UNIVERSIDADE FEDERAL DO PARANÁ

MARCELA WOLF

AVALIAÇÃO DA FUNÇÃO MIOCÁRDICA BIVENTRICULAR POR ECOCARDIOGRAFIA CONVENCIONAL E SPECKLE TRACKING EM CÃES SUBMETIDOS À QUIMIOTERAPIA COM DOXORRUBICINA

CURITIBA
2022

MARCELA WOLF

AVALIAÇÃO DA FUNÇÃO MIOCÁRDICA BIVENTRICULAR POR ECOCARDIOGRAFIA CONVENCIONAL E SPECKLE TRACKING EM CÃES SUBMETIDOS À QUIMIOTERAPIA COM DOXORRUBICINA

Tese apresentada ao Programa de Pós-graduação em Ciências Veterinárias, setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de doutor em Ciências Veterinárias.

Orientador: Prof. Dr. Marlos Gonçalves Sousa

CURITIBA

2022

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Wolf, Marcela
Avaliação da função miocárdica biventricular por ecocardiografia convencional e Speckle tracking em cães submetidos à quimioterapia com doxorrubicina/ Marcela Wolf. – Curitiba, 2022.
1 recurso online: PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.
Orientador: Prof. Dr. Marlos Gonçalves Sousa

1. Oncologia. 2. Ecocardiografia. 3. Miocárdio. 4. Quimioterapia. 5. Cães. I. Sousa, Marlos Gonçalves. II. Universidade Federal do Paraná. Programa Pós-Graduação em Ciências Veterinárias. III. Título.

Bibliotecária: Telma Terezinha Stresser de Assis CRB-9/944

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS VETERINÁRIAS - 40001016023P3

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MARCELA WOLF** intitulada: **Avaliação da função miocárdica biventricular por ecocardiografia convencional e Speckle tracking em cães submetidos à quimioterapia com doxorrubicina**, sob orientação do Prof. Dr. MARLOS GONÇALVES SOUSA, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 30 de Junho de 2022.

Assinatura Eletrônica
30/06/2022 11:44:33.0
MARLOS GONÇALVES SOUSA
Presidente da Banca Examinadora

Assinatura Eletrônica
01/07/2022 08:07:04.0
MOACIR LEOMIL NETO
Avaliador Externo (PONTIFÍCIA UNIVERSIDADE CATÓLICA DE MINAS GERAIS)

Assinatura Eletrônica
01/07/2022 09:23:51.0
RUTHNÉA APARECIDA LÁZARO MUZZI
Avaliador Externo (UNIVERSIDADE FEDERAL DE LAVRAS)

Assinatura Eletrônica
30/06/2022 13:00:09.0
SIMONE TOSTES DE OLIVEIRA STEDILE
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
30/06/2022 14:32:19.0
ANDRIGO BARBOZA DE NARDI
Avaliador Externo (UNIVERSIDADE EST.PAULISTA JÚLIO DE MESQUITA FILHO)

AGRADECIMENTOS

A Deus, por sempre estar comigo, me guiar e permitir a realização desse sonho.

À minha mãe Conceição, meu pai Tadeu e meu irmão Thiago por todo o apoio, paciência, compreensão e por serem a minha base e porto seguro.

Ao professor Marlos pela orientação, e acima de tudo pelo acolhimento fraternal, pela confiança, pelos ensinamentos, por todas as oportunidades concedidas e por ser uma inspiração para mim e para tantas pessoas. Palavras são insuficientes para expressar minha gratidão e admiração.

À equipe do Laboratório de Cardiologia Comparada da UFPR, pelo apoio, pelos ensinamentos, pela parceria e pela amizade.

Aos amigos Stephany e Vinícius, meus companheiros nessa caminhada e dois grandes irmãos que a pós-graduação me deu, agradeço por tudo.

À toda a minha família e meus amigos, que sempre estiveram comigo e foram meu apoio, incentivo e suporte, e muitas vezes acreditaram mais em mim que eu mesma.

Ao serviço de Oncologia Veterinária da UFPR, às residentes Victoria e Natália, Prof. Vinícius e Prof. Roberta por permitirem a realização desse estudo com seus pacientes e por toda a ajuda, ensinamentos, parceria e amizade.

À Dra. Cláudia Custódio, ao serviço de Oncologia Veterinária e ao Hospital Veterinário Clinivet, por permitirem a realização desse estudo, por todo suporte, ajuda, ensinamentos, parceria e amizade.

À professora, chefe e amiga Ana Paula Sarraff, por colaborar com a realização desse trabalho.

À Universidade Federal do Paraná, ao Hospital Veterinário e ao Programa de Pós-graduação em Ciências Veterinárias, pela oportunidade, ensinamentos e acolhimento.

A todos os professores e alunos, que contribuiram de forma tão importante para meu crescimento profissional e pessoal.

Aos amados animais e seus tutores pela participação, comprometimento, carinho e apoio, sem vocês não seria possível.

Por fim, agradeço de coração a todos que contribuíram direta ou indiretamente na conclusão dessa grande e importante etapa de minha vida!

“If you can dream it, you can do it.”
(WALT DISNEY)

RESUMO

A doxorrubicina é um quimioterápico da classe das antraciclinas amplamente utilizado no tratamento de diversas neoplasias em seres humanos e cães. A cardiotoxicidade dose-dependente, afetando especialmente a função miocárdica ventricular, é um dos principais efeitos colaterais da sua utilização. A detecção precoce da toxicidade é importante tendo em vista a influência na tomada de decisões, alterações no protocolo quimioterápico e no prognóstico dos pacientes humanos oncológicos. A ecocardiografia é um método acessível e não invasivo que permite o acompanhamento da função miocárdica com eficácia. As técnicas de ecocardiografia tecidual avançada por *Speckle tracking* possibilitam detecção precoce da disfunção miocárdica e são recomendadas no acompanhamento seriado do paciente humano cardio-oncológico. O objetivo de elaboração dessa tese de doutorado, foi estudar a utilização da ecocardiografia convencional e por *Speckle tracking* na avaliação na função miocárdica em cães com câncer recebendo doxorrubicina em diferentes protocolos. Para isso, essa tese foi dividida em introdução e três capítulos. Na introdução foi realizada uma abordagem geral do tema. No primeiro capítulo, foi descrita uma investigação da função biventricular em cães com linfoma multicêntrico antes do início do protocolo quimioterápico, com objetivo de avaliar a influência e repercussão da neoplasia sobre o sistema cardiovascular. Os resultados mostram que alguns parâmetros, como *strain* circunferencial global, *strain* do ventrículo direito e o TMAD do ventrículo direito foram menores nos cães com linfoma quando comparados ao grupo controle, evidenciando alteração na função miocárdica, especialmente do ventrículo direito, nesses pacientes. O segundo e o terceiro capítulo descrevem um estudo prospectivo e longitudinal de 25 cães com diferentes neoplasias que receberam quimioterapia com doxorrubicina como agente único ou associada a outros quimioterápicos. Esses cães foram avaliados com exame clínico, ecocardiografia, eletrocardiografia e aferição de pressão arterial seriadamente em intervalos de 7 dias, 21 dias, 60 dias, 120 dias e 180 dias após a primeira dose da doxorrubicina. No segundo capítulo está descrito o estudo multidirecional da função do ventrículo esquerdo, que mostrou um prejuízo da função das fibras longitudinais por uma redução no MAPSE e do TMAD global na avaliação de 180 dias. Para finalizar, no terceiro capítulo consta a análise funcional e morfométrica do ventrículo direito desses cães, o qual mostrou uma redução progressiva nos parâmetros de função sistólica identificada por parâmetros de ecocardiografia convencional e *Speckle tracking*, como TAPSE, onda S´, strain e TMAD. Essa tese trouxe informações importantes no âmbito da cardio-oncologia veterinária e abriu portas para novos estudos e investigações nesse tema.

Palavras-chave: Speckle tracking. Oncologia. Cardiotoxicidade. Ecocardiografia. Strain

ABSTRACT

Doxorubicin is a chemotherapeutic drug of the anthracycline class widely used in the treatment of various neoplasms in humans and dogs. Dose-dependent cardiotoxicity, especially affecting ventricular myocardial function, is one of the main side effects of its use. Early detection of toxicity is important due to its influence on decision making, changes in the chemotherapy protocol and the prognostic impact of human cancer patients. Echocardiography is an accessible and non-invasive method that allows the monitoring of myocardial function effectively. Speckle tracking advanced tissue echocardiography techniques allow early detection of myocardial dysfunction and are recommended in the serial follow-up of human cardio-oncological patients. The objective of this thesis was to study the myocardial function of dogs with cancer receiving doxorubicin in different protocols by conventional echocardiography and by Speckle tracking echocardiography. For this, this thesis was divided into an introduction and three chapters. The introduction provided a general approach to this topic. In the first chapter, an investigation of biventricular function in dogs with multicentric lymphoma prior to chemotherapy was described, with the objective of evaluating the influence and repercussion of the neoplasm on the cardiovascular system. The results show that some parameters, such as global circumferential strain, right ventricular strain and right ventricular TMAD were lower in dogs with lymphoma when compared to the control group, evidencing changes in myocardial function, especially of the right ventricle, in these patients. The second and third chapters describe a prospective and longitudinal study of 25 dogs with different neoplasms that received chemotherapy with doxorubicin as a single agent or associated with other chemotherapy drugs. These dogs were evaluated with clinical examination, echocardiography, electrocardiography and blood pressure measurements serially at intervals of 7 days, 21 days, 60 days, 120 days and 180 days after the first dose of doxorubicin. The second chapter is composed of the multidirectional study of left ventricular function, which showed an impairment of longitudinal fiber function by a reduction in MAPSE and global TMAD in the 180-day evaluation. Finally, the third chapter contains the functional and morphometric analysis of the right ventricle of these dogs, which showed a progressive reduction in systolic function parameters identified by conventional echocardiography and Speckle tracking parameters, such as TAPSE, S' wave, strain and TMAD. This thesis brought important information in the field of veterinary cardio-oncology and opened doors for new studies and investigations on this topic.

Keywords: Speckle tracking. Oncology. Cardiotoxicity. Echocardiography. Strain

LISTA DE ABREVIATURAS

ACVIM - American College of Veterinary Internal Medicine

AP4 - Apical four-chamber

AP2 - Apical two-chamber

AP3 - Apical three-chamber

BSA - Body surface area

BW - Body weight

CSt - Circumferential strain

CD - Cumulative dose

CHOP - Doxorubicin, cyclophosphamide, vincristine and prednisone

DoxoCarbo - Doxorubicin and carboplatin

EF - Ejection fraction

FAC - Fractional area change

FS - Fractional shortening

GCS - Global circunferencial strain

GLS - Global longitudinal strain

HF - Heart failure

IVRT - Isovolumic relaxation time

LSt - Longitudinal strain

LV - Left ventricular

$LVIDd_n$ - Normalized left ventricular internal dimension at end-diastole

$LVIDs_n$ - Normalized left ventricular internal dimension at end-systole

MAPSEi - Mitral annular plane systolic excursion indexed to the body surface area

ROI - Region of interest

RV - Right ventricular

S´ - Peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler

SBP - Systolic blood pressure

TAPSEi - Tricuspid annular plane systolic excursion indexed to the body surface area

TMAD - Tissue motion annular displacement

SUMÁRIO

1 INTRODUCTION

Doxorubicin is an anthracycline widely used in oncology because it is a potent chemotherapeutic with good results in several neoplasms in dogs [1]. On the other hand, cardiotoxicity is one of the main side effects of the use of this drug, which can directly interfere with ventricular myocardial function, and also predispose to the development of arrhythmias, valve diseases, pericardiopathies, changes in systemic blood pressure and congestive heart failure [2,3,4,5].

Doxorubicin cardiotoxicity can occur in an acute form, recognized in a short time after its administration, which in these cases shows to be less dose-dependent, potentially reversible and results mainly in hypotension and arrhythmias [1,3,6], or in the late form, with the development of ventricular dysfunction, in this case it is mostly irreversible and directly related to the cumulative dose (dose-dependent) in dogs and humans [2,4,6,7].

The etiology of doxorubicin cardiotoxicity is still not fully understood. Several mechanisms seem to be involved, such as the release of free radicals, the blocking of specific cellular pathways [9], the interference with cellular DNA by the inhibition of the topoisomerase 2β enzyme, the increase in oxidative stress [10] and increased expression of death receptors on the membrane of cardiomyocytes [11].

Doxorubicin cardiotoxicity has long been recognized in dogs [12]. Studies show an incidence of 4% to 84% in this species [5,7,13], with a cumulative dose ranging from 90 to 265 mg/kg² [5,7,13,14].

The cardiotoxicity assessment method is directly related to its incidence. Some methods, such as the fractional shortening obtained by conventional echocardiography, are later than biomarkers such as troponin I [7,15]. On the other

hand, genetic evaluation with the expression of microRNAS is even earlier than troponin I [16]. Although gold standard techniques such as MRI and histopathological evaluation provide reliable and accurate information about myocardial injury [2,17], they are less applicable in clinical routine for patient follow-up. On the other hand, echocardiography is a safe, non-invasive, non-ionizing, widely accessible method that effectively allows the assessment of myocardial function in cardio oncological patients [2,18].

Speckle tracking echocardiography techniques allow early detection of myocardium dysfunction when compared to conventional echocardiography in humans and rats receiving doxorubicin [2,19,20]. Global longitudinal strain (GLS) is a technique recommended in the serial follow-up of humans undergoing chemotherapy which, in addition to allowing the detection of early dysfunction [2,8,18], provides prognostic information in patients with preserved ejection fraction [21]. Tissue motion annular displacement (TMAD) is also a speckle tracking-based technique that has been shown to early detect left ventricular and right ventricular dysfunction in childhood cancer survivors [22]. However, there are no studies with these techniques in dogs undergoing chemotherapy.

Left ventricular function is one of the main parameters evaluated in the echocardiography of these patients and the dysfunction of this chamber may be present in the clinical form, resulting in left congestive heart failure or in the subclinical form [5,7]. However, previous studies in people show that right ventricular function is also affected and may occur before changes in the left ventricle [23], but there is no evidence to date regarding the specific evaluation of the right ventricular chamber of dogs undergoing chemotherapy.

Cardiovascular complications secondary to the use of anthracyclines are the

main causes of death in cancer survivors, therefore, early approaches are beneficial for protocol change and management of therapeutic decisions that may influence the patient's prognosis [2].

Finally, this thesis aimed to prospectively and longitudinally evaluate myocardial function and structural assessment of the left and right ventricles with conventional and Speckle tracking echocardiography, in dogs with different types of neoplasia, undergoing chemotherapy with doxorubicin as a single agent or in association with other chemotherapeutic agents determined by the oncology department.

1.1 REFERENCES

[1] Jacobs GJ. Secondary canine cardiomyopathies: their causes and characteristics. Vet Med Sci 1996;91:534-64.

[2] Hajjar LA, Costa IBSS, Lopes MACQ, Hoff PMG, Diz MPE, Fonseca SMR, Bittar CS, Rehder MHHS, Rizk SI, Almeida DR, Fernandes GS, Silva LB, Campos CAHM, Montera MW, Alves SMM, Fukushima JT, Santos MVC, Negrão CE, Silva TLF, Ferreira SMA, Malachias MVB, Moreira MCV, Neto MMRV, Fonseca VCQ, Soeiro MCF, Alves JBS, Silva CMPD, Sbano J, Pavanello R, Pinto IMF, Simão AF, Dracoulakis MDA, Hoff AO, Assunção BMBL, Novis Y, Testa L, Filho ACA, Cruz CBBV, Pereira J, Garcia DR, Nomura CH, Rochitte CE, Macedo AVS, Marcatti PTF, Junior WM, Wiermann EG, Freitas RVH, Coutinho A, Mathias CMC, Vieira FMAC, Sasse AD, Rocha V, Ramires JAF, Filho RK. Diretriz Brasileira de Cardio-oncologia. Arq Bras Cardiol 2020;115:1006-43.

[3] Souza RCA, Camacho AA. Neurohormonal, hemodynamic, and electrocardiographic evaluations of healthy dogs receiving long-term administration of doxorubicin. Am J Vet Res 2006;67:1319-25.

[4] Chung WB, Youn HJ. Pathophysiology and preventive strategies of anthracycline-induced cardiotoxicity. Korean J Intern Med 2016;31:625-633.

[5] Gallay-Lepoutre J, Bélanger MC, Nadeau ME. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. Res Vet Sci 2016;105:153-9.

[6] Narezkina A, Nasim K. Anthracycline cardiotoxicity. Circ Heart Fail 2019; 12:e:005910.

[7] Hallman BE, Hauck ML, Williams LE, Hess PR, Suter SE. Incidence and risck factors associated with development of clinical cardiotoxicity in dogs receiving doxorubicin. J Vet Intern Med 2019;33:783–91.

[8] Zamorano JL, Lancellotti P, Muñoz DR, Abovans V, Asteggiano R, Galderisi M, Habib G, Lenihan DJ, Lip GYH, Lyon AR, Fernandez TL, Mohty D, Piepoli MF, Tamargo J, Torbicki A, Suter TM. Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur Heart J 2016;37p.2768–2801.

[9] Stefani L, Pedrizzetti G, Galanti G. Clinical application of 2D Speckle tracking strain for assessing cardio-toxicity in oncology. J Funct Morphol Kinesiol 2016;1:343-354.

[10] Bloom MW, Hamo CE, Cardinale D, Ky B, Nohrja A, Baer L, Skopicki H, Lenihan DJ, Gheorghiade M, Lyon AR, Butler J. Cancer therapy-related cardiac dysfunction and heart failure: part 1: definitions, pathophysiology, risk factors, and imaging. Circ Heart Fail 2016;9:e002661.

[11] Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. Nature 2017;7:44735.

[12] Ditchey RV, LeWinter MM, Higgins CB. Acute effects of doxorrubicin (adriaycin) on left ventricular function in dogs. Int J Cardiol 1984;6:341-350.

[13] Mauldin GE, Fox PR, Patnaik AK, Bond BR, Mooney SC, Matus RE. Doxorubicin-induced cardiotoxicosis. Clinical features in 32 dogs. J Vet Intern Med 1992;6:82-88.

[14] Loar AS, Susaneck SJ. Doxorubicin-induced cardiotoxicity in five dogs. Semin

Vet Med Surg 1986;1:68-71.

[15] Surachetpong SD, Teewasutrakul P, Rungsipipat A. Serial measurements of cardiac troponin I (cTnI) in dogs treated with doxorubicin. Jpn J Vet Res 2016;64:221-233.

[16] Beumier A, Robinson SR, Robinson N, Lopez KE, Meola DM, Barber LG, Bulmer BJ, Calvalido J, Rush JE, Yeri A, Das S, Yang VK. Extracellular vesicular microRNAs as potential biomarker for early detection of doxorubicin-induced cardiotoxicity. J Vet Intern Med 2020;34:1260-71.

[17] LLesuy SF, Milei J, Flecha BSG, Boveris A. Myocardial damage induced by doxorubicins: hydroperoxide-initiated chemiluminescence and morphology. Free Radic Biol Med 1990;8:259-264.

[18] Plana JC, Galderisi M, Barac A, Ewer MS, Hy B, Scherrer-Crosbie M, Ganame J, Sebag IA, Agler DA, Badano LP, Banchs J, Cardinale D, Carver J, Cerqueira M, DeCara JM, Edvarsen T, Flamm SD, Force T, Griffin BP, Jerusalem G, Liu JE, Magalhães A, Marwick T, Sanchez LY, Sicari R, Villaraga HR, Lancelotti P. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J 2014; 15:1063-93.

[19] Oliveira MS, Melo MB, Carvalho JL, Melo IM, Lavor MSL, Gomes DA, Goes AM, Melo MM. Doxorubicin cardiotoxicity and cardiac function improvement after stem cell therapy diagnosed by strain echocardiography. J Cancer Sci Ther 2013;5:52–57.

[20] Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of anthracyclines. Front Cardiovasc Med 2020;18:7:26.

[21] Rhea IB, Uppuluri S, Sawada S, Schneider BP, Feigenbaum H. Incremental Prognostic Value of Echocardiographic Strain and Its Association With Mortality in

Cancer Patients. J Am Soc Echocardiogr 2015;28:667–73.

[22] 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;175:1379-86.

[23] Planek MIC, Manshad A, Hein K, Hemu M, Ballout F, Varandani R, Venugopal P, Okwuosa T. Prediction of doxorubicin cardiotoxicity by early detection of subclinical right ventricular dysfunction. Cardiooncology 2020;6:1-8.

2 Assessment of left and right ventricular systolic function in dogs with multicentric lymphoma

Marcela Wolf[a]*, Stephany B. Lucina[a], Vinícius B. C. Silva[a], Matheus F. Silveira[a], Victoria G. Silva[a], Ana P. Sarraff[b], Claudia C. Custódio[c], Marlos G. Sousa[a]

* Corresponding author. E-mail address: marcelawvet@gmail.com (M Wolf).

[a] Department of Veterinary Medicine, Federal University of Paraná, Curitiba, Paraná, Brazil.

[b] School of Life Sciences, Pontifical Catholic University of Paraná, Curitiba campus, Curitiba, Paraná, Brazil.

[c] Oncology Sector, Clinivet Veterinary Hospital, Curitiba, Paraná, Brazil.

Key words: canine, cardio-oncology, echocardiography, oncology, strain, speckle tracking.

2.1 Abstract

Objective: Myocardial dysfunction in cardio-oncology is generally thought to be related to the cardiotoxicity of chemotherapy treatment. However, it is known that some tumors have direct effects on myocardial function. These effects have been studied in man, but there are no published reports of the effects in dogs. Novel advanced echocardiographic techniques may allow early detection of myocardial dysfunction when compared to conventional echocardiographic techniques. This study aims to assesses myocardial systolic function in dogs with multicentric lymphoma prior to chemotherapy protocol.

Animals: Fifteen dogs with multicentric lymphoma and nineteen healthy dogs.

Methods: Prospective cross-sectional observational study. Dogs with multicentric lymphoma and healthy control dogs underwent physical examination, electrocardiography, systolic blood pressure measurement and standard and speckle tracking echocardiography to assess biventricular systolic function.

Results: There were no differences between groups in terms of ejection fraction, shortening fraction, left ventricular systolic and diastolic diameter, tricuspid annular plane systolic excursion, mitral annular plane systolic excursion and fractional area change of the right ventricle. However, there was a reduction in the values of Global Circumferential Strain (GCS) ($p=0.0003$), right ventricle (RV) Strain ($p=0.01$) and RV tissue motion annular displacement ($p<0.05$) in the dogs with lymphoma when compared to the control group.

Conclusions: Speckle tracking techniques appear to demonstrate early systolic dysfunction, primarily affecting the right ventricle, in dogs with lymphoma prior to chemotherapy treatment.

Abbreviation list

AP2 - Apical 2-chamber
AP3 - Apical 3-chamber
AP4 - Apical 4-chamber
BSA - Body surface area
CSt - Circumferential strain
EF - Ejection fraction
FAC - Fractional area change
FS - Fractional shortening
GCS - Global circumferential strain
GLS - Global longitudinal strain
IVRT - Isovolumetric relaxation time
LA:Ao - Left atrium-to-aorta ratio
LSt - Longitudinal Strain
$LVID_d$ - Left ventricular internal dimension at end-diastole
$LVID_s$ - Left ventricular internal dimension at end-systole
MAPSE - Mitral annular plane systolic excursion
ROC - Receiver operating characteristic curves
RV - Right ventricle
SBP - Systolic blood pressure
TAPSE - Tricuspid annular plane systolic excursion
TMAD - Tissue motion annular displacement
TNFa - Tumor necrosis factor alpha

2.2 Introduction

Cancer and heart diseases represent the main public health issues and cause of death in people worldwide [1]. Cardiac changes in cancer patients are generally related to the cardiotoxicity of chemotherapy treatment [2]. However, there is a more complex interrelation between cancer and heart function. Studies have shown that tumors can release several pro-inflammatory cytokines, resulting in a chronic inflammatory state with systemic effects [3], which in experimental studies have been shown to result in the development of cachexia and cardiac dysfunction [4,5]. In addition, systemic changes secondary to the presence of heart failure may favor the development of cancer [6].

Lymphoma is one of the main cancers affecting dogs. This disease can present in different stages, with or without cardiac involvement [7]. In addition to the intrinsic effects on the cardiovascular system due to mediators produced by tumors [6], multicentric lymphoma may result in myocardial infiltration which includes extensive masses and multiple microscopic infiltrative lesions [8].

The echocardiogram is an ideal non-invasive exam for the assessment of cardiac morphofunctionality, but it has some limitations for the detection of cardiac tumors [9]. However, other imaging techniques such as computed tomography are more accurate for determine the morphology, location and extent of cardiac tumors, including microscopic cardiac involvement [10]. Advanced echocardiography techniques, such as the GLS [11] and TMAD [12], allow the early detection of myocardial dysfunction due to cardiotoxicity in human patients with cancer.

In veterinary medicine, some studies have evaluated myocardial function in dogs treated with doxorubicin [13,14]. However, to our knowledge, none of these studies evaluated cardiac changes in pre-chemotherapy cancer patients. There are few studies that have looked at the interrelation of cardiovascular function in dogs with cancer. Little is known about the

intrinsic cardiovascular involvement of tumors before the start of chemotherapy. In this study we assessed the myocardial systolic function of left and right ventricles in dogs with multicentric lymphoma prior to chemotherapy.

2.3 Methods

Animals

Dogs were recruited for this prospective, cross-sectional, observational study from patients admitted for clinical evaluation at a veterinary teaching facility and at a private veterinary hospital between February 2019 and March 2020. All procedures were approved by the Institutional Animal Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Owners gave formal consent prior to the animal being enrolled on the study.

For study inclusion all dogs had a diagnosis of multicentric lymphoma by a fine needle aspirate and/or histopathological analysis and underwent an echocardiographic examination before any therapy was instituted. Inclusion criteria included that patients with lymphoma did not have any signs of cardiac involvement, such as pericardial effusion or nodules observed on echocardiography. In addition to echocardiography, all dogs underwent a complete physical examination, SBP assessment and electrocardiography. Systolic blood pressure was obtained non-invasively (vascular *Doppler*) and the recorded result was the mean of at least five measurements performed by trained observer (MW) as previously recommended [15]. A computer-based electrocardiogram (TEB ECG PC) was recorded for at least three minutes immediately prior to echocardiography in all animals to document cardiac rhythm and heart rate.

Exclusion criteria for this study included any cardiac rhythm of non-sinus origin; congenital cardiovascular disease; cardiac tumors and pericardial effusion.

Finally, dogs with no history of cardiac or oncological disease were recruited as

controls. Those animals underwent a detailed physical examination prior to inclusion in the study, as well as a complete echocardiogram, electrocardiogram, and SBP measurement to rule out any conditions that could preclude their use as controls.

Conventional echocardiography

Echocardiography was performed with Philips Affiniti 50 ultrasound system equipped with 2-4, 3-8 and 4-12 MHz phased-array transducers with continuous electrocardiogram monitoring. A single experienced operator (MW) was responsible for image acquisition and measurements. During the examination 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 [16].

Using M-mode and short axis images obtained from the right parasternal window, measurements were made of: $LVID_d$ and $LVID_s$, end-diastolic volume, end-systolic volume, FS and EF, the latter being calculated by the Teichholz formula. Normalized dimensions of the left ventricle were calculated according to the previously reported method [17]. The LA:Ao was calculated from two-dimensional short axis images obtained in early diastole.

Apical 4-chamber and AP2 views were acquired for the EF by Simpson's biplane method. The end diastolic and systolic volume obtained were also recorded. Apical 4-chamber images were also used to obtain the MAPSE with M-mode in lateral and septal regions of the mitral annulus. Mitral annular plane systolic excursion and TAPSE were indexed by BSA. The mitral early (E) and late (A) diastolic peak velocities were obtained in the transmitral flow in the AP4 chamber, while apical 5-chamber images were used for obtaining the IVRT. From these parameters the E:A and the E:IVRT ratios were calculated. Left ventricular outflow velocity was recorded to obtain the aortic valve closure time (R to

AVC), 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 used to determine the peak systolic in speckle tracking analyses.

A tissue Doppler sample gate was placed on the septal and parietal region of the mitral annulus to obtain systolic (S´), early diastolic (E´) and late diastolic (A´) velocities, which were also used to calculate E´:A´ ratio.

Finally, the AP4 view optimized for the right ventricle (RV) was performed to obtain functional evaluations of the RV. Tricuspid annular plane systolic excursion was obtained with M-mode in the free wall of the RV, and the S´, E´ and A´ wave were obtained with pulsed-wave TDI. The pulsed-Doppler was used to obtain Et (early) and At (late) to asses RV diastolic function. Fractional area change (FAC) was performed by tracing the RV endocardial border at end-diastole and at end-systole, as previously described [18].

Speckle tracking echocardiography

Longitudinal Strain

At least five cardiac cycles of apical 4- (AP4), 2- (AP2), 3- (AP3) chamber views and apical 4 chamber view optimized to the RV were recorded for subsequent off-line evaluations of speckle tracking echocardiography.

The equipment software (QLAB-automatic cardiac motion quantification) automatically detected the left ventricular myocardium to be tracked and divided the ventricle into seven segments. Manual corrections were performed where necessary. For each individual image, strain was considered as the average deformation of those seven segments. Longitudinal strain was determined as a percentage of left ventricular myocardial deformation in a heartbeat and the GLS was calculated as the average of LSt obtained in AP4, AP2 and AP3 images.

The RV Strain was obtained from three defined regions of interest (ROIs): RV septal annulus, RV lateral annulus and RV apex. Subsequently, the software automatically tracked each myocardial segment. However, only the three segments tracked on the free wall of the right ventricle were selected: basal, middle and apical. The RV Strain corresponds to the mean of deformation of these 3 segments in one cardiac cycle.

Circumferential Strain

At least five cardiac cycles were recorded on the short axis in the right parasternal window of the apical, middle (papillary plane) and mitral plane for subsequent off-line evaluations of CSt. In the same way, strain was performed in each plane and GCS was calculated as the average of CSt obtained in apical, middle and mitral plane images.

Tissue Motion Annular Displacement

Tissue motion annular displacement was calculated automatically by the software using both AP4, AP2 and AP4 optimized to the RV images. For this purpose, three ROIs were determined by the operator. To perform TMAD of the left ventricle, two of these were in the mitral annulus while the third was the epicardial region of the left ventricular apex. The RV TMAD was performed in the optimized AP4 view and three ROIs were selected: lateral and septal annulus of the tricuspid valve and the third in the epicardial region of the RV apex. Once ROIs were chosen, the software tracked the displacement of the first and second ROI (in mm) towards the third ROI. A midpoint between the two mitral ROIs was automatically created and its displacement towards the apex documented in mm and as a percentage of the total length of the ventricle.

Global TMAD was calculated in five different ways: global $TMAD_{mm}$ is the average displacement (in mm) of the midpoint of the mitral annulus obtained in AP4 and AP2 images

towards the apex for the LV and for the RV is the absolute displacement in mm of the midpoint in the AP4 optimized for the RV; global $TMAD_{\%}$ is the mean fractional displacement (in relation to the total LV length) of the midpoint towards the LV apex in the AP4 and AP2 chamber images and for the RV is a percentage value of the midpoint movement in relation to the total RV length; global $TMAD_{mm/kg}$ is the global $TMAD_{mm}$ indexed to the body weight in kilograms; global $TMAD_{mm/\sqrt[3]{BW}}$ is the global TMADmm indexed to cubic root of body weight; and the global $TMAD_{mm/m^2}$ is global $TMAD_{mm}$ indexed to BSA, as reported in a previous study [25].

2.4 Statistical Analysis

Statistical analyses were performed using Graphpad prism 8.0 software. Normality of the data was evaluated by the Shapiro-Wilk test. For parameters with normal distribution, differences between groups were evaluated with T test. For non-normally distributed data differences between groups were assessed using Mann Whitney (non-parametric approach). Pearson and Spearman correlation tests were used to assess the correlation of echocardiographic variables with weight, age, heart rate and blood pressure. Fisher's test was used to determine if there was a difference between males and females between groups. Receiver operating characteristic curves were used to determine sensitivity and specificity of several echocardiographic variables that differed the two groups.

2.5 Results

A total of 34 dogs were enrolled in this study, of which 19 were healthy dogs composing the control group and 15 were diagnosed with multicentric lymphoma.

Control group was composed of Crossbreed (n=4), Yorkshire terrier (n=4), Lhasa Apso (n=3), German Spitz (n=2), French bulldog, Labrador Retriever, Maltese, Pinscher,

Shitzu and Whippet (n=1 each). The lymphoma group was composed of Crossbreed (n=5), Golden Retriever (n=3) Fox Terrier, Rottweiller, Schnauzer, Lhasa Apso, German Shepherd, Swiss Shepherd and English Bulldog (n=1 each). The distribution of age, body weight and basic echocardiographic data of each group are shown in table 1. There were no differences in sex between the two groups (P=0.07). Four dogs in the lymphoma group had mitral valve disease with mild mitral regurgitation without cardiac remodeling (ACVIM stage B1) and one of them also had tricuspid valve disease associated with mild insufficiency without cardiac remodeling.

Nine dogs had stage IV lymphoma, one dog had medullary involvement (stage V) and five dogs had stage III lymphoma. The dogs in the lymphoma group were older (P=0.01) and heavier (P=0.01) compared to the control group (table 1).

Sinus arrhythmia was the predominant cardiac rhythm in the lymphoma group (n= 14/15) and in the control group (n= 16/19). One dog in the lymphoma group and two dogs in the control group had sinus rhythm, while one dog in the control group had sinus tachycardia. Two dogs in lymphoma group had isolated premature ventricular complexes.

There were no differences in shortening fraction (P=0.33) and left ventricular ejection fraction (P=0.54). However, GCS (P=0.03) and global $TMAD_{mm/kg}$ (P=0.02) was lower in the lymphoma group than the control group (table 2).

There was no difference between groups for the variables MAPSEi parietal (P=0.3), MAPSEi septal (P=0.3), as well as the GLS (P=0.2), LSt AP4 (P=0.8), LSt AP3 (P=0.17), LSt AP2 (P=0.06) and the global TMAD. Global $TMAD_{mm/kg}$ was lower in lymphoma patients (P=0.02) (table 1 and table 2).

Regarding the systolic function of the right ventricle, no differences were observed in the variables TAPSEi (P=0.46) and FAC (P=0.5) obtained by conventional echocardiography. However, RV Strain and RV TMAD were lower in the group with lymphoma (table 2 and

figure 1).

Global Longitudinal Strain (P<0.0001; R=-0.65), GCS (P=0.02; R=-0.38) and RV Strain (P=0.0002; R=0,6) were affected by body weight, as was biplane EF Simpson (P=0.0004; R=-0.574). Right ventricular $TMAD_{mm}$ (P=0.0011; R=0.5366), RV $TMAD_{mm/kg}$ (P=0<0.0001; R=-0.9), RV $TMAD_{mm/m^2}$ (P<0.0001;R=-0.8) and RV $TMAD_{mm/\sqrt[3]{BW}}$ (P=0.01; R=-0.4) were also correlated with body weight, as were the global $TMAD_{mm}$ (P<0.0001; R=0.68), global $TMAD_{mm/kg}$ (P=0<0.0001; R=-0.93) and global $TMAD_{mm/m^2}$ (P<0.0001;R=-0.82).

Age showed a negative correlation with the E/A ratio (P:=0.001; R:-0.5). Systemic blood pressure influenced the values of lateral MAPSEi (P=0.004; R= 0.48), septal MAPSEi (P=0.04; R=0.34), FS (P=0.04; R=0.3) and FE (P=0.03; R=0.35).

Several echocardiographic variables showed good (AUC>0.7) sensitivity and specificity to differentiate patients with lymphoma from the control group (table 3). With a cut-off of <22.85%, the RV Strain had 80% sensitivity and 73.6% specificity to differentiate the two groups.

2.6 Discussion

In this study, some parameters of assessment of myocardial function, especially RV systolic function, assessed by advanced echocardiography techniques were reduced in patients with lymphoma (table 1 and table 2). The main mechanism of myocardial dysfunction in cancer patients is thought to be related to the cardiotoxicity of chemotherapeutic drugs, especially anthracyclines. Although several factors are involved in the cardiotoxicity of these drugs the main problems are the release of free radicals and the blocking of specific cell pathways [2]. However, in this study we show that some myocardial changes can occur before chemotherapy in dogs with lymphoma.

In patients with lymphoma the myocardium can also be compromised by myocardial

infiltration [8,19] and the production of mediators by the tumor that act directly on the heart [6]. Although dogs with lymphoma in this study did not show structural changes on echocardiography, it is possible that microscopic myocardial infiltration was present, as described in human patients [8]. In a study of 196 people with lymphoma, 48 had cardiac involvement, and of these, disease was detected in 21 only on microscopic evaluation [20]. Studies in dogs show that lymphoma is one of the main metastatic tumors that affects the heart [19]. Therefore, the lack of histopathological analysis is a limitation of this study.

The study of cardiovascular impairment secondary to cancer is limited, not least because cancer patients often receive chemotherapy treatment that can compromise myocardial function and thus generate a confounding bias. Experimental studies have shown that cancer cells and the components of the tumor microenvironment produce mediators such as chemokines, hormones, metabolites and growth factors. These locally produced products have systemic effects and thus can generate cardiac dysfunction in cancer patients [6]. Tumor necrosis factor alpha is a cytokine released by tumors that causes direct myocardial dysfunction [21,22]. Studies in rats have shown that inhibition of TNFa with antibodies reduces myocardial involvement [23]. Interleukins 1β are also released by cancer cells and can also compromise cardiovascular functionality in several ways, such as reducing the response to the type L calcium channel [24].

Another important factor in this study is that dogs in the lymphoma group were heavier than dogs in the control group. Myocardial function indices, such as GLS and TMAD of the left and right ventricles, have been shown to have a negative correlation with body weight in healthy dogs [18,25,26]. However, there were no differences in the other parameters for assessment of myocardial function such as ejection fraction, shortening fraction, FAC, MAPSE and TAPSE between the two groups. One explanation for the changes recognized using advanced echocardiography techniques that were not seen using conventional

ultrasound may be a change in fibrotic index. People with cancer show intense fibrotic remodeling [27] and strain measurement, which is useful for the early detection of myocardial dysfunction, has been shown to have a negative correlation with the level of tissue fibrosis [28]. Heavier dogs tend to have lower systolic function indices. However, in this study the change in systolic function was only detected on the advanced echocardiographic techniques. This suggests that although the dogs with lymphoma in this study were heavier, the advanced techniques, especially measurement of RV systolic function and circumferential LV function, may genuinely have detected early signs of reduced systolic function since there were no differences between groups with regard to the conventional techniques (table 2).

The systolic function of the right ventricle is primarily the result of the contraction of the longitudinal fibers [29]. GLS is a technique that assesses the function of longitudinal fibers and allows the detection of early left [30] and right [31] myocardial dysfunction in human patients undergoing chemotherapy. This technique seems to be a sensitive indicator of subclinical dysfunctions in situations where there are associated metabolic disorders, in addition to allowing and assisting the cardio-oncology relationship in the early detection of individuals in the "gray zone" and thus better stratifying cardiovascular risk [2]. RV TMAD values were associated with a worse prognosis in children with leukemia and lymphoma undergoing chemotherapy [32]. TMAD is also a technique based on Speckle tracking with good repeatability for the assessment of longitudinal function of the left [25] and right [26] ventricles in dogs and which has already shown good results in detection of dysfunction myocardial in children with cancer undergoing doxorubicin chemotherapy, with good correlation with gold standard methods such as magnetic resonance imaging [12,33].

Dogs with lymphoma showed lower circumferential deformation of the apical region of the left ventricle (table 2). Some diseases result in alterations in the pattern of regional ventricular deformation, for example, amyloidosis in man produced a specific pattern called

“apical sparing”, where there is a greater impairment of the basal regions of the left ventricle [34]. Future studies with segmental myocardial evaluation and with a larger number of animals with lymphoma are necessary to investigate the possibility of specific region change in animals.

Although the slower speed of the S´ wave is related to reduced systolic ventricular function, in this study the S´ wave was greater in patients with lymphoma (table 1). However, this may be explained by the fact that there is a positive correlation of the S´ wave with body weight for both the left (P=0.004; R=0.47) and right (P=0.0011; R=0.53) ventricles, and the dogs with lymphoma were heavier than the controls [18].

In this study, two dogs in the lymphoma group had isolated premature ventricular complexes on the conventional electrocardiogram. Electrical changes can be seen in 66% of human patients with lymphoma, including changes in the ST segment and sinus tachycardia [8], and bradycardia and several arrhythmias have been reported in dogs with lymphoma [19].

This study has a number of limitations. The most important of these is the lack of histopathological evaluation of neoplastic infiltration in the heart. The weight difference between the two groups is another relevant factor. Additionally, ancillary examinations to rule out concurrent systemic diseases, i.e. infectious diseases or hormonal diseases, which might affect systolic function, were not performed unless clinically indicated. Finally, dogs with different stages of multicentric lymphoma were recruited and the number of dogs included is too small to extrapolate these results to all circumstances.

2.7 Conclusions

Dogs with multicentric lymphoma had lower speckle tracking variables of systolic function, especially in the right ventricle, when compared to the control group. Future studies with a larger number of dogs with lymphoma and other tumors are needed to better

understand cardiovascular changes in dogs with cancer.

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).

2.8 References

[1] Kocarnik J. Cancer's global epidemiological transition and growth Cancer's global epidemiological transition and growth. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(19)32046-X/fulltext, 2019 (accessed 11 December 2020).

[2] Stefani L, Pedrizzetti G, Galanti G. Clinical application of 2D Speckle tracking strain for assessing cardio-toxicity in oncology. J Funct Morphol and Kinesiol 2016;1:343-354.

[3] McAllister SS, Weinberg RA. The tumor-induced systemic environment as a critical regulator of cancer progression and metastasis. Nat Cell Biol 2014;6:717–727.

[4] Murphy KT. The pathogenesis and treatment of cardiac atrophy in cancer cachexia. Am J Physiol Heart Circ Physiol 2016;310:H466–H477.

[5] Petruzzelli M, Wagner EF. Mechanisms of metabolic dysfunction in cancer-associated cachexia. Genes Dev 2016;30:489–501.

[6] Brancaccio M, Pirozzi F, Hirsch E, et al. Mechanism underlying the cross-talk between heart and cancer. J of Physiol 2020;14:3015-3027.

[7] Zandvliet M. Canine lymphoma: a review. Vet Q 2016;36(2):76-104.

[8] Allen DC, Alderdice JM, Morton P, et al. Pathology of the heart and conduction system in lymphoma and leukaemia. J Clin Pathol 1987;40:746-750.

[9] O´Mahony D, Piekarz RL, Bandettini P, et al. Cardiac involvement with lymphoma: a review of the literature. Clin lymphoma myeloma leuk 2008; 8(4):249-252.

[10] Nguyen JD, Carrasquillo J, Little RF et al. Fluorodeoxyglucose positron emission tomography in the presence of cardiac metastases. Clin Nucl Med 2004;28(12):979-980.

[11] Wang B, Yu Y, Zhang Y, et al. Speckle tracking echocardiography in the early detection and prediction of anthracycline cardiotoxicity in diffuse large B-cell lymphoma treated with (R)-CHOP regimen. Echocardiography 2020;0:1-8.

[12] Ylänen K, Eerola A, Vettenranta K, et al. Speckle tracking echocardiography detects

decreased cardiac longitudinal function in anthracycline-exposed survivors of childhood cancer. Eur J Pediatr 2016;175:1379-1386.

[13] Souza RCA, Camacho AA. Neurohormonal, hemodynamic, and electrocardiographic evaluations of healthy dogs receiving long-term administration of doxorubicin. Am J Vet Res 2006;67:1319-1325.

[14] Gallay-Lepoutre J, Bélanger MC, Nadeau ME. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. Res Vet Sci 2016;105:153-159.

[15] Acierno MJ, Brown S, Coleman AE, et al. ACVIM consensus statement: guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J Vet Intern Med 2018;32(6):1803-1822.

[16] 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(4):247-252.

[17] 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.

[18] Visser LC, Scansen BA, Schober KE, et al. Echocardiographic assessment of right ventricular systolic function in conscious healthy dogs: repeatability and reference intervals. J Vet Cardiol 2015;17(2):83-96

[19] Mesquita LP, Abreu CC, Nogueira CI, et al. Prevalência e aspectos anatomopatológicos das neoplasias primárias do coração, de tecidos da base do coração e metastáticas, em cães do Sul de Minas Gerais (1994-2009). Pesqui Vet Bras 2012;32(11):1155-1163.

[20] Roberts WC, Glancy DL, DeVita VT. Heart in malignant lymphoma (Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma and mycosis fungoides): a study of 196 autopsy cases. Am J Card 1968;22:85-107.

[21] Belloum Y, Rannou-Bekono F, Favier FB. Cancer-induced cardiac cachexia:

Pathogenesis and impact of physical activity (Review). Oncol Rep 2017;37:2543-2552.

[22] Kadokami T, McTiernan CF, Kubota T, et al. Sex-related survival differences in murine cardiomyopathy are associated with differences in TNF-receptor expression. J Clin Invest 2000;106:589– 597.

[23] Bozkurt B, Kribbs SB, Clubb FJ Jr, et al. Pathophysiologically relevant concentrations of tumor necrosis factor-α promote progressive left ventricular dysfunction and remodeling in rats. Circulation 1998;97:1382-1391.

[24] Liu SJ, Zhou W, Kennedy RH. Suppression of β-adrenergic responsiveness of L-type Ca^{2+} current by IL-1β in rat ventricular myocytes. Am J Physiol 1999;276:H141– H148.

[25] Wolf M, Lucina SB, Brüler BC, et al. Assessment of longitudinal systolic function using tissue motion annular displacement in healthy dogs. J Vet Cardiol 2018;20(3):175-185.

[26] Silva VBC, Wolf M, Lucina SB, et al. Assessment of right ventricular systolic function by tissue motion annular displacement in healthy dogs. J Vet Cardiol 2020;32:40-48.

[27] Springer J, Tschirner A, Haghikia A, et al. Prevention of liver cancer cachexia-induced cardiac wasting and heart failure. Eur Heart J 2014;35:932–941.

[28] Badiani S, Van Zalen J, Treibel TA, et al. Aortic stenosis, a left ventricular disease: insights from advanced imaging. Curr Cardiol Rep 2016;18: 80.

[29] Rushmer RF, Crystal DK, Wagner C. The functional anatomy of ventricular contraction. Circ Res 1953;1:162-170.

[30] Plana JC, Galderisi M, Barac A, et al. Expert Consensus for Multimodality Imaging Evaluation of Adult Patients during and after Cancer Therapy: A Report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2014;27,911-939.

[31] Murbraech K, Holte E, Broch K, et al. Impaired Right Ventricular Function in Long-Term Lymphoma Survivors. J Am Soc Echocardiogr 2016;29 (6):528-536.

[32] Christiansen JR, Massey JR, Dalen H, et al. Right ventricular function in long-term adult survivors of childhood lymphoma and acute lymphoblastic leukaemia. Eur Heart J 2016;1-7.

[33] Ahmad H, Mor-avi V, Lang RM, et al. Assessment of right ventricular function using echocardiographic speckle tracking of the tricuspid annular motion: comparison with cardiac magnetic resonance. Echocardiography 2012;29:19-24.

[34] Maurer MS, Bokhari S, Damy T, et al. Expert consensus recommendations for the suspicion and diagnosis of transthyretin cardiac amyloidosis. Circ Heart Fail 2019;12(9):1-11.

Declarations of interest: the authors do not have any conflicts of interest to disclose.

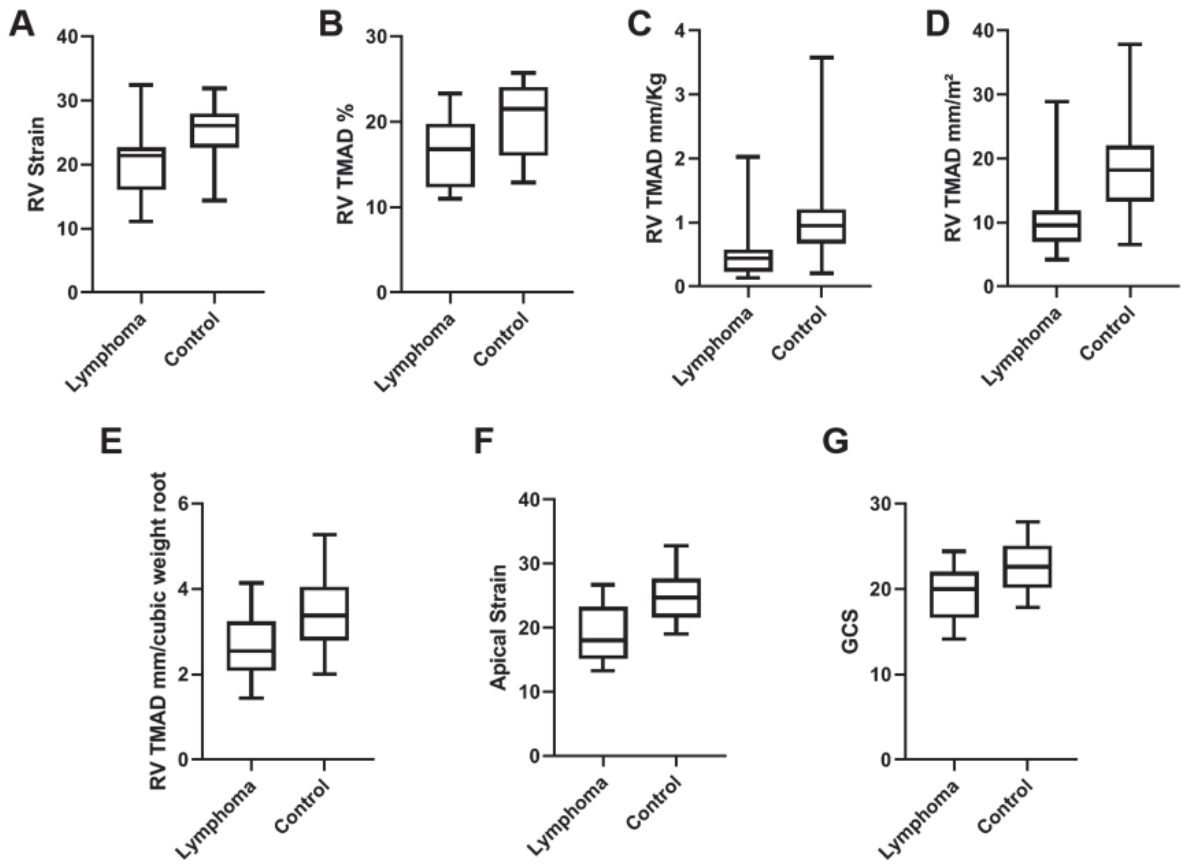

Fig. 1. Box-plots of (A) RV Strain, (B) RV $TMAD_{\%}$, (C) RV $TMAD_{mm/kg}$, (D) RV $TMAD_{mm/m^2}$, (E) RV $TMAD_{mm/\sqrt[3]{\boldsymbol{BW}}}$ (F) Strain C Apical and (G) GCS global in healthy dogs (controls) and in dogs with multicentric lymphoma.

Table 1. Comparison of conventional echocardiographic data, age, body weight, heart rate and systolic blood pressure between healthy dogs (control group) and dogs with multicentric lymphoma.

	Lymphoma	**Control**
(n)	**19**	**15**
Age (months)	84 (48-180)[a]	42 (23-144)[b]
Age (years)	7 (4-15)[a]	3.5 (1-12)[b]
SBP (mmHg)	130 (100-160)[a]	123 (100-180)[a]
HR (bpm)	116.7 (± 19.61)[a]	114.7 (± 28.87)[a]
Body Weight (Kg)	19 (2.9-41)[a]	7.3 (1.2-33)[b]
E_{mitral} (cm/s)	79.6 (42.5-129)[a]	65.8 (48-98.5)[b]
A_{mitral} (cm/s)	84.83 (± 22.17)[a]	63.57 (± 20.08)[b]
E/A	0.91 (0.64-1.6)[a]	1.1 (0.6-1.9)[a]
IVRT (ms)	63.33 (± 11.32)[a]	65.26 (± 10.84)[a]
$LVIDD_n$	1.45 (± 0.14)[a]	1.42 (± 0.17)[a]
$LVIDS_n$	0.79 (± 0.11)[a]	0.82 (± 0.14)[a]
SF	42.15 (± 6.42)[a]	39.99 (± 6.42)[a]
EF	73.81 (± 7.5)[a]	72.16 (± 7.9)[a]
LA/Ao	1.3 (1-2)[a]	1.2 (1-1.4)[a]
S'_{septal} (cm/s)	11.67 (± 1.94)[a]	9.32 (± 2.48)[b]
E'_{septal} (cm/s)	8.65 (5.67-15.3)[a]	6.48 (5.09-11.7)[b]
A'_{septal} (cm/s)	9.84 (± 2.92)[a]	7.76 (± 2.24)[b]
$S'_{lateral}$ (cm/s)	13.4 (8.41-20.1)[a]	9.95 (6.37-22.8)[b]
$E'_{lateral}$ (cm/s)	10.8 (6.07-16.1)[a]	8.95 (4.38-19.6)[a]
$A'_{lateral}$ (cm/s)	10.7 (7.05-16.8)[a]	8.36 (5.37-16.7)[b]
$MAPSE_{index}$ lateral (mm)	1.73 (± 0.52)[a]	1.91 (± 0.66)[a]
$MAPSE_{index}$ septal (mm)	1.36 (1.02-3.24)[a]	1.67 (0.98-3.79)[a]
EF Simpson AP4	74.93 (± 6.29)[a]	75.93 (± 2.73)[a]
EF Simpson AP2	71.91 (± 8.95)[a]	74.13 (± 7.12)[a]
EF Biplane Simpson	72.7 (55.6-83.7)[a]	74.7 (68.7-82.6)[a]
$TAPSE_{index}$	2.24 (1.27-6.17)[a]	2.65 (1.87-5.95)[a]
E_{tric} (cm/s)	66.9 (42-119)[a]	58 (34.9-95.7)[a]
A_{tric} (cm/s)	60.6 (34.6-140)[a]	49.5 (33.5-103)[a]
E/A	1.16 (± 0.38)[a]	1.16 (± 0.29)[a]
RV S′ (cm/s)	17.6 (± 3.74)[a]	11.91 (± 2.93)[b]
RV E′ (cm/s)	13.3 (6.86-16.5)[a]	7.9 (5.25-13.8)[b]
RV A′ (cm/s)	15.49 (± 3.06)[a]	9.15 (± 2.17)[b]
FAC	51.18 (± 8.43) [a]	49.28 (± 7.83) [a]

Data with normal distribution were expressed by the mean and standard deviation and data with abnormal distribution were expressed by the median and interquartile range. Values with different superscripted letters indicate statistically significant differences between groups. Abbreviations: (n):number of animals in each group; AP4: apical 4-chamber; AP2: apical two-chamber; EF:ejection fraction; $E_{mitral:}$ early diastolic mitral inflow velocity; $A_{mitral:}$ late diastolic mitral inflow velocity; ; $E_{tric:}$ early diastolic tricuspid inflow velocity; $A_{tric:}$ late diastolic tricuspid inflow velocity; S′: peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; E′: ′peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; A′: peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; FAC: fractional area change; FS: fractional shortening; HR: heart rate; IVRT: isovolumetric relaxation time; Kg: kilograms; LA/Ao: left atrium-to-aorta ratio; $LVIDD_{n:}$ left ventricular internal diameter at end-diastole; $LVIDS_{s:}$ left ventricular internal diameter at end-systole, MAPSE: mitral annular plane systolic excursion; RV: right ventricle; TAPSE: tricuspid annular plane systolic excursion; SBP: systolic blood pressure.

Table 2. Advanced echocardiography data comparing patients with multicentric lymphoma and the control group.

	Lymphoma	**Control**
(n)	**19**	**15**
Strain AP4	23.49 (± 5.68)[a]	23.75 (± 3.35)[a]
Strain AP3	22.85 (± 4.0)[a]	24.89 (± 4.75)[a]
Strain AP2	19.9 (12.1-26.5)[a]	21.5 (15-30.8)[a]
GLS	22.02 (± 4.21)[a]	23.75 (± 3.59)[a]
Strain C Basal	19.43 (± 4.82)[a]	21.82 (± 3.32)[a]
Strain C Middle	19.3 (14.6-27.1)[a]	20 (16.1-32.8)[a]
Strain C Apical	18.97 (± 4.54)[a]	24.97 (± 3.97)[b]
GCS	19.28 (± 3.31)[a]	22.76 (± 2.97)[b]
Global $TMAD_{\%}$	14.3 (± 3.3)[a]	14.17 (± 2.56)[a]
Global $TMAD_{mm}$	8.5 (4.4-11.75)[a]	5.8 (4-12.25)[a]
Global $TMAD_{mm/Kg}$	0.39 (0.16-2.09)[a]	0.78 (0.25-3.83)[b]
Global $TMAD_{mm/m^2}$	11.16 (5.1-29.6)[a]	14.82 (7.7-40.5)[a]
Global $TMAD_{mm/\sqrt[3]{BW}}$	3.17 (± 0.88)[a]	3.22 (± 0.69)[a]
RV *Strain*	20.35 (± 5.86)[a]	25.13 (± 4.51)[b]
RV $TMAD_{\%}$	6.64 (± 2.19)[a]	6.76 (± 2.44)[b]
RV $TMAD_{mm}$	16.8 (11-23.3)[a]	21.5 (12.9-25.7)[a]
RV $TMAD_{mm/kg}$	0.44 (0.13-2.03)[a]	0.95 (0.21-3.58)[b]
RV $TMAD_{mm/m^2}$	9.59 (4.25-28.8)[a]	18.17 (6.6-37.8)[b]
RV $TMAD_{mm/\sqrt[3]{\boldsymbol{BW}}}$	2.61 (± 0.73)[a]	3.4 (± 0.89)[b]

Data with normal distribution are expressed by the mean and standard deviation and data with abnormal distribution are expressed by the median and interquartile range. Values with different superscripted letters indicate statistically significant differences between groups.

Abbreviations: (n): number of animals in each group; AP2: apical two chamber; AP3: apical three chamber; AP4: apical four chamber; C: circumferential; GCS: Global Circumferential Strain; GLS: Global Longitudinal Strain; TMAD: Tissue Motion Annular Displacement; RV: right ventricle.

Table 3. Analysis of ROC curves and cut-off values with their respective sensitivity and specificity values of the variables that differed between the dogs with lymphoma and the control group.

	Cut-off	**Sensitivity**	**Specificity**	**AUC**	**95% IC**
E_{mitral} (cm/s)	>68.10	80	57.89	0.70	0.52-0.89
A_{mitral} (cm/s)	>61.15	93.33	52.63	0.75	0.59-0.91
S'_{septal} (cm/s)	>9.70	93.33	63.16	0.79	0.63-0.94
E'_{septal} (cm/s)	>7.41	86.67	73.68	0.78	0.62-0.94
A'_{septal} (cm/s)	>9.90	53.33	89.47	0.72	0.54-0.9
$S'_{lateral}$ (cm/s)	>12.75	60	94.74	0.8	0.64-0.95
$A'_{lateral}$ (cm/s)	>10.50	66.67	84.21	0.76	0.59-0.92
RV S′ (cm/s)	>14.85	86.67	89.47	0.91	0.81-1.0
RV E′ (cm/s)	>8.50	86.67	78.95	0.83	0.69-0.97
RV A′ (cm/s)	>12.40	86.67	94.74	0.94	0.86-1.0
Strain C Apical	<18.95	66.67	100	0.84	0.70-0.98
GCS	>13.98	93.33	47.37	0.70	0.52-0.87
Global TMAD mm/Kg	<0.43	60	89.47	0.72	0.53-0.90
RV Strain	<22.85	80	73.68	0.74	0.56-0.92
RV $TMAD_{\%}$	<21.20	93.33	57.89	0.74	0.57-0.91
RV $TMAD_{mm/kg}$	<0.63	86.67	78.95	0.8	0.65-0.96
RV $TMAD_{mm/m^2}$	<13.27	86.67	78.95	0.8	0.64-0.95
RV $TMAD_{mm/\sqrt[3]{BW}}$	<3.34	93.33	52.63	0.72	0.55-0.90

$E_{mitral:}$ early diastolic mitral inflow velocity; $A_{mitral:}$ late diastolic mitral inflow velocity; C: circumferential; S′: peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; E′: ′peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; A′: peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; FS: fractional shortening; GCS: Global Circumferential Strain; TMAD: Tissue Motion Annular Displacement RV: right ventricle.

3 Longitudinal assessment of global and segmental left ventricular systolic function in dogs undergoing chemotherapy with doxorubicin

Marcela Wolf[a]*, Stephany B. Lucina[a], Vinícius B. C. Silva[a], Matheus F. Silveira[a], Victoria G. Silva[a], Natália Kano[a], Ana P. Sarraff[b], Claudia C. Custódio[c], Bruna N. da Costa[a], Marlos G. Sousa[a]

[a] Department of Veterinary Medicine, Federal University of Paraná (UFPR), Rua dos Funcionários 1540, Curitiba, 80035-050, Brazil.

[b] School of Life Sciences, Pontifical Catholic University of Paraná (PUC-PR), Rua Rockfeller 1311, Curitiba, 80230-130, Brazil.

[c] Oncology Sector, Clinivet Veterinary Hospital, Rua Holanda 894, Curitiba, 82540-040, Brazil.

* Corresponding author. E-mail address: marcelawvet@gmail.com (M Wolf).

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: Speckle tracking echocardiography in dogs undergoing chemotherapy with doxorubicin

3.1 Abstract

Introduction: Cardiotoxicity with left ventricular dysfunction is a possible complication in patients undergoing chemotherapy. Advanced echocardiography techniques, such as the global longitudinal strain (GLS) and tissue motion annular displacement (TMAD), allow the early detection of myocardial dysfunction due to cardiotoxicity in relation to conventional echocardiography in human beings with cancer.

Animals: 25 dogs with cancer undergoing chemotherapy with doxorubicin in different protocols.

Methods: Prospective and longitudinal study. Dogs underwent conventional echocardiography and speckle tracking analysis before chemotherapy (day 0), 7 days, 60 days, 120 days and 180 (180d) days after the first administration of doxorubicin.

Results: 12 of 25 dogs completed all evaluations of the study. There was no difference in volume variables by Simpson's method, as well as fractional shortening, ejection fraction, global circumferential strain and GLS at different evaluation times. However, a reduction was observed in the lateral MAPSEi (P=0.0233) and in the global TMAD% (P=0.046) when compared to the pre-chemotherapy evaluation with 180d in the paired analysis of the 12 dogs. The reduction in E' wave velocity at 180d compared to the initial assessment and the negative correlation of the doxorubicin dose with the E:A ratio may demonstrate a dose-dependent influence of this chemotherapy drug on diastolic function.

Conclusions: A reduction in longitudinal systolic function was detected by MAPSE and global TMAD in dogs at a safe cumulative dose of doxorubicin. Speckle tracking echocardiography did not detect a reduction in cardiac function

46 early on conventional echocardiography in this study.

47 *Key words:* cardiotoxicity, echocardiography, oncology, strain, speckle tracking.

48

3.2 List of Abbreviations

AP4	apical four-chamber
AP2	apical two-chamber
AP3	apical three-chamber
CSt	circumferential strain
CD	cumulative dose
CHOP protocol	doxorubicin, cyclophosphamide, vincristine and prednisone
DoxoCarbo	doxorubicin and carboplatin
EF	ejection fraction
FS	fractional shortening
GCS	global circunferencial strain
GLS	global longitudinal strain
HF	heart failure
IVRT	isovolumic relaxation time
LSt	longitudinal strain
LV	left ventricular
$LVIDd_n$	normalized left ventricular internal dimension at end-diastole
$LVIDs_n$	normalized left ventricular internal dimension at end-systole
MAPSEi	mitral annular plane systolic excursion indexed to the body surface area
ROI	region of interest
S´	peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler

SBP	systolic blood pressure
TMAD	tissue motion annular displacement

3.3 INTRODUCTION

Doxorubicin is a chemotherapeutic agent of the anthracycline class widely used in veterinary medicine for the treatment of various neoplasms [1]. However, one of its main side effects is cardiotoxicity [2] reported in several species, such as humans [3], dogs [4], cats [5] and rats [6]. The mechanism of doxorubicin cardiotoxicity is still not fully understood, however the inhibition of topoisomerase resulting in alteration in cellular DNA, increase in cellular oxidative stress [2] and increase in death receptors are the main points reported [7].

Doxorubicin cardiotoxicity is dose-dependent. Studies shown a cardiotoxic effect in dogs at a cumulative dose (CD) of 90 to 265 mg/m² [1,4,8,9]. The timing of cardiotoxicity detection is directly related to the analysis methodology. Biomarkers such as troponin I [10,11], and microRNAs [12], have been shown to be early to conventional echocardiography in detecting myocardial injury in dogs and humans. However, echocardiography is a non-invasive, non-ionizing method, with good accessibility that allows the assessment of cardiac function and an important tool in the follow-up of these patients, assisting in the therapeutic management and decision-making in the cardio-oncology field [13]. Speckle tracking techniques such as the global longitudinal strain (GLS) provide early information and are recommended in the follow-up of people with cancer [13]. Tissue motion annular displacement is also

a speckle tracking technique that assesses longitudinal systolic function that has already been studied in dogs [14,15,16] and that has demonstrated early detection of cardiotoxicity in childhood surviving cancer [17]. Therefore, this study aims to evaluate the cardiotoxicity of doxorubicin in dogs during different chemotherapy protocols with doxorubicin, through conventional echocardiography and speckle tracking analysis.

3.4 MATERIALS AND METHODS

Animals

Dogs with different types of neoplasia treated at the oncology sector of the veterinary teaching facility and at a private veterinary hospital between February 2019 and March 2021, which received at least one dose of doxorubicin were included in this prospective and longitudinal study. All procedures were approved by the Institutional Animal Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Owners gave formal consent prior to the animal being enrolled on the study.

Animals that received chemotherapy with doxorubicin alone or in combination with other drugs (according to the protocol determined by the responsible veterinary oncologist) were selected. The initial assessment (day 0) was performed before the start of the chemotherapy protocol. The following evaluations were performed based on the administration of doxorubicin, these being 7, 21, 60, 120 and 180 days after the first application of doxorubicin (Figure 1).

In addition to echocardiography, in all evaluations, patients underwent a

complete physical examination, at least 3 minutes of computerized electrocardiography[a] to determine heart rhythm and rate immediately before echocardiography, and non-invasive blood pressure measurement[b], as previously recommended [18].

Exclusion criteria were the presence of systolic dysfunction in the initial evaluation, cardiomegaly, arrhythmias (except arrhythmias of sinus origin), the use of cardiovascular drugs, congenital heart diseases, cardiac neoplasms, pericardial effusion and systemic arterial hypertension [systolic blood pressure (SBP)>160 mmHg] or systemic arterial hypotension (SBP <80 mmHg). Dogs whose owner does not formally agree with the procedure by signing the informed consent form will also not be admitted to the research.

Conventional Echocardiography

Echocardiography[c] was performed by an experienced observer (MW) with continuous electrocardiogram monitoring. The study was carried out with unsedated dogs in left and right lateral recumbency, as recommended by the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine [19].

Left ventricular ejection fraction (EF) was obtained by the Teichholz method with M-mode in the short axis and also by Simpson's biplanar method in the apical 4-chamber and 2-chamber view, in which the systolic and diastolic volumes of the left ventricle were also recorded. Fractional shortening (FS), as well as left ventricular systolic and diastolic diameters were obtained by M-mode in the short axis. Normalized dimensions of the left ventricle were calculated according to the previously reported method [20]. The left atrium and aorta ratio was obtained in early diastole by the two-dimensional mode [21].

The mitral early (E) and late (A) diastolic peak velocities were obtained with pulsed Doppler on the transmitral flow in the apical four-chamber view, while the isovolumetric relaxation time (IVRT) was performed with pulsed Doppler in the apical 5-chamber view. From these parameters the E:A and the E:IVRT ratios were calculated.

The aortic valve closure time (R to AVC) was obtained with pulsed Doppler in the aortic flow in the 5-chamber apical view and consists of the time, in milliseconds, from the beginning of the QRS complex to the end of the aortic flow spectral. This time is used by the software to determine the systolic period in speckle tracking analyses.

Tissue Doppler evaluation was performed in the septal and parietal region of the mitral annulus to obtain systolic (S´), early diastolic (E´) and late diastolic (A´) velocities, which were also used to calculate E´:A´ ratio. Mitral annulus systolic excursion (MAPSE) was obtained with M-mode in the septal (MAPSE septal) and lateral (MAPSE lateral) region of the mitral annulus. Mitral annular plane systolic excursion and left ventricular systolic and diastolic volume were indexed by body surface area (BSA), using the following formula:

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

K= constant (10.1 for dogs)

Speckle tracking echocardiography

All speckle tracking techniques were performed offline[d] with the images previously obtained.

Global longitudinal strain

At least five cardiac cycles of apical 4- (AP4), 2- (AP2), 3- (AP3) chamber

views were recorded for subsequent off-line evaluations of longitudinal strain. The software automatically tracking the left ventricle and divided the ventricle into seven segments, to which manual corrections were made if necessary.

Longitudinal strain (LSt) was obtained by averaging the percentage of deformation of the 7 segments of the left ventricle in a heartbeat. In addition to the AP4, AP3 and AP2 longitudinal strain values, the segmental strain was also evaluated. Therefore, the deformation of the 21 segments resulting from the 3 analyses were recorded and compared.

Global longitudinal strain was calculated as the average of LSt obtained in AP4, AP2 and AP3 images, and with these segmented sections, the software provides a diagram of the left ventricle, called "bulls eye".

Global circumferential strain

At least five cardiac cycles were recorded of the apical, middle (papillary plane) and mitral plane on the short axis in the right parasternal window for subsequent off-line evaluations of circumferential strain (CSt). Circumferential strain was obtained as the percentage of CSt in a heartbeat and global circumferential strain (GCS) was calculated as the average of CSt obtained in apical, middle and mitral plane images.

Tissue motion annular displacement

The tissue motion annular displacement (TMAD) was performed on AP4 and AP2 images, from the definition of three regions of interest (ROI) by the operator, two of them in the mitral annulus region and the third in the epicardial region of the left ventricle. The software automatically tracks the displacement

of the mitral annulus ROIs towards the third ROI in the apex region (in mm). In addition, a midpoint is created between the two ROIs of the mitral annulus, and the software provides the displacement of the midpoint towards the third ROI in mm and in percentage in relation to the length of the left ventricle.

Global TMAD of the left ventricle is an average of the measurements obtained in the AP4 and AP2 chambers and was calculated in 2 different ways: global $TMAD_{\%}$ is the mean fractional displacement (in relation to the total LV length) of the midpoint towards the LV apex in the AP4 and AP2 chamber images and the global $TMAD_{mm/m^2}$ is global $TMAD_{mm}$ [which is the average displacement (in mm) of the midpoint of the mitral annulus obtained in AP4 and AP2 images towards the apex] indexed to BSA, as previous described [14].

3.5 Statistical Analysis

Statistical analyses were performed[e] and normality of the data was evaluated by the Shapiro-Wilk test. For parameters with normal distribution, differences between groups were evaluated with ANOVA, followed by the post hoc Tukey's multiple comparisons test. For non-normally distributed data differences between groups were assessed using Kruskal-Wallis test followed by Dunn´s multiple comparisons test. Fisher's test was used to assess categorical data (sex and rhythm). A paired analysis of the 12 dogs that completed all the evaluations proposed by the study was performed. For this, the test ANOVA, followed by the post hoc Tukey's multiple comparisons test was used for the parametric data, and Friedman test followed by Dunn´s multiple comparisons test to non-parametric data.

The correlation of the CD of doxorubicin with the echocardiographic

parameters was evaluated by the Pearson correlation test (normal distribution) or Spearman (anormal distribution). T test or the Mann-Whitney test were used to compare the echocardiographic variables between the different chemotherapy protocols (doxorubicin with carboplatin and doxorubicin with cyclophosphamide, vincristine and prednisone). The Chi-Square test was performed to compare the difference between the number of animals at different times of evaluation.

Receiver operating characteristic (ROC) curves were performed to determine the cut-off values with the best combination of sensitivity and specificity of the variables to differentiate patients who died and survived. Kaplan-Meier curves were used to assess the prognostic value of this variables for the all-cause mortality. Breslow's test was used to assess differences between curves. A value of $P<0.05$ was considered significant.

3.6 RESULTS

Initially, 32 dogs that would undergo chemotherapy with doxorubicin were recruited for this study. However, seven were excluded from the longitudinal analysis, because one of them had mitral valve disease ACVIM B2 stage in pre doxorrubicin assessment and would receive pimobendan, five due to tutors' withdrawal and one of them presented valve neoformation during treatment. Therefore, 25 dogs were included: 13 females and 12 males aged from 2 to 16 years (median: 10 years; mean: 9.9 years); weighting (median: 7.9 kg; mean: 12.7 kg), most of them neutered (N=18). Of the 25 dogs recruited, 12 dogs completed the 6 assessments (pre-dox, 7d, 21d, 60d, 120d and 180d), and although there is a progressive reduction in the number of animals, there was

224 no difference (P=0.998) between the number of animals at different times of
225 analysis.

226 The chemotherapy protocol was determined by the oncology service.
227 Doxorubicin was used in the protocols in association with cyclophosphamide,
228 vincristine and prednisone (CHOP protocol) in 10 patients (n=10), in association
229 with carboplatin (DoxoCarbo) (n=10) and as a single agent (n=5). Lymphoma
230 was the most common neoplasm, affecting 10 of the 25 dogs, followed by
231 mammary adenocarcinoma (n=10), mesothelioma, leiomyosarcoma,
232 chondrosarcoma, melanoma and inflammatory carcinoma (n=1 each).

233 Doxorubicin dose was calculated to 30 mg/m² for dogs ≥15 kg and 1
234 mg/kg for dogs <15 kg. However, for standardization, the milligram per kg was
235 used in this study and the administration was at an interval of between 20 to 30
236 minutes. Total CD of dox of 12 dogs that completed all assessments range from
237 3.45 mg/kg to 5 mg/kg (median:4 mg/kg; mean:4,26 mg/kg).

238 The breeds included were Crossbreed (n=9), Golden Retriever (n=3),
239 Poodle (n=3), Dachshund (n=2), Lhasa Apso (n=2), Rottweiler, Schnauzer,
240 German Shepherd, Pinscher, Fox Terrier and English Bulldog (n=1 each).

241 The most frequent rhythm was sinus arrhythmia (n=14/56%), followed by
242 sinus rhythm (n=10/40%) and sinus tachycardia (n=1/4%). An English bulldog
243 presented sinus arrhythmia with ventricular premature complexes alone and in
244 pairs at reassessment 60 days after the first dose of doxorubicin. Of the 25
245 dogs included in the study, 4 had degenerative disease of the mitral and
246 tricuspid valves and 3 only had mitral valve disease, all with mild valvular
247 insufficiency, without cardiac remodeling and without hemodynamic
248 repercussions (ACVIM Stage B1).

Comparisons were also made between the animals that received the different chemotherapy protocols (CHOP and DoxoCarbo). No differences were found regarding rhythm (P=0.069), body weight (P=0.477), heart rate (P=0.559) and SBP (P=0.308). However, patients in the CHOP group were younger (P=0.0013) compared to animals in the DoxoCarbo group, as well as the CHOP group had more males (n=8/80%) than the DoxoCarbo group (N=1/10%). Of the 12 patients who completed all study evaluations, half of them received the DoxoCarbo protocol (N=6/50%), followed by CHOP (N=5/41.7%) and Doxo as only agent (N=1/8.3%). Patients in the CHOP group presented higher values of E wave (P=0.013), E' wave (P=0.005) and E:A ratio (P=0.024) in the pre-chemotherapy echocardiographic evaluation when compared to the DoxoCarbo group. At the end of the study (180d), the animals that received the CHOP protocol showed less GCS (P=0.0104) and greater LVIDdn (P=0.015) when compared to the DoxoCarbo group.

The comparison of the evolution of the animals throughout the treatment was performed in 4 different ways. An unpaired evaluation involving all study animals (Table 1) (N=25), as well as only patients who received CHOP protocol (N=10) and DoxoCarbo (N=10) were compared over time. In addition, a paired analysis was performed only comparing the 12 patients who completed the last evaluation (Table 2) (180d).

The unpaired evaluation of the 25 animal dogs over time revealed a reduction in the lateral S' wave velocity (P=0.039), however, in the post hoc analysis there was no significant difference. A reduction in E' wave velocity (P=0.047) was observed when compared to the pre-chemotherapy assessment of the 180d assessment. No variables differed over time in patients receiving

CHOP protocol. However, smaller values of S', E' lateral waves and EF by the biplanar method were found in the 180d evaluation when compared to the initial evaluation in the dogs receiving the DoxoCarbo protocol. In the paired evaluation of the 12 animals that completed all evaluations, there was a reduction in lateral MAPSEi (P=0.0023) and global TMAD% (P=0.003) in the 180-day evaluation, as well as a reduction in septal MAPSEi at the 21-day assessment compared to pre-chemotherapy evaluation (Figure 2). No difference was found in the segmental assessment of the left ventricle.

A negative correlation was found between the CD of doxorubicin and the E:A ratio (P=0.004; R=-0.785), with the septal S' wave velocity (P=0.0016; R=-0.692) and lateral E' wave (P=0.034; R=-0.621).

None of the patients in this study developed signs of heart failure (cough, dyspnea, tachypnea, syncope, easy tiredness) or died from a cardiac cause. No differences were found in the echocardiographic variables of the surviving animals from those that died. Therefore, neither parameter was able to accurately predict all-cause mortality in this study and showed a difference in the survival analysis (Table 3).

The median survival time of the study animals (N=25) was 201 days, for patients who received a protocol with DoxoCarbo (N=10) it was 411, CHOP was 181.5 and only Doxo (N=5) was 117 days. There was no difference in patient survival in the different protocols (P=0.139).

3.7 DISCUSSION

Cardiotoxicity is one of the main complications of treatment and an important impact factor in the survival of cancer patients. The early detection of

299 cardiotoxicity is an object of study for optimal patient follow-up and appropriate
300 therapeutic approach in the cardio-oncological field [10,13].

301 In this study, no patient showed clinical signs during treatment related to
302 cardiovascular diseases, and none of them developed important systolic
303 dysfunction and significant cardiac remodeling. This may be related to the CD of
304 doxorubicin, in this study the mean final CD of the 12 patients was 4.26 mg/kg,
305 approximately 130 mg/m². In the literature, studies show a 4% incidence of
306 clinical cardiotoxicity in dogs, with a median of 144.78 mg/m² [4]. However,
307 another study with a mean CD of 90 mg/m² (30 – 180 mg/m²) shows an
308 incidence of 7.4%, with signs of congestive heart failure, arrhythmias and
309 systolic dysfunction [22].

310 A reduction in longitudinal systolic function (detected by MAPSE and
311 global TMAD%) was observed at the 21d and 180d assessment (Figure 2 and 3
312 and Table 2). Longitudinal myocardial function can be affected prior to radial
313 function in several diseases, such as in dogs with endocardiosis [16],
314 pulmonary hypertension [23], hyperadrenocorticism [24], and in cats with
315 cardiomyopathy [25]. The speckle tracking techniques, such as TMAD and
316 GLS, allows the detection of reduced longitudinal myocardial function earlier
317 than conventional parameters, such as EF, in humans undergoing
318 chemotherapy [10,26,27]. Global longitudinal strain is a recommended
319 technique in the echocardiographic follow-up of cardio-oncology patients
320 [13,28], in addition to providing prognostic information in patients with preserved
321 EF [29]. Experimentally, strain has also been shown to detect early reduction in
322 myocardial function in rats receiving doxorubicin [6]. Interestingly, no difference
323 in GLS was observed in this investigation, as well as speckle tracking

echocardiography techniques were not early in detecting the drop in myocardial function. This can be explained by a few factors. The incidence of doxorubicin cardiotoxicity is known to be dose-dependent in humans and dogs [4,28]. Cumulative doses less than 150 mg/m² are considered safe [30]. In this study, the mean final CD of the 12 patients was approximately 130 mg/m², which may explain why these patients did not present severe echocardiographic alterations. Another factor to consider is the possibility that these tissue techniques are not sensitive enough to detect very slight changes in the myocardium [30], which could perhaps be detected by histopathology, biomarkers or molecular methods [4]. Furthermore, in this study no patient was of a breed predisposed to the development of cardiomyopathy, it is known that heavier dogs and dogs of breeds predisposed to the development of cardiomyopathy are at greater risk of developing cardiotoxicity [4]. Our results partially corroborate those of another prospective study involving 14 dogs with lymphoma with a final CD of doxorubicin of 120 mg/m² associated with other chemotherapeutic agents, which did not detect changes in myocardial function parameters by conventional echocardiography, as well as tissue echocardiography and in the troponin I concentration [30].

In this study, no differences were observed in volumetric, as well as in shortening and EF. These results are similar to another longitudinal study with 13 dogs in different protocols with doxorubicin, in which one dog had a CD of 30 mg/m², 3 dogs with 60 mg/m², 2 with 90 mg/m², 6 with 120 mg/m². m² and 1 with 150 mg/m² [31]. Surachetpong et al. [11] also found no difference in the volumetric assessment of the left ventricle in dogs with a final CD 120 mg/m². Fractional shortening is a later parameter of cardiotoxicity detection [4].

However, another investigation showed that reductions in FS and EF can occur in dogs with a CD considered safe (90 mg/m²) [11].

Although differences in segmental deformation of the left ventricular strain have been reported in humans undergoing chemotherapy [26], no differences were found in the dogs in this study, as previously described in a tissue Doppler study in dogs with a similar CD of doxorubicin [30].

Comparison between patients who received different protocols (DoxoCarbo and CHOP) reveal lower systolic indices in dogs who receive the CHOP protocol in the 180d assessment. However, in the longitudinal evaluation over time, there was a reduction in the parameters of systolic (S' wave, EF) and diastolic (E' wave) function parameters in patients who received the DoxoCarbo protocol at 180 days. As there is no difference between body weight in these groups, the influence of other chemotherapeutic agents in the protocol cannot be ruled out, as well as the neoplasm (mostly lymphoma in the CHOP group), due to the fact that patients in the CHOP group ended the study with a lower average CD (3.84 mg/kg) than the DoxoCarbo group (4.5 mg/kg) and the small number of animals in each group at this point of evaluation (5 dogs each) must be taken into account.

In this study, the infusion rate of doxorubicin recommended by the oncology department was 20 to 30 minutes. It is known that in people, the rate of doxorubicin infusion is directly related to the incidence of cardiotoxicity [32]. Slower infusions (6 hours) are related to a lower incidence of cardiovascular complications [32] and it was speculated that slower infusions could generate a lower rate of cardiotoxicity in dogs [30]. However, one recent retrospective study found no difference in the incidence of clinical cardiotoxicity in dogs when

comparing a 10- to 15-minute and a 1-hour doxorubicin infusion rate [4], demonstrating that this may not be a factor that may have influenced our results considerably.

The reduction in E' wave velocity at 180 days compared to the initial assessment and the negative correlation of the doxorubicin dose with the E:A ratio may demonstrate a dose-dependent influence of this chemotherapy drug on diastolic function. An alteration in diastolic function detected by a reduction in the E:A ratio has already been observed in a study with longitudinal evaluation of dogs in different protocols with doxorubicin, in which no differences were detected in the parameters of systolic function [31].

The velocity of the E wave, the E´ wave and the E:A ratio was higher in the pre-chemotherapy evaluation of patients who received the CHOP protocol, this is possibly due to the fact that this group is composed of younger patients and in this study a correlation was observed age with the E:A ratio (P=0.0011; R=-0.613). A negative influence of age on the early diastolic phase detected by mitral flow velocities using a conventional Doppler examination as well as tissue Doppler have already been reported [33].

A higher prevalence of males was observed in patients who received the CHOP protocol. Although most patients were neutered, it is known that male dogs are more predisposed to lymphoma than females, as well as neutered animals are at greater risk, than not neutered [34].

In this study, only one dog had premature ventricular complexes after starting the chemotherapy protocol, which corroborates other studies that also found development of ventricular and supraventricular arrhythmias in dogs treated with doxorubicin [4,12]. The appearance of ventricular arrhythmias

correlates with the incidence of clinical cardiotoxicity in dogs [4]. On the other hand, other studies with a similar CD did not find significant electrocardiographic changes in dogs undergoing chemotherapy with doxorubicin, in which the development of arrhythmias and differences in the measurements of waves and electrocardiographic intervals were not observed [11,30].

Changes in blood pressure, such as the development of arterial hypertension, have been described in humans as a factor of cardiotoxicity [13]. However, in this study, no differences were observed in the value of systolic blood pressure during chemotherapy treatment.

Global longitudinal strain value less than 18% with preserved ejection fraction it is related to lower survival in people on chemotherapy [29]. However, GLS>22.5 had 66.6% sensitivity and 66.6% specificity and low accuracy to separate dogs who lived from those who died, and no echocardiographic parameter was shown to be a good predictor of mortality and survival analysis in this study. This can be explained by the fact that none of the patients in this study developed significant clinical cardiotoxicity and cardiac death.

This study must be interpreted in the context of its several limitations. The small number of animals is one of the most important limiting factors to be considered. Another important limitation is the non-standardization of doxorubicin and chemotherapy protocols, since the patients are from the hospital routine and in some cases needed a change in the protocol for specific reasons determined by the veterinary oncologist. In addition, the effects of other medications and chemotherapy on cardiac parameters assessed by echocardiography is not known. These drugs could not only affect myocardial

performance but also alter cardiovascular loading conditions and interfere with some echocardiographic parameters. Also, ancillary examinations to rule out concurrent systemic diseases, i.e. infectious diseases, which might affect systolic function, were not performed unless the animal's clinical condition required it. Heart rhythm was monitored for only 3 minutes with conventional electrocardiography, however, a Holter analysis would provide more accurate information about heart rhythm and incidence of cardiotoxicity with arrhythmias in these patients. The different types of neoplasia included in the study may also have an influence on the results obtained, especially in the survival analysis, and the absence of a comparison with a gold standard, as a histopathological evaluation of myocardium, is also an important limitation.

In conclusion, a reduction in longitudinal systolic function can be detected by MAPSE and global TMAD in dogs undergoing chemotherapy with considered safe doses of doxorubicin. Speckle tracking echocardiography techniques (Strain and TMAD) did not detect a reduction in cardiac function early on conventional echocardiography in this study.

Conflict of Interest Statement

The authors do not have any conflicts of interest to disclose.

Footnotes

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

[b] - MedMega DV 6108 - Vascular *Doppler,* 10 MHz, Franca, Brazil.

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

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

[e] - Graphpad prism 5.0 Software

3.8 REFERENCES

[1] Mauldin GE, Fox PR, Patnaik AK, Bond BR, Mooney SC, Matus RE. Doxorubicin-induced cardiotoxicosis. Clinical features in 32 dogs. J Vet Intern Med 1992;6:82-88.

[2] Bloom MW, Hamo CE, Cardinale D, Ky B, Nohrja A, Baer L, Skopicki H, Lenihan DJ, Gheorghiade M, Lyon AR, Butler J. Cancer therapy-related cardiac dysfunction and heart failure: part 1: definitions, pathophysiology, risk factors, and imaging. Circ Heart Fail 2016;9:e002661.

[3] Henriksen PA. Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention. Heart 2018;104:971-7.

[4] Hallman BE, Hauck ML, Williams LE, Hess PR, Suter SE. Incidence and risck factors associated with development of clinical cardiotoxicity in dogs receiving doxorubicin. J Vet Intern Med 2019;33:783–91.

[5] Keefe DO, Sisson D, Gelberg HB, Schaeffer DJ, Krawiec DR. Systemic toxicity associated with doxorrubicin administration in cats. J Vet Intern Med 1993;7:309-17.

[6] Oliveira MS, Melo MB, Carvalho JL, Melo IM, Lavor MSL, Gomes DA, Goes AM, Melo MM. Doxorubicin cardiotoxicity and cardiac function improvement after stem cell therapy diagnosed by strain echocardiography. J Cancer Sci Ther 2013;5:52–57.

[7] Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. Nature 2017;7:44735.

[8] Susaneck SJ. Doxorubicin therapy in the dog. J Am Vet Med Assoc

1983;182:70-72.

[9] Loar AS, Susaneck SJ. Doxorubicin-induced cardiotoxicity in five dogs. Semin Vet Med Surg 1986;1:68-71.

[10] Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of anthracyclines. Front Cardiovasc Med 2020;18:7:26.

[11] Surachetpong SD, Teewasutrakul P, Rungsipipat A. Serial measurements of cardiac troponin I (cTnI) in dogs treated with doxorubicin. Jpn J Vet Res 2016;64:221-233.

[12] Beumier A, Robinson SR, Robinson N, Lopez KE, Meola DM, Barber LG, Bulmer BJ, Calvalido J, Rush JE, Yeri A, Das S, Yang VK. Extracellular vesicular microRNAs as potential biomarker for early detection of doxorubicin-induced cardiotoxicity. J Vet Intern Med 2020;34:1260-71.

[13] Hajjar LA, Costa IBSS, Lopes MACQ, Hoff PMG, Diz MPE, Fonseca SMR, Bittar CS, Rehder MHHS, Rizk SI, Almeida DR, Fernandes GS, Silva LB, Campos CAHM, Montera MW, Alves SMM, Fukushima JT, Santos MVC, Negrão CE, Silva TLF, Ferreira SMA, Malachias MVB, Moreira MCV, Neto MMRV, Fonseca VCQ, Soeiro MCF, Alves JBS, Silva CMPD, Sbano J, Pavanello R, Pinto IMF, Simão AF, Dracoulakis MDA, Hoff AO, Assunção BMBL, Novis Y, Testa L, Filho ACA, Cruz CBBV, Pereira J, Garcia DR, Nomura CH, Rochitte CE, Macedo AVS, Marcatti PTF, Junior WM, Wiermann EG, Freitas RVH, Coutinho A, Mathias CMC, Vieira FMAC, Sasse AD, Rocha V, Ramires JAF, Filho RK. Diretriz Brasileira de Cardio-oncologia. Arq Bras Cardiol 2020;115:1006-43.

[14] Wolf M, Lucina SB, Brüler BC, Tuleski GLR, Silva VBC, Sousa MG. Assessment of longitudinal systolic function using tissue motion annular

500 displacement in healthy dogs. J Vet Cardiol 2018;20:175-85.
501 [15] Silva VBC, Wolf M, Lucina SB, Sarraff-Lopes AP, Sousa MG. Assessment
502 of right ventricular systolic function by tissue motion annular displacement in
503 healthy dogs. J Vet Cardiol 2020;32:40-48
504 [16] Wolf M, Lucina SB, Silva VB, Tuleski GLR, Sarraff AP, Komatsu EY,
505 Sousa MG. Assessment of longitudinal systolic function using tissue motion
506 annular displacement in dogs with degenerative mitral valve disease. J Vet
507 Cardiol 2021;38:44-58.
508 [17] Ylänen K, Eerola A, Vettenranta K, Poutanen T. Speckle tracking
509 echocardiography detects decreased cardiac longitudinal function in
510 anthracycline-exposed survivors of childhood cancer. Eur J of Pediatr
511 2016;1379-1386.
512 [18] Acierno MJ, Brown S, Coleman AE, Jepson RE, Papich M, Stepien RL,
513 Syme HM. ACVIM consensus statement: guidelines for the identification,
514 evaluation, and management of systemic hypertension in dogs and cats. J Vet
515 Intern Med 2018;32:1803-22.
516 [19] Thomas WP, Gaber CE, Jacobs GJ. Recommendations for standards in
517 transthoracic Two-Dimensional echocardiography in the dog and cat. J Vet
518 Intern Med 1993;7:247-52.
519 [20] Cornell CC, Kittleson MD, Torre PD, Häggström J, Lombard CW, Pedersen
520 HD, Vollmar A, Wey A. Allometric scaling of M-mode cardiac measurements in
521 normal adult dogs. J Vet Intern Med 2004;18:311-21.
522 [21] Hansson K, Häggström J, Kvart C, Lord P. Left atrial to aortic root indices
523 using two-dimensional and M-mode echocardiography in cavalier king Charles
524 spaniels with and without left atrial enlargement. Vet Radiol Ultrasound

525 2002;43:568-75.
526 [22] Ratterree W, Gieger T, Pariaut R, Saelinger C, Strickland K. Value of
527 echocardiography and electrocardiography as screening tools prior to
528 Doxorubicin administration. J Am Anim Hosp Assoc 2012;48:89-96.
529 [23] Morita T, Nakamura K, Osuga T, Morishita K, Sasaki N, Ohta H, Takiguchi
530 M. Right ventricular function and dyssynchrony measured by echocardiography
531 in dogs with precapillary pulmonary hypertension. J Vet Cardiol 2019; 23:1-14.
532 [24] Chen HY, Lien YU, Huang HP. Assessment of left ventricular function by
533 two-dimensional speckle-tracking echocardiography in small breed dogs with
534 hyperadrenocosticism. Acta Vet Scand 2014;56:1-10.
535 [25] Spalla I, Boswood A, Cannolly DJ, Fuentes VL. Speckle tracking
536 echocardiography in cats with preclinical hypertrophic cardiomyopathy. J Vet
537 Intern Med 2019;33:1232-41.
538 [26] Plana JC, Galderisi M, Barac A, Ewer MS, Hy B, Scherrer-Crosbie M,
539 Ganame J, Sebag IA, Agler DA, Badano LP, Banchs J, Cardinale D, Carver J,
540 Cerqueira M, DeCara JM, Edvarsen T, Flamm SD, Force T, Griffin BP,
541 Jerusalem G, Liu JE, Magalhães A, Marwick T, Sanchez LY, Sicari R, Villaraga
542 HR, Lancelotti P. Expert consensus for multimodality imaging evaluation of
543 adult patients during and after cancer therapy: a report from the American
544 Society of Echocardiography and the European Association of Cardiovascular
545 Imaging. Eur Heart J 2014; 15:1063-93.
546 [27] Ylänen K, Eerola A, Vettenranta K, Poutanen T. Speckle tracking
547 echocardiography detects decreased cardiac longitudinal function in
548 anthracycline-exposed survivors of childhood cancer. Eur J Pediatr
549 2016;175:1379-86.

[28] Zamorano JL, Lancellotti P, Muñoz DR, Abovans V, Asteggiano R, Galderisi M, Habib G, Lenihan DJ, Lip GYH, Lyon AR, Fernandez TL, Mohty D, Piepoli MF, Tamargo J, Torbicki A, Suter TM. Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur Heart J 2016;37p.2768–2801.

[29] Rhea IB, Uppuluri S, Sawada S, Schneider BP, Feigenbaum H. Incremental Prognostic Value of Echocardiographic Strain and Its Association With Mortality in Cancer Patients. J Am Soc Echocardiogr 2015;28:667–73.

[30] Tater G, Eberle N, Hungerbuehler S, Joetzke A, Nolte I, Wess G, Betz D. Assessment of cardiac troponin I (cTnI) and tissue velocity imaging (TVI) in 14 dogs with malignant lymphoma undergoing chemotherapy treatment with doxorubicin. Vet Comp Oncol 2017;15:55-64.

[31] Gallay-Lepoutre J, Bélanger MC, Nadeau ME. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. Res Vet Sci 2016;105:153-9.

[32] Legha SS, Benjamin RS, Mackay B, Ewer M, Wallace S, Valdivieso M, Rasmussen SL, Blumenschein GR, Freireich EJ. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. *Ann Intern Med*. 1982;96:133-139.

[33] Wess G, Killich M, Hartmann K. Comparison of pulsed wave and color Doppler myocardial velocity imaging in healthy dogs. J Vet Intern Med 2010;24:360–66.

575 [34] Bennett PF, Taylor R, Williamson P. Demographic risk factors for
576 lymphoma in Australian dogs: 6201 cases. J Vet Intern Med 2018;32:20

Table 1 – Descriptive data, cumulative dose of doxorubicin and the main echocardiographic variables compared over time in all animals of the study.

	Pre (Day 0)	7d	21d	60d	120d	180d	P
N	25	23	21	19	15	12	0.998
N (CHOP/DoxoCarbo/Doxo)	10/10/5	10/10/3	10/9/2	9/9/1	7/7/1	5/6/1	0.998
CD of doxorubicin (mg/kg)	0	0.99 (0.88 – 1)	1.09 (0.88 – 2.17)	2.09 (1.74 – 4.3)	3.48 (2.6 – 4)	4.26 (3.45 – 5)	
E:A	0.88(0.58-1.38)	0.99 (0.57-1.56)	0.99 (0.58-1.29)	0.96 (0.57-1.80)	1.0 (0.60-1.30)	0.92 (0.50-1.70)	0.986
LA:Ao	1.24 (1.0-2.0)	1.22 (1.0-1.45)	1.29 (1.0-1.56)	1.30 (1.0-1.53)	1.27 (1.0-1.67)	1.27 (1.0-1.67)	0.615
$LVIDD_n$	1.45 (1.21-2.63)	1.44 (1.22-1.71)	1.41 (0.45-1.78)	1.43 (1.20-1.95)	1.41 (1.28-1.91)	1.45 (1.24-1.73)	0.8205
$LVIDS_n$	0.81 (0.59-1.30)	0.81 (0.61-1.08)	0.80 (0.60-1.30)	0.78 (0.62-1.29)	0.79 (0.60-1.15)	0.85 (0.56-0.96)	0.833
FS (%)	44.03 (±6.97)	42.77 (±6.04)	43.39 (±7.09)	41.99 (±7.85)	42.82 (±6.79)	40.08 (±7.26)	0.699
EF (AP4 SM) (%)	75.40 (61.50-85.80)	75.30 (56.70-81.5)	72.60 (59.10-83.40)	72.70 (61.40-80.80)	73.30 (59.20-77.30)	70.35 (60.50-79.0)	0.201
EF (AP2 SM) (%)	74.85 (±6.42)	74.58 (±7.03)	74.12 (±7.68)	74.16 (±5.53)	74.53 (±6.13)	69.89 (±3.99)	0.350
EF Biplane SM (%)	74.74 (±5.24)	73.60 (±6.54)	73.01 (±6.37)	73.83 (±3.95)	73.36 (±4.64)	70.31 (±4.29)	0.344
EDVi AP4 (ml/m²)	45.97 (16.60-117.80)	37.55 (15.20-83.40)	37.38 (23.19-63.41)	35.86 (17.70-68.88)	36.84 (18.34-84.61)	37.85 (22.80-64.91)	0.462
ESVi AP4 (ml/m²)	9.85 (5.01-45.35)	9.87 (3.07-24.25)	11.45 (4.97-25.44)	9.29 (4.14-26.59)	9.88 (4.16-20.49)	11.63 (5.84-15.22)	0.906
S´septal (cm/s)	11.06 (±2.11)	10.42 (±1.80)	10.35 (±1.75)	10.87 (±2.74)	9.58 (±1.88)	10.33 (±2.10)	0.360
E´ septal (cm/s)	7.86 (4.93-15.3)	7.09 (4.87-9.57)	7.45 (4.54-11.80)	7.88 (4.38-10.40)	6.29 (5.17-9.55)	6.66 (4.77-9.33)	0.316
A´septal (cm/s)	10.35 (±2.0)	9.28 (±1.85)	9.30 (±2.11)	9.31 (±1.90)	8.81 (±2.42)	9.32 (±2.66)	0.297
S´ lateral (cm/s)	13.0 (8.41-20.1)	11.10 (7.96-14.50)	10.90 (6.45-18.50)	10.50 (6.29-17.40)	10.30 (7.76-18.10)	9.36 (6.96-13.80)	**0.039**
E´ lateral (cm/s)	8.7 (5.57-16.10)	8.67 (4.77-13.20)	7.66 (4.62-12.90)	8.14 (5.10-12.20)	7.46 (5.57-14.30)	6.79 (4.89-11.30)[a]	**0.0471**
A´lateral (cm/s)	11.10 (7.11-16.80)	10.20 (4.54-21.0)	10.40 (6.67-14.60)	10.40 (6.47-13.90)	9.85 (6.96-15.10)	10.40 (6.68-12.40)	0.196
MAPSEi lateral (cm/m²)	1.89 (1.17-8.07)	2.01 (1.0-3.33)	1.80 (0.70-2.88)	1.75 (0.93-3.38)	1.64 (0.72-3.20)	1.64 (0.83-2.77)	0.789
MAPSEi septal (cm/m²)	1.82 (0.97-7.51)	1.88 (0.76-2.81)	1.76 (0.85-2.73)	1.83 (0.71-3.79)	1.90 (0.78-2.64)	1.73 (0.75-2.68)	0.921
Strain AP4 (%)	23.31 (±5.39)	22.66 (±4.72)	23.03 (±4.17)	21.89 (±4.75)	21.19 (±4.23)	20.32 (±4.85)	0.442
Strain AP3 (%)	19.44 (±4.60)	20.17 (±5.99)	19.41 (±3.46)	19.13 (±3.85)	19.57 (±3.66)	18.38 (±4.25)	0.926
Strain AP2 (%)	22.62 (±4.61)	23.31 (±6.43)	22.92 (±4.81)	23.74 (±5.04)	23.20 (±4.20)	20.27 (±4.68)	0.555
GLS (%)	21.85 (±4.42)	22.17 (±5.43)	21.79 (±3.77)	21.59 (±4.04)	21.32 (±3.65)	19.65 (±4.29)	0.711
GCS (%)	19.74 (±4.37)	19.24 (±3.53)	19.57 (±3.70)	18.29 (±3.07)	19.57 (±2.09)	19.11 (±3.14)	0.845
Global TMAD%	14.12 (±3.0)	12.91 (±2.13)	13.53 (±2.81)	13.29 (±2.43)	13.03 (±2.25)	12.07 (±2.34)	0.295
Global TMAD mm/m²	15.28 (6.98-29.65)	14.29 (6.09-24.72)	14.29 (6.76-25.48)	15.07 (6.02-27.08)	14.42 (5.73-24.11)	14.06 (5.28-25.28)	0.944

(N):number of animals in each group; AP4: apical 4-chamber; AP2: apical two-chamber; EF: ejection fraction; $E_{mitral:}$ early diastolic mitral inflow velocity; EDVi: end-diastolic volume indexed to the body surface area; ESVi: end-systolic volume indexed to the body surface area; $A_{mitral:}$ late diastolic mitral inflow velocity; CD: cumulative dose; E´: ´peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; A´: peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; FS: fractional shortening; GCS: Global Circumferential Strain; GLS: Global Longitudinal Strain; LA:Ao: left atrium-to-aorta ratio; $LVIDd_n$: normalized left ventricular internal dimension at end-diastole; $LVIDs_n$: normalized left ventricular internal dimension at end-systole; MAPSEi: mitral annular plane systolic excursion indexed to the body surface area; S´: peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; SM: Simpson method; TMAD: Tissue Motion Annular Displacement. Data with normal distribution were expressed by the mean and standard deviation and data with abnormal distribution were expressed by the median and interquartile range. Values with superscripted letter [a] indicate statistically significant differences between pre (day 0) evaluation.

Table 2 – Descriptive data, cumulative dose of doxorubicin and the main echocardiographic variables compared over time from the paired analysis of the 12 animals that reached the end of the study.

	Pre (Day 0)	7d	21d	60d	120d	180d	P
N	12	12	12	12	12	12	0.998
N (CHOP/DoxoCarbo)	5/6	5/6	5/6	5/6	5/6	5/6	0.998
CD of doxorubicin (mg/kg)	0	0.98 (0.88 – 1)	1.06 (0.88 – 1)	2.12 (1.74 – 3)	3.52 (2.6 – 4)	4.26 (3.45 – 5)	
E:A	0.84 (0.58-1.28)	0.90 (0.57-1.18)	0.95 (0.64-1.50)	0.94 (0.57-1.80)	0.95 (0.60-1.30)	0.92 (0.50-1.70)	0.772
LA:Ao	1.15 ^{ab} (1.08-1.40)	1.14^{a} (1.0-1.38)	1.29^{b} (1.06-1.56)	1.30^{b} (1.10-1.53)	1.23^{ab} (1.0-1.67)	1.27^{ab} (1.0-1.67)	**0.0086**
LVIDD$_n$	1.45 (1.21-1.75)	1.47 (1.29-1.71)	1.40 (0.45-1.78)	1.49 (1.30-1.70)	1.41 (1.33-1.91)	1.45 (1.24-1.73)	0.8652
LVIDS$_n$	0.82 (0.59-1.07)	0.82 (0.61-0.96)	0.77 (0.71-1.30)	0.78 (0.70-0.98)	0.81 (0.60-1.15)	0.85 (0.56-0.96)	0.7526
FS (%)	41.89 (±6.65)	42.51 (±6.67)	43.76 (±6.29)	42.41 (±7.36)	42.24 (±6.60)	40.08 (±7.26)	0.5960
EF (AP4 SM) (%)	75.45 (62.2-80.5)	72.55 (61.0-81.50)	71.70 (59.10-75.80)	73.0 (68.30-80.80)	72.65 (59.20-77.3)	70.35 (60.50-79.0)	0.0970
EF (AP2 SM) (%)	74.76 (±7.12)	75.87 (±6.42)	72.28 (±6.51)	73.79 (±6.33)	73.56 (±6.41)	69.89 (±3.99)	0.0609
EF Biplane SM (%)	74.24 (±5.05)	73.75 (±6.41)	71.66 (±5.67)	74.15 (±3.26)	72.62 (±4.92	70.31 (±4.29)	0.1440
EDVi AP4 (ml/m²)	38.34 (18.98-80.21)	36.49 (20.65-72.07)	37.18 (23.19-63.20)	38.50 (17.70-57.85)	37.9 (18.34-84.61)	37.85(22.80-64.91)	0.4159
ESVi AP4 (ml/m²)	8.75 (5.94-24.20)	9.90 (5.04-22.97)	11.63 (5.61-22.80)	9.71 (4.14-18.37)	11.16 (4.16-20.49)	11.63 (5.84-15.22)	0.2837
S´septal (cm/s)	10.97 (±2.54)	10.33 (±1.74)	10.16 (±1.46)	10.60 (±2.88)	9.60 (±2.07)	10.33 (±2.10)	0.4119
E´ septal (cm/s)	7.41 (4.93-15.30)	6.49 (5.07-8.59)	7.34 (4.54-11.80)	7.61 (4.87-9.63)	6.62 (5.17-9.55)	6.66 (4.77-9.33)	0.3696
A´septal (cm/s)	10.09 (±1.85)	9.11 (±1.98)	9.29 (±1.93)	9.60 (±2.14)	8.94 (±2.54)	9.32 (±2.66)	0.4179
S´ lateral (cm/s)	11.40 (8.41-20.10)	11.05 (7.96-14.30)	11.05 (6.45-13.70)	10.80 (6.29-17.40)	10.30 (7.76-18.10)	9.36 (6.96-13.80)	0.2693
E´ lateral (cm/s)	8.45 (5.57-15.60)	7.80 (4.77-12.60)	7.30 (4.62-11.60)	7.60 (5.10-12.20)	7.47 (5.57-14.30)	6.79 (4.89-11.30)	0.1519
A´lateral (cm/s)	11.0 (8.43-15.80)	9.77 (6.27-21.0)	8.82 (6.67-14.0)	9.78 (6.77-13.90)	9.66 (7.16-15.10)	10.40 (6.68-12.40)	0.1673
MAPSEi lateral (cm/m²)	2.06^{a} (1.17-3.19)	1.93^{a} (1.0-3.33)	1.82^{ab} (0.70-2.88)	1.83^{ab} (0.93-3.38)	1.66^{ab} (0.88-3.20)	1.64^{b} (0.83-2.77)b	**0.0041**
MAPSEi septal (cm/m²)	2.18^{a} (0.97-3.24)	1.97^{ab} (0.76-2.81)	1.81^{b} (0.85-2.73)	1.87^{ab} (0.71-3.79)	1.84^{ab} (0.89-2.62)	1.73^{ab} (0.75-2.68)	**0.0174**
Strain AP4 (%)	22.80 (±4.46)	21.91 (±4.55)	22.10 (±4.05)	20.64 (±4.28)	21.58 (±4.33)	20.32 (±4.85)	0.0945
Strain AP3 (%)	18.69 (±3.10)	19.85 (±6.10)	19.13 (±4.21)	17.83 (±3.19)	19.24 (±3.65)	18.38 (±4.25)	0.4309
Strain AP2 (%)	22.43 (±4.32)	22.39 (±6.43)	21.36 (±4.43)	22.96 (±5.42)	22.62 (±4.42)	20.27 (±4.68)	0.3066
GLS (%)	21.30 (±3.24)	21.38 (±5.40)	20.86 (±3.95)	20.48 (±3.82)	21.14 (±3.87)	19.65 (±4.29)	0.2889
GCS (%)	18.62 (±4.60)	18.16 (±4.03)	19.20 (±3.41)	18.04 (±3.54)	18.92 (±1.48)	19.11 (±3.14)	0.183
Global TMAD%	14.52 (±2.72)^{a}	13.16 (±2.15)^{ab}	13.48 (±2.35)^{ab}	13.37 (±2.23)^{ab}	13.18 (±2.23)^{ab}	12.07 (±2.34)^{b}	**0.0465**
Global TMAD mm/m²	15.54 (6.98-29.61)	14.77 (6.09-24.72)	14.53 (7.65-25.48)	15.19 (6.02-27.08)	15.25 (5.73-24.11)	14.06 (5.28-25.28)	0.145

(N):number of animals in each group; AP4: apical 4-chamber; AP2: apical two-chamber; EF: ejection fraction; E_{mitral}: early diastolic mitral inflow velocity; EDVi: end-diastolic volume indexed to the body surface area; ESVi: end-systolic volume indexed to the body surface area; A_{mitral}: late diastolic mitral inflow velocity; CD: cumulative dose; E´: ´peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; A´: peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; FS: fractional shortening; GCS: Global Circumferential Strain; GLS: Global Longitudinal Strain; LA:Ao: left atrium-to-aorta ratio; LVIDd$_n$: normalized left ventricular internal dimension at end-diastole; LVIDs$_n$: normalized left ventricular internal dimension at end-systole; MAPSEi: mitral annular plane systolic excursion indexed to the body surface area; S´: peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; SM: Simpson method; TMAD: Tissue Motion Annular Displacement. Data with normal distribution were expressed by the mean and standard deviation and data with abnormal distribution were expressed by the median and interquartile range. Values with different superscripted letters indicate statistically significant differences between groups and equal letters represents equality between group.

Table 3 – Cut-off values from ROC curve analyzes with sensitivity, specificity and AUC to differentiate dogs that survived from those that died during the study.

	Cut-off	Sensitivity %	Specificity %	AUC	95% CI
E:A	> 0.845	50	76.92	0.6122	0.3862 - 0.8382
$LVIDd_n$	< 1.330	25	92.31	0.5032	0.2668 - 0.7396
$LVIDs_n$	< 0.660	91.67	30.77	0.5673	0.3368 - 0.7978
FS	> 45.30	75	61.54	0.6731	0.4566 - 0.8895
EDVi	< 84.98	91.67	30.77	0.5321	0.2959 - 0.7682
ESVi	< 8.645	91.67	30.77	0.5449	0.3125 - 0.7772
LA:Ao	< 1.295	61.54	75	0.6538	0.4294 - 0.8782
S' septal	> 9.165	25	100	0.5128	0.2738 - 0.7518
E' septal	> 8.975	83.33	38.46	0.6058	0.3790 - 0.8325
A' septal	> 11.65	91.67	30.77	0.5609	0.3296 - 0.7921
S' lateral	> 11.80	66.67	84.62	0.6603	0.4225 - 0.8980
E' lateral	> 7.310	33.33	92.31	0.6154	0.3871 - 0.8437
A' lateral	> 10.28	41.67	84.62	0.5513	0.3173 - 0.7853
MAPSEi lateral	> 1.955	58.33	61.54	0.5032	0.2650 - 0.7415
MAPSEi septal	> 2.105	58.33	76.92	0.6090	0.3771 - 0.8409
EF AP4 SM	> 79.25	91.67	30.77	0.5417	0.3073 - 0.7760
EF AP2 SM	> 76.90	50	76.92	0.5256	0.2880 - 0.7633
EF Biplane SM	> 71.40	41.67	92.31	0.5385	0.2975 - 0.7794
GLS	> 22.52	66.67	66.67	0.6111	0.3685 - 0.8537
GCS	> 16.72	84.62	50	0.6282	0.3986 - 0.8578
Global TMADmm/m2	> 19.12	84.62	41.67	0.5609	0.3199 - 0.8019
Global TMAD %	> 14.08	61.54	66.67	0.5897	0.3585 - 0.8210

AP4: apical 4-chamber; AP2: apical two-chamber; EF: ejection fraction; EDVi: end-diastolic volume indexed to the body surface area; ESVi: end-systolic volume indexed to the body surface area; E´: ´peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; A´: peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; FS: fractional shortening; GCS: Global Circumferential Strain; GLS: Global Longitudinal Strain; LA:Ao: left atrium-to-aorta ratio; $LVIDd_n$: normalized left ventricular internal dimension at end-diastole; $LVIDs_n$: normalized left ventricular internal dimension at end-systole; MAPSEi: mitral annular plane systolic excursion indexed to the body surface area; S´: peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; SM: Simpson method; TMAD: Tissue Motion Annular Displacement.

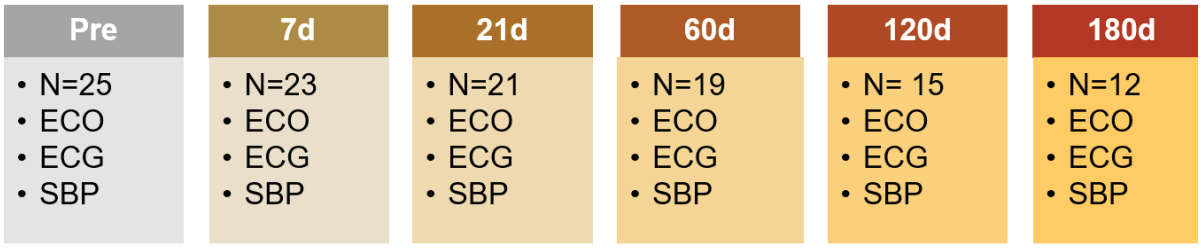

Figure 1 - Schematic representation of follow-up assessments and number of dogs before chemotherapy initiation (pre) and 7 days (7d), 21d, 60d, 120d and 180d after the first application of doxorubicin.

N: number of animals in each group; ECO: echocardiography; ECG: electrocardiography; SBP: systolic blood pressure.

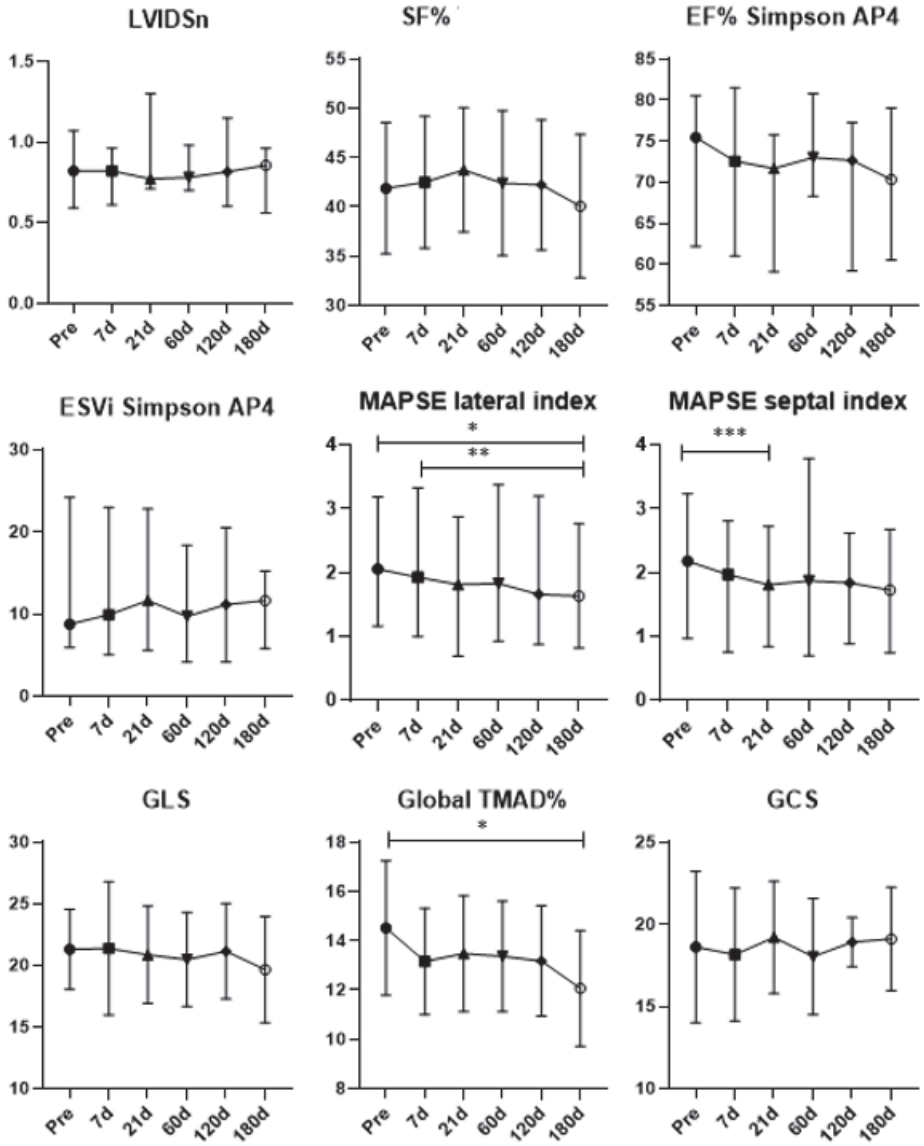

Figure 2 - Graphs representing the main echocardiographic variables of systolic function compared over time from the paired analysis of the 12 animals that reached the end of the study. The differences between the groups are represented by the bars, in which (*) represents the difference between the pre-chemotherapy and 180-day assessment, (**) between the 7-day and 180-day assessment, and (***) between the pre- and 21-day assessment.

AP4: apical 4-chamber; AP2: apical two-chamber; EF: ejection fraction; FS: fractional shortening; GCS: Global Circumferential Strain; GLS: Global Longitudinal Strain; $LVIDs_n$: normalized left ventricular internal dimension at end-systole; MAPSEi: mitral annular plane systolic excursion indexed to the body surface area; TMAD: Tissue Motion Annular Displacement.

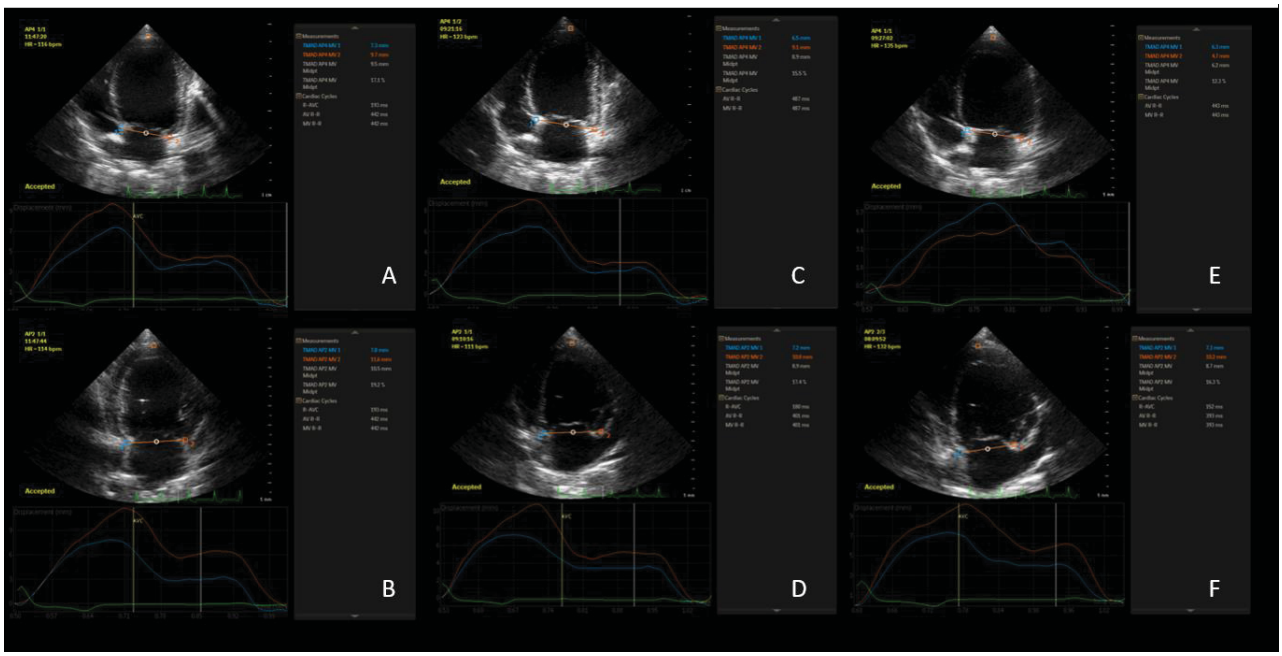

Figure 3 – Echocardiographic images of the tissue motion annular displacement (TMAD) technique of a Fox Terrier dog with lymphoma before the start of the CHOP chemotherapy protocol (A,B), 120 days (C, D) and 180 days (E,F) after the first application of doxorubicin, showing a progressive reduction in tissue displacement of the mitral annulus.

4 Longitudinal assessment of global and segmental right ventricular systolic function in dogs undergoing chemotherapy with doxorubicin

Marcela Wolf[a*], Stephany B. Lucina[a], Vinícius B. C. Silva[a], Matheus F. Silveira[a], Victoria G. Silva[a], Natália Kano[a], Ana P. Sarraff[b], Claudia C. Custódio[c], Marlos G. Sousa[a]

[a] Department of Veterinary Medicine, Federal University of Paraná (UFPR), Rua dos Funcionários 1540, Curitiba, 80035-050, Brazil.

[b] School of Life Sciences, Pontifical Catholic University of Paraná (PUC-PR), Rua Rockfeller 1311, Curitiba, 80230-130, Brazil.

[c] Oncology Sector, Clinivet Veterinary Hospital, Rua Holanda 894, Curitiba, 82540-040, Brazil.

[*] Corresponding author. E-mail address: marcelawvet@gmail.com (M Wolf).

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: Cardiotoxicity on the right ventricle in dogs undergoing chemotherapy with doxorubicin.

4.1 Abstract

Introduction: Left ventricular function is an important factor evaluated in the investigation of cardiotoxicity in patients undergoing chemotherapy with doxorubicin. However, human studies show that global cardiac function can be compromised, and right ventricular (RV) function may have the potential to be an early marker of myocardial dysfunction in cardio-oncology patients.

Animals: 25 dogs with cancer undergoing chemotherapy with doxorubicin as a single agent or in combination with other chemotherapeutic drugs.

Methods: Prospective and longitudinal study. Dogs underwent conventional echocardiography and speckle tracking analysis before chemotherapy (day 0), 7 days, 21 days, 60 days, 120 days and 180 days after the first administration of doxorubicin for morphometric and functional assessment of the RV.

Results: 12 of 25 dogs completed all evaluations of the study. A reduction in TAPSEi values ($P<0.001$) was observed progressively from the 60 days assessment, as well as a reduction in S' wave ($P=0.036$) at 120 days and FAC ($P=0.04$), global longitudinal strain (GLS) ($P=0.002$) and TMAD ($P=0.019$) at 180d when compared to the initial evaluation. GLS and TMAD were the only echocardiographic parameters that reduced to median values below the widely used normality reference. No differences were observed in RV morphometric measurements ($P>0.05$).

Conclusions: A subclinical reduction in right ventricular function in dogs undergoing chemotherapy with doses considered safe of doxorubicin. Speckle tracking techniques, although they were not early in the detection of reduced function, proved to be important in the punctual assessment of these patients.

Key words: cardiotoxicity, echocardiography, oncology, tmad, speckle tracking.

4.2 Abbreviation list

ACVIM	American College of Veterinary Internal Medicine
BSA	body surface area
BW	body weight
CD	cumulative dose
CHOP	doxorubicin, cyclophosphamide, vincristine and prednisone
DoxoCarbo	doxorubicin and carboplatin
FAC	fractional area change
GLS	global longitudinal strain
TAPSEi	tricuspid annular plane systolic excursion indexed to the body surface area
ROI	region of interest
RV	right ventricular
SBP	systolic blood pressure
TMAD	tissue motion annular displacement

4.3 Introduction

Doxorubicin is an anthracycline widely used in several chemotherapy protocols in humans and dogs [1,2]. However, by mechanisms not yet fully understood, its administration is related to dose-dependent cardiotoxicity [3]. Early detection of cardiotoxicity is a key approach that can affect outcomes and is directly linked to cardio-oncological therapeutic management in humans [4].

Left ventricular function is one of the main parameters evaluated to detect cardiotoxicity in dogs and its dysfunction may be present in a subclinical form or

resulting in left congestive heart failure [5,6]. However, little is known about right ventricular (RV) function in these patients. Preliminary studies in humans and experimental models show that the right ventricle is also affected [7,8,9,10,11], and may occur before left ventricular dysfunction [12]. Therefore, an echocardiographic evaluation of this chamber can provide early information on anthracycline-induced cardiotoxicity [10].

Furthermore, RV function has a prognostic implication in patients with or without LV systolic dysfunction in various settings in humans [13,14,15,16] and dogs [17,18,19], but there are limited data on the effect of anthracyclines on the RV. There are several studies evaluating the performance of the left ventricle in dogs undergoing chemotherapy with doxorubicin [6,20,21], however, to our knowledge, no study has specifically evaluated RV performance in these patients. Therefore, this study aimed to longitudinally evaluate the function of the right ventricle of dogs undergoing chemotherapy with doxorubicin, using conventional echocardiography and speckle tracking echocardiography.

4.4 MATERIALS AND METHODS

Animals

This is a prospective and longitudinal study in which dogs with different types of neoplasia treated at the oncology sector of the at a veterinary teaching facility and at a private veterinary hospital between February 2019 and March 2021, which received at least one dose of doxorubicin were included. This study was approved by the Institutional Animal Use Committee (protocol 099/2018) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all owners gave formal consent prior to the animal being enrolled on the study.

The chemotherapy protocol was determined by the institution's oncology service and dogs that received chemotherapy with doxorubicin alone or in combination with other drugs were selected. All animals were submitted to an evaluation before (day 0) the start of the chemotherapy protocol and 7, 21, 60, 120 and 180 days after the first application of doxorubicin.

In all visits the dogs were submitted to a complete physical examination, echocardiographic examination, at least 3 minutes of computerized electrocardiography[a] to determine heart rate and cardiac rhythm and non-invasive systolic blood pressure (SBP) measurement[b] as previously recommended [22].

Dogs that had systolic dysfunction, cardiomegaly, arrhythmias (except arrhythmias of sinus origin), congenital heart diseases, cardiac neoplasms, pulmonary hypertension, pericardial effusion, systemic arterial hypertension (SBP>160 mmHg), systemic arterial hypotension (SBP <80 mmHg), that received medications for the cardiovascular system, such as pimobendan or whose owner does not formally agree with the procedure by signing the informed consent form were excluded from the study.

Conventional Echocardiography

All echocardiographic exams[c] were performed with no sedation, by an experienced observer (MW), with unsedated dogs in left and right lateral recumbency, as recommended by the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine (ACVIM) [23].

For the echocardiographic evaluation of the right ventricle that will be described, an apical four-chamber view optimized for the right ventricle was performed.

The morphological evaluation of the right ventricle was performed in two-

dimensional mode with the following measurements, obtained in systole and diastole: internal basal diameter (measured immediately below the tricuspid annulus, from the free wall to the interventricular septum); mean diameter (in the medial third of the right ventricle); and length, from the ventricular apex to the tricuspid annular plane, as previously described [24]. All morphometric measurements were indexed by body surface area (BSA) using the following formula:

BSA= K x (body weight (BW) in grams$^{2/3}$) x 10^{-4}

K= constant (10.1 for dogs)

To assess right ventricular systolic function by conventional echocardiography, the following parameters were obtained: the tricuspid annulus systolic excursion (TAPSE), which was obtained with the M-mode cursor on the tricuspid annulus in the region of the RV free wall and TAPSEi was calculated by TAPSE indexed by the BSA; the right ventricular fractional area change (FAC) was calculated as the percentage difference between the RV systolic and diastolic area; and the S' wave, which was obtained with the tissue Doppler cursor positioned at the junction of the tricuspid plane with the RV free wall.

Right ventricular diastolic function was evaluated with pulsed Doppler in the transtricuspid flow, determining the early (Et) and late (At) diastolic peak velocities, and the Et:At ratio, and also by tissue Doppler, measuring the early (Et´) and late (At') RV diastolic velocities, with the cursor positioned in the tricuspid annulus on the free wall of the right ventricle.

Speckle tracking Echocardiography

Two-dimensional images of the apical 4-chamber view optimized for the right ventricle were obtained for at least 5 cardiac cycles for further off-line analysis of

speckle tracking techniques[d].

Global longitudinal strain (free wall)

The global longitudinal strain (GLS) of the RV was obtained with an adaptation of the software prepared for longitudinal strain 4 chambers of the left ventricle. Three regions of interest (ROI) were defined by the operator: RV septal annulus, RV lateral annulus and RV apex. The software automatically starts tracking each myocardial segment and manual corrections were made if necessary. Only the three segments (apical, middle and basal) of the RV free wall were considered and the GLS of the free wall was determined an average of the deformation of these three segments.

Tissue motion annular displacement

The RV TMAD was also obtained with a software adaptation for the TMAD apical 4 chamber of the left ventricle. For the analysis, three ROIs were determined by the operator, two of them in the septal and lateral region of the tricuspid annulus and the third in the epicardial region of the apex of RV. The TMAD was terminated as the displacement in mm of the midpoint, created virtually between the two ROIs of the tricuspid annulus, towards the third point and as a percentage of the displacement of the midpoint in relation to the length of the right ventricle (global TMAD%). The global TMAD in mm was indexed in by BSA (mm/m²) as previously described [25].

4.5 Statistical Analysis[e]

The Shapiro-Wilk test was used to investigated data normality. Parametric data are represented by mean and standard deviation and non-parametric by median and quartile interval. The differences between groups in evaluated times were compared with ANOVA, followed by the post hoc Tukey's multiple comparisons test

(in data with normal distribution) and using Kruskal-Wallis test followed by Dunn´s multiple comparisons test (non-parametric approach). A paired analysis of the 12 dogs that completed all the evaluations. For this, the test ANOVA. followed by the post hoc Tukey's multiple comparisons test was used for the parametric data, and Friedman test followed by Dunn´s multiple comparisons test to non-parametric data. The variables sex and rhythm were evaluated using Fisher's test and the Chi-Square test was performed to compare the difference between the number of animals at different times of evaluation.

To investigate the correlation of the echocardiographic parameters with cumulative dose (CD) of doxorubicin, Pearson (for the parametric data), and Spearman (for non-parametric data) tests were use. T test or the Mann-Whitney test were used to compare the echocardiographic variables between the different chemotherapy protocols [doxorubicin with carboplatin (DoxoCarbo) and doxorubicin with cyclophosphamide, vincristine and prednisone (CHOP)].

A comparison was made between the echocardiographic variables of the group that survived with the group that died and receiver operating characteristic (ROC) curves were performed to determine the cut-off values with the best combination of sensitivity and specificity of the variables to differentiate this groups. Kaplan-Meier curves were used to assess the prognostic value of this variables for the all-cause mortality. Breslow's test was used to assess differences between curves. A value of $P<0.05$ was considered statistically significant.

4.6 RESULTS

A total of 32 dogs were selected for this study. However, 7 were excluded from the analyzes due to the presence of cardiac disease requiring treatment with

pimobendan (endocardiosis stage ACVIM B2) (N=1), owner withdrawal (N=5) and the development of intracardiac neoformation during the study (N=1). The selected dogs comprised of Crossbreed (N=9), Golden Retriever (N=3), Poodle (N=3), Dachshund (N=2), Lhasa Apso (N=2), Rottweiler, Schnauzer, German Shepherd, Pinscher, Fox Terrier and English Bulldog (n=1 each). Of these, 13 females and 12 males, most of them neutered (N=18), aged from 2 to 16 years (median: 10 years; mean: 9.9 years); weighting (median: 7.9 kg; mean: 12.7 kg).

Of the 25 dogs recruited, 13 died during the course of the study and 12 completed all assessments (pre-dox, 7d, 21d, 60d, 120d and 180d), and although the number of dogs is smaller in each evaluation, there was no difference (P=0.998) between the number of animals at different times of analysis.

Doxorubicin was used as a single agent (N=5), associated with carboplatin (N=10) or associated with cyclophosphamide, vincristine and prednisone (CHOP protocol) (N=10), depending on the protocol that was determined by the oncology sector. The established dose of doxorubicin was 30 mg/m² for dogs ≥15 kg and 1 mg/kg for dogs <15 kg with an infusion time of 20 to 30 minutes. For standardization, the milligram per kg was used in this study. Total CD of dox of 12 dogs that completed all assessments range from 3.45 mg/kg to 5 mg/kg (median:4 mg/kg; mean:4.26 mg/kg). Lymphoma was the most common neoplasm, affecting 10 of the 25 dogs, followed by mammary adenocarcinoma (n=10), mesothelioma, leiomyosarcoma, chondrosarcoma, melanoma and inflammatory carcinoma (n=1 each).

In terms of heart rhythm, sinus arrhythmia (N=14/56%) was the most found, followed by sinus rhythm (N=10/40%) and sinus tachycardia (N=1/4%). Only one dog had ventricular arrhythmias (single and paired complexes) at reassessment 60 days

after the first dose of doxorubicin. Of the 25 dogs included in the study, 4 had degenerative disease of the mitral and tricuspid valves and 3 only had mitral valve disease, all with mild valvular insufficiency, without cardiac remodeling and without hemodynamic repercussions (ACVIM Stage B1).

General and echocardiographic data of patients who received CHOP and DoxoCarbo protocol were compared. At the end of the study (180d), six dogs received the DoxoCarbo protocol (N=6/50%), five CHOP protocol (N=5/41.7%) and one only doxorubicin (N=1/8.3%). Dogs that received the CHOP protocol were younger (P=0.0013) compared to animals in the DoxoCarbo group, as well as the CHOP group had more males (n=8/80%) than the DoxoCarbo group (N=1/10%) and no differences were found regarding rhythm (P=0.069), body weight (P=0.477), heart rate (P=0.559) and SBP (P=0.308). The Et' wave velocity (P=0.028) was higher in dogs than in the CHOP group than in the DoxoCarbo group in the pre-chemotherapy evaluation and at the end of the study (180d), the animals in the DoxoCarbo group presented S' wave values lower than the CHOP group (P=0.0007).

The comparison of the evolution of the data over time was performed with a non-paired analysis of all animals (N=25), as well as the patients who received the CHOP (N=10) and DoxoCarbo(N=10) protocol were also evaluated separately. In addition, a paired evaluation of the 12 animals that reached the end of the study was performed.

The unpaired analysis of all dogs revealed a reduction in the values of GLS (P=0.0020), global TMAD% (P=0.0016), FAC (P=0.0012) and less deformation of the basal segment of the RV free wall (P=0.0009) at 180 days when compared to the pre-chemotherapy evaluation (Table 1). A drop in S' wave velocity was detected at the assessment of 120d (P=0.0045) and 180d (P=0.0148) compared to the initial

examination. In addition, a progressive reduction in TAPSEi values was observed at 60d (P=0.019), 120d (P=0.0009) and 180d (P=0.001) when compared to day 0 (Table 2; Figure 1).

No variables differed over time in patients receiving CHOP protocol. However, patients who received the DoxoCarbo protocol demonstrated a reduction in the S' wave (P=0.044) and TAPSEi (P=0.032) at 120d, and less deformation of the basal segment of the right ventricle (P=0.0041) at 180 days when compared to assessment before treatment.

In the paired analysis of the 12 dogs that completed the study, a reduction in the values of GLS (P=0.011), FAC (P=0.013), TAPSEi (P=0.007), as well as a reduction in the deformation of the RV basal segment (P=0.046) at 180d was observed. No significant changes were found morphometric measurements and parameters related to diastolic function of the RV in any of the assessments (P>0.05) (Table 2).

Age showed a negative correlation with the Et wave (P=0.038; R=-0.41) and with the Et' wave (P<0.0001; R=-0.75). Interestingly, the cumulative dose of doxorubicin showed a positive correlation with the At wave (P=0.044; R=0.59)

None of the patients in this study developed signs of right cardiac heart failure (ascites, limb edema, effusions, dyspnea, tachypnea) or died due to cardiac alteration. The most echocardiographic parameters were not able to accurately predict all-cause mortality in this study (P>0.05). However, FAC with a cut-off value of >47.05%, presented 53.8% sensitivity, 91.6% specificity and AUC 0.71 in differentiating patients who survived from patients who died. The dogs that had an FAC > 47.05% at baseline lived longer (median 335 days) than patients who had a lower value (median 102 days) (P=0.0045) (Table 3; Figure 2).

The median survival time of the study animals (N=25) was 201 days. No difference was observed in the survival of patients receiving different protocols, for patients who received a protocol (DoxoCarbo=411 days, CHOP=181.5 days and only Doxo=117 days). There was no difference in patient survival in the different protocols (P=0.139).

4.7 DISCUSSION

It is known that left ventricular function can be affected clinically and subclinically in dogs and humans during anthracycline therapy [4,6]. However, the impairment of cardiac function in chemotherapy toxicity is global [26]. Magnetic resonance imaging study shows lower muscle mass, diffuse interstitial fibrosis and RV atrophy on a scale similar to that of the left ventricle in people on anthracycline chemotherapy [11], however, data evaluating right ventricular function in dogs undergoing chemotherapy are scarce. The function of this chamber plays an important role in the prognostic assessment of various diseases in people and dogs [14-19] including in patients with left ventricular dysfunction [27].

The RV myocardial anatomy and kinetics are complex. The RV is composed of approximately 75% of the longitudinally arranged fibers located especially in the endocardial region. In addition, it shares the epicardial layer with the left ventricle, which associated with the interventricular septum and pericardium promote ventricular interdependence [28]. Histological evaluation in an experimental study of rats undergoing therapy with anthracyclines showed greater tissue damage to the subendocardial layer of the right ventricle [29], this justifies the fact that echocardiographic techniques that mainly assess the function of longitudinal fibers (such as TAPSE, TMAD, GLS and S' wave) have detected a reduction in myocardial

function in this study. In addition, the lower right ventricular muscle mass and thinner walls may support earlier detection of this chamber dysfunction [10].

Speckle tracking techniques appear to be less affected by loading conditions than conventional techniques [10] and are shown to be early in the detection of right ventricular myocardial dysfunction in childhood cancer survivors [30]. The results of this research show a reduction in the median RV GLS from 23.2% (pre) to 17.08% at 180d with a mean cumulative dose of 4.6 mg/kg, corroborating studies in humans on chemotherapy with anthracyclines in which a reduction in the RV GLS from 16.2% to 13.81% [10]. Similar results were also obtained in a longitudinal study of humans evaluated before and 6 months after the start of chemotherapy with doxorubicin, in which a reduction of 22.3% to 20.1% in RV GLS and in FAC from 47% to 42% was observed, with no significant changes in left ventricular ejection fraction at cumulative doses greater than 200 mg/m² [12].

The RV TMAD was shown to be reduced in human survivors of childhood cancer and with good correlation with magnetic resonance and three-dimensional echocardiography in a study that did not observe a difference in the values of TAPSE [30]. Interestingly, conventional techniques such as TAPSE and S' wave demonstrated a reduction in myocardial function before the advanced techniques in this study. No patient showed severe systolic dysfunction or clinical cardiotoxicity until the last evaluation (180d), so this reduction can be considered as mild and subclinical. However, an important factor to be considered is that in a punctual evaluation of the patient in the 180d, the values of conventional echocardiographic parameters, such as TAPSE, FAC and S' wave, although they reduced with time, would be considered within the normality of reference values previously suggested and used in routine [31]. On the other hand, speckle tracking techniques (GLS and

306 TMAD) presented a median below the values usually considered as normal [25,31],
307 which would allow detection of reduced right ventricular function in the punctual
308 evaluation of these dogs. This result demonstrates the importance of patient follow-
309 up, the advantage of using advanced techniques in routine, especially in a punctual
310 evaluation of dogs undergoing chemotherapy, and also imposes a questioning of the
311 normality values routinely used for the detection of mild cardiotoxicity in dogs. Similar
312 results were seen in a previous study, in which a progressive subclinical reduction in
313 right ventricular systolic indices (TAPSE, FAC, S' wave) was observed in the
314 longitudinal evaluation of humans undergoing chemotherapy in a short period of time,
315 although still with values within normal ranges [7].

316 In this study, less deformation of the basal segment of the free wall was
317 observed in the RV. Some diseases affect specific regions of the myocardium, for
318 example humans with amyloidosis have greater apical deformation and less basal
319 deformation [32]. Future studies with segmental echocardiographic evaluation and
320 histopathological analysis will be able to clarify whether there is really a greater
321 involvement of the basal fibers with the administration of anthracyclines.

322 Increases in right ventricular [7] and right atrial dimensions have been
323 observed in people receiving anthracyclines [10]. In this study, no differences were
324 found in the morphometric assessment of the right ventricle, probably due to the
325 slight and subclinical reduction in myocardial function.

326 A progressive and subclinical reduction of the Et´/At´ ratio is seen in humans
327 on chemotherapy [7] and lower Et´ wave velocity in people who survived childhood
328 cancer treated with anthracyclines [30]. The positive correlation of the At wave with
329 the DC of doxorubicin in this study may demonstrate the dose-dependence of this
330 chemotherapy agent influencing diastolic function. In addition, the higher velocity of

the Et' wave in the patients in the CHOP group is due to the fact that these patients were younger than the DoxoCarbo group, since age was negatively correlated with the Et wave (P=0.038; R:-0.41) and with the Et' wave (P<0.0001; R=-0.75).

Only one dog had isolated and paired ventricular premature complexes after doxorubicin administration in this study. An experimental electrophysiological study with rats showed that the administration of doxorubicin causes a heterogeneous increase in ventricular repolarization times, generating local and interregional dispersions, interfering especially with the repolarization of the right ventricle, which has been shown to be more sensitive to doxorubicin than the left ventricle [33].

In this study, three dogs had mitral valve degeneration and four had mitral and tricuspid degeneration. It is known that right ventricular function can be affected in dogs with valvular disease [34] and pulmonary hypertension [35]. However, the patients in this study had mild regurgitation without hemodynamic repercussions, a tricuspid regurgitation velocity less than 3 m/s, and a low probability of pulmonary hypertension according to previously established criteria [36]. Previous studies show that right ventricular function is affected in more advanced stages of valvular disease, when heart failure is present (ACVIM stage C) [34], therefore, this does not seem to be a factor that could compromise our results.

Studies show that right ventricular function can provide important prognostic information in people and dogs with a variety of diseases [14-19]. Right ventricular strain was a good predictor of dyspnea in a prospective study of people with cancer receiving anthracycline [37], as well as RV volumetric assessment was a good predictor of left ventricular systolic dysfunction in humans on chemotherapy [11]. In this study, most parameters showed poor accuracy in predicting all-cause mortality, this may be due to the fact that none of the patients progressed to clinical

cardiotoxicity, significant systolic dysfunction or died from cardiac causes, and also because different protocols and neoplasms are being compared. However, the FAC >47.05%, presented 53.8% sensitivity, 91.6% specificity and AUC 0.71 in differentiating patients who survived from patients who died and dogs with lower FAC at baseline (pre) lived shorter than those with higher values.

Several limitations must be considered in this study. The low number of animals is an important factor in the interpretation of results. In addition, the non-standardization of chemotherapy protocols, the inclusion of different types of neoplasia are important points to be considered in the echocardiographic results obtained, as well as in the analysis of survival. In addition, the echocardiographic analysis of the speckle tracking techniques (GLS and TMAD) of the right ventricle were used with an adaptation of the software made for the left ventricle. The detection of arrhythmias may have been underestimated as the rhythm assessment was performed with 3 minutes of ambulatory electrocardiography. A Holter analysis would provide more accurate information about the cardiac rhythm. The lack of a comparative gold standard assessment such as magnetic resonance imaging and histopathological analysis are also limitations to be considered.

In conclusion, this study shows a subclinical reduction in right ventricular function in dogs undergoing chemotherapy with doses considered safe of doxorubicin. Speckle tracking techniques, although they were not early in the detection of reduced function, proved to be important in the punctual assessment of these patients, as they presented values lower than those considered normal reference at the time of reduction. New studies with a greater number of animals and standardization of protocols and neoplasms are necessary to evaluate the use of these techniques in the detection of cardiotoxicity in dogs.

381

382 **Conflict of Interest Statement**

383 The authors do not have any conflicts of interest to disclose.

384

385 **Footnotes**

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

387 [b] - MedMega DV 6108 - Vascular *Doppler,* 10 MHz. Franca, Brazil.

388 [c] - Philips Affiniti 50 ultrasound system equipped with 2-4. 3-8 and 4-12 MHz phased-

389 array transducers. Andover, MA, USA.

390 [d] - QLAB Software - automatic cardiac motion quantification (aCMQ)

391 [e] - Graphpad prism 5.0 Software

392

393 **4.8 REFERENCES**

394 [1] Mauldin GE, Fox PR, Patnaik AK, Bond BR, Mooney SC, Matus RE. Doxorubicin-

395 induced cardiotoxicosis. Clinical features in 32 dogs. J Vet Intern Med 1992;6:82-88.

396 [2] Henriksen PA. Anthracycline cardiotoxicity: an update on mechanisms. monitoring

397 and prevention. Heart 2018;104:971-7.

398 [3] Chung WB, Youn HJ. Pathophysiology and preventive strategies of anthracycline-

399 induced cardiotoxicity. Korean J Intern Med 2016;31:625-633.

400 [4] Hajjar LA, Costa IBSS, Lopes MACQ, Hoff PMG, Diz MPE, Fonseca SMR, Bittar

401 CS, Rehder MHHS, Rizk SI, Almeida DR, Fernandes GS, Silva LB, Campos CAHM,

402 Montera MW, Alves SMM, Fukushima JT, Santos MVC, Negrão CE, Silva TLF,

403 Ferreira SMA, Malachias MVB, Moreira MCV, Neto MMRV, Fonseca VCQ, Soeiro

404 MCF, Alves JBS, Silva CMPD, Sbano J, Pavanello R, Pinto IMF, Simão AF,

405 Dracoulakis MDA, Hoff AO, Assunção BMBL, Novis Y, Testa L, Filho ACA, Cruz

406 CBBV, Pereira J, Garcia DR, Nomura CH, Rochitte CE, Macedo AVS, Marcatti PTF,

Junior WM, Wiermann EG, Freitas RVH, Coutinho A, Mathias CMC, Vieira FMAC, Sasse AD, Rocha V, Ramires JAF, Filho RK. Diretriz Brasileira de Cardio-oncologia. Arq Bras Cardiol 2020;115:1006-43.

[5] Gallay-Lepoutre J, Bélanger MC, Nadeau ME. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. Res Vet Sci 2016;105:153-9.

[6] Hallman BE, Hauck ML, Williams LE, Hess PR, Suter SE. Incidence and risck factors associated with development of clinical cardiotoxicity in dogs receiving doxorubicin. J Vet Intern Med 2019;33:783–91.

[7] Tanindi A, Demirci U, Tacoy G, Buyukberber S, Alsancak Y, Coskun U, Yalcin R, Benekli M. Assessment of right ventricular functions during cancer chemotherapy. Eur J Echocardiogr 2011;12:834–840.

[8] Kharin SN, Krandycheva VV, Strelkova MV, Tsvetkova AS, Shmakov DN. Doxorubicin-induced changes of ventricular repolarization heterogeneity: results of a chronic rat study. Cardiovasc Toxicol 2012;12:312–317.

[9] Oliveira GH, Dupont M, Naftel D, Myers SL, Yuan Y, Tang WH, Gonzalez-Stawinski G, Young JB, Taylor DO, Starling RC. Increased need for right ventricular support in patients with chemotherapy-induced cardiomyopathy undergoing mechanical circulatory support: outcomes from the INTERMACS Registry (Interagency Registry for Mechanically Assisted Circulatory Support). J Am Coll Cardiol 2014;63:240–248.

[10] Boczar KE, Aseyey O, Sulpher J, Johnson C, Burwash IG, Turek M, Dent S, Dwivedi G. Right heart function deteriorates in breast cancer patients undergoing anthracycline-based chemotherapy. Echo Res Pract 2016;3:79-84.

[11] Souza TF, Silva TQ, Antunes-Correa L, Drobni ZD, Costa FO, Dertkigil SSJ, Nadruz W, Brenelli F, Sposito AC, Matos-Souza Jr JR, Coelho OR, Neilan TG, Jerosch-Herold M, Coelho-Filho OR. Cardiac magnetic resonance assessment of right ventricular remodeling after anthracycline therapy. Sci Rep 2021;11:17123.

[12] Planek MIC, Manshad A, Hein K, Hemu M, Ballout F, Varandani R, Venugopal P, Okwuosa T. Prediction of doxorubicin cardiotoxicity by early detection of subclinical right ventricular dysfunction. Cardiooncology 2020;6:1-8.

[13] Fine NM, Chen L, Bastiansen PM, Frantz RP, Pellikka PA, Oh JK, Kane GC. Outcome prediction by quantitative right ventricular function assessment in 575 subjects evaluated for pulmonary hypertension. Circ Cardiovasc Imaging 2013;6:711–721.

[14] Ghio S, Guazzi M, Scardovi AB, Klersy C, Clemenza F, Carluccio E, Temporelli PL, Rossi A, Faggiano P, Traversi E, Vriz O, Dini FL. Different correlates but similar prognostic implications for right ventricular dysfunction in heart failure patients with reduced or preserved ejection fraction. Eur J Heart Fail 2017;19:873–879.

[15] Carluccio E, Biagioli P, Alunni G, Murrone A, Zuchi C, Coiro S, Riccini C, Mengoni A, D´Antonio A, Ambrosio G. Prognostic value of right ventricular dysfunction in heart failure with reduced ejection fraction: Superiority of longitudinal strain over tricuspid annular plane systolic excursion. Circ Cardiovasc 2018;11:e006894.

[16] Li Y, Li H, Zhu S, Xie Y, Wang B, He L, Zhang D, Zhang Y, Yuan H, Wu C, Suri W, Zhang Y, Li M, Cui Li, Cai Y, Wang J, Yang Y, Lv Q, Zhang L, Xie M. Prognostic Value of Right Ventricular Longitudinal Strain in Patients With COVID-19. JACC:Cardiovasc Imaging 2020;13:2287-99.

[17] Kaye BM, Borgeat K, Motskula PF, Fuentes VL, Connolly DJ. Association of

457 tricuspid annular plane systolic excursion with survival time in Boxer dogs with
458 ventricular arrhythmias. J Vet Intern Med 2015;29:582-8.
459 [18] Nakamura K, Morita T, Osuga T, Morishita K, Ohta H, Takiguchi M. Prognostic
460 Value of Right Ventricular Tei Index in Dogs with Myxomatous Mitral Valvular Heart
461 Disease. J Vet Intern Med 2016; 30: 69-75.
462 [19] Morita T, Nakamura K, Osuga T, Takiguchi M. Incremental predictive value of
463 echocardiographic indices of right ventricular function in the assessment of long-term
464 prognosis in dogs with myxomatous mitral valve disease. J Vet Cardiol 2022;39:51-
465 62.
466 [20] Susaneck SJ. Doxorubicin therapy in the dog. J Am Vet Med Assoc
467 1983;182:70-72.
468 [21] Loar AS, Susaneck SJ. Doxorubicin-induced cardiotoxicity in five dogs. Semin
469 Vet Med Surg 1986;1:68-71.
470 [22] Acierno MJ, Brown S, Coleman AE, Jepson RE, Papich M, Stepien RL, Syme
471 HM. ACVIM consensus statement: guidelines for the identification, evaluation, and
472 management of systemic hypertension in dogs and cats. J Vet Intern Med
473 2018;32:1803-22.
474 [23] Thomas WP, Gaber CE, Jacobs GJ. Recommendations for standards in
475 transthoracic Two-Dimensional echocardiography in the dog and cat. J Vet Intern
476 Med 1993;7:247-52.
477 [24] Gentile-Solomon JM. Abbott JA. Conventional echocardiographic assessment of
478 the canine right heart: reference intervals and repeatability. J Vet Cardiol 2016;18:
479 234e47.
480 [25] Silva VBC, Wolf M, Lucina SB, Sarraff-Lopes AP, Sousa MG. Assessment of
481 right ventricular systolic function by tissue motion annular displacement in healthy

482 dogs. J Vet Cardiol 2020;32:40-48.
483 [26] Oliveira GH, Dupont M, Naftel D, Myers SL, Yuan Y, Tang WH, Gonzalez-
484 Stawinski G, Young JB, Taylor DO, Starling RC. Increased need for right ventricular
485 support in patients with chemotherapy-induced cardiomyopathy undergoing
486 mechanical circulatory support: outcomes from the INTERMACS Registry
487 (Interagency Registry for Mechanically Assisted Circulatory Support). J Am Coll
488 Cardiol 2014;63:240–248.
489 [27] Anavekar NS, Skali H, Bourgoun M, Ghali JK, Kober L, Maggioni AP, McMurray
490 JJV, Velazquez E, Califf R, Pfeffer MA, Solomon SD. Usefulness of right ventricular
491 fractional area change to predict death , heart failure, and stroke following myocardial
492 infarction (from the VALIANT ECHO Study). Am J Cardiol 2008;101:607-12.
493 .[28] Sanz J; Sánchez-Quintana D, Bossone E, Bogaard HJ, Naeije R. Anatomy,
494 function, and dysfunction of the right ventricle: JACC State-of-the-art review. J Am
495 Coll Cardiol 2019;73:1463-82.
496 [29] LLesuy SF, Milei J, Flecha BSG, Boveris A. Myocardial damage induced by
497 doxorubicins: hydroperoxide-initiated chemiluminescence and morphology. Free
498 Radic Biol Med 1990;8:259-264.
499 [30] Ylänen K, Eerola A, Vettenranta K, Poutanen T. Speckle tracking
500 echocardiography detects decreased cardiac longitudinal function in anthracycline-
501 exposed survivors of childhood cancer. Eur J Pediatr 2016;175:1379-86.
502 [31] Visser LC, Scansen BA, Schober KE, Bonagura JD. Echocardiographic
503 assessment of right ventricular systolic function in concious healthy dogs:
504 repeatability and reference intervals. J Vet Cardiol 2015;17:83-96.
505 [32] Maurer MS, Bokhari S, Damy T, Dorbala S, Drachman BM, Fontana M, Grogan
506 M, Kristen AV, Lousada I, Nativi-Nicolau J, Quarta CC, Rapezzi C, Ruberg FL,

Witteless R, Merlini G. Expert consensus recommendations for the suspicion and diagnosis of transthyretin cardiac amyloidosis. Circ Heart Fail 2019;12:1-11.

[33] Kharin SN, Krandycheva VV, Strelkova MV, Tsvetkova AS, Shmakov DN. Doxorubicin-induced changes of ventricular repolarization heterogeneity: results of a chronic rat study. Cardiovasc Toxicol 2012;12:312–317.

[34] Chapel EH, Scansen BA, Schober KE, Bonagura JD. Echocardiographic Estimates of Right Ventricular Systolic Functionin Dogs with Myxomatous Mitral Valve Disease. J Vet Intern Med 2018;32:64–71.

[35] Vezzosi T, Domenech O, Costa G, Marchesotti F, Venco L, Zini E, Palacio MJF, Tognetti R. Echocardiographic evaluation of the right ventriculardimension and systolic function in dogs with pulmonary hypertension. J Vet Intern Med 2018;32:1541–48.

[36] Reinero C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, Williams K, Aboott J, Borgarelli M, Scansen BA. ACVIM consensus statement guidelines for the diagnosis,classification, treatment, and monitoring of pulmonary hypertension in dogs.J Vet Intern Med 2020;34:549–73.

[37] Chang WT, Shih JY, Feng YH, Chiang CY, Kuo YH, Chen WY, Wu HC, Cheng JT, Wang JJ, Chen ZC. The early predictive value of right ventricular strain in epirubicin -induced cardiotoxicity in patients with breast cancer. Acta Cardiol Sin 2016;32:550-559.

Table 1 – Descriptive data, cumulative dose of doxorubicin and the main echocardiographic variables compared over time in all animals of the study.

	Pre (Day 0)	7d	21d	60d	120d	180d	P
N	25	23	21	19	15	12	0.998
N (CHOP/DoxoCarbo)	10/10/5	10/10/3	10/9/2	9/9/1	7/7/1	5/6/1	0.998
CD of doxorubicin (mg/kg)	0	0.99 (0.88 – 1)	1.09 (0.88 – 2.17)	2.09 (1.74 – 4.3)	3.48 (2.6 – 4)	4.26 (3.45 – 5)	
Et	62.80 (42.50 - 119.0)	60.80 (50.50 - 82.70)	65.30 (45.0 - 85.90)	59.40 (34.0 - 99.40)	58.25 (37.60 - 106.0)	59.75 (42.50 - 76.70)	0.5061
At	67.30 (34.60 - 140.0)	55.90 (38.60 - 101.0)	66.30 (35.70 - 104.0)	70.20 (36.60 - 86.10)	57.65 (41.70 - 83.60)	56.05 (46.0 - 88.80)	0.2096
Et:At	1.14 (0.58 - 1.82)	1.11 (0.62 - 1.69)	1.03 (0.47 - 1.67)	0.80 (0.40 - 1.60)	1.15 (0.60 - 9.0)	1.05 (0.70 - 1.30)	0.7314
S´	17.10 (± 3.48)^{a}	15.0 (± 3.34)^{a,b}	15.10 (± 4.01)^{a,b}	15.42 (± 3.24)^{a,b}	12.85 (± 3.12)^{b}	13.08 (± 3.03)^{b}	**0.0036**
Et´	10.10 (6.27 - 16.50)	8.95 (4.77 - 17.70)	9.40 (6.17 - 14.70)	8.59 (6.74 - 13.40)	9.24 (4.69 - 14.90)	8.19 (4.67 - 13.90)	0.4374
At´	15.64 (± 3.07)	13.89 (± 3.67)	13.89 (± 3.58)	13.69 (± 2.29)	12.96 (± 2.81)	13.65 (± 2.69)	0.1336
Area$_d$ index	7.61 (4.69 - 40.17)	7.13 (4.60 - 11.79)	7.82 (4.27 - 11.02)	7.84 (5.17 - 11.17)	7.71 (5.33 - 13.13)	7.75 (4.62 - 13.02)	0.8017
Area$_s$ index	3.95 (1.56 - 28.15)	3.86 (2.12 - 7.15)	3.96 (1.86 - 6.46)	3.88 (2.58 - 6.89)	4.10 (3.03 - 8.20)	5.44 (2.65 - 8.28)	0.4306
FAC	52.05 (± 10.48)^{a}	46.70 (± 9.57)^{a,b}	45.89 (± 9.49)^{a,b}	46.38 (± 12.47)^{a,b}	42.76 (± 9.56)^{a,b}	37.07 (± 11.34)^{b}	**0.004**
TAPSEi	7.04 (4.83 - 9.82)^{a}	6.44 (4.95 - 8.86)^{a,b}	6.0 (4.44 - 7.60)^{a,b}	5.94 (4.39 - 7.76)^{b}	5.81 (3.77 - 6.46)^{b}	5.57 (4.51 - 6.93)^{b}	**<0.0001**
Basal diameter index	3.79 (1.31 - 15.20)	3.10 (1.57 - 5.83)	3.34 (1.99 - 4.73)	3.45 (1.63 - 5.01)	3.66 (1.71 - 4.92)	3.74 (1.94 - 6.46)	0.631
Medio diameter index	3.25 (1.35 - 11.28)	3.06 (1.58 - 5.87)	3.34 (1.52 - 4.45)	3.26 (1.48 - 5.32)	3.28 (1.67 - 4.94)	3.30 (1.75 - 7.32)	0.964
Length index	5.96 (2.83 - 26.31)	6.30 (2.85 - 11.21)	5.87 (3.19 - 8.15)	6.24 (2.86 - 8.44)	5.99 (2.72 - 10.21)	6.85 (3.04 - 8.95)	0.906
GLS	23.22 (± 5.18)^{a}	23.61 (± 4.44)^{a}	23.07 (± 3.86)^{a}	21.31 (± 5.42)^{a,b}	20.59 (± 4.89)^{a,b}	17.08 (± 3.82)^{b}	**0.002**
BIS	21.60 (± 4.27)^{a}	22.25 (± 4.96)^{a}	20.27 (± 4.59)^{a}	19.46 (± 5.83)^{a,b}	17.89 (± 4.18)^{a,b}	14.61 (± 4.54)^{b}	**0.0002**
MIS	24.73 (± 7.85)	25.24 (± 5.89)	25.0 (± 5.33)	22.40 (± 7.20)	22.56 (± 6.82)	19.58 (± 4.99)	0.1333
APS	24.88 (± 6.83)	24.48 (± 5.96)	24.56 (± 4.89)	22.88 (± 7.72)	22.39 (± 6.61)	18.25 (± 6.24)	0.063
TMADmm/m²	13.23 (4.02 - 39.25)	12.25 (4.77 - 28.39)	14.24 (4.65 - 21.59)	13.54 (5.97 - 25.11)	11.25 (5.28 - 27.45)	11.33 (4.95 - 22.49)	0.9476
TMAD%	17.79 (± 3.50)^{a}	17.55 (± 3.46)^{a}	17.62 (± 2.77)^{a}	17.80 (± 3.26)^{a}	16.55 (± 2.82)^{a,b}	14.07 (± 3.16)^{b}	**0.0198**

(N):number of animals in each group; Area$_d$: RV diastolic area; Area$_s$: RV systolic area; APS: middle segment of the free wall; BIS: basal segment of the free wall; Et: early diastolic tricuspid inflow velocity; At:: late diastolic tricuspid inflow velocity; CD: cumulative dose; Et´: ´peak velocity of early diastolic tricuspid annular motion as determined by pulsed wave Doppler; At´: peak velocity of diastolic tricuspid annular motion as determined by pulsed wave Doppler; FAC: fractional area change; GLS: global longitudinal strain; MIS: middle segment of the free wall; TAPSEi: tricuspid annular plane systolic excursion indexed to the body surface area; S´: peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler; TMAD: Tissue Motion Annular Displacement. Data with normal distribution were expressed by the mean and standard deviation and data with abnormal distribution were expressed by the median and interquartile range. Values with different superscripted letters indicate statistically significant differences between groups and equal letters represents equality between group

Table 2 – Descriptive data. cumulative dose of doxorubicin and the main echocardiographic variables compared over time from the paired analysis of the 12 animals that reached the end of the study.

	Pre (Day 0)	7d	21d	60d	120d	180d	P
N	12	12	12	12	12	12	0.998
N (CHOP/DoxoCarbo)	5/6	5/6	5/6	5/6	5/6	5/6	0.998
CD of doxorubicin (mg/kg)	0	0.98 (0.88 – 1)	1.06 (0.88 – 1)	2.12 (1.74 – 3)	3.52 (2.6 – 4)	4.26 (3.45 – 5)	
Et	63.30 (50.30 - 91.70)	60.20 (51.90 - 82.70)	65.05 (49.0 - 80.0)	60.85 (34.0 - 75.90)	58.25 (37.60 - 106.0)	59.75 (42.50 - 76.70)	0.4159
At	67.80 (34.60 - 97.50)	58.35 (46.0 - 100.0)	63.65 (35.70 - 84.70)	64.45 (36.60 - 86.10)	56.40 (41.70 - 79.60)	56.05 (46.0 - 88.80)	0.4831
Et:At	1.04 (0.59 - 1.82)	1.07 (0.62 - 1.35)	1.11 (0.69 - 1.67)	0.80 (0.40 - 1.60)	1.20 (0.70 - 9.0)	1.05 (0.70 - 1.30)	0.238
S´	16.34 (± 3.65)	15.94 (± 3.25)	14.37 (± 4.25)	14.78 (±3.18)	12.86 (± 3.28)	13.08 (± 3.03)	0.0704
Et´	10.35 (7.56 - 16.50)	9.62 (5.65 - 16.40)	8.03 (6.17 - 14.20)	8.71 (6.74 - 13.40)	8.99 (4.69 - 14.90)	8.19 (4.67 - 13.90)	0.310
At´	15.32 (± 3.48)	13.53 (± 3.32)	13.12 (± 3.73)	14.08 (± 2.14)	12.73 (± 2.89)	13.65 (± 2.69)	0.221
$Area_d$ index	7.82 (4.69 - 14.34)	7.52 (4.60 - 11.0)	7.24 (4.27 - 9.57)	7.94 (5.17 - 10.39)	7.72 (5.33 - 13.13)	7.75 (4.62 - 13.02)	0.098
$Area_s$ index	3.61 (1.95 - 6.31)	3.92 (2.12 - 5.25)	3.50 (1.86 - 5.20)	3.89 (3.09 - 6.16)	4.10 (2.58 - 8.20)	5.44 (2.65 - 8.28)	0.169
FAC	55.51 (± 8.72)^{a}	47.73 (± 7.93)^{a}	48.19 (± 8.88)^{a}	46.94 (± 12.58)^{a}	43.86 (± 11.46)^{a}	37.07 (± 11.34)^{b}	**0.0131**
TAPSEi	6.81 (4.83 - 9.82)^{a}	7.03 (5.20 - 8.08)^{a}	6.03 (4.44 - 7.60)^{a,b}	5.97 (4.58 - 7.76)^{a,b}	5.81 (3.77 - 7.44)^{a,b}	5.57 (4.51 - 6.93)^{b}	**0.0079**
Basal diameter index	4.0 (1.31 - 5.97)	3.27 (1.57 - 5.16)	3.39 (1.99 - 4.73)	3.73 (1.76 - 4.84)	3.77 (1.71 - 4.92)	3.74 (1.94 - 6.46)	0.283
Medio diameter index	3.33 (1.35 - 6.56)	3.08 (1.58 - 4.86)	3.32 (1.52 - 4.45)	3.58 (1.48 - 5.07)	3.05 (1.67 - 4.94)	3.30 (1.75 - 7.32)	0.6706
Length index	6.10 (2.83 - 11.40)	6.55 (2.85 - 11.21)	5.95 (3.19 - 8.15)	6.70 (2.86 - 8.44)	6.34 (2.72 - 8.90)	6.85 (3.04 - 8.95)	0.502
GLS	21.55 (± 4.08)^{a}	22.46 (± 5.19)^{a}	22.52 (± 3.97)^{a}	20.60 (± 5.17)^{a}	20.17 (± 4.80)^{a}	17.08 (± 3.82)^{b}	**0.023**
BIS	20.18 (± 2.56)^{a}	20.57 (± 4.67)^{a}	19.86 (± 4.93)^{a,b}	18.07 (± 5.98)^{a,b}	17.38 (± 4.22)^{a,b}	14.61 (± 4.54)^{b}	**0.029**
MIS	24.18 (± 9.40)	24.99 (± 7.14)	24.0 (± 4.99)	22.63 (± 8.75)	22.07 (± 6.74)	19.58 (± 4.99)	0.347
APS	23.13 (± 6.16)	22.77 (± 7.11)	24.06 (± 4.30)	21.64 (± 6.51)	21.46 (± 6.09)	18.25 (± 6.24)	0.136
TMADmm/m²	13.04 (4.02 - 28.88)	13.43 (4.77 - 28.39)	14.28 (4.65 - 21.59)	13.51 (5.97 - 18.49)	11.25 (5.28 - 27.45)	11.33 (4.95 - 22.49)	0.605
TMAD%	17.40 (± 3.41)	17.14 (± 4.03)	17.19 (± 3.14)	17.13 (± 1.92)	16.26 (± 2.97)	14.07 (± 3.16)	0.139

(N):number of animals in each group; $Area_d$: RV diastolic area; $Area_s$: RV systolic area; APS: middle segment of the free wall; BIS: basal segment of the free wall; Et: early diastolic tricuspid inflow velocity; At:: late diastolic tricuspid inflow velocity; CD: cumulative dose; Et´: ´peak velocity of early diastolic tricuspid annular motion as determined by pulsed wave Doppler; At´: peak velocity of diastolic tricuspid annular motion as determined by pulsed wave Doppler; FAC: fractional area change; GLS: global longitudinal strain; MIS: middle segment of the free wall; TAPSEi: tricuspid annular plane systolic excursion indexed to the body surface area; S´: peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler; TMAD: Tissue Motion Annular Displacement. Data with normal distribution were expressed by the mean and standard deviation and data with abnormal distribution were expressed by the median and interquartile range. Values with different superscripted letters indicate statistically significant differences between groups and equal letters represents equality between group.

Table 3 – Cut-off values from ROC curve analyzes with sensitivity, specificity and AUC to differentiate dogs that survived from those that died during the study.

	Cut-off	Sensitivity %	Specificity %	AUC	95% CI
Et:At	> 1.210	46.15	75	0.5128	0.2747 - 0.7510
S'	> 13.40	100	25	0.6090	0.3824 - 0.8356
Et'	< 9.405	53.85	75	0.5545	0.3184 - 0.7906
At'	> 17.35	46.15	83.33	0.5897	0.3571 - 0.8224
FAC	> 47.05	53.85	91.67	0.7147	0.5017 - 0.9278
TAPSEi	> 6.605	76.92	50	0.5833	0.3476 - 0.8191
GLS	> 23.35	53.85	75	0.6250	0.4006 - 0.8494
BIS	> 23.50	46.15	91.67	0.6186	0.3846 - 0.8526
MIS	> 19.50	84.62	41.67	0.5513	0.3148 - 0.7878
APS	> 34.50	23.08	100	0.5865	0.3582 - 0.8149
TMADmm/m2	> 17.60	92.31	25	0.5288	0.2960 - 0.7616
TMAD%	> 18.95	76.92	41.67	0.5032	0.2681 - 0.7383

APS: middle segment of the free wall; BIS: basal segment of the free wall; Et´: ´peak velocity of early diastolic tricuspid annular motion as determined by pulsed wave Doppler; At´: peak velocity of diastolic tricuspid annular motion as determined by pulsed wave Doppler; FAC: fractional area change; GLS: global longitudinal strain; MIS: middle segment of the free wall; TAPSEi: tricuspid annular plane systolic excursion indexed to the body surface area; S´: peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler; TMAD: Tissue Motion Annular Displacement.

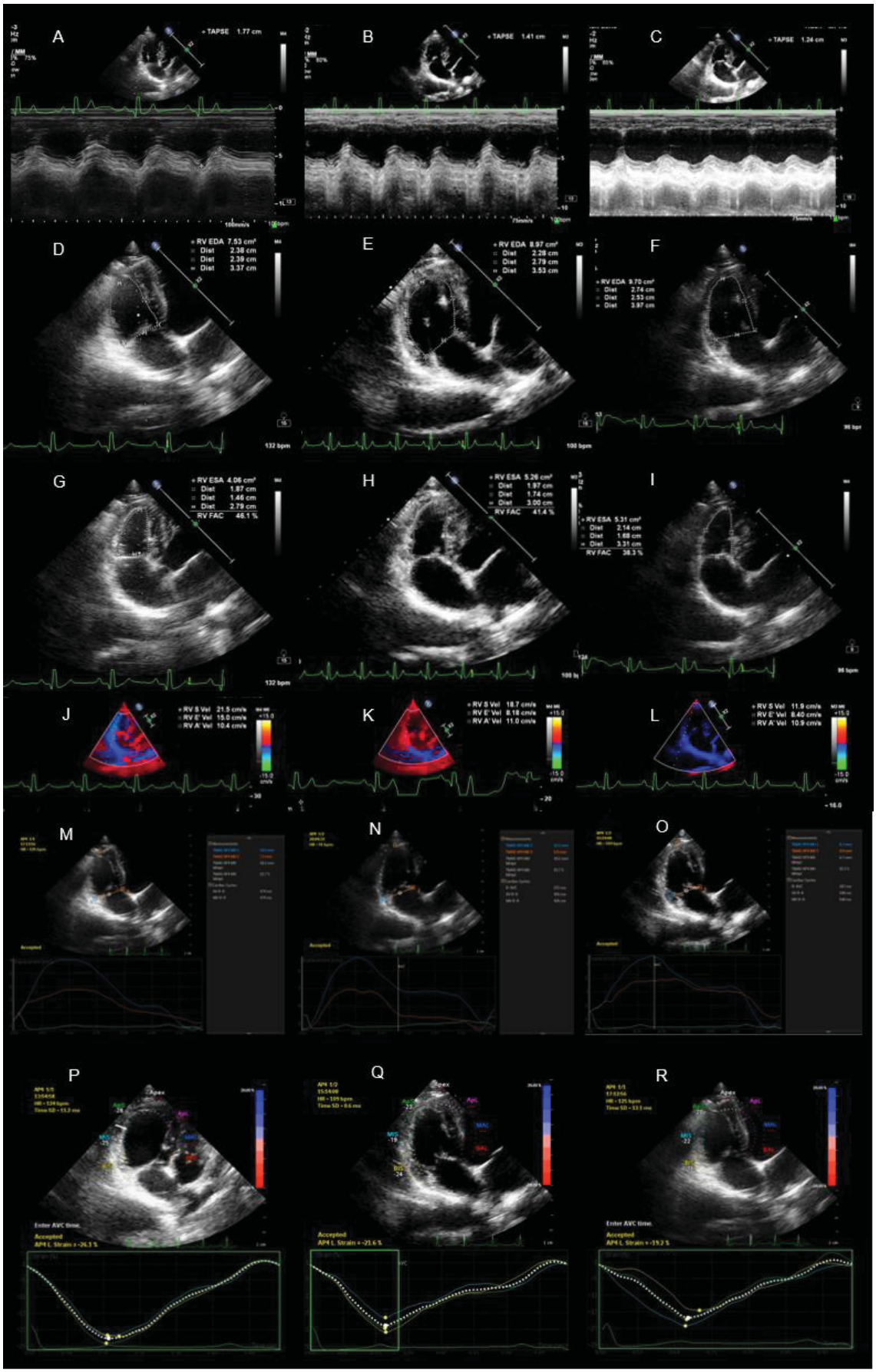

Figure 1 - Image showing echocardiographic evolution of a male golden retriever dog

with multicentric lymphoma undergoing chemotherapy with CHOP protocol. Images show the TAPSE (A,B,C), the morphometric indices and area in diastole (D,E,F) and in systole (G,H,I), FAC (G,H,I), S´ wave (J,K,L), TMAD (M,N,O) and GLS (P,Q,R) pre-chemotherapy (A,D,G,J,M,P), 120 days after starting doxorubicin (B,E,H,K,N,Q) and in the evaluation at 180 days (C,F,I,L,O,R). Note the progressive reduction in systolic function parameters.

FAC: fractional area change; GLS: global longitudinal strain; TAPSE: tricuspid annular plane systolic excursion; S´: peak velocity of systolic tricuspid annular motion as determined by pulsed wave Doppler; TMAD: Tissue Motion Annular Displacement.

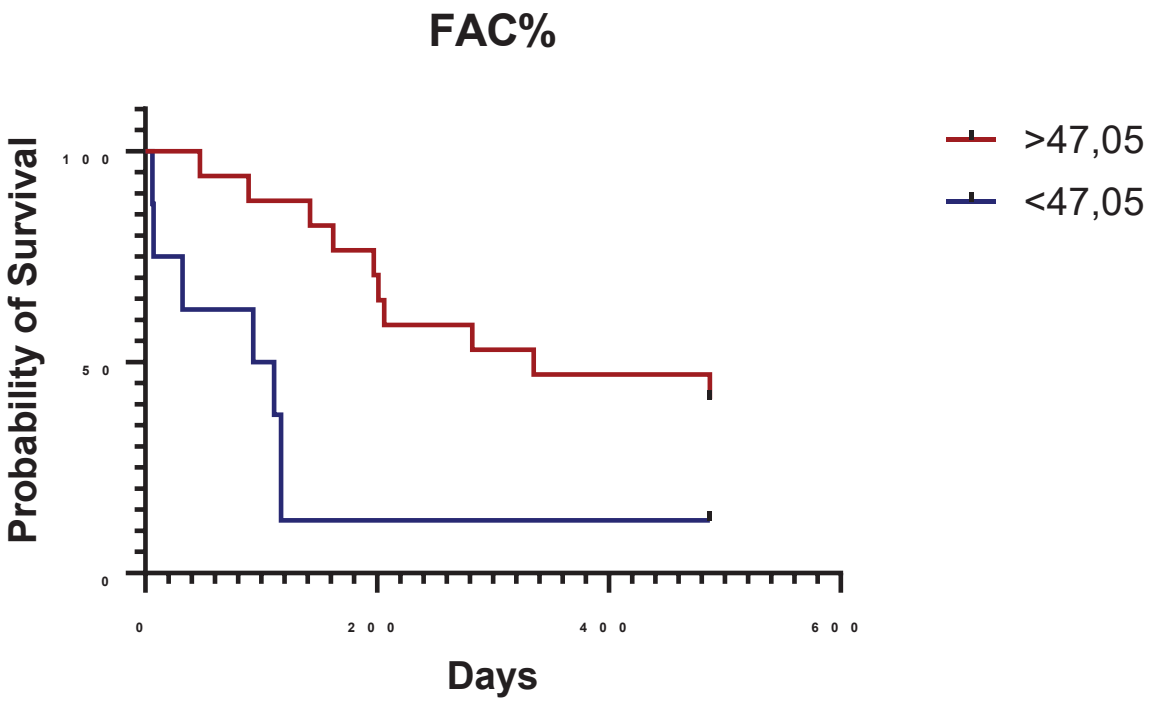

Figure 2 - Kaplan Meyer curve showing that dogs that had an FAC > 47.05% lived longer (median 335 days) than patients who had a lower FAC value (median 102 days) (P=0.0045) in the initial assessment (pre).

FAC: fractional area change

5 VITA

Médica veterinária graduada pela Pontifícia Universidade Católica de Minas Gerais (PUC MG), Campus Poços de Caldas no ano de 2013.

Concluiu o curso de Aperfeiçoamento em Cardiologia Veterinária pela Unesp, Campus Jaboticabal em 2014.

Realizou o programa de Aprimoramento Profissional em Serviço na área de Clínica Médica de Pequenos Animais na Faculdade de Jaguaruna no ano de 2015.

Concluiu o curso de Pós-graduação *lato sensu* – especialização – em Cardiologia Veterinária, pela Universidade Cruzeiro do Sul (ANCLIVEPA SP) em 2017.

Concluiu como bolsista integral o curso de Pós-graduação *lato sensu* em Clínica Médica e Cirúrgica de Pequenos Animais pelo Centro Universitário Cesmac (EQUALIS) no ano de 2020.

Obteve o título de Mestre pelo Programa de Pós-graduação em Ciências Veterinárias da Universidade Federal do Paraná (UFPR), com a linha de pesquisa Medicina Experimental e Comparada no ano de 2018.

Cursou o Doutorado no Programa de Pós-graduação em Ciências Veterinárias da Universidade Federal do Paraná (UFPR), com a linha de pesquisa Medicina Experimental e Comparada no ano de 2018 a 2022.

6 REFERÊNCIAS

[1] Jacobs GJ. Secondary canine cardiomyopathies: their causes and characteristics. Vet Med Sci 1996;91:534-64.

[2] Hajjar LA, Costa IBSS, Lopes MACQ, Hoff PMG, Diz MPE, Fonseca SMR, Bittar CS, Rehder MHHS, Rizk SI, Almeida DR, Fernandes GS, Silva LB, Campos CAHM, Montera MW, Alves SMM, Fukushima JT, Santos MVC, Negrão CE, Silva TLF, Ferreira SMA, Malachias MVB, Moreira MCV, Neto MMRV, Fonseca VCQ, Soeiro MCF, Alves JBS, Silva CMPD, Sbano J, Pavanello R, Pinto IMF, Simão AF, Dracoulakis MDA, Hoff AO, Assunção BMBL, Novis Y, Testa L, Filho ACA, Cruz CBBV, Pereira J, Garcia DR, Nomura CH, Rochitte CE, Macedo AVS, Marcatti PTF, Junior WM, Wiermann EG, Freitas RVH, Coutinho A, Mathias CMC, Vieira FMAC, Sasse AD, Rocha V, Ramires JAF, Filho RK. Diretriz Brasileira de Cardio-oncologia. Arq Bras Cardiol 2020;115:1006-43.

[3] Souza RCA, Camacho AA. Neurohormonal, hemodynamic, and electrocardiographic evaluations of healthy dogs receiving long-term administration of doxorubicin. Am J Vet Res 2006;67:1319-25.

[4] Chung WB, Youn HJ. Pathophysiology and preventive strategies of anthracycline-induced cardiotoxicity. Korean J Intern Med 2016;31:625-633.

[5] Gallay-Lepoutre J, Bélanger MC, Nadeau ME. Prospective evaluation of Doppler echocardiography, tissue Doppler imaging and biomarkers measurement for the detection of doxorubicin-induced cardiotoxicity in dogs: A pilot study. Res Vet Sci 2016;105:153-9.

[6] Narezkina A, Nasim K. Anthracycline cardiotoxicity. Circ Heart Fail 2019; 12:e:005910.

[7] Hallman BE, Hauck ML, Williams LE, Hess PR, Suter SE. Incidence and risck factors associated with development of clinical cardiotoxicity in dogs receiving doxorubicin. J Vet Intern Med 2019;33:783–91.

[8] Zamorano JL, Lancellotti P, Muñoz DR, Abovans V, Asteggiano R, Galderisi M, Habib G, Lenihan DJ, Lip GYH, Lyon AR, Fernandez TL, Mohty D, Piepoli MF, Tamargo J, Torbicki A, Suter TM. Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur Heart J 2016;37p.2768–2801.

[9] Stefani L, Pedrizzetti G, Galanti G. Clinical application of 2D Speckle tracking strain for assessing cardio-toxicity in oncology. J Funct Morphol Kinesiol 2016;1:343-354.

[10] Bloom MW, Hamo CE, Cardinale D, Ky B, Nohrja A, Baer L, Skopicki H, Lenihan DJ, Gheorghiade M, Lyon AR, Butler J. Cancer therapy-related cardiac dysfunction and heart failure: part 1: definitions, pathophysiology, risk factors, and imaging. Circ Heart Fail 2016;9:e002661.

[11] Zhao L, Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. Nature 2017;7:44735.

[12] Ditchey RV, LeWinter MM, Higgins CB. Acute effects of doxorrubicin (adriaycin) on left ventricular function in dogs. Int J Cardiol 1984;6:341-350.

[13] Mauldin GE, Fox PR, Patnaik AK, Bond BR, Mooney SC, Matus RE. Doxorubicin-induced cardiotoxicosis. Clinical features in 32 dogs. J Vet Intern Med 1992;6:82-88.

[14] Loar AS, Susaneck SJ. Doxorubicin-induced cardiotoxicity in five dogs. Semin

Vet Med Surg 1986;1:68-71.

[15] Surachetpong SD, Teewasutrakul P, Rungsipipat A. Serial measurements of cardiac troponin I (cTnI) in dogs treated with doxorubicin. Jpn J Vet Res 2016;64:221-233.

[16] Beumier A, Robinson SR, Robinson N, Lopez KE, Meola DM, Barber LG, Bulmer BJ, Calvalido J, Rush JE, Yeri A, Das S, Yang VK. Extracellular vesicular microRNAs as potential biomarker for early detection of doxorubicin-induced cardiotoxicity. J Vet Intern Med 2020;34:1260-71.

[17] LLesuy SF, Milei J, Flecha BSG, Boveris A. Myocardial damage induced by doxorubicins: hydroperoxide-initiated chemiluminescence and morphology. Free Radic Biol Med 1990;8:259-264.

[18] Plana JC, Galderisi M, Barac A, Ewer MS, Hy B, Scherrer-Crosbie M, Ganame J, Sebag IA, Agler DA, Badano LP, Banchs J, Cardinale D, Carver J, Cerqueira M, DeCara JM, Edvarsen T, Flamm SD, Force T, Griffin BP, Jerusalem G, Liu JE, Magalhães A, Marwick T, Sanchez LY, Sicari R, Villaraga HR, Lancelotti P. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J 2014; 15:1063-93.

[19] Oliveira MS, Melo MB, Carvalho JL, Melo IM, Lavor MSL, Gomes DA, Goes AM, Melo MM. Doxorubicin cardiotoxicity and cardiac function improvement after stem cell therapy diagnosed by strain echocardiography. J Cancer Sci Ther 2013;5:52–57.

[20] Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of anthracyclines. Front Cardiovasc Med 2020;18:7:26.

[21] Rhea IB, Uppuluri S, Sawada S, Schneider BP, Feigenbaum H. Incremental Prognostic Value of Echocardiographic Strain and Its Association With Mortality in

Cancer Patients. J Am Soc Echocardiogr 2015;28:667–73.

[22] 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;175:1379-86.

[23] Planek MIC, Manshad A, Hein K, Hemu M, Ballout F, Varandani R, Venugopal P, Okwuosa T. Prediction of doxorubicin cardiotoxicity by early detection of subclinical right ventricular dysfunction. Cardiooncology 2020;6:1-8.

7 ANEXOS

ANEXO 1 – CERTIFICADO DA COMISSÃO DE ÉTICA NO USO E EXPERIMENTAÇÃO DE ANIMAIS

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

CERTIFICADO

Certificamos que o protocolo número 099/2018, referente ao projeto **"Avaliação da função miocárdica biventricular por ecocardiografia convencional e *speckle tracking* em cães submetidos à quimioterapia com doxorrubicina",** sob a responsabilidade **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 2 de invasividade, em reunião de 05/12/2018.

Vigência do projeto	Fevereiro/2019 até Fevereiro/2022
Espécie/Linhagem	*Canis familiaris* (cão)
Número de animais	150
Peso/Idade	Variável/Variável
Sexo	Macho e fêmea
Origem	Hospital Veterinário da Universidade Federal do Paraná, Curitiba, Paraná, Brasil

CERTIFICATE

We certify that the protocol number 099/2018, regarding the project **"Assessment of the biventricular myocardial function by conventional and speckle tracking ecocardiography in dogs submitted to doxorrubicine chemotherapy"** 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 2 of invasiveness, in session of 05/12/2018.

Duration of the project	February/2019 until February/2022
Specie/Line	*Canis familiaris* (canine)
Number of animals	150
Wheight/Age	Variable /Variable
Sex	Male and Female
Origin	Veterinary Hospital of Federal University of Paraná, Curitiba, Brazil

Curitiba, 05 de dezembro de 2018

Chayane da Rocha

Coordenadora CEUA-SCA

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