

Conventional vs. Advanced Echocardiographic Parameters for Predicting Fluid Responsiveness in Healthy Dogs

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ABSTRACT

Background: Volume replacement is considered an essential component in the treatment of critically ill patients, but excess fluid administration can be as harmful as the lack of fluid therapy. Therefore, the volume replacement is only beneficial in patients deemed to be fluid responsive, that is, when the individual has an increase in their stroke volume after administration of a volume challenge. This study aim to assess whether conventional and advanced echocardiographic parameters for assessing left ventricular systolic function are able to predict fluid responsiveness in healthy spontaneously breathing dogs. The hypothesis was that some of these parameters would differ between responsive and non-responsive animals and could be used as a complementary measure for assessment of fluid responsiveness.

Materials, Methods & Results: A prospective study was carried out in which 22 client-owned healthy dogs over 1 year of age that presented to the Veterinary Medical Teaching Hospital for elective neutering procedure were included. All dogs underwent conventional and advanced echocardiographic examination to obtain the left ventricular internal diameter in diastole normalized (LVIDDn), left ventricular internal diameter in systole normalized (LVIDSn), ejection fraction (EF) and shortening fraction (SF) according to the Teichholz methodology, global longitudinal strain (GLS) and tissue motion annular displacement (TMAD). These parameters were evaluated before and after administration of a volume challenge with 10 mL/kg lactate ringer intravenously for 20 min. Based on the variation in aortic velocity integral time, 31.82% of dogs were considered responsive and 68.18% were non-responsive to the volume challenge. For advanced echocardiography, TMAD indexed by body weight (mm/kg) > 0.89 ($P = 0.004$) and body surface area (mm/m²) > 18.9 ($P = 0.004$) after volume challenge had the best area under the curve values (both 0.895) and smaller gray zone intervals (0.52 - 0.81 and 14.89 - 17.88) for the identification of responsive dogs. Although, TMAD (mm/kg and mm/m²) before volume challenge was also higher in the responsive dogs ($P = 0.041$ and $P = 0.029$). As for conventional echocardiography, the LVIDDn < 1.39 ($P = 0.003$) before volume challenge had the best area under the curve value (0.866) and the smallest gray zone interval (1.4 - 1.57) for the identification of responsive dogs. The GLS and the TMAD in millimeters (mm), percentage (%) and indexed by cubic root of weight (mm/³/kg) did not differ between the responsive and non-responsive dogs before and after volume challenge.

Discussion: TMAD indexed by body weight (mm/kg) and body surface area (mm/m²) before and after volume challenge and the LVIDDn before volume challenge may be useful measures to complement the assessment of fluid responsiveness in spontaneously breathing dogs. This is the first study to use TMAD as a predictive parameter for fluid responsiveness in healthy, spontaneously breathing, dogs. Even it is an advanced echocardiographic technique, TMAD has advantages in terms of execution time and the fact that it is less dependence on image quality or operator experience. It is possible that in the future such variables may form a useful part of the evaluation of fluid responsiveness in dogs. However, more studies are needed to better understand the applicability of these techniques, especially in dogs with hemodynamic changes.

Keywords: canine, global longitudinal strain, stroke volume, tissue motion annular displacement, TMAD, VTI.

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INTRODUCTION

Volume replacement is considered an essential component in the treatment of critically ill patients, but excess fluid administration can be harmful [24]. In spontaneously breathing patients it is particularly difficult to assess fluid responsiveness [12,18], since the heart-lung interaction causes a cyclic variation in stroke volume [15]. However, this group represents the vast majority of critically ill individuals who require intravenous fluid [18].

The concept of fluid responsiveness can be explained by the Frank-Starling curve [23]. When individuals are in the ascending portion of the curve, they are deemed “preload dependent” or “responsive”, and the individuals on the plateau of the curve are called “non-preload dependent” or “non-responsive” [9,22]. Volume replacement is only beneficial in patients deemed to be fluid responsive as defined by an $\geq 15\%$ increase in stroke volume [4,8,25]. Stroke volume is dependent on systolic function which is influenced by factors such as preload and afterload [3,7]. Therefore, fluid responsiveness is not a binary or static condition, but varies according to dynamic interactions between intravascular volume, vascular tone and systolic function [7].

Left ventricular systolic function can be assessed by measurement of several parameters in conventional and advanced echocardiography. Within this context, the aim of this study was to assess whether conventional and advanced echocardiographic assessment of left ventricular systolic function could predict fluid responsiveness in healthy spontaneously breathing dogs.

MATERIALS AND METHODS

Study design and animals

A prospective study that included healthy dogs from 1 year of age presented to a Veterinary Medical Teaching Hospital for elective neutering procedure. Owners gave informed consent for inclusion of their pets in the study. The animals were considered healthy if there were no history of disease, no alteration on the physical examination, complete blood count and echocardiographic and electrocardiographic examinations.

Interventions

Standardized Doppler technique was used for indirect measurement of SBP. The mean of 5 stable values of SBP (with a variation $< 10\%$) was recorded

and animals were considered normotensive when the SBP was between 80 and 160 mmHg [1]. Electrocardiographic evaluation was performed simultaneously with the echocardiographic examination, to identify heart rate and rhythm. Hypotensive or hypertensive dogs and those with cardiac rhythms of non-sinus origin, bradycardia or tachycardia were excluded from the study.

The first echocardiographic evaluation (T0) was performed prior to the volemic challenge, which was performed using an infusion pump administering lactated ringer's at a dose of 10 mL/kg intravenously for 20 min. The second echocardiographic evaluation was performed (T1) within 5 min of completion of fluid administration. This entire procedure was performed before neutering.

The echocardiographic examination was performed in all dogs without sedation, with dogs breathing spontaneously and positioned in left and right lateral recumbency according to pre-established recommendations [27]. All examinations were performed by the same operator (SL) using standard equipment Philips Affiniti 50 equipped with 2-4, 3-8 MHz and 4-12 phased-array transducers¹.

Fluid responsiveness classification

Apical 5 chamber images obtained through the left parasternal window were used to measure the aortic velocity time integral (VTI Ao). Three measurements of the VTI Ao over a respiratory cycle were performed and the mean of these was used for the calculation of the variation of the VTI Ao (Δ VTI Ao), according to the formula: Δ VTI Ao = (VTI Ao T1 – VTI Ao T0) / VTI Ao T0 x 100

The classification of patients as responsive and non-responsive to the fluid challenge was determined by the value of Δ VTI Ao, in which responsiveness were considered those with Δ VTI Ao $\geq 15\%$ and non-responders with Δ VTI Ao $< 15\%$ [4,25]. For statistical analysis, dogs were subdivided into 2 groups, responsive and non-responsive to the volemic challenge, and evaluations were made at T0 and T1.

Conventional echocardiographic evaluation

Transverse images of the heart in the chordal plane were obtained for M-mode measurements of the left ventricle. The left ventricular internal diameter in diastole (LVIDD), at the beginning of the QRS complex, and the left ventricular internal diameter in systole (LVIDS), after the T wave on the surface electrocardiogram, were evaluated. From these data, ejection fraction (EF) and shortening fraction (SF) were

calculated according to the Teischholz methodology. In addition, LVIDD and LVIDS were normalized by body weight (LVIDDn and LVIDSn) [11].

Advanced echocardiographic evaluation

Videos of apical 4-chamber (A4C) and apical 2-chamber (A2C) images were obtained over at least 5 cardiac cycles for offline measurement of global longitudinal strain (GLS) and tissue motion annular displacement (TMAD). The echocardiographic equipment software automatically detected the left ventricular myocardium for assessment of longitudinal strain. Manual adjustments were performed when necessary to ensure accurate identification of the limits of the myocardium. This parameter was defined as the percentage of myocardial deformation of the left ventricle in the

longitudinal plane during a cardiac cycle. From these analyzes, the GLS in percentage (%) was calculated, using the mean longitudinal strain value obtained in the A4C and A2C images (Figure 1).

To obtain the TMAD, three regions of interest were manually established: the first, at the insertion of the anterior mitral leaflet on the A4C image and at the anterior portion of the mitral annulus on the A2C image; the second, at the insertion of the posterior mitral leaflet on the A4C image and at the inferior portion of the mitral annulus on the A2C image; and the third, at the epicardial region of the apex of the left ventricle on the A4C and A2C images. After selecting the regions of interest, myocardial screening was performed automatically by the equipment's software (Figure 2).

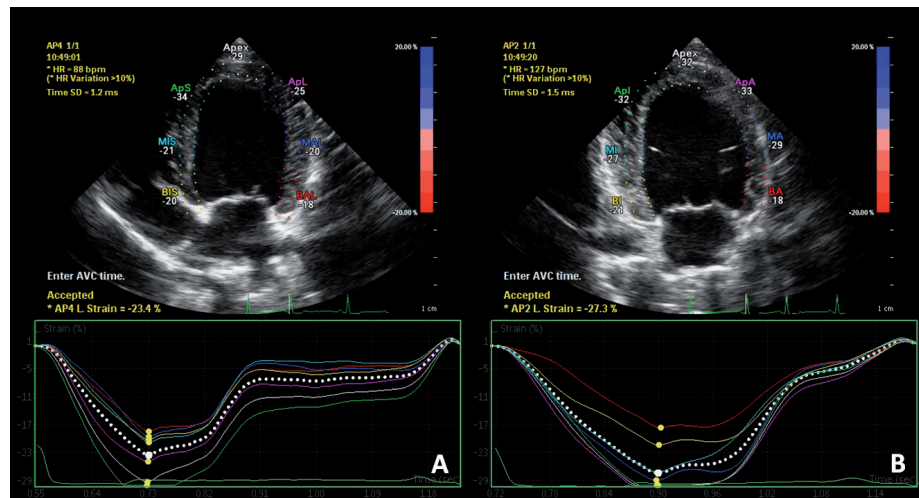


Figure 1. Advanced echocardiography showing the apical 4-chamber (A) and apical 2-chamber (B) images used to obtain the global longitudinal strain (GLS) in percentage (%) of a healthy dog non-responsive to the fluid challenge.

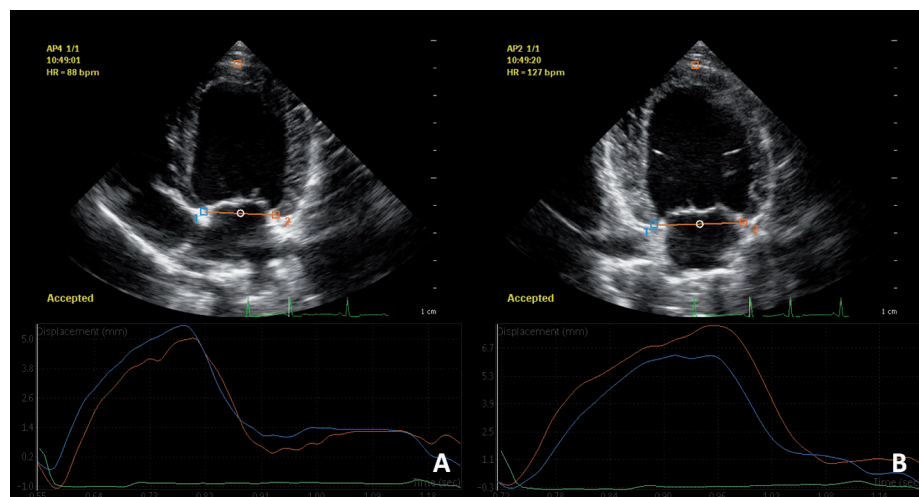


Figure 2. Advanced echocardiography showing the apical 4-chamber (A) and apical 2-chamber (B) images used to obtain the global tissue motion annular displacement (TMAD), in millimeters (mm) and percentage (%), of a healthy dog non-responsive to the fluid challenge.

The TMAD obtained from the A4C and A2C images was calculated from the apex-base displacement of the virtual midpoint between the 2 regions of interest of the mitral valve annulus in relation to the total length of the left ventricle. To obtain the global TMAD, the mean values, in millimeters (mm) and percentage (%), of the A4C and A2C images were calculated. In addition, global TMAD (mm) was indexed by body weight (mm/kg), by the cubic root of body weight (mm/ $\sqrt[3]{\text{kg}}$) and by body surface area (mm/m²) according to the formula:

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

$$K = \text{constant (10.1 for dogs)}$$

Statistical analysis

The descriptive statistical analysis was performed with the estimate of the frequencies (%) of the qualitative variables, calculation of the mean and standard deviation or median and range of the quantitative variables depending on the distribution of the samples. For the distribution analysis, the Shapiro-Wilk test was used.

Comparisons of means were performed between the group responsive in T0 *versus* T1, non-responsive in T0 *versus* T1, responsive versus non-responsive in T0 and responsive versus non-responsive in T1. The paired *t* test or Student *t* test was used for data with normal distribution (parametric) and Wilcoxon or Mann-Whitney test for data with non-normal distribution (non-parametric).

Positive (PPV) and negative (NPV) predictive values were calculated, ROC curves were plotted to obtain the area under the curve (AUC) and 95% confidence interval (CI), the Youden index was calculated to identify the cut-off value with the best combination of sensitivity and specificity and the gray zone of the echocardiographic parameters that differed between the responsive and non-responsive dogs at T0 or T1 was identified. The gray zone was calculated with the aim of identifying the inconclusive zone of the studied cutoff values, and this zone was defined by cutoff values with 90% sensitivity and specificity (10% diagnostic tolerance).

In addition, dogs were subdivided into 2 groups according to heart rhythm, sinus rhythm when the heart rate varied by less than 10%, or sinus arrhythmia when the heart rate varied by more than 10%. Dogs were compared according to heart rhythm to verify the influence of rhythm on the echocardiographic variables

studied. Finally, Pearson correlation (parametric data) or Spearman correlation (non-parametric data) were calculated to verify the influence of age, body weight, average heart rate and SBP in all the studied echocardiographic parameters. All analysis were performed using the same software² and the level of statistical significance adopted was $P < 0.05$.

RESULTS

A total of 22 dogs were included in the study, 54.5% (12) were females and 45.5% (10) males. The age of the animals ranged between 1 and 9 years (median: 2 years). The dogs were of different sizes with a mean and standard deviation of body weight of 12.8 (± 6.4) kg. The most commonly represented breeds were: mixed breed dogs (13), followed by Lhasa Apso (5), Australian Cattle (2), Shih-tzu (1) and Yorkshire Terrier (1).

SBP was measured in 77.3% (17) of the dogs, with a mean of 113 (± 15.7) mmHg. The most prevalent heart rhythm was sinus arrhythmia (SA), observed in 72.7% (32) of the evaluations, followed by sinus rhythm (SR), observed in 27.3% (12) of the evaluations. At T1, animals with SA showed significantly higher values of LVIDSn and LVIDDn compared to animals with SR [$P = 0.021$ and $P = 0.036$]. Heart rhythm did not affect any of the advanced echocardiographic parameters evaluated.

Of the 22 dogs selected, 31.8% (7) were considered responsive and 68.2% (15) non-responsive to the volume challenge based on the value of $\Delta\text{VTI}_{\text{Ao}}$ (Figure 3). The mean and standard deviation or median and range of all echocardiographic parameters evaluated are shown in Table 1.

EF and SF increased in responsive dogs at T1 [$P = 0.019$ and $P = 0.017$]. LVIDSn was higher in non-responsive animals at T1 [$P = 0.018$], while LVIDDn was lower in responsive individuals at T0 [$P = 0.003$] (Figure 4). The GLS and the TMAD (mm, % and mm/ $\sqrt[3]{\text{kg}}$) did not differ between the responsive and non-responsive dogs at T0 and T1. However, TMAD (mm/kg and mm/m²) was higher in the responsive dogs at T0 [$P = 0.041$ and $P = 0.029$] and T1 [$P = 0.004$ and $P = 0.004$] (Figure 5).

The conventional echocardiographic parameters that differed between responsive and non-responsive dogs were LVIDSn at T1 and LVIDDn at T0. For the advanced echocardiographic parameters

only TMAD (mm/kg and mm/m²) at T0 and T1 varied between groups. The best cut-off values of the cited parameters, with their respective PPV, NPV, sensitivity, specificity, AUC, 95% CI and gray zone interval are described in Table 2. The ROC curves are presented in Figure 6 and the sensitivity and specificity graphs with the demarcation of the gray zone are shown in Figure 7.

There were no correlations between EF and SF with heart rate and SBP. However, these pa-

rameters correlated with body weight and age. The LVIDSn correlated with all variables studied with the exception of PAS, while the LVIDDn correlated with body weight and heart rate. No correlations were observed between advanced echocardiographic parameters with age, heart rate and SBP. However, TMAD (mm, mm/kg and mm/m²) showed a correlation with body weight. The results of the correlation analysis are described in Table 3.

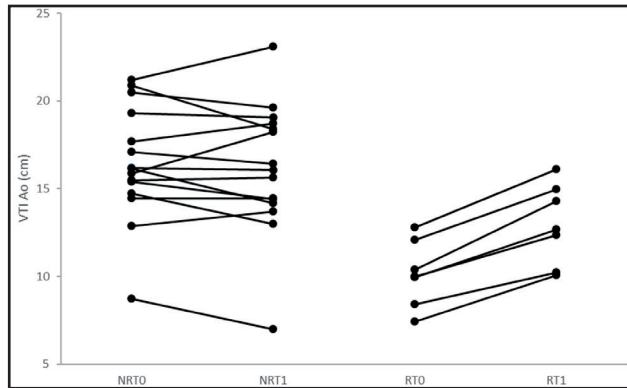


Figure 3. Point-graphs showing the values of the aortic velocity time integral (VTI Ao) before (T0) and after (T1) the administration of the fluid challenge of dogs classified as responsive (n = 7) and non-responsive (n = 15). Ao: aortic; NRT0: non-responsive time 0; NRT1: non-responsive time 1; RT0: responsive time 0; RT1: responsive time 1.

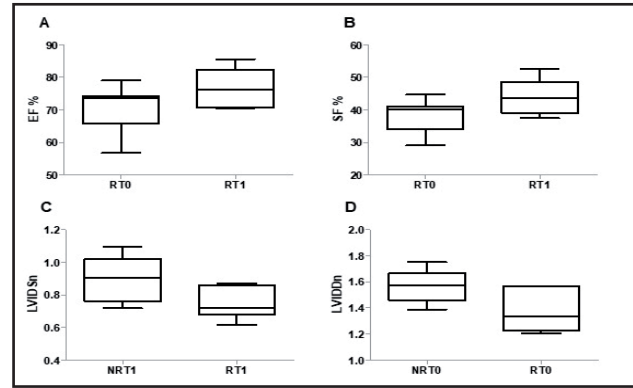


Figure 4. Box-plot graphs showing the minimum, maximum, mean and interquartile ranges of conventional echocardiographic parameters that differed between responsive (n = 7) and non-responsive (n = 15) dogs. EF: ejection fraction. LVIDDn: left ventricle internal diameter in diastole normalized. LVIDSn: left ventricle internal diameter in systole normalized. NRT0: non-responsive time 0; NRT1: non-responsive time 1; RT0: responsive time 0; RT1: responsive time 1; SF: shortening fraction.

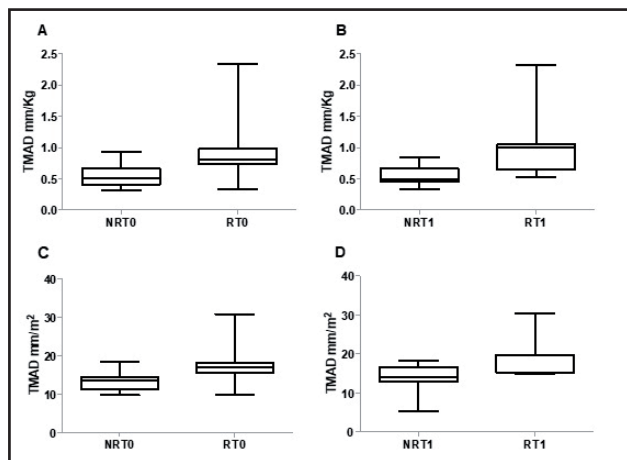


Figure 5. Box-plot graphs showing the minimum, maximum, mean and interquartile ranges of advanced echocardiographic parameters that differed between responsive (n = 7) and non-responsive (n = 15) dogs. NRT0: non-responsive time 0; NRT1: non-responsive time 1; RT0: responsive time 0; RT1: responsive time 1; TMAD: tissue motion annular displacement.

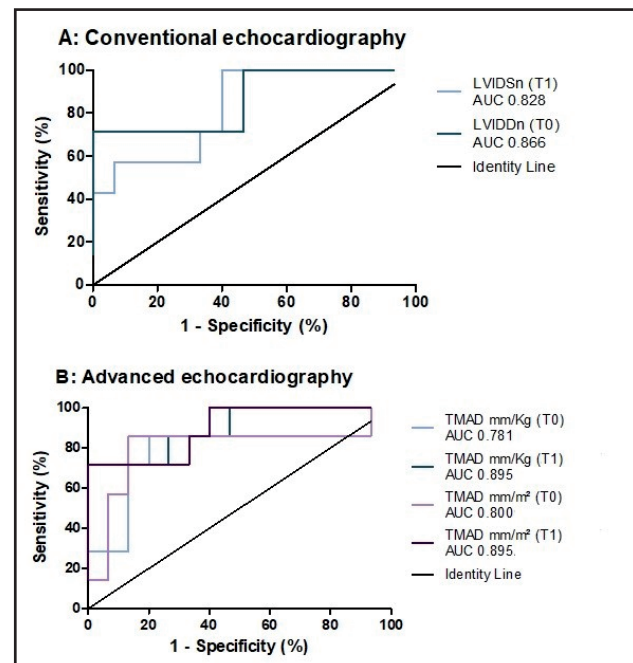


Figure 6. ROC curves and AUC values of conventional (A) and advanced (B) echocardiographic parameters that differed between responsive (N = 7) and non-responsive (N = 15) dogs. AUC: area under the curve; LVIDDn: left ventricle internal diameter in diastole normalized; LVIDSn: left ventricle internal diameter in systole normalized; TMAD: tissue motion annular displacement; T0: time 0; T1: time 1.

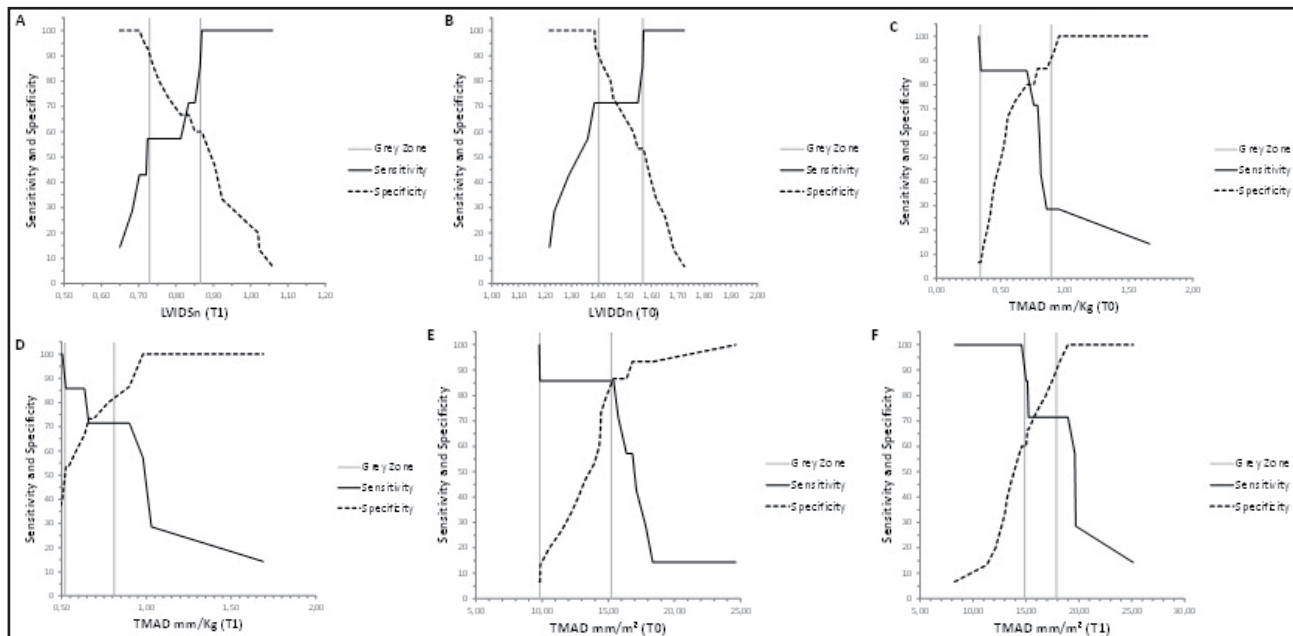


Figure 7. Sensitivity and specificity graphs with the gray zone interval demarcation of conventional (A and B) and advanced (C, D, E and F) echocardiographic parameters that differed between the responsive (n = 7) and non-responsive (n = 15) dogs. LVIDSn: left ventricle internal diameter in systole normalized. LVIDDn: left ventricle internal diameter in diastole normalized; TMAD: tissue motion annular displacement; T0: time 0; T1: time 1.

Table 1. Mean and standard deviation or median and range of all echocardiographic parameters assessed for dogs classified as responsive (n = 7) and non-responsive (n = 15) dogs.

Conventional echocardiographic parameters				
	Non-responsive		Responsive	
	T0	T1	T0	T1
EF (%)	70.86 (± 8.29)	70.11 (± 8.07)	70.91 (± 7.36)	77.04 (± 5.89)
SF (%)	39.68 (± 6.58)	38.98 (± 6.36)	38.57 (± 5.25)	44.04 (± 5.59)
LVIDSn	0.89 (± 0.11)	0.89 (± 0.12)	0.80 (± 0.12)	0.75 (± 0.10)
LVIDDn	1.56 (± 0.11)	1.53 (± 0.11)	1.36 (± 0.15)	1.41 (± 0.21)
Advanced echocardiographic parameters				
	Non-responsive		Responsive	
	T0	T1	T0	T1
GLS (%)	24.34 (± 3.33)	24.78 (± 2.90)	25.43 (± 4.26)	26.35 (± 2.25)
TMAD (mm)	7.24 (± 1.65)	7.94 (± 2.64)	5.99 (± 1.21)	6.94 (± 1.83)
TMAD (%)	13.54 (± 2.02)	14.70 (3.15-18.65)	15.31 (± 1.57)	16.35 (14.20-19.60)
TMAD (mm/kg)	0.51 (0.32-0.93)	0.49 (0.33-0.84)	0.81 (0.34-2.35)	1 (0.52-2.33)
TMAD (mm/m²)	13.64 (9.76-18.43)	114 (5.34-18.33)	16.87 (9.82-30.84)	19.64 (14-30.51)
TMAD (mm/√kg)	2.99 (± 0.47)	3.27 (0.78-4.50)	3.11 (± 0.45)	3.52 (3.21-3.87)

EF: ejection fraction; GLS: global longitudinal strain; LVIDDn: left ventricle internal diameter in diastole normalized; LVIDSn: left ventricle internal diameter in systole normalized; SF: shortening fraction; TMAD: tissue motion annular displacement; T0: time 0; T1: time 1. 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.

Table 2. Cut-off values for identification of the responsive patient with their respective positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity, area under the curve (AUC), 95% confidence interval (CI) and gray zone interval of echocardiographic parameters that differed between responsive (n = 7) and non-responsive (n = 15) dogs.

Conventional echocardiographic parameters	Cutoff Value	PPV	NPV	Sensitivity %	Specificity %	AUC (95% CI)	Gray Zone
LVIDSn T1	< 0.87	0.85	0.60	100	60	0.828 (0.648-1.008)	0.73-0.87
LVIDDn T0	< 1.39	0.71	0.93	71.43	100	0.866 (0.689-1.044)	1.40-1.57
Advanced echocardiographic parameters	Cutoff Value	PPV	NPV	Sensitivity %	Specificity %	AUC (95% CI)	Gray Zone
TMAD (mm/kg) T0	> 0.7	0.85	0.80	85.71	80	0.781 (0.533-1.028)	0.34-0.90
TMAD (mm/kg) T1	> 0.89	0.71	1.0	71.43	100	0.895 (0.746-1.044)	0.52-0.81
TMAD (mm/m ²) T0	> 15.4	0.85	0.86	85.71	86.67	0.800 (0.553-1.047)	9.82-15.22
TMAD (mm/m ²) T1	> 18.9	0.71	1.0	71.43	100	0.895 (0.748-1.042)	14.89-17.88

AUC: area under the curve; CI: confidence interval; LVIDDn: left ventricle internal diameter in diastole normalized; LVIDSn: left ventricle internal diameter in systole normalized; NPV: negative predictive value; PPV: positive predictive value; TMAD: tissue motion annular displacement.

Table 3. Correlation coefficient R and the *P*-value of all the echocardiographic parameters evaluated in relation to age, body weight, heart rate and SBP of the dogs (n = 22) selected for the study.

Conventional echocardiographic parameters	Age (months)		Weight (kg)		HR (bpm)		SAP (mmHg)	
	R	P	R	P	R	P	R	P
EF (%) T0	0.076	0.749	-0.468	0.028	0.259	0.243	0.076	0.772
EF (%) T1	0.563	0.010	-0.511	0.015	0.349	0.111	0.001	0.998
SF (%) T0	0.050	0.833	-0.368	0.092	0.242	0.277	0.107	0.683
SF (%) T1	0.552	0.012	-0.451	0.035	0.298	0.178	0.255	0.323
LVIDSn T0	-0.262	0.264	0.652	0.001	-0.349	0.112	0.087	0.739
LVIDSn T1	-0.526	0.017	0.459	0.032	-0.510	0.015	0.054	0.836
LVIDDn T0	-0.290	0.215	0.625	0.002	-0.239	0.283	0.208	0.423
LVIDDn T1	-0.310	0.184	0.296	0.181	-0.465	0.029	0.098	0.708
Advanced echocardiographic parameters	Age (months)		Weight (kg)		HR (bpm)		SAP (mmHg)	
	R	P	R	P	R	P	R	P
GLS (%) T0	0.091	0.702	-0.312	0.157	0.123	0.584	0.407	0.104
GLS (%) T1	0.081	0.733	-0.332	0.131	-0.049	0.829	0.285	0.267
TMAD (mm) T0	-0.403	0.078	0.746	<0.001	-0.223	0.318	0.089	0.734
TMAD (mm) T1	-0.271	0.248	0.836	<0.001	-0.338	0.124	0.166	0.524
TMAD (%) T0	-0.074	0.757	-0.225	0.315	0.319	0.148	0.055	0.833
TMAD (%) T1	0.008	0.972	0.204	0.362	0.285	0.198	0.381	0.131
TMAD (mm/kg) T0	0.348	0.132	-0.906	<0.001	0.255	0.251	-0.044	0.866
TMAD (mm/kg) T1	0.159	0.502	-0.581	0.005	0.233	0.297	0.011	0.966
TMAD (mm/m ²) T0	0.352	0.128	-0.768	<0.001	0.271	0.223	-0.063	0.807
TMAD (mm/m ²) T1	0.225	0.340	-0.451	0.035	0.185	0.410	-0.009	0.970
TMAD (mm/ $\sqrt[3]{\text{kg}}$) T0	-0.116	0.626	-0.095	0.673	0.056	0.806	0.076	0.771
TMAD (mm/ $\sqrt[3]{\text{kg}}$) T1	0.144	0.544	0.311	0.159	0.050	0.825	0.373	0.139

EF: ejection fraction; GLS: global longitudinal strain; HR: heart rate; LVIDDn: left ventricle internal diameter in diastole normalized; LVIDSn: left ventricle internal diameter in systole normalized; SF: shortening fraction; SAP: systolic arterial pressure; TMAD: tissue motion annular displacement; T0: time 0; T1: time 1.

DISCUSSION

This is the first study to use TMAD as a predictive parameter for fluid responsiveness in healthy, spontaneously breathing, dogs. Some echocardiographic parameters used for assessment of left ventricular systolic function, in particular TMAD (mm/kg and mm/m²), were higher in responsive dogs before and after fluid challenge (Figure 5). TMAD is part of an advanced echocardiographic examination but is widely studied in individuals with heart disease to assess longitudinal myocardial fibers [6,10,17,29,30]. This group of muscle fibers seems to be the first to be affected in systolic dysfunction [16].

Since the classification of fluid responsiveness depends on the increase in stroke volume after a fluid challenge, it can be said that ventricular systolic function directly influences fluid responsiveness [24]. Studies have shown that the parameters that measure left ventricular longitudinal myocardial fibers are sufficiently sensitive to detect changes in myocardial performance or subclinical systolic dysfunction [13,14,26,28]. TMAD was higher in responsive dogs, and since the dogs in this study were healthy with no subclinical changes in ventricular systolic function, it can be assumed that responsive individuals had a better myocardial performance.

TMAD was higher in responsive dogs even before the fluid challenge, therefore, this parameter could be used as a predictor of fluid responsiveness without the need for fluid administration. Even it is an advanced echocardiographic technique, TMAD has advantages in terms of execution time and the fact that it is less dependence on image quality or operator experience [30].

TMAD (mm/kg) > 0.89 and TMAD (mm/m²) > 18.9 after volume challenge can be considered the best advanced echocardiographic parameters, as they provided good predictive values, higher AUC values and smaller gray zone intervals (Table 2, Figures 6 & 7). In many cases, the fluid responsiveness indices fall within a range called the gray zone, in which false positive or negative results can occur. This is because such indices represent the simplification of a complex system that does not have a binary nature after fluid administration [5]. In this context, it is suggested that the gray zone interval be taken into account when interpreting any parameter that is predictive of fluid responsiveness. Values that fall outside the gray zone

can be considered reliable, whereas values that fall within this interval should be interpreted with greater caution [25].

Although no deleterious effects of the volume challenge were identified in the dogs of the present study, it is known that this intervention is potentially harmful in patients who do not need fluid [25]. In cases where the fluid challenge may harm the individual, the measurement of TMAD (mm/kg and mm/m²) before fluid challenge may provide an alternative measure of myocardial performance that is directly related to fluid responsiveness (Table 2, Figures 6 & 7).

Advanced echocardiographic parameters were less influenced by age, body weight, heart rate, SBP and heart rhythm. There was a positive correlation of TMAD (mm) with body weight (Table 3), because the larger the size of the patient, the greater the absolute displacement in millimeters of the mitral annulus towards the cardiac apex. However, the indexed TMAD (mm/kg and mm/m²) was negatively correlated to body weight (Table 3). The physiological behavior of systolic function in dogs predicts that systolic indices tend to decrease in larger animals, that is, these individuals have a smaller proportional displacement of the mitral annulus towards the cardiac apex [20,30].

GLS did not differ between responsive and non-responsive individuals in the same way as TMAD. A previous study carried out in healthy dogs identified a positive correlation of these parameters [30]. Although GLS and TMAD are correlated, GLS assesses the change in the length of the myocardial segments between systole and diastole providing accurate measurements of myocardial deformation [2]. TMAD assesses the degree of displacement of the valve annulus in relation to the cardiac apex [30]. In this case, the GLS is probably less influenced by the increment in the preload, as it directly assesses myocardial deformation.

LVIDDn < 1.39 before the fluid challenge provided good predictive values, sensitivity and specificity, the best AUC value and the smallest gray zone interval (Table 2, Figures 6 & 7), proving to be the most effective conventional echocardiographic parameter for identifying the responsive patient. A previous study that evaluated dogs with a variety of clinical conditions found a similar result, with LVIDDn ≤ 1.34 having 76.9% sensitivity, 100% specificity and 0.85 AUC in identifying the responsive patient [25]. In

addition, LVIDDn has the advantage that potentially responsive individuals can be identified before the volume challenge.

In the present study, the LVIDDn gray zone interval was 1.40 to 1.57, so a dog with LVIDDn > 1.57 can be considered non-responsive with a greater degree of reliability. In the study mentioned above, dogs with LVIDDn > 1.67 were considered to be in a hypervolemic state [25]. Animals in these conditions may not be fluid tolerant, which means that after fluid challenge, stroke volume may remain the same or decrease substantially [23,25]. Hypervolemic patients with severe clinical conditions are more likely to develop negative sequelae secondary to excess fluid administration [21,25].

The increase in LVIDSn seen after the fluid challenge in non-responsive dogs (Figure 4) may be linked to the concept of fluid responsiveness. Non-responsive individuals are those who, after administering a volume challenge do not produce an increase in stroke volume with an increase in preload (plateau of the Frank-Starling curve) [9,22]. Consequently, the extra volume administered affects the internal diameter of the left ventricle. After the volume challenge, responsive dogs had an increase in EF and SF (Figure 4), showing an increase in stroke volume in response to increased preload (ascending portion of the Frank-Starling curve) [9,22].

The indices for assessment of systolic function in dogs tend to decrease as with increasing size [20,30] which explains the correlations between EF and SF and body weight (Table 3). LVIDSn and LVIDDn also correlated with weight (Table 3), despite the fact that these parameters are indexed. However, non-responsive dogs had higher mean values of LVIDSn and LVIDDn, which may have influenced the correlation analyzes.

LVIDSn, EF and SF were influenced by age. Although the majority of dogs were young adults (median age 2 years), 3 animals were more than 6-years-old and these individuals were all small breed dogs (Lhasa Apso and Shih-tzu) and, therefore, had greater indices of systolic function. Finally, LVIDSn and LVIDDn were influenced by heart rate and heart rhythm, that is, when dogs had SA with lower heart rates, the ventricular filling time increased and, consequently, the diastolic and systolic ventricular diameters also increased [3].

Only 31.8% of the dogs in this study were considered responsive (Figure 3). Theoretically, a normovolemic individual without myocardial dysfunction should be responsive. However, it has been shown that only half of people with hemodynamic instability, and less than 40% of human patients with sepsis and hypotension, are able to respond adequately to a fluid challenge [22]. In addition to underlying disease, fluid responsiveness is also affected by the method of fluid challenge (type of fluid and administration rate). It is possible that different clinical conditions require different techniques for fluid challenge [19]. In this case, the management of fluid therapy should also be based on the presence of other signs of hemodynamic instability (or peripheral hypoperfusion) and the absence of risks related to volume overload [24].

There are a number of limitations to this study which must be considered when interpreting the results. The use of the volume challenge to classify the patient with regard to fluid responsiveness requires that the stroke volume be inferred using a precise method, and transthoracic echocardiography is not the gold standard technique for this assessment. Although TMAD has a number of advantages regarding its execution, the authors recognize that this technique is difficult to implement in clinical practice by anesthesiologists or intensivists with limited experience in echocardiography. Also, this echocardiographic technique requires an expensive software. Since spontaneously breathing dogs were studied, the echocardiographic parameters evaluated were static in nature (based on a single observation over time), and it is known that fluid responsiveness depends on the dynamic interaction between preload, afterload, systolic and diastolic function, heart rate and rhythm, vascular physiology and autonomic tone. Finally, only healthy dogs were included in this study and it is likely that animals with hemodynamic instability caused by different clinical conditions will react differently in terms of fluid responsiveness.

CONCLUSIONS

In conclusion, some of the echocardiographic parameters for assessing left ventricular systolic function differed between dogs responsive and non-responsive to the fluid challenge. TMAD (mm/kg) > 0.89 and TMAD (mm/m²) > 18.9 after the volume challenge were considered the best advanced echocar-

diography parameters, while LVIDDn < 1.39 before the volume challenge was considered the best conventional echocardiographic parameter for the identification of the responsive individuals. It is possible that in the future such variables may form a useful part of the evaluation of fluid responsiveness in dogs. However, more studies are needed to better understand the applicability of these techniques, especially in dogs with hemodynamic changes.

MANUFACTURERS

¹Philips Affiniti 50 ultrasound system. Andover, MA, USA.

²Software GraphPad Prism for Windows. La Jolla, CA, USA.

Ethical approval. The study was approved by the Animal Use Ethics Committee of the Federal University of Paraná (UFPR), Curitiba, PR, Brazil with the protocol number 058/2019.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

- 1 Ancierno M.J., Brown S., Coleman A.E., Jepson R.E., Papich M., Stepien R.L. & Syme H.M. 2018. ACVIM consensus statement: guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *Journal of Veterinary Internal Medicine*. 32: 1803-1822.
- 2 Blessberger H. & Binder T. 2010. Two dimensional speckle tracking echocardiography: basic principles. *Heart*. 96: 716-722.
- 3 Boon J.A. 2011. Chapter four: evaluation of size, function, and hemodynamics. In: *Veterinary Echocardiography*. 2nd edn. Ames: WileyBlackwell, pp.266-277.
- 4 Bucci M., Rabozzi R., Guglielmini C. & Franci P. 2017. Respiratory variation in aortic blood peak velocity and caudal vena cava diameter can predict fluid responsiveness in anaesthetised and mechanically ventilated dogs. *The Veterinary Journal*. 277: 30-35.
- 5 Cannesson M., Manach Y., Hofer C.K., Goarin J.P., Lehot J.J., Vallet B. & Tavernier B. 2011. Assessing the diagnostic accuracy of pulse pressure variations for the prediction of fluid responsiveness: a “gray zone” approach. *Anesthesiology*. 115(2): 231-341.
- 6 Carnabuci C., Hanas S., Ljungvall I., Tidholm A., Haggstrom J. & Hoglund K. 2013. Assessment of cardiac function using global and regional left ventricular endomyocardial and epimyocardial peak systolic strain and strain rate in healthy labrador retriever dogs. *Research in Veterinary Science*. 95(1): 241-248.
- 7 Cecconi M., Hofer C., Teboul J.L., Pettita V., Wilkman E., Molnar Z., Rocca G.D., Aldecoa C., Artigas A., Jog S., Sander M., Spies C., Lefrant J.Y. & Backer D. 2015. Fluid challenges in intensive care: the FENICE study: a global inception cohort study. *Intensive Care Medicine*. 41(9): 1529-1537.
- 8 Celeita-Rodríguez N., Teixeira-Neto F.J., Garofalo N.A., Dalmagro T.L., Giroto C.H., Oliveira G.C. & Santos I.F. 2019. Comparison of the diagnostic accuracy of dynamic and static preload indexes to predict fluid responsiveness in mechanically ventilated, isoflurane anesthetized dogs. *Veterinary Anaesthesia and Analgesia*. 46(3): 276-288.
- 9 Chaves R.C.F., Corrêa T.D., Serpa Neto A., Bravim B.A., Cordioli R.L., Moreira F.T., Timenetsky K.T. & Assunção M.S.C. 2018. Assessment of fluid responsiveness in spontaneously breathing patients: a systematic review of literature. *Annals of Intensive Care*. 8(21): 1-10.
- 10 Chetboul V., Sampedrano C.C., Gouni V., Nicolle A.P., Pouchelon J.L. & Tissier R. 2006. Ultrasonographic assessment of regional radial and longitudinal systolic function in healthy awake dogs. *Journal of Veterinary Internal Medicine*. 20(4): 885-893.
- 11 Cornell C.C., Kittleson M.D., Torre P.D., Häggström J., Lombard C.W., Pedersen H.D., Vollmar A. & Wey A. 2004. Allometric scaling of M-mode cardiac measurements in normal adult dogs. *Journal of Veterinary Internal Medicine*. 18(3): 311-321.
- 12 Coudray A., Romand J.A., Treggiari M., Bendjelid K. 2005. Fluid responsiveness in spontaneously breathing patients: a review of indexes used in intensive care. *Critical Care Medicine*. 33(12): 2757-2762.
- 13 Dalen H., Thorstensen A., Romundstad P.R., Aase S.A., Stoylen A. & Vatten L.J. 2011. Cardiovascular risk factors and systolic and diastolic cardiac function: a tissue Doppler and speckle tracking echocardiographic study. *Journal of the American Society of Echocardiography*. 24(3): 322-332.

- 14 Grue J.F., Storve S., Dalen H., Salvesen O., Mjølstad O.C., Samstad S.O., Torp H. & Haugen B.O. 2017. Automatic measurements of mitral annular plane systolic excursion and velocities to detect left ventricular dysfunction. *Ultrasound in Medicine and Biology*. 12(1): 924-930.
- 15 Heenen S., Backer D. & Vincent J.L. 2006. How can the response to volume expansion in patients with spontaneous respiratory movements be predicted? *Critical Care*. 10 (4): 1-7.
- 16 Jones C.J.H., Raposo L. & Gibson D.G. 1990. Functional importance of the long axis dynamics of the human left ventricle. *British Heart Journal*. 63(4): 215-220.
- 17 Kusunose K., Zhang Y., Mazgalev T.N., Thomas J.D. & Popovic Z.B. 2013. Left ventricular strain distribution in healthy dogs and in dogs with tachycardia-induced dilated cardiomyopathy. *Cardiovascular Ultrasound*. 11: 1-11.
- 18 Lamia B., Ochagavia A., Monnet X., Chemla D., Richard C. & Teboul J.L. 2007. Echocardiographic prediction of volume responsiveness in critically ill patients with spontaneously breathing activity. *Intensive Care Medicine*. 33: 1125-1132.
- 19 Lammi M.R., Aiello B., Burg G.T., Rehman T., Douglas I.S., Wheeler A.P. & Boisblanc B.P. 2015. Response to fluid boluses in the fluid and catheter treatment trial. *Chest*. 148(4): 919-926.
- 20 Locatelli C., Spalla I., Zanaboni A.M., Brambilla P.G. & Bussadori C. 2016. Assessment of right ventricular function by feature-tracking echocardiography in conscious healthy dogs. *The Research Veterinary Science*. 105: 103-110.
- 21 Marik P.E. & Lemson J. 2014. Fluid responsiveness: an evolution of our understanding. *British Journal of Anaesthesia*. 112(4): 617-620.
- 22 Michard F. 2002. Predicting fluid responsiveness in ICU patients: a critical analysis of the evidence. *Chest*. 121(6): 2000-2008.
- 23 Miller A. & Mandeville J. 2016. Predicting and measuring fluid responsiveness with echocardiography. *Echocardiography Research and Practice*. 3(2): 1-12.
- 24 Monnet X., Marik P.E. & Teboul J.L. 2016. Prediction of fluid responsiveness: an update. *Annals of Intensive Care*. 6: 1-11.
- 25 Oricco S., Rabozzi R., Meneghini C. & Franci P. 2019. Usefulness of focused cardiac ultrasonography for predicting fluid responsiveness in conscious, spontaneously breathing dogs. *American Journal of Veterinary Research*. 80(4): 369-377.
- 26 Thavendiranathan P., Poulin F., Lim K.D., Plana J.C., Woo A. & Marwick T.H. 2014. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy. *Journal of American College Cardiology*. 63(25): 2751-2768.
- 27 Thomas W.P., Gaber C.E., Jacobs G.J., Kaplan P.M., Lombard C.W., Moise N.S. & Moses B.L. 1993. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine. *Journal of Veterinary Internal Medicine*. 7(4): 247-252.
- 28 Thorstensen A., Dalen H., Amundsen B.H. & Stoylen A. 2011. Peak systolic velocity indices are more sensitive than end-systolic indices in detecting contraction changes assessed by echocardiography in young healthy humans. *European Journal of Echocardiography*. 12(12): 924-930.
- 29 Westrup U. & McEvoy F.J. 2013. Speckle tracking echocardiography in mature Irish Wolfhound dogs: technical feasibility, measurement error and reference intervals. *Acta Veterinaria Scandinavica*. 55(41): 1-11.
- 30 Wolf M., Lucina S.B., Bruler B.C., Tuleski G.L.R., Silva V.B.C. & Sousa M.G. 2018. Assessment of longitudinal systolic function using tissue motion annular displacement in healthy dogs. *Journal of Veterinary Cardiology*. 20(3): 175-185.