotes shortening of the left ventricle in the longitudinal direction and consequently the mitral annulus moves towards the apex [6]. Some techniques performed with conventional echocardiography can be used to evaluate the longitudinal systolic function as well, such as the longitudinal shortening fraction and the MAM [9,10]. Speckle tracking echocardiography is becoming available for general veterinary practice and several studies have used it to evaluate longitudinal systolic function in healthy dogs [11-14], dogs with mitral valve disease [23-25], induced-cardiomyopathy [26], patent ductus arteriosus [27] and hyperadrenocorticism [28], but none of these studies used TMAD as an evaluation technique, at least to our knowledge.

Tissue motion annular displacement is a tracking technique that provides information on systolic function, from the degree of longitudinal deformation of the mitral annulus [17], not previously investigated in dogs. Thus, TMAD and GLS are rapid techniques for assessment of longitudinal function, complementing the information obtained from the regular echocardiographic procedure [15,17,23].

Global longitudinal strain is the sum of myocardial deformations in the longitudinal plane during systole [29] and provides accurate and angle-independent measurements when compared with magnetic resonance imaging [30]. Previous studies have determined GLS in healthy dogs [11,13,26]. The GLS recorded by Kusunose et al.[26] in 25 healthy animals was 18 ± 4%. In our study, higher values

were obtained, which is probably related to the greater BW variability of our population. Nonetheless, when we compared our results obtained in dogs with the same BW (21 to 35 kg), our GLS is similar (18.5%) to that reported by Kusunose et al. Interestingly, the results of this study are in agreement with another study which included a more heterogeneous population, in terms of weight and size [11].

Studies show that GLS has a strong correlation with TMAD (mm) in healthy people [18], in people with hypertrophic cardiomyopathy [31] and in individuals undergoing hemodialysis [32]. In this study, a negative correlation existed between GLS and TMAD (mm), probably attributable to the negative relationship between strain and BW. This fact might be explained by the physiological behavior of systolic function in dogs, with decreased systolic indices in large dogs [33]. While heavier animals have lower myocardial deformation [33-34], their larger heart size results in a greater absolute mitral displacement towards the apex [9]. Because the population of this study was heterogeneous, and a representative number of large dogs were enrolled. This fact also explains the disagreement with the medical literature, since heart size is relatively constant in adult human beings in contrast to the variations seen between dog breeds. Therefore the proposal values of TMAD are divided in quartiles of body weight, as shown in table 1.

To minimize the effect of BW, GLS was then compared with the global TMAD% and indexed TMAD (mm/m² and mm/kg, the least being derived from the cube root body weight). In all cases, positive correlations were found to exist. Therefore, despite higher values in mm, the percentage of annular displacement is lower in larger dogs. Similar results were found by Schober and Fuentes [9] with the MAM technique. A positive correlation of MAM (in cm) with body weight was observed, and when MAM was indexed by body surface area a negative correlation

was demonstrated. Although our study showed that BW plays a major role in TMAD and LSt, another study found no differences in the LSt between Beagle and Cavalier King Charles Spaniel [23]. These contrasting results are explained by the minimal variation in BW in that comparison, which did not include large dogs.

Significant correlations were documented between the global TMAD (mm), HR, and SBP. However, when the BSA-index TMAD was used, none of these parameters influenced TMAD. This is an interesting observation, probably attributable to the fact that small dogs are usually more nervous and often have an associated elevation in HR and SBP than larger dogs. Indexing TMAD to body surface area removed this bias. A study in dogs with mitral valve disease showed that HR affected strain rate values [23] possibly due to the compensatory mechanisms in response to disease, which were eliminated in this study as only healthy animals were evaluated. The same effect occurs for systemic arterial blood pressure. Although the increase in afterload was shown to produce lower strain values [35], no hypertensive dogs were included in this study.

Interestingly, TMAD was not affected by heart rhythm, whereas LSt was higher in animals with sinus arrhythmia. Due to the high numbers of dogs with sinus arrhythmia, this result might represent another benefit of TMAD over LSt in clinical practice.

In this study, a moderate correlation was shown between either GLS or global TMAD (mm, %, mm/m² and mm/kg) and left ventricular internal dimension at end - sistole and diastole (P <0,001), which indicate they are preload- and afterload-dependent surrogates. Other studies have demonstrated that the increase of preload and the decrease of afterload can directly increase LSt values [23,35]. Future studies

in animals with volume and pressure overload might characterize the behavior of TMAD in these circumstances.

The weak correlations between the systolic indices obtained from standard echocardiography and either the GLS or the indexed-TMAD (%, mm/m² or mm/kg) observed in this study are likely to be related to both EF or FS being surrogates of the radial myocardial shortening. Since both are obtained from the left-ventricular internal diameter measured in short-axis images, they do not effectively reflect the longitudinal contraction as observed by LSt and TMAD [11,18]. Similar results were obtained in people undergoing hemodialysis [32] and individuals with structural heart disease, probably due to the fact the TMAD is a technique with lower error bias, less dependent on image quality than EF [18,31].

In this study, a negative correlation between age and global TMAD was observed. In people, a physiological decrease in longitudinal contractility has been shown with age progression[36], but this has not been validated for dogs. The best way to validate this data in dogs would be a prospective evaluation of the same animal with advancing age [11].

Previous studies have shown that TMAD in people and LSt in people and dogs have a low inter- and intra-observer variability [15,18,23,28,31-32]. In this study, there was a good repeatability between duplicate measurements of TMAD, probably by the fact that TMAD does not require a good definition of endocardial borders [15,17,18,32]. This may be particularly important in patients with pulmonary edema, dyspnea and in situations in which a good echocardiographic images may be difficult to acquire.

A study in patients undergoing hemodialysis demonstrated that TMAD cannot provide prognostic information such as likelihood of cardiac death or cardiac events

[32]. In contrast, GLS allows the early detection of longitudinal systolic dysfunction in patients with acute myocardial infarction with preserved EF evaluated by magnetic resonance imaging [37]. Future studies in dogs may show the prognostic capacity of TMAD and the GLS in various heart diseases.

The results of this study also showed that TMAD is a more rapid technique when compared with LSt [17,18]. Tsang et al.[16] evaluated 118 human patients and found that all TMAD analyses were concluded in less than 10 seconds. In this study, the median time needed to complete TMAD was less than 20 seconds, while LSt required a median of approximately 45 seconds. In a single dog the time required to complete TMAD AP2 analysis was significantly longer (116.3 sec). However, in that case, unusually the definition of the lateral mitral annulus was of low quality and several manual corrections were necessary to allow the software to track the point towards the cardiac apex in an appropriate fashion. Of note, the TMAD technique itself does not impact on the overall exam time, since the analytical procedure is performed offline. The only exception to the conventional examination is acquiring the apical 2-chamber image, which may be done without difficulties. Subsequently, the analyzes performed offline take approximately 20 seconds each to be performed, being therefore a plausible technique to be included in routine examinations.

Some limitations of this study must be considered. The animals were considered healthy based on clinical evaluation, echocardiogram, electrocardiogram and blood pressure measurement. However, we cannot exclude the possibility of asymptomatic comorbidities that were not identified by specific ancillary examinations. Systemic blood pressure was obtained by a non-invasive method that is not considered the gold standard. The number and variability of animals may be too small to extrapolate the results obtained for all breeds. This study compared

TMAD and GLS with the systolic surrogates obtained by conventional echocardiography, which are influenced by several factors. Therefore, the absence of a gold standard noninvasive technique for comparison is a limitation to be considered.

In conclusion, global TMAD (%, mm/m² and mm/kg) shows positive correlation with GLS and with several echocardiographic parameters of systolic function. Also, TMAD allowed the assessment of longitudinal systolic function in a very straightforward fashion, and with a reliable intra- and inter-observer repeatability. It is, therefore, a new and promising tool in veterinary cardiology as an alternative to GLS for assessment of longitudinal systolic function. However, further studies are warranted to validate the real clinical applicability of TMAD in animals with heart diseases.

Footnotes

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

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

[c] - QLAB Software - automatic cardiac motion quantification (aCMQ).

[d] - Graphpad Prism® (v.5.0).

Conflict of Interest: Authors disclose no conflict of interest.

REFERENCES

[1] Atkins CE, Curtis MB, Mcguirk SM, Kittleson MD, Sato T, Snyder PS. The Use of M-Mode Echocardiography in Determining Cardiac output in dogs with normal, low,

and high output states: comparison to thermodilution method. Vet Radiol Ultrasound 1992;33:5:297-304.

[2] Wu EX, Wu Y, Tang H, Wang J, Yang J, Ng MC, Yang ES, Chan CW, Zhu S, Lau CP, Tse HF. Study of myocardial fiber pathway using magnetic resonance diffusion tensor imaging. J Magn Reson Imaging 2007;25:1048-1057.

[3] Sengupta PP, Korinek J, Belohlavek M, Narula J, Vannan MA, Jahangir A, Khandheria BK. Left Ventricular Structure and Function - Basic Science for Cardiac Imaging. J Am Coll Cardiol 2006; 48:1988-2001.

[4] Aurigemma GP, Silver KH, Priest MA, Gaasch WH. Geometric Changes Allow Normal Ejection Fraction Despite Depressed Myocardial Shortening in Hypertensive Left Ventricular Hypertrophy. J Am Coll Cardiol 1995;26:195-202.

[5] Bogaert J, Rademakers FE. Regional nonuniformity of normal and adult left ventricle. Am J Physiol Heart Circ Physiol 2001;280:H610-H620.

[6] Jones CJH, Raposo L, Gibson DG. Functional importance of the long axis dynamics of the human left ventricle. Br Heart J 1990;63:215-220.

[7] Kittleson MD, Eyster GE, Knowlen GG, Bari Olivier N, Anderson LK. Myocardial function in small dogs with chronic mitral regurgitation and severe congestive heart failure. J Am Vet Med Assoc 1984;184:455–459.

[8] Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Häggström J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med 2009;23:1142-1150.

[9] Schober KE, Fuentes VL. Mitral annulus motion as determined by M-mode echocardiography in normal dogs and dogs with cardiac disease. Vet Radiol Ultrasound 2001;42:52-61.

[10] Sousa MG, Gava FN, Filho JCS, Silva SNS, Camacho RR, Carareto R, Wolf M, Camacho AA. Long-axis fractional shortening and mitral annulus motion in dogs. Semina: Ciências Agrárias 2016;37:3115-3124.

[11] Chetboul V, Sampedrano CC, Gouni V, Nicolle AP, Pouchelon JL, Tissier R. Ultrasonographic assessment of regional radial and longitudinal systolic function in healthy awake dogs. J Vet Intern Med 2006;20:885-893.

[12] Wess G, Keller LJM, Klausnitzer M, Killich M, Hartmann K. Comparasion of longitudinal myocardial tissue velocity strain, and strain rate measured by two-dimensional speckle tracking and by color tissue doppler imaging in healthy dogs. J Vet Cardiol 2011;13:31-43.

[13] Westrup U, McEvoy FJ. Speckle tracking echocardiography in mature Irish Wolfhound dogs: technical feasibility, measurement error and reference intervals. Acta Vet Scand 2013;55:1-11.

[14] Carnabuci C, Hanas S, Ljungvall I, Tidholm A, Bussadori C, Häggström J, Höglund K. Assessment of cardiac function using global and regional left ventricular endomyocardial and epimyocardial peak systolic strain and strain rate in healthy Labrador retriever dogs. Res Vet Sci 2013; 95:241-248.

[15] Suzuki K, Akashi YJ, Mikukoshi K, Kou S, Takai M, Izumo M, Hayashi A, Ohtaki E, Nobuoka S, Miyake F. Relationship between left ventricular ejection fraction and mitral annular displacement by speckle tracking echocardiography in patients with different heart diseases. J Cardiol 2012;60:55-60.

[16] Tsang W, Ahmad H, Patel AR, Patel AR, Sugeng L, Salgo IS, Weinert L, Mor-Avi V, Lang RM. Rapid estimation of left-ventricular function using echocardiographic speckle-tracking of mitral annular displacement. J Am Soc Echocardiogr 2010;23:511-515.

[17] Iwakura K, Okamura A, Koyama Y, Inoue K, Nagai H, Toyoshima Y, Tanaka K, Oka T, Iwamoto M, Fujii K. Assessment of left ventricular global systolic function by tissue mitral annular displacement: comparison with global longitudinal strain [Abstract]. J Am Coll Cardiol 2016;67.

[18] Buss SJ, Mereles D, Emami M, Korosoglou G, Riffel JH, Bertel D, Schonland SO, Hegenbart U, Katus HA, Hardt SE. Rapid assessment of longitudinal systolic left ventricular function using speckle tracking of the mitral annulus. Clin Res Cardiol 2012;101:273-280.

[19] Mizuguchi Y, Oishi Y, Miyoshi H, Iuchi A, Nagase N, Oki T. The functional role of longitudinal, circumferential and radial myocardial deformation for regulating the early impairment of left ventricular contraction and relaxation in patients with cardiovascular risk factors: a study with two-dimensional strain imaging. J Am Soc Echocardiogr 2008;21:1138-144.

[20] Brown S, Atkins C, Bagley R, Carr A, Cowgill L, Davidson M, Egner B, Elliot J, Henik R, Labato M, Littman M, Polzin D, Ross L, Snyder P, Stepien. Guidelines for the Identification, Evaluation, and Management of Systemic Hypertension in Dogs and Cats. J Vet Intern Med 2007;21:542-558.

[21] Thomas WP, Gaber CE, Jacobs GJ. Recommendations for standards in transthoracic Two-Dimensional echocardiography in the dog and cat. J Vet Intern Med 1993;7:247-252

[22]Cornell CC, Kittleson MD, Torre Pd, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311-321.

[23] Zois NE, Tidholm A, Nagga KM, Moesgaard SG, Rasmussen CE, Häggstrröm J, Pedersen HD, Ablad B, Nilsen HY, Olsen LH. Radial and longitudinal strain and

strain rate assessed by speckle-tracking echocardiography in dogs with myxomatous mitral valve disease. J Vet Intern Med, 2012;26:1309-1319.

[24] Suzuki R, Matsumoto H, Teshima T, Koyama H. Clinical assessment of systolic myocardial deformations in dogs with chronic mitral valve insufficiency using two-dimensional speckle-tracking echocardiography. J Vet Cardiol 2013;15:41-49.

[25] Mantovani MM, Muzzi RAL, Pereira GG, Yamato RJ, Silva AC, Reis GF, Muzzi LAL, Guimarães EC. Systolic cardiac function assessment by feature tracking echocardiography in dogs with myxomatous mitral valve disease. J Small Anim Pract 2015:1-10.

[26] Kusunose K, Zhang Y, Mazgalev TN, Thomas JD, Popovic ZB. Left ventricular strain distribution in healthy dogs and in dogs with tachycardia-induced dilated cardiomyopathy. J Cardiovasc Ultrasound 2013; 11:1-9.

[27] Spalla I, Locatelli C, Zanaboni AM, Brambilla P, Bussadori C. Echocardiographic assessment of cardiac function by conventional and speckle-tracking echocardiography in dogs with patent ductus arteriosus. J Vet Intern Med 2016;30:706-713.

[28] Chen HY, Lien YH, Huang HP. Assessment of left ventricular function by two-dimensional speckle-tracking echocardiography in small breed dogs with hyperadrenocorticism. Acta Vet Scand 2014;56:1-10.

[29] Blessberger H, Binder T. Non-invasive imaging: two dimensional speckle tracking echocardiography: basic principles. Heart 2010;96:716-722.

[30] Amundsen BH, Helle-Velle T, Edvardsen T, Torp H, Crosby J, Lyseggen E, Stoylen A, Ihlen H, Lima JAC, Smiseth OA, Slordahl SA. Noninvasive myocardial strain measurement by speckle tracking echocardiography. J Am Coll Cardiol 2006;47:789-793.

[31] Liu L, Tuo S, Zhang J, Zuo L, Liu F, Hao L, Sun Y, Yang L, Shao H, Qi W, Zhou X, GE S. Reduction of left ventricular longitudinal global and segmental systolic functions in patients with hypertrophic cardiomyopathy: Study of two-dimensional tissue motion annular displacement. Exp Ther Med 2014;7:1457-1464.

[32] Chiu DYY, Abidin N, Hughes J, Sinha S, Kalra PA, Green D. Speckle tracking determination of mitral tissue annular displacement: comparison with strain and ejection fraction, and association with outcomes in haemodialysis patients. Int J Cardiovasc Imaging 2016;32:1511-1518.

[33] Takano H, Fujii Y, Ishikawa R, Aoki T, Wakao Y. Comparison of left ventricular contraction profiles among small, medium and large dogs by use of two-dimensional speckle tracking echocardiography. Am J Vet Res 2010;71:421-427.

[34] Locatelli C, Spalla I, Zanaboni AM, Brambilla PG, Bussadori C. Assessment of right ventricular function by feature-tracking echocardiography in conscious healthy dogs. Res vet Sci 2016;105:103-110.

[35] Weidemann F, Jamal F, Sutherland GR, Sutherland GR, Claus P, Kowalski M, Hatle L, Schherder I, Bijnens B, Rademakers FE. Myocardial function defined by strain rate and strain during alterations in inotropic states and heart rate. Am J Physiol-Heart C 2002;283:H792–H799.

[36] Andersen NH, Poulsen SH. Evaluation of the longitudinal contraction of the left ventricle in normal subjects by doppler tissue tracking and strain rate. J Am Soc Echocardiogr 2003;16:716-723.

[37] Fente GJ, Garg P, Foley JRJ, Dobson LE, Musa TA, Erhayiem B, Greenwood JP, Plein S, Swoboda PP. The utility of global longitudinal strain in the identification

of prior myocardial infarction in patients with preserved left ventricular ejection fraction. Int J Cardiovasc Imaging 2017;1-9.

TABLES AND FIGURES

Table 1 - Comparison of TMAD and longitudinal strain in accordance with body weight in dogs.

	Body Weight (quartiles)				*P*
	1.70 - 8.55 kg	**8.56 – 11.60 kg**	**11.61– 21.55 kg**	**21.56 – 50.0 kg**	
(n)	**38**	**40**	**37**	**38**	
Age (years)	3.0 (1.0-7.0)	1.4 (1.4-7.2)	3 (1.9 - 7.0)	4.0 (1.9 - 9.0)	0.3194
TMAD (mm)					
AP4 MV1	4.6 (3.7-5.7) ^{A}	6.2 (5.4-7.1) ^{B}	6.0 (5.5-7.9) ^{BC}	7.3 (6.5-8.0) ^{C}	<0.0001
AP4 MV2	4.4 (3.5-6.0) ^{A}	6.5 (5.5-7.5) ^{B}	6.2 (5.1-7.5) ^{B}	7.2 (6.3-8.8) ^{B}	<0.0001
AP4 PM	5.0 (3.9-6.2) ^{A}	7.0 (6.0-7.8) ^{B}	6.6 (6.0-7.7) ^{B}	7.7 (6.6-8.8) ^{B}	<0.0001
AP4 PM%	12.6 (11.7-14.2) ^{AB}	13.5 (12.5-14.7) ^{A}	12.5 (11.5-14.0) ^{AB}	11.8 (10.1-13.4) ^{B}	0.0025
AP2 MV1	5.4 (3.9-5.8) ^{A}	6.6 (5.5-7.7) ^{B}	6.1 (5.7-7.3) ^{B}	7.1 (5.8-8.6) ^{B}	<0.0001
AP2 MV2	6.1 (4.7-7.0) ^{A}	7.5 (6.3-8.6) ^{BC}	6.9 (5.6-8.0) ^{AB}	9.1 (7.2-10.7) ^{C}	<0.0001
AP2 PM	5.7 (4.7-6.6) ^{A}	6.9 (5.7-8.5) ^{B}	6.8 (5.8-8.7) ^{B}	7.9 (6.8-8.7) ^{B}	<0.0001
AP2 PM%	14.3 (12.6-15.7) ^{A}	13.5 (11.7-15.1) ^{AB}	12.3 (11.3-13.9) ^{BC}	11.5 (10.2-13.4) ^{C}	<0.0001
Global TMAD	5.4 (4.5-6.3) ^{A}	7.1 (6.0-8.0) ^{B}	6.7 (6.2-7.6) ^{B}	7.7 (6.8-8.7) ^{B}	<0.0001
Longitudinal Strain (%)					
AP4	25.1 (22.5-27.5) ^{A}	23.9 (21.9-25.8) ^{A}	21.2 (19.5-22.9) ^{B}	19.6 (16.7-21.2) ^{B}	<0.0001
AP2	27.1 (24.0-28.9) ^{A}	23.2 (21.7-26.2) ^{B}	21.6 (18.9-23.9) ^{B}	17.8 (14.9-20.3) ^{C}	<0.0001
GLS	26.3 (24.0-28.3) ^{A}	24.0 (21.5-24.9) ^{B}	21.3 (19.0-24.1) ^{B}	18.5 (16.1-20.8) ^{C}	<0.0001

TMAD, tissue motion annular displacement; AP4, apical 4-chamber ; AP2, apical 2-chamber; MV1, point of septal mitral annulus; MV2, point of lateral mitral annulus; PM, midpoint; GLS: global longitudinal strain; kg, kilograms; (n), number of animals in quartile.
Data are expressed as median (interquartile range). Values with different superscripted letters indicate statistically significant differences between groups.

Table 2 - Correlation between global BSA-indexed TMAD (mm/m²), global TMAD (%), cube root BW global TMAD or the GLS (%) with data obtained by conventional echocardiography.

	Global TMAD (mm/m²)		**Global TMAD (%)**		**Cube root BW global TMAD (mm/kg)**		**GLS (%)**	
	P	**R**	***P***	**R**	***P***	**R**	***P***	**R**
FS	0.0003	0.2876	0.0158	0.1948	0.0651	0.1500	0.0014	0.2555
EF	<0.0001	0.3497	0.0078	0.2144	0.0399	0.1669	<0.0001	0.3103
LA/Ao	0.4635	0.0599	0.0157	0.1950	0.0631	0.1511	0.3383	0.0779
E_{mitral}	0.9876	0.0013	0.8475	0.0158	0.0312	0.1760	0.2715	-0.0900
E/A_{mitral}	0.6308	0.0395	0.1537	0.1167	0.0150	0.1983	0.4852	0.0572
IVRT	0.0004	-0.285	0.0785	-0.143	0.0017	-0.253	0.0050	-0.2264

TMAD, tissue motion annular displacement; BW: body weight; GLS, global longitudinal strain; FS, fractional shortening; EF, ejection fraction; E, peak early diastolic mitral inflow velocity; A, late diastolic mitral inflow velocity; LA/Ao, left atrial to aortic root ratio ; IVRT, isovolumic relaxation time.

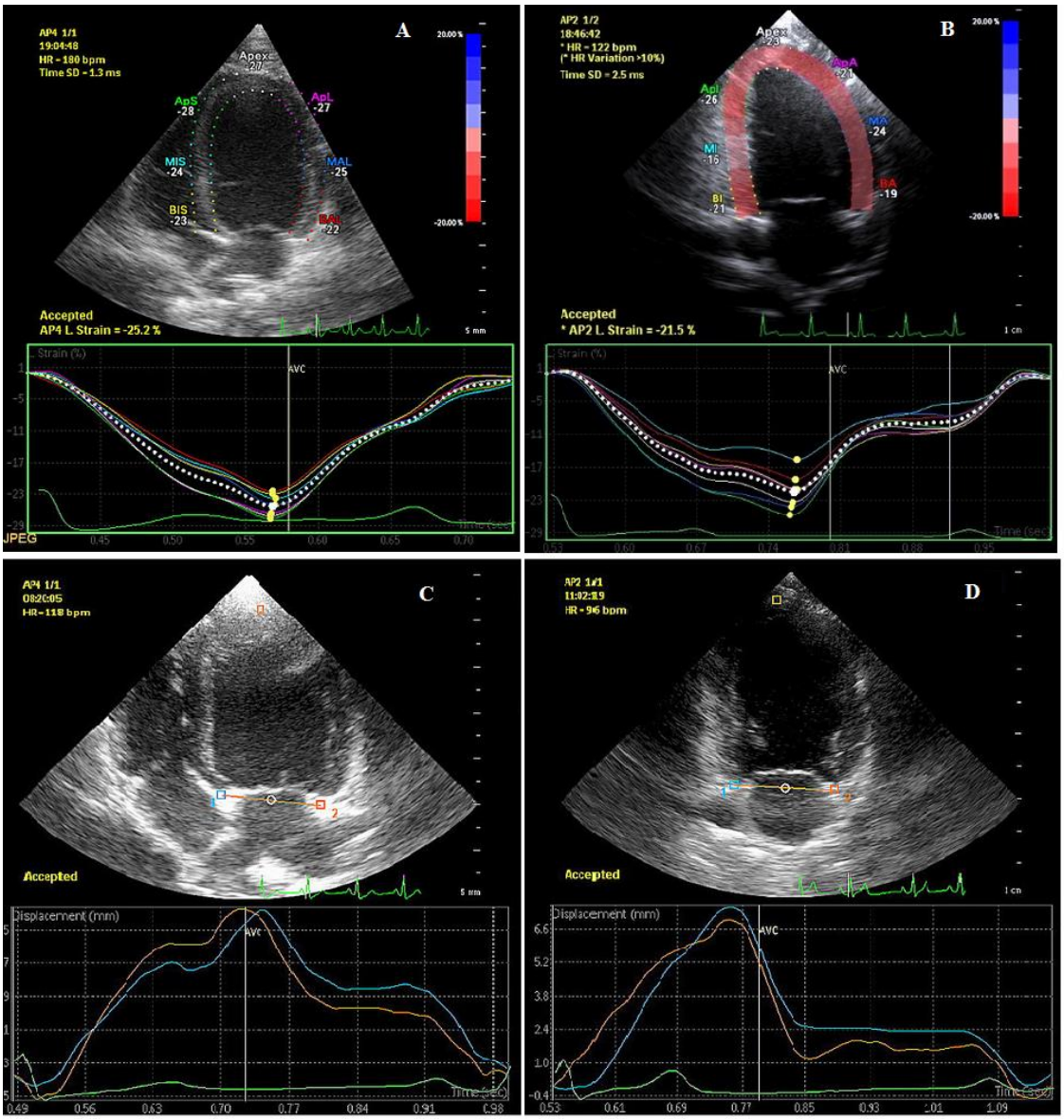

Figure 1 - Longitudinal Strain is determined by the software in 4-chamber (A) and 2-chamber (B) images. The mean of both results represents the global longitudinal strain (GLS). Tissue motion annular displacement is automatically determined by the software. However, the operator needs to identify three points: medial and lateral mitral annulus and left ventricular epicardial apex in both 4-chamber (C) and 2-chamber (D) images.

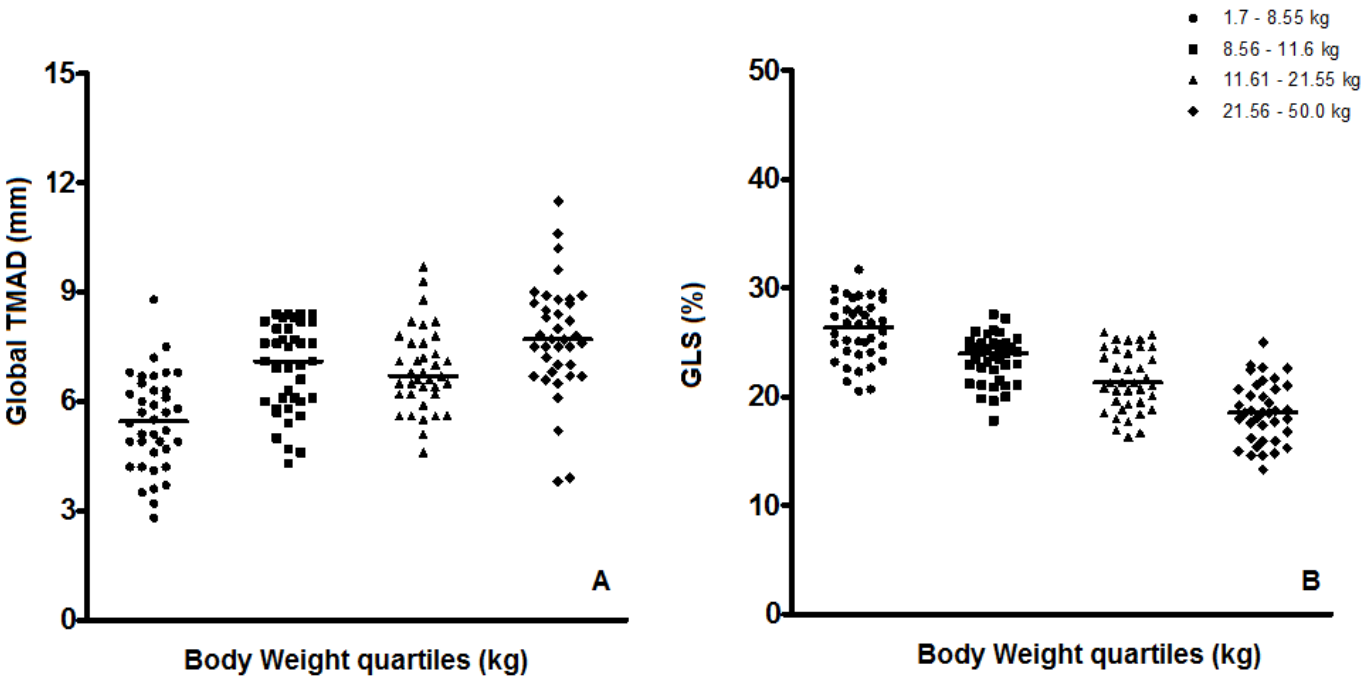

Figure 2 - Median and individual values of global TMAD (mm) (A) and GLS (%) (B) obtained in dogs subdivided according to body weight.

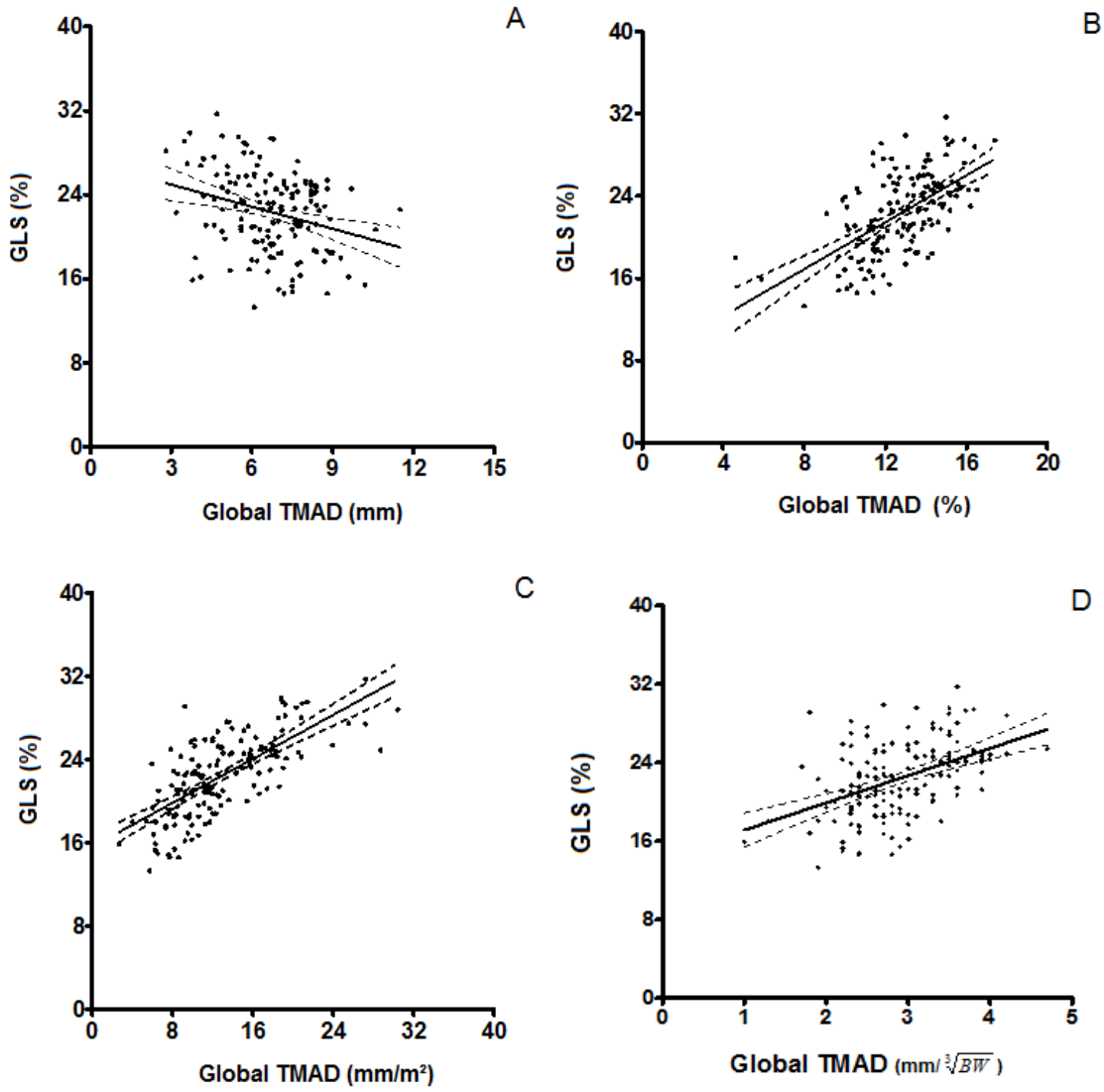

Figure 3 - Correlation between global TMAD and GLS. A negative correlation (R:-0.2552; *P*:0.0015) existed when GLS was compared with the global TMAD (mm) (A). Positive correlations were found when comparing GLS and global TMAD (%) (R:0.5898; *P*<0.0001) (B), GLS and global BSA-TMAD (mm/m²) (R:0.7153; *P*<0.0001) (C); GLS and global cube root BW-indexed TMAD (R:0.4502; *P*<0.0001) (D).

CHAPTER 2 - Assessment of Longitudinal Systolic Function Using Tissue Motion Annular Displacement and Global Longitudinal Strain in Dogs with Mitral Valve Disease

Acknowledgments

The authors are grateful for the financial support provided by Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Running head: Tissue Motion Annular Displacement and Global Longitudinal Strain in Dogs with Mitral Valve Disease.

Abstract

Introduction: Although mitral valve disease (MVD) is an essentially valve disorder, the myocardium undergoes structural changes with the progression of the disease, that may compromise left ventricular systolic function. Longitudinal systolic function, poorly explored in conventional echocardiography, may be affected before circumferential function. Tissue motion annular displacement (TMAD) is a speckle tracking technique that evaluates the function of longitudinal fibers by displacing the mitral annulus towards the apex during systole, which has never been studied in dogs with MVD.

Animals: One hundred twenty-six dogs with MVD of varying severity and forty six healthy dogs.

Methods: Prospective study. One hundred twenty-six client-owned dogs with MVD of various stages and forty six healthy dogs as control group underwent physical examination, electrocardiography, systolic blood pressure (SBP) measurement, as

well as a standard and speckle tracking echocardiography. Global longitudinal strain (GLS) and TMAD were used to assessment the longitudinal systolic function. These parameters were compared with the conventional echocardiography in the various stages of MVD, in order to evaluate the use of TMAD as a diagnostic method and the behavior of longitudinal function with the progression of MVD.

Results: The global TMAD values were higher in the animals in stage B2 and in the symptomatic group, while GLS were higher in B2 group. GLS, global TMAD% and global TMAD mm/m² were correlated with several parameters of conventional echocardiography, such as ejection fraction and fractional shortening. GLS was influenced by gender (P: 0.04) and SBP (P: 0.02; R: -0.17). Curiously, global TMAD mm/m² (P: 0.002; R: 0.234) and global TMAD% (P: 0.007; R: 0.205) presented a weak correlation with age. TMAD is a faster technique to perform than GLS (*P*: 0.0007) and presents good intra- and inter-observer repeatability in dogs with MVD.

Conclusions: An evident impairment in longitudinal systolic function was not observed in this study by GLS and TMAD. TMAD is repeatable and rapid technique for evaluation of longitudinal systolic function in dogs with MVD.

Key words: speckle tracking, longitudinal contractility, endocardiosis, left ventricle, echocardiography.

List of Abbreviations

ACVIM	American College of Veterinary Internal Medicine
AP4	Apical 4-chamber
AP2	Apical 2-chamber
BSA	Body surface area

EF	Ejection fraction
FS	Fractional shortening
GLS	Global longitudinal strain
HF	Heart failure
ISACHC	International Small Animal Cardiac Health Council
IVRT	Isovolumic relaxation time
LA:Ao	Left atrium-to-aorta ratio
LSt	Longitudinal strain
LV	Left ventricle
MAM	Mitral annulus motion
ROI	Region of interest
SBP	Systolic blood pressure
STE	Speckle tracking echocardiography
TMAD	Tissue motion annular displacement

INTRODUCTION

The assessment of left ventricle (LV) performance depends on intrinsic cardiac pump factors and myocardial fiber function [1]. The heart is composed of fibers oriented longitudinally and circumferentially. In the canine myocardium, the circumferential myocardial fibers are expressed in greater quantity than the longitudinal, with a ratio of approximately 1:10 [2] and are considered the main responsible for ventricular systole [3]. However, longitudinal fibers, which are located mainly in the subepicardial and subendocardial layers, play a fundamental role in systolic efficiency [4,5]. The function of these fibers is thought to be extremely important for the long-term survival of patients with cardiac heart failure [6].

Mitral valve disease (MVD) is recognized as the main acquired heart disease in dogs [7]. Although it is a primarily valve disease [8], with its progression the myocardium undergoes contractile and cellular damages [9] resulting in areas of fibrosis and arterial narrowing [10] that may culminate with impairment of systolic function in more advanced stages [11].

Some studies have demonstrated that longitudinal fibers may be the first to be impaired in the development of systolic dysfunction, prior to the circumferential fibers [5,12-13]. A study that used speckle tracking echocardiography (STE) showed that in more advanced stages of the MVD the longitudinal myocardial deformation is smaller whereas radial strain is similar to that of healthy dogs [14].

In standard echocardiography, the main parameters that provide information on systolic function are obtained from short axis images [15], and consequently reflect the function of the circumferential fibers. This exclusive evaluation of the transverse shortening compromises an early diagnosis of systolic dysfunction, since circumferential fibers can optimize their function thereby masking a longitudinal deficit and maintaining the normal values when only evaluated from the short axis [5]. In addition, these parameters are highly influenced by load changes [16] and poorly correlated with values obtained by invasive methods of volume assessment in dogs with heart disease [15]. Therefore, chronic volume overload and other hemodynamic changes compromise the use of these conventional variables for the evaluation of systolic function in MVD [17].

For an adequate assessment of ventricular function in MVD, multidirectional myocardial evaluation [17], using techniques that also evaluate the longitudinal systolic function are necessary [13]. During systole, contraction of the longitudinal fibers promotes the excursion of the atrioventricular plane towards the apex of the left

ventricle [18]. This displacement can be evaluated by M mode, with the technique called mitral annulus motion (MAM). Animals with MVD have lower MAM% values than healthy animals, pointing to contractile deficit of longitudinal fibers in this disease [19].

In addition to MAM, other techniques with good correlation with invasive methods can be used, such as techniques that study myocardial deformation by STE [20,21]. Studies with healthy dogs and dogs with MVD using the longitudinal strain (LSt) and Strain rate, which evaluate the deformation and velocity of the myocardial deformation respectively, also show the existence of changes in longitudinal deformation during the course of MVD [17,22].

Tissue motion annular displacement is a STE-based technique that studies the displacement of the mitral annulus toward the ventricular apex during systole, and consequently provides information on the longitudinal fibers' systolic function [23,24] with good correlation with gold-standard methods, such as MRI [25]. TMAD is an easy and quick technique that has good repeatability and requires little operator experience. This technique has shown to be a good and practical method of evaluating the longitudinal systolic function in healthy humans as well as in people with several cardiac diseases [23,26-27].

Our research group previously studied TMAD in healthy dogs in an unpublished study. This technique showed correlation with global longitudinal strain (GLS) and several echocardiographic parameters. However, to the best of our knowledge, no study was performed using TMAD in dogs with mitral valve disease so far. Therefore, the aims of this prospective study were three-fold: (1) to evaluate the longitudinal systolic function in dogs with various stages of mitral valve disease; (2) to investigate whether TMAD and GLS correlate with the standard echocardiographic

parameters of systolic function; (3) to investigate whether there longitudinal parameters are influenced by age, gender, cardiac rhythm and systolic blood pressure (SBP) in dogs with MVD.

MATERIALS AND METHODS

Animals

This observational cross-sectional study included 172 client-owned dogs of various breeds and ages, being 126 dogs with MVD and 46 healthy dogs (control group) enrolled prospectively at the cardiology section of a veterinary teaching hospital.

The animals assigned to the control group had no history of cardiovascular disorders and were absolutely normal on the physical examination, electrocardiography and echocardiography. In contrast, several echocardiographic findings such as thickening and/or prolapse of the valvular leaflets and valvular insufficiency were considered to diagnose MVD. Patients with MVD were further divided into three groups: asymptomatic animals with no cardiac enlargement (stage B1; n=42); subclinical MVD and cardiac remodeling (LA:Ao ≥1.6) (stage B2; n=38); and dogs with overt heart failure (HF) (either stage C or D; n=46).

In addition to echocardiography, all dogs underwent a thorough physical examination, SBP assessment and electrocardiography. Systolic blood pressure was obtained non-invasivally (vascular Doppler) and the final result was considered as the average of at least five measurements performed by trained observers (MW, SBL, VBCS) as previously recommended [28]. Also, at least a computer-based electrocardiography[a] was recorded for at least three minutes immediately prior to echocardiography in all animals in order to document cardiac rhythm and heart rate.

The exclusion criteria of this study included a systolic arterial blood pressure >150 mmHg; any cardiac rhythm of non-sinus origin; another acquired or congenital cardiovascular disease and cardiac neoplasms. The use of medications for cardiac treatment was allowed whenever necessary. All owners gave a formal consent prior to the animal being enrolled into the investigation. Also, the study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all procedures were previously approved by the Institutional Animal Use Committee (protocol 072-2016).

Conventional echocardiography

The echocardiography was performed using a Phillips Affinit 50[b] with continuous ECG monitoring. A single experienced operator (MW) was responsible for imaging acquisition and measurements. During the exam, the unsedated dogs were positioned in left and right lateral recumbency, in accordance with the recommendations of the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine [29].

Using M-mode and short axis images obtained from the right parasternal window, we documented the left ventricular internal diameter at end-diastole ($LVID_d$) and at end-systole ($LVID_s$), fractional shortening (FS) and ejection fraction (EF), the latter being calculated by the Teichholz formula. The left atrium-to-aorta ratio (LA/Ao) was calculated from two-dimensional short axis images.

Apical 4-chamber images were used to obtain the mitral early (E_{mitral}) and late (A_{mitral}) diastolic peak velocities, while apical 5-chamber images were required for obtaining the isovolumic relaxation time. These parameters allowed the calculation of the E_{mitral}-to-A_{mitral} and the E_{mitral}-to-IVRT ratios. The left ventricular outflow was

recorded to obtain the aortic valve closure time (R to AV closure), which corresponded to the time (in milliseconds) from the beginning of the QRS complex to the end of the aortic flow spectral envelope. That time was required to determine the peak systolic in STE analyses (LSt and TMAD).

Finally, the tissue Doppler sample gate was used on the septal region of the mitral annulus to determine early diastolic (E´), late diastolic (A´) velocity, and E´/A´ ratio.

Left ventricle longitudinal systolic function

Longitudinal Strain

At least five cardiac cycles of the apical 4- (AP4) and 2- (AP2) chamber views were recorded for subsequent off-line evaluations of STE. The equipment software[c] automatically detected the left ventricular myocardium to be screened and divided the ventricle in seven segments. For each individual image, the strain was considered as the average of the deformation of these seven segments. In some cases the software was not able to track the myocardium correctly, which required manual corrections. The LSt was determined as a percentage (%) of left ventricle myocardial deformation in a heartbeat and the GLS was calculated as the mean of LSt obtained in both AP4 and AP2 images (Fig. 1 A and B).

Tissue Motion Annular Displacement

Similar to strain, TMAD was calculated automatically by software using Speckle Tracking in both AP4 and AP2 images. TMAD is based on the degree of displacement of the mitral annulus towards the cardiac apex during systole. For this purpose, three regions of interest (ROIs) need to be determined by operator: two of

them in the mitral annulus (AP4 chamber: lateral and septal region; AP2 chamber: anterior and posterior region of the mitral annulus) while the third one is the epicardial region of the left ventricular apex (Fig. 1 C and D).

Once the ROIs are determined by the operator, the software tracks the displacement of the first and second ROI (in mm) towards the third ROI. A midpoint between the two mitral ROIs is automatically created and its displacement towards the apex is documented in mm and as a percentage (%) of the total length of the left ventricle.

Global TMAD was calculated in three different ways: global TMAD (mm) is the average of the midpoint value obtained in AP4 and AP2 images; global TMAD (%) is the average of the percentage value of midpoint in the AP4 and AP2 chamber images; and the global TMAD (mm/m²) is global TMAD (mm) indexed to the patient's body surface area (BSA), according to the following formula:

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

K = constant (10.1 for dogs)

Intra- and interobserver variability study and time requirement

To assess the repeatability of the STE analysis, 20 animals in different stages of MVD which had been studied at least 30 days before were randomly selected for reevaluation by the first investigator (MW) to provide the intraobserver variability. In order to evaluate interobserver variability, the same 20 dogs were examined by a co-investigator (SBL), who was blinded to the results of the first investigation. The required time for offline analyses (without image acquisition time) for LSt and TMAD was documented from another 20 studies.

Statistical Analysis

The statistical analysis was performed with the software R in the free distribution version i386 3.4.1 that can be found at: www.r-project.org. Firstly, a descriptive analysis was performed with the objective of visualizing aspects of the data contained in this study. The normality of the data was evaluated by the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare the execution time of the techniques and to investigate differences in global TMAD and GLS in accordance with cardiac rhythm. The same test was used to search for differences between disease stages concerning the parameters of conventional echocardiography, age, body weight, heart rate, as well TMAD and GLS values. Following, the Dunn test was performed to identify these differences between the groups and the Chi-square test for identify whether any association existed with gender and cardiac rhythm. The correlations between the echocardiographic variables were evaluated by the Spearman test. The degrees of sensitivity and specificity of GLS and global TMAD to differentiate control group from dogs with MVD, control group from dogs with asymptomatic MVD, control group from symptomatic group, dogs with asymptomatic MVD from symptomatic group and dogs without cardiac remodeling from animals with cardiac remodeling were assessed with ROC curves. Finally, kappa was calculated to evaluate intra- and inter-observer repeatability. A value of $P < 0.05$ was considered significant.

RESULTS

A total of 172 animals were enrolled in this study, of which 46 were healthy dogs ascribed to the control group. The remaining 126 dogs were diagnosed with MVD, and were further subdivided according to the severity of the disease. Stage B1

was represented by 42 dogs, whereas 38 were in stage B2. The groups of animals with overt signs of heart failure had 46 dogs (stage C: 38 dogs; stage D: 8 dogs) [7].

Although crossbred animals were the majority of the studied population, several breeds were also represented. The control group was composed by Mixed breed dogs (n=13), Lhasa Apso (n=7), Miniature Schnauzer (n=7), Beagles (n=5), Dachshund (n=4), Poodle (n=2), Yorkshire Terrier (n=2), English Cocker Spaniel, French Bulldog, Border Collie, Pekingese, Pug and Shi-Tzu (n=1 each). The MVD groups were represented by Mixed breed dogs (n=39), Poodle (n=23), Lhasa Apso (n=15), Dachshund (n= 13), Miniature Pinscher (n=9), Shi-tzu (n=6), Maltese (n=5), Beagle (n=3), Yorkshire Terrier (n=3), Bichon Frisé (n=2), Miniature Schnauzer (n=2), Brazilian Terrier, English Cocker Spaniel, German Shepherd, Jack Russell Terrier, Pomeranian and Pug (n=1 each). The distribution of age, gender, body weight and basic echocardiographic data of each group are shown in table 1.

Sinus arrhythmia was the predominant cardiac rhythm of the control group (58.7%), ACVIM B1 (76.2%) and ACVIM B2 (71%) groups. In the symptomatic group, the predominant rhythm was sinus rhythm (39.13%) followed by sinus arrhythmia (32.3%) and sinus tachycardia (28.26%). Forty-three dogs with MVD had postcapillary pulmonary hypertension (gradient of tricuspid regurgitation>35 mmHg).

Global TMAD mm/m² ($P<0.0001$; R:0.5580) and global TMAD% ($P<0.0001$; R:0.5182) correlated with GLS (Fig. 2) and other echocardiographic surrogates of systolic function, such as EF and FS. The animals of the symptomatic group have a LA/Ao, E mitral, EF and FS higher than other groups. The comparisons between groups of the other echocardiographic parameters are shown in table 1.

Our results show that the values of global TMAD are higher in stage B2 animals and in animals with clinical signs of HF as compared to control group and

dogs in stage B1, while GLS was higher in stage B2 (Table 2). There was no significant difference in gender between groups (*P*:0.85), but there was a difference in cardiac rhythm (*P*<0.0005). The medians for GLS and TMAD values in each group are represented by gender in figure 3.

Global TMAD mm (*P*:0.85), global TMAD mm/m² (*P*:0.49), global TMAD% (*P*:0.41) and GLS (*P*:0.59) were not influenced by cardiac rhythm. GLS demonstrated a gender (*P*:0.04) and SBP (*P*:0.02; R:-0.17) influence, while the global TMAD mm (*P*:0.39), global TMAD% (*P*:0.09) and global TMAD mm/m² (*P*:0.15) did not. Males had lower GLS values than females (Fig. 3). Interestingly, in this study we found a weak positive correlation between age and both global TMAD mm/m² (*P*:0.002; R:0.234) and global TMAD% (*P*:0.007; R:0.205).

GLS and TMAD demonstrated good repeatability in inter- (Kappa´s value 0.623 for GLS and 0.45 for TMAD) and intra-observer analyses (Kappa´s value 0.677 for GLS and 0.6 for TMAD). However, both techniques demonstrated low sensitivity and specificity to differentiate control group from dogs with MVD, control group from dogs with asymptomatic MVD, control group from symptomatic group, dogs with asymptomatic MVD from symptomatic group and dogs without cardiac remodeling from animals with cardiac remodeling (Table 4; Fig. 4). TMAD was found to be a faster technique to be performed as compared to LSt in both AP4 (P:0.0007) and AP2 images (P:0.0019). The median processing time for TMAD was 16.5 seconds in the AP4 and 18.4 seconds in the AP2 view, while for LSt we needed 37.3 and 33.1 seconds, respectively.

DISCUSSION

In this study we sought to compare TMAD with the GLS and standard echocardiography data in dogs with MVD with the aim of evaluating LV systolic function. The global myocardial performance is directly associated to the function of the heart pump and loading conditions [30]. The parameters routinely used to evaluate LV function are known to be affected by both preload and afterload changes [17], which compromises their use in dogs with MVD. Therefore, new functional surrogates are warranted [26], including those intended for the evaluation of longitudinal function, which can provide early data on systolic dysfunction [13].

Left ventricular longitudinal function has already been evaluated in dogs with MVD by techniques that evaluate the excursion of the mitral annulus towards the cardiac apex during systole using M-mode echocardiography [19,31] and with more elaborate techniques based on STE, such as strain and strain rate [22,32-33] .

TMAD is a speckle tracking technique that evaluates the displacement of the mitral annulus towards the ventricular apex. This technique has a good correlation with EF [34] and GLS in people, besides being quickly executed and requiring less image quality and operator experience than GLS and EF. Echocardiographic images of low quality allow a complete analysis of TMAD in more patients than the strain analyses [24,26], which might be an advantage in dogs with pulmonary edema and dyspnea.

TMAD has been studied in healthy children [25], children cancer survivors [35], healthy adults [26] and people with various heart diseases [22,26,36,37]. This technique was shown to be an early surrogate for systolic dysfunction in heart diseases [26]. LSt is another STE-based technique that provides information on the percentage myocardial deformation relative to the original dimension [14]. Also, it is

considered more accurate to document systolic deficit than conventional echocardiography [14,38] in addition to being repeatable and reproducible in dogs [39].

The median LSt values found by Suzuki et al. (2013) [22] were 20% for dogs with asymptomatic valve disease, 23% for patients with mild to moderate HF and 24% for animals with advanced HF. Zois et al. (2012) [32] found a mean LSt of 21.8% for animals of various breeds with overt HF signs, 22.5% for asymptomatic Cavalier King Charles Spaniel with moderate to severe mitral regurgitation and approximately 18% for healthy Beagles or Cavaliers with minimal valve regurgitation. Similar values were found in this study, with a median of approximately 23% for the both the control and stage B1 groups, 26% for stage B2 animals and 24.9% in dogs already presenting signs of HF (Table 2). In contrast, Mantovani et al. (2015) [33] found slightly lower values. In his study, the animals in stages B1 and B2 had an endomyocardial LSt of approximately 18% while in stage C dogs LSt was 15%. These differences might be justified by differences in the study population.

Chetboul and Tisser (2012) [14] found that strain values have a wide variety in dogs with heart failure, suggesting that systolic changes also depend on individual factors and not only on the HF category. This finding could be a justication why some studies found larger longitudinal deformations [32], smaller [22] and some documented no difference at all [33] as MVD progresses. Significantly decreased LV function before the onset of clinical signs of HF was not detected in our study, since the animals in the symptomatic group had global TMAD values higher than controls and B1 dogs, as well as similar values stage B2 animals (Table 2). This curious finding is in agreement with studies that point to clinical signs of MVD not being primarily caused by ventricular dysfunction [16].

In this investigation, we observed higher values of GLS and global TMAD (mm, mm/m² and %) in stage B2 dogs (Table 2), as previously documented by Zois et al. (2012), whose study showed an augmentation in LV function in animals with moderate to severe MVD [32]. According to their study, Cavalier King Charles Spaniel with moderate to severe mitral regurgitation had an increased LSt as compared to healthy dogs and/or those with only minimal regurgitation. In people with structural heart disease, studies have shown an association between TMAD and HF class, as well as with the serum concentration of natriuretic peptides [26].

The activation of compensatory mechanisms and water retention [40] results in a progressive volume overload and promotes an increase in overall cardiac function and sympathetic tonus, which eventually result in a hyperdynamic LV [11,14,17,22]. Such increase in myocardial fiber distension explains the higher EF, FS, global TMAD and GLS values found in B2 and C/D dogs.

Studies have shown that myocardial strain increase in response to volume overload and high heart rate, and decrease with an augmented afterload [41,42], explaining the negative correlation found in our study between GLS and SBP (P:0.02; R:-0.17) and $LVID_s$ (P:0.0001; R:-0.28). This is also a reasonable explanation for the increase in strain values in MVD animals, in which a decrease in afterload occurs in conjunction with an increase in preload [32].

Some investigations showed that animals with MVD had a smaller longitudinal deformation when compared to healthy animals [17,19]. Interestingly, a study evaluating myocardial strain in 74 dogs with MVD showed no difference in the radial strain, but the LSt values were lower in the animals with ISACHC class 3 compared to healthy animals and animals in others ISACHC classes. That curious finding points

to a deficit in the longitudinal function before any impairment in radial function becomes apparent in animals with MVD [14].

Some studies did not find differences in LSt among healthy animals and dogs with stage B1, B2 and C MVD [33]. Suzuki et al. (2013) demonstrated that animals in ISACHC class II and III had larger circumferential strain values than class I and control animals in spite of the similarity in LSt between groups. Similar finding were observed by Tidholm et al. (2009), who found no differences in TDI-derived strain between animals with and without signs of CHF secondary to MVD [43]. These results contrast with ours, but may be justified by the individual variation of each animal in response to valve disease [14] and also by the fact that some studies excluded animals receiving pimobendan therapy [22,33]. Also, we must consider that our symptomatic group included only eight dogs refractory to the standard therapy (stage D MVD). Some studies report longitudinal systolic dysfunction mainly in the more advanced stages of the disease [14]. Therefore, we speculate that if more animals in the terminal stage of MVD had been enrolled, some degree of longitudinal systolic dysfunction might have been detected.

This study showed a higher GLS value in dogs in stage B2 compared to healthy dogs and dogs in stage B1. However, the symptomatic dogs had a GLS similar to the controls, B1 and B2 dogs. In the symptomatic stage of the MVD, volume overload generates a greater stretch of the myocardial fibers and higher values of GLS and TMAD would be expected as a compensatory response. However, the equalization of the values in group B2 and in animals with HF, in addition to a slightly lower GLS median in this group, might indicate an onset of longitudinal systolic impairment.

In this study, we observed that the global TMAD% and global TMAD mm/m² correlated with both longitudinal (GLS) and radial systolic function (EF and FS) in dogs with MVD (Table 3). This is in agreement with other studies in healthy people, people with structural heart disease [26], hypertrophic cardiomyopathy [36] and individuals undergoing hemodialysis [37]. In contrast, the global TMAD mm showed no correlation with GLS and EF, and only a very weak correlation with FS. The global TMAD mm is the mean of the midpoint displacement in mm. The use of global TMAD mm is less acceptable than the global TMAD% and global TMAD mm/m², mainly because it is dependent on body weight. Although MVD mainly affects small dogs [7], the use of global TMAD mm may face limitations because of the wide variety in dogs´ size and consequently heart size.

In a yet unpublished study conducted by our research group with a heterogeneous group of healthy dogs, we observed that heavier dogs had larger global TMAD mm values, but when TMAD was indexed by the body surface area or by the LV length, the mitral annular displacement was smaller than in the smaller animals. This information may also justify the strong correlation between global TMAD mm and $LVID_d$.

FE and FS obtained from M mode measurements correspond to an one-dimensional evaluation of the myocardium. Therefore, those parameters are highly influenced by changes in preload and afterload [14,17,45], which compromise their use for evaluation of systolic function in dogs with MVD. Our study is in agreement with others which documented higher FS and EF in animals with MVD [11]. Interestingly, previous studies did not find correlation between FS and LSt, but a positive correlation could be identified with the radial strain. FS obtained from the short axis essentially reflects radial shortening [39], which may explain the poor

correlations found in our study between GLS and both FS and EF. In contrast, studies have shown that TMAD is correlated with EF obtained by 3D echocardiography [26], magnetic resonance imaging [25] and the Simpson method [37]. In children, TMAD midpoint displacement had a high correlation with the EF obtained by magnetic resonance imaging and a poor correlation with the EF obtained by M-mode [25], which is usually calculated by the Teichholz equation. Besides overestimating LV volume [44], that ignores the increase in ventricular sphericity that occurs during the course of the disease [45], which makes it imprecise for the volumetric assessment of dogs with cardiac remodeling [14,17].

The progressive increase in preload results in an increase in $LVID_d$ that is proportional to ventricular remodeling and volume overload in MVD [46]. The increase in LV filling pressure is one of the cornerstones of the development of HF and may be assessed indirectly by echocardiography as an increase in mitral E wave velocity, a decrease in IVRT [48,49] and consequently an increase in the E/IVRT ratio [49]. The correlation observed between GLS with both $LVID_d$ and $LVID_s$ in our study point to its preload and afterload dependency, such as previously indicated by Zois et al. (2012) [32] and Weidmann et al (2002) [41]. In contrast, global TMAD% and global TMAD mm/m² did not correlate with $LVID_d$, which might suggest they are less preload-dependent than GLS and surrogates obtained from the standard echocardiogram. However, we found a correlation between both GLS and global TMAD with mitral E wave peak velocity and E/IVRT ratio, which suggests that both parameters are influenced by ventricular filling and loading conditions.

A positive correlation between global TMAD, GLS and LA/Ao was found in this study. Zois et al. (2012) also found a curvilinear relationship between LA/Ao and LSt,

and speculated that a morphodynamic change occurs in LV with the progression of the disease [32].

A deficit in longitudinal function was already observed in human beings with advancing age [50]. Curiously, in this study only a weak positive correlation between age and global TMAD mm/m² and global TMAD% was found. On the other hand, GLS was not influenced by age, which is in agreement with other studies in dogs [39,51].

In people, aging generates a difference in the amount and cellular morphology of cardiac myocytes of women and men. By mechanisms not yet fully clarified, women's have the most preserved myocardium and no loss of cells when compared to men's [52]. Although this has not yet been validated for dogs, in this study we observed higher GLS values in females than males. Nonetheless, the female population (n=103) included in our study was higher than the male population (n=69) and the gender did not play a role in the global TMAD.

The results of this study showed that the repeatability and reproducibility of GLS and TMAD measurements in dogs with MVD are adequate for routine clinical use, because the kappa coefficient (>0.6) demonstrated a substantial agreement to GLS and a moderate agreement to TMAD (>0.4) in the intra- and inter- observer analysis. Other studies have demonstrated that TMAD in humans [23,26,36,37] and strain in people and dogs [26,32,37,51] also have a low intra- and inter-observer variability.

Concerning time to perform the analyses, this study is in agreement with others that indicated that TMAD is a faster technique to be performed than LSt [26,27]. Studies required less than 10 to 15 seconds to obtain TMAD [24,26]. Similar values were found in our study, in which the median for TMAD acquisition was 16.5

seconds in the AP4 and 18.4 seconds in the AP2, while some TMAD analyses required less than 10 seconds to be performed.

In this study, neither GLS nor TMAD not attain an acceptable sensitivity and specificity to differentiate the control group from dogs with MVD, control group from dogs with asymptomatic MVD, control group from symptomatic group, dogs with asymptomatic MVD from symptomatic group and dogs without cardiac remodeling from animals with cardiac remodeling, which is similar to findings of a medical study [37]. In contrast, Buss et al. (2012) [26] demonstrated that using a cut-off of 14.2%, TMAD had a good sensitivity (92.1%) and specificity (95.7%) for detecting patients with structural heart disease. Also, TMAD obtained in the first week of hospitalization in patients with septic heart disease was shown to be related to mortality and time of hospitalization [53], while a good ability to detect systolic impairment in patients with myocardial infarction and preserved EF was found for GLS [54].

There are some limitations in this study that need to be considered. We can not rule out the influence played by medication on cardiac parameters assessed by echocardiography. These medications could affect myocardial performance and cardiovascular loading conditions. For example, some animals received pimobendan, which has a recognized positive inotropic effect that may have interfered with our results. Also, ancillary exams to rule out any systemic disease, i.e. infectious diseases, which may affect systolic function, were not included in the screening assessment of the patients unless their clinical conditions required that. Since the study had a cross-sectional design, no patient was reassessed for deterioration of myocardial function along the progression of MVD. Forty-three dogs with postcapillary pulmonary hypertension were not excluded from this investigation. However, it is well known that pulmonary hypertension may compromise LV function

[55] and affect some outcomes. The absence of comparison with a gold standard is also a limitation to be considered. Future studies, maybe with a larger population of dogs refractory to the standard therapy or with myocardial diseases may provide more information about TMAD as a prognostic and diagnostic evaluator and better elucidate the behavior of these variables in dogs in terminal stage of MVD.

In conclusion, no impairment was observed in longitudinal systolic function assessed by TMAD and GLS in dogs with various stages of MVD. TMAD is a fast technique with good repeatability and applicable in the routine for the evaluation of longitudinal function in dogs with MVD. GLS was influenced by gender and SBP, while TMAD was influenced by age. Both techniques showed correlation with several echocardiographic surrogates of systolic function, ventricular filling and cardiac remodelling, and were influenced by changes in preload and afterload. Future studies may clarify the behavior of both TMAD and GLS in the terminal stages of MVD and in dogs with others cardiac diseases.

Footnotes

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

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

[c] - QLAB Software - automatic cardiac motion quantification (aCMQ).

Conflict of Interest: Authors disclose no conflict of interest.

REFERENCES

[1] Hawthorne EW. Instantaneous dimensional changes of the left ventricle in dogs. Circ Res 1961;9:110-119.

[2] Streeter DD, Sportnitz HM. Fiber orientation in the canine left ventricle during diastole and systole. Circ Res 1969;14:339-347.

[3] Sengupta PP, Korinek J, Belohlavek M, Narula J, Vannan MA, Jahangir A, Khandheria BK. Left Ventricular Structure and Function - Basic Science for Cardiac Imaging. J Am Coll Cardiol 2006; 48:1988-2001.

[4] Greenbaum RA, Ho SY, Gibson DG, Becker AE, Anderson RH. Left ventricular fibre architecture in man. Br Heart J 1981;45:248-263.

[5] Aurigemma GP, Silver KH, Priest MA, Gaasch WH. Geometric Changes Allow Normal Ejection Fraction Despite Depressed Myocardial Shortening in Hypertensive Left Ventricular Hypertrophy. J Am Coll Cardiol 1995;26:195-202.

[6] Sveälv BG, Olofsson EL, Andersson B. Ventricular long-axis is of major importance for long-term survival in patients with heart failure. Heart 2008;94:284-289.

[7] Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Häggström J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med 2009;23:1142-1150.

[8] Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. Adv Vet Sci Comp Med 1977;21:75–106.

[9] Urabe Y, Mann DL, Kent RL, Nakano K, Tomanek RJ, Carabello BA, Cooper G. Cellular and ventricular contractile dysfunction in experimental canine mitral regurgitation. Circ Res 1992;70:131-147.

[10] Falk T, Jönsson L, Olsen LH, Pedersen HD. Arteriosclerotic changes in the myocardium, lung, and kidney in dogs with chronic congestive heart failure and myxomatous mitral valve disease. Cardiovasc Pathol 2006;15:185–193.

[11] Borgarelli M, Tarducci A, Zannata R, Haggstrom J. Decreased Systolic Function and Inadequate Hypertrophy in Large and Small Breed Dogs with Chronic Mitral Valve Insufficiency. J Vet Intern Med 2007,21:61-67.

[12] Jones CJH, Raposo L, Gibson DG. Functional importance of the long axis dynamics of the human left ventricle. Br Heart J 1990;63:215-220.

[13] Mizuguchi Y, Oishi Y, Miyoshi H, Iuchi A, Nagase N, Oki T. The functional role of longitudinal, circumferential and radial myocardial deformation for regulating the early impairment of left ventricular contraction and relaxation in patients with cardiovascular risk factors: a study with two-dimensional strain imaging. J Am Soc Echocardiogr 2008;21:1138-144.

[14] Chetboul V, Tissier R. Echocardiographic assessment of canine degenerative mitral valve disease. J Vet Intern Med 2012;14:127-148.

[15] Atkins CE, Curtis MB, Mcguirk SM, Kittleson MD, Sato T, Snyder PS. The Use of M-Mode Echocardiography in Determining Cardiac output in dogs with normal, low, and high output states: comparison to thermodilution method. Vet Radiol *Ultrasound* 1992;33:5:297-304.

[16] Kittleson MD, Eyster GE, Knowlen GG, Bari Olivier N, Anderson LK. Myocardial function in small dogs with chronic mitral regurgitation and severe congestive heart failure. J Am Vet Med Assoc 1984;184:455–459.

[17] Bonagura JD, Schober KE. Can ventricular function be assessed by echocardiography in chronic canine mitral valve disease? J Small Anim Pract 2009;50:12-24.

[18] Komeda M, Glasson JR, Bolger AF, Daughters GT, Niczyporuk MA, Ingels NB, Miller DC. Three-dimensional dynamic geometry of the normal canine mitral annulus and papillary muscles. Circulation 1996;94:159-163.

[19] Schober KE, Fuentes VL. Mitral annulus motion as determined by M-mode echocardiography in normal dogs and dogs with cardiac disease. Vet Radiol Ultrasound 2001;42:52-61.

[20] Culwell NM, Bonagura JD, Schober KE. Comparison of echocardiographic indices of myocardial strain with invasive measurements of left ventricular systolic function in anesthetized healthy dogs. Am J Vet Res 2011;72:650–660.

[21] Steflik D, Butts RJ, Baker GH, Bandisode V, Savage A, Atz AM, Chowdhury SM. A preliminary comparasion of two-dimensional speckle tracking echocardiography and pressure-volume loop analysis in patients with Fontan physiology: The role of left ventricular morphology. Echocardiography 2017;34:1353-1359.

[22] Suzuki R, Matsumoto H, Teshima T, Koyama H. Clinical assessment of systolic myocardial deformations in dogs with chronic mitral valve insufficiency using two-dimensional speckle-tracking echocardiography. J Vet Cardiol 2013;15:41-49.

[23] Suzuki K, Akashi YJ, Mikukoshi K, Kou S, Takai M, Izumo M, Hayashi A, Ohtaki E, Nobuoka S, Miyake F. Relationship between left ventricular ejection fraction and mitral annular displacement by speckle tracking echocardiography in patients with different heart diseases. Journal of Cardiology 2012;60:55-60.

[24] Tsang W, Ahmad H, Patel AR, Patel AR, Sugeng L, Salgo IS, Weinert L, Mor-Avi V, Lang RM. Rapid estimation of left-ventricular function using echocardiographic speckle-tracking of mitral annular displacement. J Am Soc Echocardiogr 2010;23:511-515.

[25] Black DE, Bryant J, Peebles C, Godfrey KM, Hanson M, Vettukattil JJ. Tissue motion annular displacement of the mitral valve using two-dimensional speckle tracking echocardiography predicts the left ventricle ejection fraction in normal chindren. Cardiol young 2014;24:640-648.

[26] Buss SJ, Mereles D, Emami M, Korosoglou G, Riffel JH, Bertel D, Schonland SO, Hegenbart U, Katus HA, Hardt SE. Rapid assessment of longitudinal systolic left ventricular function using speckle tracking of the mitral annulus. Clin Res Cardiol 2012;101:273-280.

[27] Iwakura K, Okamura A, Koyama Y, Inoue K, Nagai H, Toyoshima Y, Tanaka K, Oka T, Iwamoto M, Fujii K. Assessment of left ventricular global systolic function by tissue mitral annular displacement: comparison with global longitudinal strain [Abstract]. J Am Coll Cardiol 2016;67.

[28] Brown S, Atkins C, Bagley R, Carr A, Cowgill L, Davidson M, Egner B, Elliot J, Henik R, Labato M, Littman M, Polzin D, Ross L, Snyder P, Stepien. Guidelines for the Identification, Evaluation, and Management of Systemic Hypertension in Dogs and Cats. J Vet Intern Med 2007;21:542-558.

[29] Thomas WP, Gaber CE, Jacobs GJ. Recommendations for standards in transthoracic Two-Dimensional echocardiography in the dog and cat. J Vet Intern Med 1993;7:247-252

[30] Higgins CB, Vatner SF, Franklin D, Braunwald E. Extent of regulation of the heart´s contractile state in the conscious dogs by alteration in the frequency of contration. J Clin Invest 1973;52:1187-1194.

[31] Sousa MG, Gava FN, Filho JCS, Silva SNS, Camacho RR, Carareto R, Wolf M, Camacho AA. Long-axis fractional shortening and mitral annulus motion in dogs. Semina: Ciências Agrárias 2016;37:3115-3124

[32] Zois NE, Tidholm A, Nagga KM, Moesgaard SG, Rasmussen CE, Häggstrröm J, Pedersen HD, Ablad B, Nilsen HY, Olsen LH. Radial and longitudinal strain and strain rate assessed by speckle-tracking echocardiography in dogs with myxomatous mitral valve disease. J Vet Intern Med, 2012;26:1309-1319.

[33] Mantovani MM, Muzzi RAL, Pereira GG, Yamato RJ, Silva AC, Reis GF, Muzzi LAL, Guimarães EC. Systolic cardiac function assessment by feature tracking echocardiography in dogs with myxomatous mitral valve disease. J Small Anim Pract 2015:1-10.

[34] DeCara JM, Toledo E, Salgo IS, Lammertin G, Weinert L, Lang RM. Evaluation of left ventricular systolic function using automated angle-independent motion tracking of mitral annular displacement. J Am Soc Echocardiogr 2005;18:1266-1269.

[35] Ylänen K, Eerola A, Vettenranta K, Poutanen T. Speckle tracking echocardiography detects decreased cardiac longitudinal function in anthracycline-exposed survivors of childhood cancer. Eur J Pediatr 2016;175:1379-1386.

[36] Liu L, Tuo S, Zhang J, Zuo L, Liu F, Hao L, Sun Y, Yang L, Shao H, Qi W, Zhou X, GE S. Reduction of left ventricular longitudinal global and segmental systolic functions in patients with hypertrophic cardiomyopathy: Study of two-dimensional tissue motion annular displacement. Exp Ther Med 2014;7:1457-1464.

[37] Chiu DYY, Abidin N, Hughes J, Sinha S, Kalra PA, Green D. Speckle tracking determination of mitral tissue annular displacement: comparison with strain and ejection fraction, and association with outcomes in haemodialysis patients. Int J Cardiovasc Imaging 2016;32:1511-1518.

[38] Marciniak A, Claus P, Sutherland GR, Marciniak M, Karu T, Baltabaeva A, Merli E, Bijnens B, Jahangiri M. Changes in systolic left ventricular function in isolated mitral regurgitation. A strain rate imaging study. Eur Heart J 2007;28: 2627-2636.

[39] Chetboul V, Sampedrano CC, Gouni V, Nicolle AP, Pouchelon JL, Tissier R. Ultrasonographic assessment of regional radial and longitudinal systolic function in healthy awake dogs. J Vet Intern Med 2006;20:885-893.

[40] Katayama K, Tajimi T, Guth BD, Matsuzaki M, Lee JD, Seitelberger R, Peterson KL. Early diastolic filling dynamics during experimental mitral regurgitation in the conscious dog. Circulation 1988;78:390-400.

[41] Weidemann F, Jamal F, Sutherland GR, Sutherland GR, Claus P, Kowalski M, Hatle L, Schherder I, Bijnens B, Rademakers FE. Myocardial function defined by strain rate and strain during alterations in inotropic states and heart rate. Am J Physiol-Heart C 2002;283:H792–H799.

[42] Ferferieva V, Van den Bergh A, Claus P, et al. The relative value of strain and strain rate for defining intrinsic myocardial function. Am J Physiol Heart C 2012;302:H188-H195.

[43] Tidholm A, Ljungvall I, Höglund K, Westling AB, Häggström J. Tissue doppler and strain imaging in dogs with myxomatous mitral valve disease in different stages of congestive heart failure. 2009;23:1197-1207.

[44] Tidholm A, Westling AB, Höglund K, Ljungvall I, Häggström J. Comparisons of 3-, 2-dimensional, and M mode echocardiographical methods for estimation of left chamber volumes in dogs with and without acquired heart disease. J Vet Intern Med 2010;24:1414-1420.

[45] Ljungvall I, Höglund K, Carnabuci C, Tidholm A, Häggström J. Assessment of global and regional left ventricular volume and shape by real-time 3-dimensional echocardiography in dogs with myxomatous mitral valve disease. J Vet Intern Med 2011;25:1036-1043.

[46] O'gara P, Sugeng L, Lang R, Sarano M, Hung J, Raman S, Fischer G, Carabello B, Adams D, Vannan M. The role of imaging in chronic degenerative mitral regurgitation. JACC Cardiovasc Imaging 2008;1:221-237.

[47] Borgarelli M, Savarino P, Crosara S. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. J Vet Intern Med 2008;22:120–128.

[48] Ohno M, Cheng CP, Little WC. Mechanism of altered patterns of left ventricular filling during the development of congestive heart failure. Circulation 1994;89:2241–2250.

[49] Schober KE, Hart TM, Stern JA, Li X, Samii VF, Zekas LJ, Scansen BA, Bonagura JD. Detection of congestive heart failure in dogs by doppler echocardiography. J Vet Intern Med 2010;24:1358-1368.

[50] Andersen NH, Poulsen SH. Evaluation of the longitudinal contraction of the left ventricle in normal subjects by doppler tissue tracking and strain rate. J Am Soc Echocardiogr 2003;16(7):716-723.

[51] Chetboul V, Serres F, Gouni V, Tissier R, Pouchelon JL. Radial strain and strain rate by two-dimensional speckle tracking echocardiography and the tissue velocity based technique in the dogs. J Vet Cardiol 2007;9:69-81.

[52] Olivetti G, Giordano G, Corradi D, Melissari M, Lagrasta C, Gambert SR, Anversa P. Gender differences and aging: effects on the human heart. J Am Coll Cardiol 1995;26:1068-1079.

[53] Zaky A, Gill E, Paul C, Bendjelid K, Treggiari M. Characteristics of sepsis-induced cardiac dysfunction using speckle tracking echocardiography: a feasibility study. Anaesth Intensive Care 2016;44(1):65–76.

[54] Fente GJ, Garg P, Foley JRJ, Dobson LE, Musa TA, Erhayiem B, Greenwood JP, Plein S, Swoboda PP. The utility of global longitudinal strain in the identification of prior myocardial infarction in patients with preserved left ventricular ejection fraction. Int J Cardiovasc Imaging 2017;1-9.

[55] Chiavegato D, Borgarelli M, D'Agnolo G, Santilli RA. Pulmonary hypertension in dogs with mitral regurgitation attributable to myxomatous valve disease. Vet Radiol Ultrasound 2009;50:253e258.

TABLES AND FIGURES

Table 1 - Comparison of echocardiographic data, age, body weight and heart rate between healthy dogs (control group) and dogs in the various stages of mitral valve disease (B1, B2 and symptomatic).

	Controls	Stage B1	Stage B2	Symptomatic Stages C/D
(n)	**46**	**42**	**38**	**46**
Gender (F/M)	27/19	24/18	22/16	30/16
Age (months)	48 ^{C} (24-96)	132 ^{B} (120-144)	143 ^{AB} (132-156)	155 ^{A} (139-156)
Body weight (kg)	9.425 ^{A} (7.9-10.5)	7.025 ^{A} (5.8-9)	6.35 ^{B} (5.2-9.6)	6.3 ^{B} (5.6-7.3)
HR (bpm)	129 ^{B} (121-136)	127.5 ^{B} (119-137)	124 ^{B} (119-137)	160.5 ^{A} (141-173)
LA/Ao	1.14 ^{C} (1.1-1.17)	1.14 ^{C} (1.07-1.21)	1.73 ^{B} (1.69-1.8)	2.08 ^{A} (1.93-2.19)
$LVID_d$	28.1 ^{B} (26.3-29.7)	27 ^{B} (25.8-30.5)	31.05 ^{A} (29-35.4)	34.1 ^{A} (31.8-36.2)
$LVID_s$	16.2 (14.3-17.5)	17.15 (14.7-18)	17.5 (14.7-18.9)	16.95 (14.5-18.3)
EF	77.85 ^{BC} (71.4-79.8)	71.9 ^{C} (69.2-76.3)	80.55 ^{B} (76.1-82.2)	84 ^{A} (80.1-86.3)
FS	44.35 ^{BC} (40-46.7)	39.8 ^{C} (37.6-43.3)	47.8 ^{B} (43.5-50)	51.45 ^{A} (48.4-54)
E_{mitral}	74.7 ^{C} (69.7-84.6)	65.45 ^{D} (63.3-66.8)	90 ^{B} (78-101)	137.5 ^{A} (122-153)
E_{mitral}/A_{mitral}	1.2 ^{A} (0.9-1.4)	0.8 ^{B} (0.8-1)	0.95 ^{B} (0.9-1.3)	1.3 ^{A} (1.2-1.6)
E´/A´	1.2 ^{A} (0.9-1.4)	0.6 ^{C} (0.6-0.7)	0.7 ^{BC} (0.6-0.8)	0.72 ^{B} (0.7-1.2)
IVRT	63 ^{B} (58-69)	74 ^{A} (69-79)	63 ^{B} (58-69)	51.5 ^{C} (48-55)
E_{mitral}/IVRT	1.17 ^{B} (1.05-1.37)	0.87 ^{C} (0.78-1)	1.445 ^{B} (1.11-1.66)	2.76 ^{A} (2.09-3.2)

Kg, kilograms; (n), number of animals in quartile; EF, ejection fraction; FS, fractional shortening; HR, heart rate; F, female; M, male; IVRT, isovolumic relaxation time; E_{mitral}, early diastolic mitral inflow velocity; LA/Ao, left atrium-to-aorta ratio; $LVID_d$, left ventricular internal diameter at end-diastole; $LVID_s$, left ventricular internal diameter at end-systole. Data are expressed as median (interquartile range). Values with different superscripted letters indicate statistically significant differences between groups.

Table 2 - Comparison of STE data between healthy dogs (control group) and dogs in the various stages of mitral valve disease (B1, B2 and symptomatic).

	Controls	**Stage B1**	**Stage B2**	**Symptomatic Stages C/D**
(n)	**46**	**42**	**38**	**46**
GLS	23.18 ^{B} (21.5-24.2)	23.5 ^{B} (22.3-25.25)	26.35 ^{A} (24.3-27.25)	24.9 ^{AB} (22.25-26.55)
Global TMAD (mm)	6.35 ^{B} (5.95-6.8)	5.425 ^{B} (5.05-5.95)	7.525 ^{A} (6.95-8.05)	7.25 ^{A} (6.7-8.35)
Global TMAD (%)	13.45 ^{B} (12.55-14.1)	13.15 ^{B} (12.3-14.05)	16.875 ^{A} (15.5-17.6)	15.925 ^{A} (14.6-17)
Global TMAD (mm/m²)	13.88 ^{B} (12.8-16.13)	15.49 ^{B} (13.11-17.03)	19.315 ^{A} (16.98-22.02)	20.62 ^{A} (18.44-23.25)

GLS, global longitudinal strain; TMAD, tissue motion annular displacement; STE, speckle tracking echocardiography. Data are expressed as median (interquartile range). Values with different superscripted letters indicate statistically significant differences between groups.

Table 3 - Correlations between GLS, global TMAD mm, global TMAD% and global TMAD mm/m² with the variables of conventional echocardiography, heart rate and blood pressure.

	Global TMAD mm		Global TMAD %		Global TMAD mm/m²		GLS	
	P	**R**	***P***	**R**	***P***	**R**	***P***	**R**
SBP	0.41	-0.063	0.681	-0.031	0.136	-0.1140	0.02	-0.17
HR	0.647	-0.035	0.0657	0.14	0.05	0.1471	0.5126	0.05
LA/Ao	<0.0001	0.39	<0.000001	0.46	<0.000001	0.4794	0.001	0.236
LVIDd	<0.000001	0.56	0.107	0.12	0.612	-0.0390	0.0006	-0.568
LVIDs	0.0025	0.35	0.5	-0.04	0.007	-0.2026	0.0001	-0.286
EF	0.19	0.09	0.0004	0.26	0.009	0.2927	0.022	0.174
FS	0.05	0.14	0.00034	0.003	0.00016	0.2830	0.049	0.139
E_{mitral}	<0.000001	0.53	<0.000001	0.436	<0.000001	0.4300	0.01	0.182
E_{mitral}/A_{mitral}	0.0006	0.37	0.147	0.11	0.09	0.1285	0.306	0.079
E´/A´	0.03	0.16	0.844	-0.015	0.5	-0.0518	0.47	0.056
IVRT	0.046	-0.15	0.03	-0.16	0.0006	-0.2573	0.04	-0.15
E_{mitral}/IVRT	<0.000001	0.44	0.0003	0.37	0.00003	0.4052	0.18	0.189

TMAD, tissue motion annular displacement; GLS, global longitudinal strain; EF, ejection fraction; FS, fractional shortening; HR, heart rate; IVRT, isovolumic relaxation time; E, early diastolic mitral inflow velocity; LA/Ao, left atrium-to-aorta ratio; $LVID_d$, left ventricular internal diameter at end-diastole; $LVID_s$, left ventricular internal diameter at end-systole.

Table 4 - Cut-off values and best combinations of sensitivity, specificity and accuracy to differentiate: (A) control group from dogs with MVD (ACVIM B1, B2 and symptomatic group); (B) control group from dogs with asymptomatic MVD (ACVIM B1 and B2 groups); (C) control group from symptomatic group; (D) dogs with asymptomatic MVD (ACVIM B1 and B2 groups) from symptomatic group and (E) dogs without cardiac remodeling (ACVIM B1) from animals with cardiac remodeling (ACVIM B2 and symptomatic group).

	Variable	Cut-offs	Sensibility	Specificity	Acuracy
A	GLS (%)	15.45	0.5397	0.5853	0.6267
	Global TMAD (mm)	3.00	0.5280	0.5463	0.5829
	Global TMAD (%)	7.95	0.5580	0.6324	0.6951
	Global TMAD (mm/m^2)	8.44	0.5629	0.5463	0.7136
B	GLS (%)	21.60	0.5513	0.5664	0.6189
	Global TMAD (mm)	3.65	0.5144	0.4930	0.5179
	Global TMAD (%)	14.00	0.5695	0.5942	0.6688
	Global TMAD (mm/m^2)	14.42	0.5647	0.5907	0.6556
C	GLS (%)	25.90	0.5809	0.5578	0.6404
	Global TMAD (mm)	6.95	0.6087	0.5788	0.6959
	Global TMAD (%)	15.60	0.6680	0.6461	0.7410
	Global TMAD (mm/m^2)	17.79	0.6313	0.6044	0.8145
D	GLS (%)	31.15	0.5116	0.4920	0.5057
	Global TMAD (mm)	8.25	0.5926	0.5365	0.6334
	Global TMAD (%)	19.55	0.5634	0.5195	0.5875
	Global TMAD (mm/m^2)	20.38	0.6122	0.5518	0.6644
E	GLS (%)	20.95	0.5421	0.5588	0.6145
	Global TMAD (mm)	5.65	0.5989	0.6698	0.5625
	Global TMAD (%)	14.15	0.5989	0.6696	0.5592
	Global TMAD (mm/m^2)	14.31	0.5864	0.6489	0.5799

TMAD, tissue motion annular displacement; GLS, global longitudinal strain.

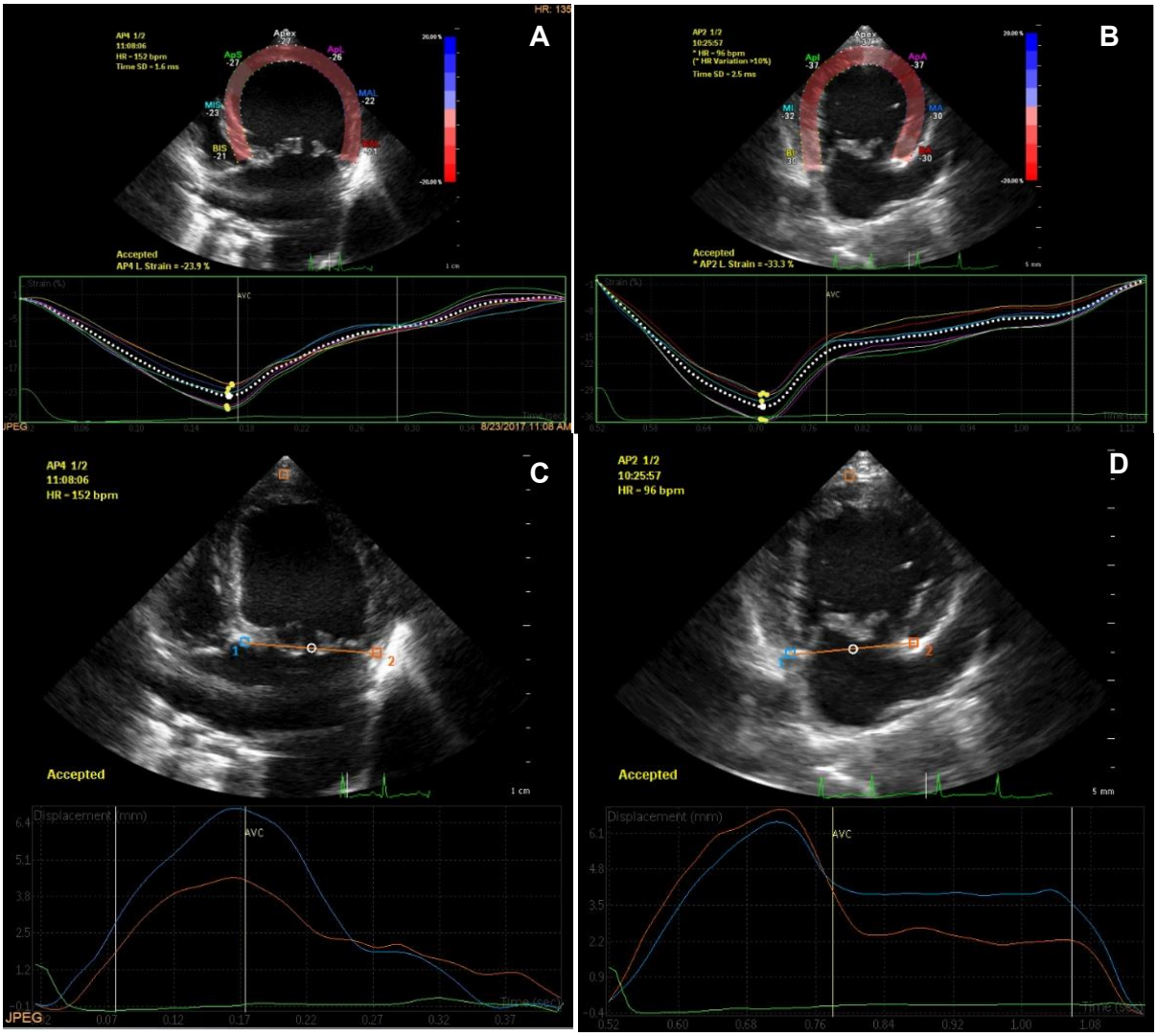

Figure 1 - Longitudinal strain is a result of myocardial tracking performed by the software, providing the percentage of deformation in AP4 (A) and AP2 (B) chambers view. Tissue motion annular displacement (TMAD) is also automatically detected by the software after the operator identifies the three regions of interest (ROIs) deformation in AP4 (C) and AP2 (D) chambers view.

A B C

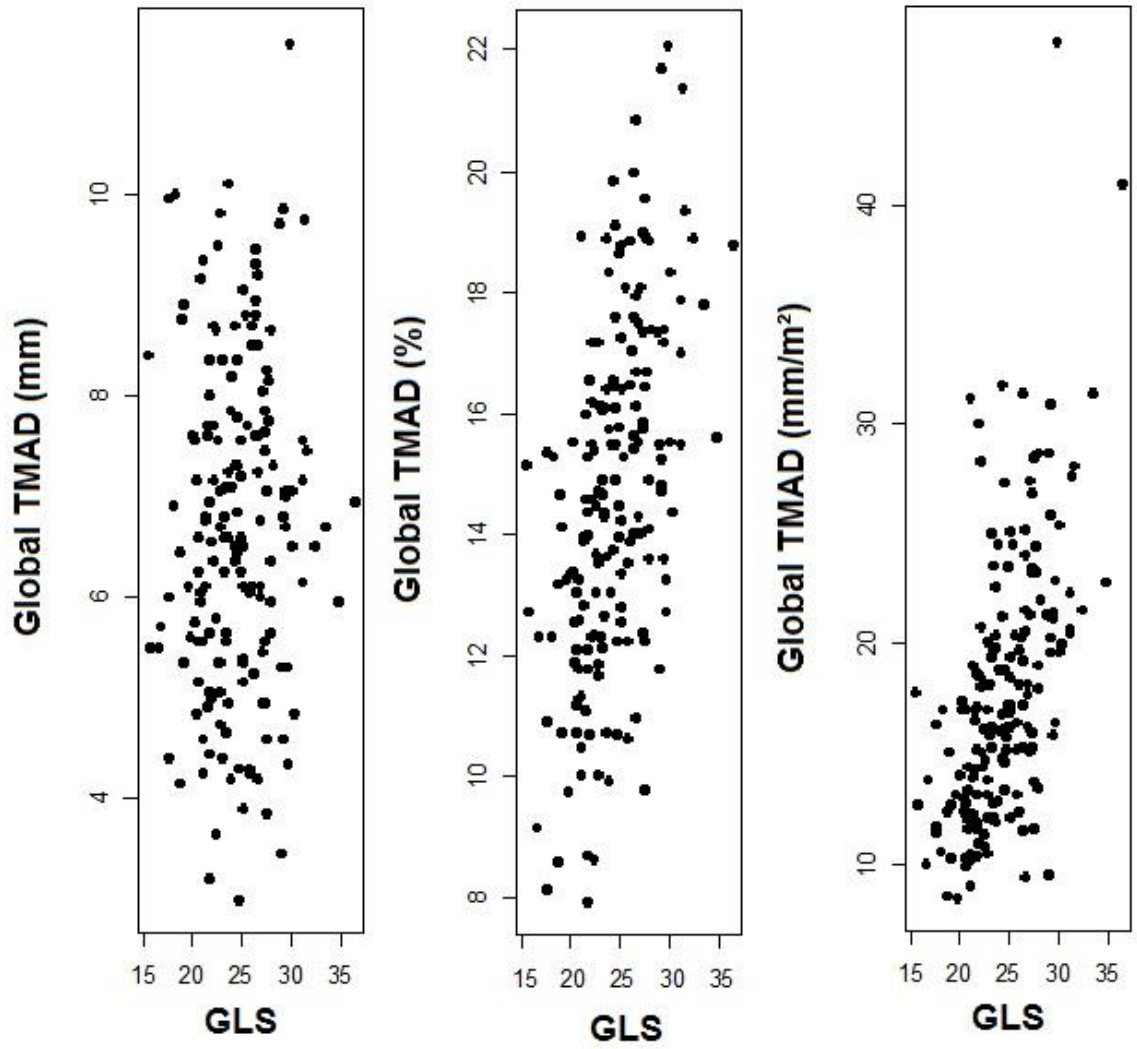

Figure 2 - Correlation between GLS and global TMAD mm (A), global TMAD% (B) e global TMAD mm/m² (C). There is no correlation between GLS and global TMAD (mm) (*P*:0.33) and moderate positive correlations were observed from GLS with global TMAD (%) (*P*:<0.00001; R:0.51) and global TMAD (mm/m²) (*P*:<0.00001; R:0.558).

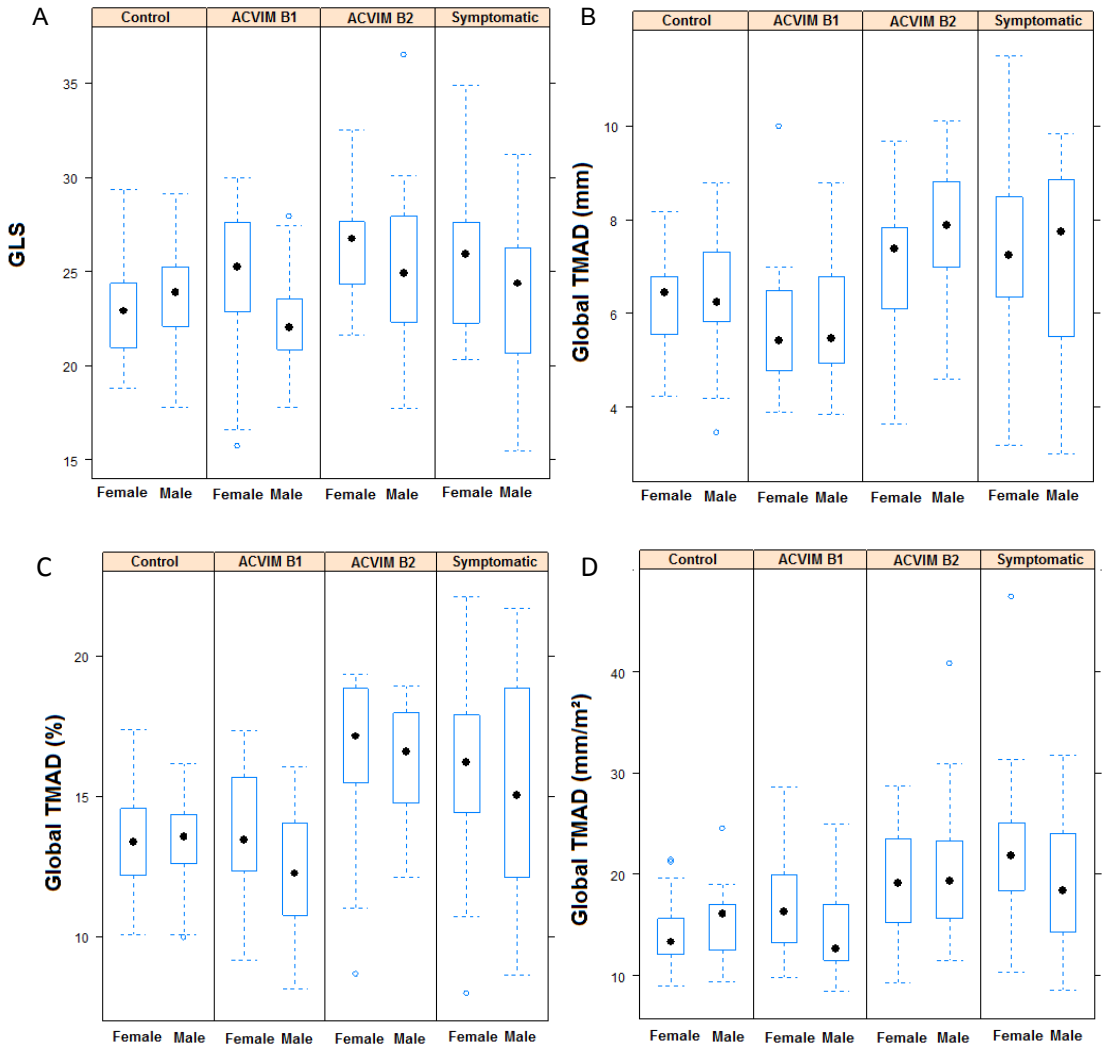

Figure 3 - Boxplots representing the values of GLS (A), global TMAD mm (B), global TMAD % (C) and global TMAD (mm/m²) in healthy dogs (control group) and in dogs with mitral valve disease (stage B1, stage B2 and symptomatic dogs) according to gender.

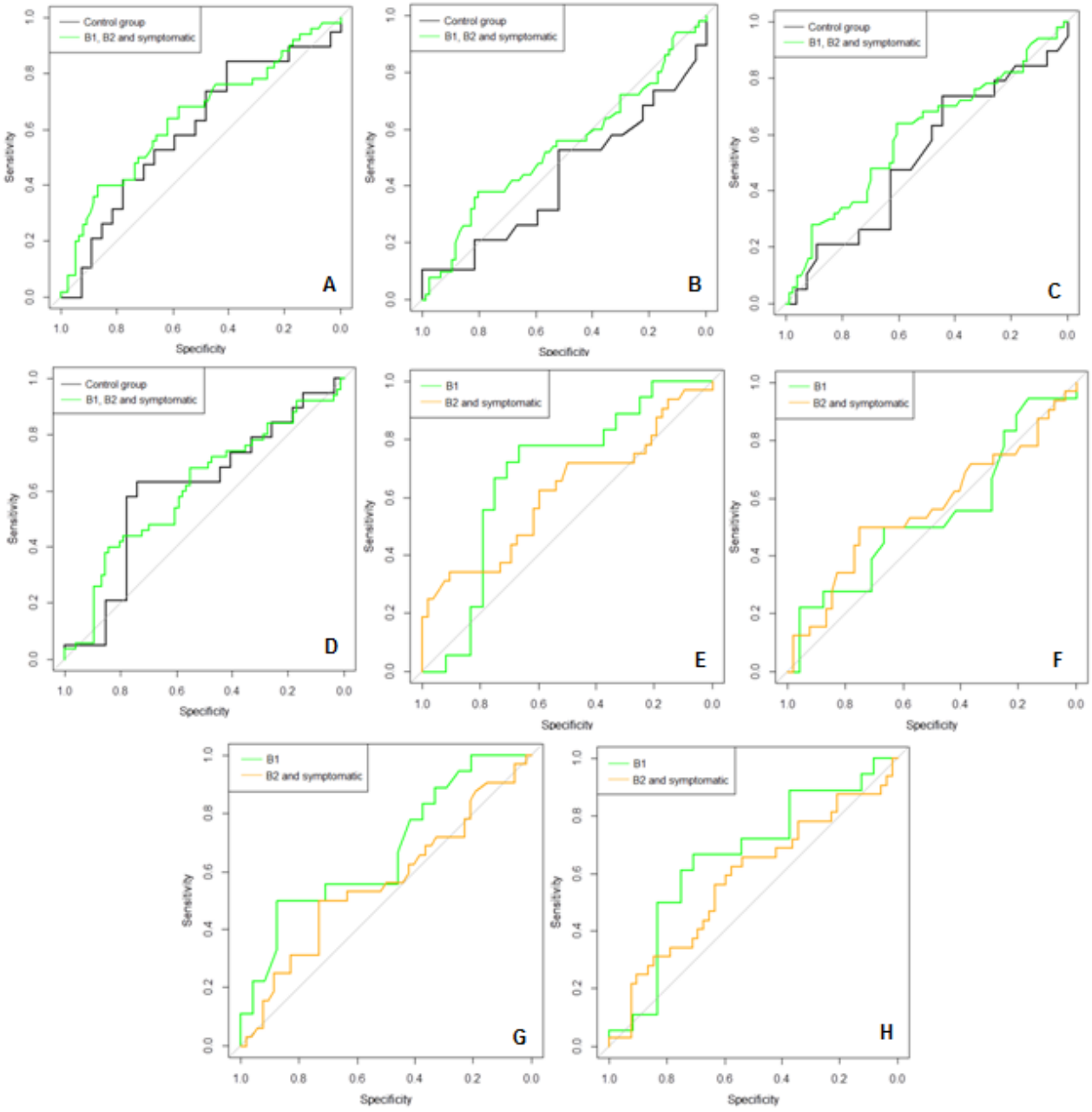

Figure 4 - ROC curves demonstrating sensitivity and specificity of GLS (A), global TMAD mm (B), global TMAD% (C) and global TMAD mm/m² (D) in differentiating the control group and the animals with MVD (B1, B2 and symptomatic); and the GLS (E), global TMAD mm (F), global TMAD% (G) and global TMAD mm/m² (H) in differentiating dogs with MVD with (stages B2, C, D) and without (stage B1) cardiac remodeling.

FINAL CONSIDERATIONS

Tissue motion annular displacement (TMAD) is a technique performed with no difficulties which can be added to the routine examination to evaluate the longitudinal systolic function of healthy dogs and dogs with mitral valve disease. This technique demonstrated a correlation with the Global Longitudinal Strain (GLS) and several parameters of systolic function obtained by conventional echocardiography in healthy dogs and in dogs with mitral valve disease. Both TMAD and GLS presented good repeatability in inter- and intra-observer evaluation. In this study, dogs with mitral valve disease had no impairment in longitudinal systolic function, as demonstrated by the higher TMAD and GLS documented in dogs with remodeled hearts as compared to both the healthy dogs and those without cardiac remodeling. TMAD is a less time-consuming technique that may be an alternative to GLS whenever longitudinal systolic function needs to be evaluated in dogs.

Anexo 1. Aprovação do estudo no Comitê de Ética do Setor de Ciências Agrárias da UFPR.

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 072/2016, referente ao projeto **"AVALIAÇÃO DA FUNÇÃO SISTÓLICA POR DESLOCAMENTO TECIDUAL DO ANEL MITRAL COMPARADO AO STRAIN LONGITUDINAL E ECOCARDIOGRAFIA CONVENCIONAL EM CÃES"**, sob a responsabilidade de **Marlos Gonçalves Sousa** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em reunião de 14/09/2016.

Vigência do projeto	Outubro/2016 até Julho/2017
Espécie/Linhagem	*Canis familiaris* (cão)
Número de animais	75
Peso/Idade	Variado
Sexo	Ambos (38 machos e 37 fêmeas)
Origem	Pacientes da rotina do Hospital Veterinário da Universidade Federal do Paraná e de alunos e/ou funcionários da instituição

CERTIFICATE

We certify that the protocol number 072/2016, regarding the project **"SYSTOLIC FUNCTION ASSESSMENT BY TISSUE SHIFTING OF MITRAL RING COMPARED TO LONGITUDINAL STRAIN AND CONVENTIONAL ECHOCARDIOGRAPHY IN DOGS"** under **Marlos Gonçalves Sousa** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October, 2008, of Decree nº 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 1 of invasiveness, in session of 14/09/2016.

Duration of the project	October/2016 until July/2017
Specie/Line	*Canis familiaris* (dog)
Number of animals	75
Wheight/Age	Diverse
Sex	Both (38 males and 37 females)
Origin	Routine of Veterinary Hospital at the Federal University of Paraná Hospital and students and / or staff of the institution

Curitiba, 14 de setembro de 2016.

Simone Tostes de Oliveira Stedile

Coordenadora CEUA-SCA

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR

Anexo 2. Ofício do Comitê de Ética do Setor de Ciências Agrárias da UFPR autorizando a solicitação do aumento do número de animais utilizados no estudo.

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OFÍCIO Nº 113/2016

Para: **Marlos Gonçalves Sousa**
Assunto: Protocolo 072/2016

Prezado(a) pesquisador(a),

Após avaliação do pedido de alteração referente ao protocolo número 072/2016, intitulado "**AVALIAÇÃO DA FUNÇÃO SISTÓLICA POR DESLOCAMENTO TECIDUAL DO ANEL MITRAL COMPARADO AO STRAIN LONGITUDINAL E ECOCARDIOGRAFIA CONVENCIONAL EM CÃES**", pela Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias – UFPR, aprovamos o aumento do número de animais usados para 300, sendo esses 150 cães saudáveis e 150 cães com doença valvar mitral.

Curitiba, 7 de dezembro de 2016.

Atenciosamente,

Simone Tostes de Oliveira Stedile
Coordenadora CEUA SCA

CIENTE:

Nome e assinatura do proponente

DATA: ____/____/_____

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR

VITA

Médica veterinária formada pela Pontifícia Universidade Católica de Minas Gerais - *campus* Poços de Caldas no ano de 2013. Realizou aprimoramento profissional na área de clínica médica de pequenos animais na Faculdade de Jaguariúna (2015). Cursou aperfeiçoamento em cardiologia veterinária na FUNEP/UNESP Jaboticabal (2014) e especialização em Cardiologia Veterinária pela Associação Nacional dos Clínicos Veterinários de Pequenos Animais (ANCLIVEPA-SP) (2016). Atualmente é aluna de mestrado no Programa de Pós-graduação em Ciências Veterinárias da Universidade Federal do Paraná (2018) e da pós-graduação em clínica médica de pequenos animais EQUALIS (2019).

REFERÊNCIAS

Amundsen BH, Helle-Velle T, Edvardsen T, Torp H, Crosby J, Lyseggen E, Stoylen A, Ihlen H, Lima JAC, Smiseth OA, Slordahl SA. Noninvasive myocardial strain measurement by speckle tracking echocardiography. J Am Coll Cardiol 2006;47:789-793.

Andersen NH, Poulsen SH. Evaluation of the longitudinal contraction of the left ventricle in normal subjects by doppler tissue tracking and strain rate. J Am Soc Echocardiogr 2003;16:716-723.

Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Häggström J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med 2009;23:1142-1150.

Atkins CE, Curtis MB, Mcguirk SM, Kittleson MD, Sato T, Snyder PS. The Use of M-Mode Echocardiography in Determining Cardiac output in dogs with normal, low, and high output states: comparison to thermodilution method. Vet Radiol *Ultrasound* 1992;33:5:297-304.

Aurigemma GP, Silver KH, Priest MA, Gaasch WH. Geometric Changes Allow Normal Ejection Fraction Despite Depressed Myocardial Shortening in Hypertensive Left Ventricular Hypertrophy. J Am Coll Cardiol 1995;26:195-202.

Black DE, Bryant J, Peebles C, Godfrey KM, Hanson M, Vettukattil JJ. Tissue motion annular displacement of the mitral valve using two-dimensional speckle tracking echocardiography predicts the left ventricle ejection fraction in normal chindren. Cardiol young 2014;24:640-648.

Blessberger H, Binder T. Non-invasive imaging: two dimensional speckle tracking echocardiography: basic principles. Heart 2010;96:716-722.

Bogaert J, Rademakers FE. Regional nonuniformity of normal and adult left ventricle. Am J Physiol Heart Circ Physiol 2001;280:H610-H620.

Bonagura JD, Schober KE. Can ventricular function be assessed by echocardiography in chronic canine mitral valve disease? J Small Anim Pract 2009;50:12-24.

Borgarelli M, Savarino P, Crosara S. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. J Vet Intern Med 2008;22:120–128.

Borgarelli M, Tarducci A, Zannata R, Haggstrom J. Decreased Systolic Function and Inadequate Hypertrophy in Large and Small Breed Dogs with Chronic Mitral Valve Insufficiency. J Vet Intern Med 2007,21:61-67.

Brown S, Atkins C, Bagley R, Carr A, Cowgill L, Davidson M, Egner B, Elliot J, Henik R, Labato M, Littman M, Polzin D, Ross L, Snyder P, Stepien. Guidelines for the Identification, Evaluation, and Management of Systemic Hypertension in Dogs and Cats. J Vet Intern Med 2007;21:542-558.

Brown S, Atkins C, Bagley R, Carr A, Cowgill L, Davidson M, Egner B, Elliot J, Henik R, Labato M, Littman M, Polzin D, Ross L, Snyder P, Stepien. Guidelines for the Identification, Evaluation, and Management of Systemic Hypertension in Dogs and Cats. J Vet Intern Med 2007;21:542-558.

Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. Adv Vet Sci Comp Med 1977;21:75–106.

Buss SJ, Mereles D, Emami M, Korosoglou G, Riffel JH, Bertel D, Schonland SO, Hegenbart U, Katus HA, Hardt SE. Rapid assessment of longitudinal systolic left ventricular function using speckle tracking of the mitral annulus. Clin Res Cardiol 2012;101:273-280.

Carnabuci C, Hanas S, Ljungvall I, Tidholm A, Bussadori C, Häggström J, Höglund K. Assessment of cardiac function using global and regional left ventricular endomyocardial and epimyocardial peak systolic strain and strain rate in healthy Labrador retriever dogs. Res Vet Sci 2013; 95:241-248.

Chen HY, Lien YH, Huang HP. Assessment of left ventricular function by two-dimensional speckle-tracking echocardiography in small breed dogs with hyperadrenocorticism. Acta Vet Scand 2014;56:1-10.

Chetboul V, Sampedrano CC, Gouni V, Nicolle AP, Pouchelon JL, Tissier R. Ultrasonographic assessment of regional radial and longitudinal systolic function in healthy awake dogs. J Vet Intern Med 2006;20:885-893.

Chetboul V, Serres F, Gouni V, Tissier R, Pouchelon JL. Radial strain and strain rate by two-dimensional speckle tracking echocardiography and the tissue velocity based technique in the dogs. J Vet Cardiol 2007;9:69-81.

Chetboul V, Tissier R. Echocardiographic assessment of canine degenerative mitral valve disease. J Vet Intern Med 2012;14:127-148.

Chiavegato D, Borgarelli M, D'Agnolo G, Santilli RA. Pulmonary hypertension in dogs with mitral regurgitation attributable to myxomatous valve disease. Vet Radiol Ultrasound 2009;50:253e258.

Chiu DYY, Abidin N, Hughes J, Sinha S, Kalra PA, Green D. Speckle tracking determination of mitral tissue annular displacement: comparison with strain and ejection fraction, and association with outcomes in haemodialysis patients. Int J Cardiovasc Imaging 2016;32:1511-1518.

Cornell CC, Kittleson MD, Torre Pd, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311-321.

Culwell NM, Bonagura JD, Schober KE. Comparison of echocardiographic indices of myocardial strain with invasive measurements of left ventricular systolic function in anesthetized healthy dogs. Am J Vet Res 2011;72:650–660.

DeCara JM, Toledo E, Salgo IS, Lammertin G, Weinert L, Lang RM. Evaluation of left ventricular systolic function using automated angle-independent motion tracking of mitral annular displacement. J Am Soc Echocardiogr 2005;18:1266-1269.

Falk T, Jönsson L, Olsen LH, Pedersen HD. Arteriosclerotic changes in the myocardium, lung, and kidney in dogs with chronic congestive heart failure and myxomatous mitral valve disease. Cardiovasc Pathol 2006;15:185–193.

Fente GJ, Garg P, Foley JRJ, Dobson LE, Musa TA, Erhayiem B, Greenwood JP, Plein S, Swoboda PP. The utility of global longitudinal strain in the identification of prior myocardial infarction in patients with preserved left ventricular ejection fraction. Int J Cardiovasc Imaging 2017;1-9.

Ferferieva V, Van den Bergh A, Claus P, et al. The relative value of strain and strain rate for defining intrinsic myocardial function. Am J Physiol Heart C 2012;302:H188-H195.

Greenbaum RA, Ho SY, Gibson DG, Becker AE, Anderson RH. Left ventricular fibre architecture in man. Br Heart J 1981;45:248-263.

Hawthorne EW. Instantaneous dimensional changes of the left ventricle in dogs. Circ Res 1961;9:110-119.

Higgins CB, Vatner SF, Franklin D, Braunwald E. Extent of regulation of the heart´s contractile state in the conscious dogs by alteration in the frequency of contration. J Clin Invest 1973;52:1187-1194.

Iwakura K, Okamura A, Koyama Y, Inoue K, Nagai H, Toyoshima Y, Tanaka K, Oka T, Iwamoto M, Fujii K. Assessment of left ventricular global systolic function by tissue

mitral annular displacement: comparison with global longitudinal strain [Abstract]. J Am Coll Cardiol 2016;67.

Jones CJH, Raposo L, Gibson DG. Functional importance of the long axis dynamics of the human left ventricle. Br Heart J 1990;63:215-220.

Katayama K, Tajimi T, Guth BD, Matsuzaki M, Lee JD, Seitelberger R, Peterson KL. Early diastolic filling dynamics during experimental mitral regurgitation in the conscious dog. Circulation 1988;78:390-400.

Kittleson MD, Eyster GE, Knowlen GG, Bari Olivier N, Anderson LK. Myocardial function in small dogs with chronic mitral regurgitation and severe congestive heart failure. J Am Vet Med Assoc 1984;184:455–459.

Komeda M, Glasson JR, Bolger AF, Daughters GT, Niczyporuk MA, Ingels NB, Miller DC. Three-dimensional dynamic geometry of the normal canine mitral annulus and papillary muscles. Circulation 1996;94:159-163.

Kusunose K, Zhang Y, Mazgalev TN, Thomas JD, Popovic ZB. Left ventricular strain distribution in healthy dogs and in dogs with tachycardia-induced dilated cardiomyopathy. J Cardiovasc Ultrasound 2013; 11:1-9.

Liu L, Tuo S, Zhang J, Zuo L, Liu F, Hao L, Sun Y, Yang L, Shao H, Qi W, Zhou X, GE S. Reduction of left ventricular longitudinal global and segmental systolic functions in patients with hypertrophic cardiomyopathy: Study of two-dimensional tissue motion annular displacement. Exp Ther Med 2014;7:1457-1464.

Ljungvall I, Höglund K, Carnabuci C, Tidholm A, Häggström J. Assessment of global and regional left ventricular volume and shape by real-time 3-dimensional echocardiography in dogs with myxomatous mitral valve disease. J Vet Intern Med 2011;25:1036-1043.

Locatelli C, Spalla I, Zanaboni AM, Brambilla PG, Bussadori C. Assessment of right ventricular function by feature-tracking echocardiography in conscious healthy dogs. Res vet Sci 2016;105:103-110.

Mantovani MM, Muzzi RAL, Pereira GG, Yamato RJ, Silva AC, Reis GF, Muzzi LAL, Guimarães EC. Systolic cardiac function assessment by feature tracking echocardiography in dogs with myxomatous mitral valve disease. J Small Anim Pract 2015:1-10.

Marciniak A, Claus P, Sutherland GR, Marciniak M, Karu T, Baltabaeva A, Merli E, Bijnens B, Jahangiri M. Changes in systolic left ventricular function in isolated mitral regurgitation. A strain rate imaging study. Eur Heart J 2007;28: 2627-2636.

Mizuguchi Y, Oishi Y, Miyoshi H, Iuchi A, Nagase N, Oki T. The functional role of longitudinal, circumferential and radial myocardial deformation for regulating the early impairment of left ventricular contraction and relaxation in patients with cardiovascular risk factors: a study with two-dimensional strain imaging. J Am Soc Echocardiogr 2008;21:1138-144.

O'gara P, Sugeng L, Lang R, Sarano M, Hung J, Raman S, Fischer G, Carabello B, Adams D, Vannan M. The role of imaging in chronic degenerative mitral regurgitation. JACC Cardiovasc Imaging 2008;1:221-237.

Ohno M, Cheng CP, Little WC. Mechanism of altered patterns of left ventricular filling during the development of congestive heart failure. Circulation 1994;89:2241–2250.

Olivetti G, Giordano G, Corradi D, Melissari M, Lagrasta C, Gambert SR, Anversa P. Gender differences and aging: effects on the human heart. J Am Coll Cardiol 1995;26:1068-1079.

Schober KE, Fuentes VL. Mitral annulus motion as determined by M-mode echocardiography in normal dogs and dogs with cardiac disease. Vet Radiol Ultrasound 2001;42:52-61.

Schober KE, Hart TM, Stern JA, Li X, Samii VF, Zekas LJ, Scansen BA, Bonagura JD. Detection of congestive heart failure in dogs by doppler echocardiography. J Vet Intern Med 2010;24:1358-1368.

Sengupta PP, Korinek J, Belohlavek M, Narula J, Vannan MA, Jahangir A, Khandheria BK. Left Ventricular Structure and Function - Basic Science for Cardiac Imaging. J Am Coll Cardiol 2006; 48:1988-2001.

Sousa MG, Gava FN, Filho JCS, Silva SNS, Camacho RR, Carareto R, Wolf M, Camacho AA. Long-axis fractional shortening and mitral annulus motion in dogs. Semina: Ciências Agrárias 2016;37:3115-3124

Spalla I, Locatelli C, Zanaboni AM, Brambilla P, Bussadori C. Echocardiographic assessment of cardiac function by conventional and speckle-tracking echocardiography in dogs with patent ductus arteriosus. J Vet Intern Med 2016;30:706-713.

Steflik D, Butts RJ, Baker GH, Bandisode V, Savage A, Atz AM, Chowdhury SM. A preliminary comparasion of two-dimensional speckle tracking echocardiography and pressure-volume loop analysis in patients with Fontan physiology: The role of left ventricular morphology. Echocardiography 2017;34:1353-1359.

Streeter DD, Sportnitz HM. Fiber orientation in the canine left ventricle during diastole and systole. Circ Res 1969;14:339-347.

Suzuki K, Akashi YJ, Mikukoshi K, Kou S, Takai M, Izumo M, Hayashi A, Ohtaki E, Nobuoka S, Miyake F. Relationship between left ventricular ejection fraction and

mitral annular displacement by speckle tracking echocardiography in patients with different heart diseases. J Cardiol 2012;60:55-60.

Suzuki R, Matsumoto H, Teshima T, Koyama H. Clinical assessment of systolic myocardial deformations in dogs with chronic mitral valve insufficiency using two-dimensional speckle-tracking echocardiography. J Vet Cardiol 2013;15:41-49.

Sveälv BG, Olofsson EL, Andersson B. Ventricular long-axis is of major importance for long-term survival in patients with heart failure. Heart 2008;94:284-289.

Takano H, Fujii Y, Ishikawa R, Aoki T, Wakao Y. Comparison of left ventricular contraction profiles among small, medium and large dogs by use of two-dimensional speckle tracking echocardiography. Am J Vet Res 2010;71:421-427.

Thomas WP, Gaber CE, Jacobs GJ. Recommendations for standards in transthoracic Two-Dimensional echocardiography in the dog and cat. J Vet Intern Med 1993;7:247-252

Tidholm A, Ljungvall I, Höglund K, Westling AB, Häggström J. Tissue doppler and strain imaging in dogs with myxomatous mitral valve disease in different stages of congestive heart failure. 2009;23:1197-1207.

Tidholm A, Westling AB, Höglund K, Ljungvall I, Häggström J. Comparisons of 3-, 2-dimensional, and M mode echocardiographical methods for estimation of left chamber volumes in dogs with and without acquired heart disease. J Vet Intern Med 2010;24:1414-1420.

Tsang W, Ahmad H, Patel AR, Patel AR, Sugeng L, Salgo IS, Weinert L, Mor-Avi V, Lang RM. Rapid estimation of left-ventricular function using echocardiographic speckle-tracking of mitral annular displacement. J Am Soc Echocardiogr 2010;23:511-515.

Urabe Y, Mann DL, Kent RL, Nakano K, Tomanek RJ, Carabello BA, Cooper G. Cellular and ventricular contractile dysfunction in experimental canine mitral regurgitation. Circ Res 1992;70:131-147.

Weidemann F, Jamal F, Sutherland GR, Sutherland GR, Claus P, Kowalski M, Hatle L, Schherder I, Bijnens B, Rademakers FE. Myocardial function defined by strain rate and strain during alterations in inotropic states and heart rate. Am J Physiol-Heart C 2002;283:H792–H799.

Wess G, Keller LJM, Klausnitzer M, Killich M, Hartmann K. Comparasion of longitudinal myocardial tissue velocity strain, and strain rate measured by two-dimensional speckle tracking and by color tissue doppler imaging in healthy dogs. J Vet Cardiol 2011;13:31-43.

Westrup U, McEvoy FJ. Speckle tracking echocardiography in mature Irish Wolfhound dogs: technical feasibility, measurement error and reference intervals. Acta Vet Scand 2013;55:1-11.

Wu EX, Wu Y, Tang H, Wang J, Yang J, Ng MC, Yang ES, Chan CW, Zhu S, Lau CP, Tse HF. Study of myocardial fiber pathway using magnetic resonance diffusion tensor imaging. J Magn Reson Imaging 2007;25:1048-1057.

Ylänen K, Eerola A, Vettenranta K, Poutanen T. Speckle tracking echocardiography detects decreased cardiac longitudinal function in anthracycline-exposed survivors of childhood cancer. Eur J Pediatr 2016;175:1379-1386.

Zaky A, Gill E, Paul C, Bendjelid K, Treggiari M. Characteristics of sepsis-induced cardiac dysfunction using speckle tracking echocardiography: a feasibility study. Anaesth Intensive Care 2016;44(1):65–76.

Zois NE, Tidholm A, Nagga KM, Moesgaard SG, Rasmussen CE, Häggstrröm J, Pedersen HD, Ablad B, Nilsen HY, Olsen LH. Radial and longitudinal strain and

strain rate assessed by speckle-tracking echocardiography in dogs with myxomatous mitral valve disease. J Vet Intern Med, 2012;26:1309-1319.