**Assessment of left ventricular function in healthy Great Danes and in Great Danes with Dilated Cardiomyopathy using Speckle Tracking Echocardiography**

**Authors**

Brigite Pedroa,c, MSc

Hannah Stephensona,b BVMS

Christopher Linneya,c BVSc

Peter Crippsa,d, BVSc, PhD

Joanna Dukes-McEwana BVMS, MVM, PhD

**Affiliations**

a University of Liverpool School of Veterinary Science, Leahurst, Chester High Road, Neston, CH64 7TE, UK

###### b HS Cardiology, Dalton House, 9 Dalton Square, Lancaster LA1 1WD, UK

c Willows Veterinary Centre and Referral Service, Highlands Road, Solihull, West Midlands B90 4NH, UK

d PJC Clinical Epidemiology, 68 Marshlands Road, Little Neston, Neston, Cheshire CH64 0SW, UK

**Corresponding author**

Brigite Pedro (brigite.pedro@willows.uk.net)

**Key words**

Dogs, myocardial function, radial strain, circumferential strain.

**ABSTRACT**

**Objectives**: Assess global circumferential and radial systolic and diastolic myocardial function with speckle tracking echocardiography (STE) in healthy Great Danes (GD) and in GD diagnosed with dilated cardiomyopathy (DCM).

**Animals**: Eighty-nine GD were included in the study: 39 healthy (NORMg) and 50 diagnosed with DCM (DCMg).

**Methods**: This was a retrospective study. Signalment and echocardiographic diagnosis were obtained from the medical records of GD assessed between 2008 and 2012. STE analysis of circumferential (C) and radial (R) strain (St) and strain rate (SR) in systole (S), early (E) and late (A) diastole was performed at the levels of the mitral valve (MV), papillary muscles (PM) and apex (Ap) of the left ventricle (LV). Univariable and multivariable analysis was performed to identify differences between groups.

**Results**: STE variables increase from the MV towards the Ap of the LV in both NORMg and DCMg dogs, some reaching statistical significance. Most of the variables (28/31) were lower in DCMg than in NORMg dogs: statistically significant variables included radial SR at the Ap in systole (p=0.029), radial St at the PM (p=0.012), circumferential SR at the PM in systole (p=0.031), circumferential and radial SR at the MV in early diastole (p=0.019 and p=0.049, respectively).

**Conclusions**: There are significant differences in STE variables between NORMg and DCMg Great Danes, although the overlap between the two groups may indicate that these variables are not sufficiently discriminatory. STE variables are not sufficiently sensitive to use in isolation as a screening method.

**ABBREVIATIONS**

|  |  |
| --- | --- |
| A | Late diastole |
| ApL | Apical level |
| C | Circumferential |
| CSRA | Circumferential strain rate in late diastole |
| CSRE | Circumferential strain rate in early diastole |
| CSRS | Circumferential strain rate in systole |
| CSt | Circumferential strain  |
| DCM  | Dilated Cardiomyopathy  |
| DCMg | Group considered affected by dilated cardiomyopathy |
| E | Early diastole |
| ESVC | European Society of Veterinary Cardiology |
| fps | Frames per second |
| GD | Great Danes |
| LV | Left ventricle |
| MVL | Mitral valve level |
| NORMg | Group considered healthy |
| PML | Papillary muscles level |
| R | Radial |
| ROI | Region of interest |
| RSRA | Radial strain rate in late diastole |
| RSRE | Radial strain rate in early diastole |
| RSRS | Radial strain rate in systole |
| RSt | Radial strain  |
| S | Systole |
| SR | Strain rate |
| SRA  | Strain rate in late diastole  |
| St | Strain |
| STE | Speckle tracking echocardiography |

**INTRODUCTION**

Dilated cardiomyopathy (DCM) is one of the most common acquired cardiac diseases in dogs.1 It is characterized by ventricular dilation and systolic dysfunction with normal left ventricular wall thickness.2 There are two stages of DCM: a clinical or *overt* phase, that is preceded by an initial pre-clinical or *occult* phase (detected only by careful screening).3 Progression to congestive heart failure commonly occurs after a variable but prolonged asymptomatic period.4

Great Danes are a commonly affected breed and it has recently been shown that the prevalence of DCM in the United Kingdom population of GD is higher than previously suggested.5 Little is known about the underlying genetic mutations that lead to the disease and its progression from sub-clinical to clinical stages.5-7 Identification of animals with preclinical DCM is important and due to proposed mechanisms of inheritance, affected dogs should be excluded from the breeding pool. Furthermore, early medical therapy may slow the progression from preclinical to clinical DCM.8,9 In the absence of a reliable genetic test, identification of preclinical animals is accomplished by Holter and echocardiography.5,10

Speckle tracking echocardiography (STE) is a recent technique for myocardial function assessment.11,12 The magnitude of myocardial deformation is described by systolic strain (St) and the rate of deformation is described by strain rate (SR).13 Radial St (RSt) represents myocardial thickening and thinning; circumferential St (CSt) is defined as the change of circumference in the short axis, perpendicular to the radial and long axis.14-16 St and SR can be measured in three different axes, from 2D echo images for longitudinal, radial and circumferential myocardial motion.11,12 In addition, from 3D echo, area strain can be calculated.17 The main advantages of STE are related to the fact that it is less load dependent than other echocardiographic variables,18 semi-automated and not dependent on insonation angle, unlike tissue Doppler imaging.19,20 In humans, STE has been shown to be useful not only in patients with clinical signs (reduced RSt21-23 and CSt23), but also in the detection of sub-clinical left ventricular dysfunction in patients with normal ejection fraction.24,25 26 In veterinary medicine, STE differences were noted in dogs with different classes of congestive heart failure due to myxomatous degenerative valvular disease;27-30 St and SR may be useful in detecting early myocardial impairment in dogs with Duchenne muscular dystrophy.31

To the authors’ knowledge, no studies using STE to assess left ventricular mechanics in dogs with naturally occurring DCM have been published. The purpose of this study was to assess global circumferential and radial myocardial function of the left ventricle (LV) in healthy GD using STE and to identify and report differences in the LV mechanics between healthy GD and GD diagnosed with DCM. It was hypothesized that the magnitude and rate of radial and circumferential deformation was lower in dogs with DCM than in healthy dogs, it was also hypothesized these variables would increase from the base towards the apex of the LV in healthy and affected GD, as reported in humans.32-37

**MATERIAL AND METHODS**

This longitudinal study was performed at the Small Animal Teaching Hospital, University of Liverpool, between 2008 and 2012. This study was funded initially by the LUPAe  project, and afterwards by the Kennel Club Charitable Trust and the UK Great Dane Breed Council. The study was approved by the University of Liverpool Committee on Research Ethics and by the LUPA consortium.

The dogs included in the study were client-owned or breeder-owned and were examined as part of a screening program for DCM. All dogs underwent a full physical examination and had hematology and biochemistry analyses. Thyroid function was initially tested only in dogs with clinical suspicion of hypothyroidism and later in all dogs as previously described.5 Dogs were excluded if other cardiac or systemic disease was identified. Dogs were manually restrained in lateral recumbency with no sedation for echocardiographic examination using an echocardiographic systemf equipped with a 2–4 MHz multifrequency matrix transducer. A simultaneous ECG was recorded in all dogs.

Conventional echocardiography

The echocardiographic examinations were performed by three different board-certified cardiologists or supervised residents, with the majority (85%) performed by one echocardiographer (HS). Echocardiographic views were obtained following standard recommendations,38,39 and stored as cine-loops of three cardiac cycles for offline analysis.g All the obtained measurements were used to allocate the patient to a group according to the European Society of Veterinary Cardiology taskforce guidelines.5,10 Dogs scoring 3 points or fewer were considered healthy and included in the normal group (NORMg), dogs scoring 4 or 5 were considered equivocal, and dogs scoring 6 or more were considered affected and included in the group of dogs with DCM (DCMg). For the purpose of this study, only NORMg and DCMg dogs were included; all the dogs considered equivocal were excluded from the study. For dogs with multiple echocardiographic examinations (performed as part of the LUPA project), data and score from the most recent assessment were used in the analysis. A single lead electrocardiogram was simultaneously acquired during each echocardiographic study.

Speckle-tracking echocardiography

Analysis of the 2D-STE data was performed offlineg using stored cineloops. Images had been acquired using highest frame rate possible for the transducer, depth and sector width. Images were analyzed by a single observer (BP), blinded to the results of the conventional echocardiographic examinations. The software only analysed images with appropriate frame rate (mean 51 fps, ranging from 29 to 64 fps in NORMg and from 29 to 58 fps in DCMg). Radial (R) and circumferential (C) St and SR were evaluated by 2D-STE from a right parasternal short-axis view at three different levels: at the mitral valve level (MV), at the papillary muscle level (PM) and at the apex (Ap). Each one of three acquired consecutive cardiac cycles was selected and the endocardial border of the myocardium was carefully traced to select the appropriate region of interest (ROI), which was automatically detected by the software and adjusted by the observer to incorporate the entire myocardial thickness as the ROI. The observer ensured that ROI was visually synchronized with the cardiac movement throughout the cardiac cycle. The computer software automatically created six segments in each image, and evaluated whether it reliably tracked myocardial speckles through the cardiac cycle. Segments marked by the software as inadequate tracking quality were retraced and manually corrected, as needed. If more than three to four attempts failed, the entire image was excluded from analysis. Only cardiac cycles where systolic and diastolic waves could be clearly identified and with no signs of arrhythmia, except sinus arrhythmia, were included.

Radial St values were positive with an increase in thickness (systole) and negative with a decrease (diastole) (Figure 1). Circumferential St values were positive with increase in LV radius and wall thinning (diastole) and negative with decrease in radius and wall thickening (systole) (Figure 2). For R and C deformations, the segmental values were averaged to obtain global systolic St (Sts) and SR in systole (S), early (E) and late (A) diastole. Systolic strain and SRs were defined as the maximal deflections of the respective curves during the ejection phase, defined as the peak systolic deflections recorded between the QRS complex and end of T wave of the simultaneously acquired ECG. Strain rate in early diastole and SRA were defined as the maximal curve deflections during early and late diastole, respectively, and timed from the ECG. All measurements were averaged from 3 heart cycles.

The different types of St and SR were compared between the Ap, PM and MV in the NORMg and in the DCMg. The St and SR gradient between Ap and MV was assessed in NORMg and DCMg dogs.

To assess the intra-observer measurement variability, the images of six dogs were analysed on two different days by the main observer (BP). To assess the inter-observer measurement variability, the images of six dogs were assessed by another observer (CL).

**STATISTICAL ANALYSIS**

Data were collected into an Excel spreadsheet h and analyzed using standard commercial software.i,j,k,l Standard basic descriptive statistics were performed. For further analyses the covariates of age and body weight were categorized into quartiles.

For comparison between the variables at the Ap, PM and MV, the groups were coded according to the expected order of magnitude (MV 1, PM 2, Ap 3) and a simple least-squares regression was used to assess evidence of a linear relationship. The Kruskal-Wallis test was used for the overall comparison between the three different levels and if this suggested a difference, individual levels were compared with a Mann-Whitney test and a Bonferroni correction40 was applied to account for multiple comparisons. Univariable investigation of the possible effect on each echocardiographic variable of group and sex, age and weight was investigated in the NORMg using both parametric and non-parametric methods: two-sample t tests, one-way ANOVA and Kruskal-Wallis tests were used as appropriate.

For multivariable analyses each variable was checked for normality and if necessary transformed to meet the required assumptions. A general linear model was then generated with group, sex, age and weight in the model. The Wald statistic was examined to assess statistical significance. The model assumptions were then checked using graphical displays of the residuals.

From archived echocardiographic images from six dogs, STE variables (3 DCMg, 3 NORMg) were measured on two separate occasions by one observer, for intra-observer comparison, and by a second observer to allow inter-observer comparison. For all six dogs to determine their population coefficient of variation, the mean and standard deviations were determined for both sets of observations, and the coefficient of variation was calculated, as previously described.41 For the six variables with the lowest CV%, Bland-Altman Plots were constructed, to show bias and the limits of agreements between the two sets of measurements. No adjustments were made for multiple statistical testing, apart from the use of Bonferroni corrections as stated above.

Statistical significance was defined as P < 0.05.

**RESULTS**

Eighty-nine dogs were included in this study: 39 (43.8%) were NORMg and 50 (56.2%) DCMg. In the NORMg males represented 33.3% (13/39) of the population and in the DCMg 54.0% (27/50) (p=0.053). The median age at presentation in NORMg was 75 months and in DCMg was 79.5 months (p=0.340). The median body weight for NORMg dogs was 63.0 kg and for DCMg was 64.6 kg (p=0.240) (Table 1). None of the dogs were receiving cardiac medications at the time of screening. One dog was identified during the screening to have CHF although the owner had not recognised any clinical signs. This dog was excluded from STE analysis as it was in atrial fibrillation at the time of initial admission. The other dogs were pre-clinical (stage B of the American College of Veterinary Internal Medicine classification for heart disease and heart failure42 – amended and applied to DCM).

Conventional echocardiographic results from this population have been previously published and a summary is shown in Table 2.5 Circumferential and R St and SR at the Ap could be obtained in 33/39 (85%) NORMg and 39/50 (78%) DCMg dogs. Circumferential and R St and SR at the PM could be obtained in 23/39 (59%) NORMg and 22/50 (44%) DCMg dogs. Circumferential and R St and SR at the MV could be obtained in 34/39 (87%) NORMg and 37/50 (74%) DCMg dogs.

In the NORMg, there was no identified influence of age, weight or sex on the STE variables. In the same group, a statistically significant progressive increase from base to apex was noted for CSt, RSt, CSRS, CSRE and RSRA (Table 3).

In the DCMg, significant progressive increases in the magnitude and rate of deformation from the base towards the apex of the LV were noted for CSt, RSt, CSRS, CSRE, CSRA and RSRE (Table 3).

The Ap-MV gradient was overall lower in the DCMg dogs compared with the NORMg, with exception of CSRA and RSRE. This difference was significant for RSt and CSRS (Table 4).

When considering the whole population (NORMg and DCMg), the effect of group (NORMg or DCMg) was investigated using the same variables. The NORMg had higher systolic [RSRApS (3.05sec-1 vs 2.54sec-1; p=0.048), CSRPMS (-2.05 sec-1 vs -1.71 sec-1; p=0.006)] and diastolic parameters [CSRPMA (1.24 sec-1 vs 0.98 sec-1; p=0.017), CSRMVE (1.52 sec-1 vs 1.27 sec-1; p=0.005), RSRMVE (-1.73 sec-1 vs -1.22; p=0.007), RSRMVA (-1.85 sec-1 vs -1.50 sec-1; p=0.045)] than DCMg dogs. When the weight was considered, RSRApE was significantly decreased in the animals with the highest weight (p=0.021). When considering the effect of age, CSRMVA (p=0.045) and RSRMVE (p=0.006) were significantly lower in the older dogs. The effect of sex appeared to have an impact in more variables: males showed lower values in CSRApS (p=0.014), CSRApE (p=0.026), RSRApE (p=0.044), CStPM (p=0.044), CSRPMS (p=0.041), CSRPME (p=0.032) and CStMV (p=0.047).

After adjusting for age, weight and sex, the mean values of 28/31 of the assessed variables were lower in DCMg than in NORMg dogs, but the difference was only statistically significant for RSRApS (p=0.029), RStPM (p=0.012), CSRPMS (p=0.031), CSRMVE (p=0.019) and RSRMVE (p=0.049) (Table 3, Figure 3).

Six dogs were used to assess the intra and inter-observer measurement variability. The intra-observer CV% ranged from a minimum of 12.1% for CStMV up to a maximum of 45.4% for RSRApA. Only six other variables (as well as CStMV) had CV% of less than 20% (CStPM, CSRPMS, CSRPME, CSRMVS and CSRMVE). The inter-observer CV% ranged from a minimum of 13.3% (also for CStMV) up to a maximum of 48.6% (also for RSRApA) (Table 5). The same variables as listed for intra-observer variability also had CV% <20%, except CSRMVE (which was 21.6%). Bland Altman (BA) analysis giving the bias and limits of agreements for the six variables with lowest CV% for intra- and inter-observer repeatability are shown in Table 6.

**DISCUSSION**

To the author’s knowledge, this is the first study where STE is used to assess the LV function of giant breed dogs with naturally occurring DCM. As previously shown in humans,34-36 some variables measuring magnitude and rate of deformation in the circumferential and radial axes increase from the base towards the apex of the LV in GD. When compared to NORMg dogs, an overall decrease in St and SR is noted in DCMg dogs; some of these variables achieved a statistically significant difference. The results of this study show that both systolic and diastolic function appear to be affected with DCM. In addition, when compared to St, variables associated with SR show more significant differences between the groups, which may indicate SR variables may be more reliable indicators of early myocardial dysfunction in preclinical DCM. Of the variables reaching a statistically significant difference, CSRMVE and CSRPMS were also among the ones that showed a lower CV and therefore may be among the most promising for clinical assessment. Although the direct assessment of myocardial function offered by STE was thought to hold promise in early detection of GD with preclinical DCM, the high inter- and intra-observer measurement variability in this study suggests most STE variables cannot be relied on as a screening tool.

This study shows that the magnitude and rate of deformation tend to increase from the base towards the apex of the LV in NORMg dogs: as reported in humans and dogs radial and circumferential strain are significantly higher at the level of the apex.32-37 In DCMg dogs, a similar increase from base towards apex is seen, however radial and circumferential strain show a lower Ap to MV gradient than in NORMg. This reflects the reduced systolic function in DCMg dogs, where reduced apical deformation may have a significant impact on LV function,43,44 possibly related to the fact that relative contribution to ejection increases toward the apex. Although the myocardium significantly thins from base to apex, the absolute volume contribution of the apex is smaller due to the tapering of the LV walls.45,46 During the cardiac cycle, myocardial shortening in the apex precedes the mid and basilar regions, therefore the apical region may be more strongly affected by wall stress, as previously suggested;47 on the other hand, other cardiac magnetic resonance imaging studies assessing regional wall stress have not noted significant differences between apex and other regions in healthy humans.48 Previous STE studies in humans show that circumferential shortening in the apex may be more dynamic than in the mid and basilar regions.36 This same explanation could potentially be applied to the radial deformation in this study. This theory is supported by some reports,33,34 although converse to others.35,36

Radial and circumferential strain were generally decreased in DCMg dogs when compared to NORMg dogs. Systolic function and diastolic function parameters were also generally lower in DCMg then NORMg.

Significant differences between the two groups were evident (RSRApS, RStPM, CSRPMS, CSRMVE and RSRMVE), consistent with some human studies, where deformational variables were decreased in DCM patients when compared to healthy controls.21-23,49

The only variable related to the magnitude of deformation that appears to be significantly different between these two groups was the radial strain at the PM. Similar findings have been reported in children with DCM.21 The largest degree of myocardial deformation occurs in the radial direction,21 which explains why this is one of the main differences between NORMg and DCMg dogs and makes radial function crucial to left ventricular ejection.21 As systolic dysfunction is one of the main features of DCM, decreased radial strain is expected in this population. All the other significantly different variables are related to the rate of deformation, which has also been reported in humans.23 In our study, radial SR appears to be most significant at the level of the Ap (in systole) and the MV (in early diastole), while circumferential SR was most significant at the level of the PM (in systole) and MV (in early diastole). The reason for having different types of systolic SR affecting different levels of the LV remains unclear. However an interesting finding is that diastolic variables appear to be potentially more clinically relevant at the level of the MV than anywhere else in the LV (as diastolic variables were significantly different between DCMg and NORMg only at the MV level). One can therefore speculate that the base of the LV may have a more significant impact on the diastolic phase of the cardiac cycle while the Ap and PM may contribute more to the systolic phase, which could be justified by the increasing relative contribution to ejection from the base towards the apex of the LV.45,46 As these variables are significantly different between the two groups, they potentially could be considered indicators of myocardial dysfunction in the breed. Future prospective, longitudinal studies with a larger population are needed to investigate this further.

This study has several limitations. The total number of dogs included was small and all of them were GD, which makes it impossible to extrapolate these findings to other breeds. In addition, due to inadequate image quality during three consecutive cycles, not every variable was analysed in all the dogs. Most of the dogs did not have a Holter monitor, therefore intermittent arrhythmias could not be completely ruled out, which can affect LV mechanics. As far as possible, concurrent systemic diseases were excluded, but unfortunately abdominal ultrasound and thyroid function testing were not carried out in all dogs. Additionally, a genetic test for DCM was not available and therefore the definition of the groups was based on a scoring system that has not so far been prospectively evaluated.10 In addition, the vast majority of these dogs did not have a follow-up examination and therefore some of NORMg dogs could in fact progress into equivocal or DCMg over time, although this risk was minimised by screening dogs over 4 years old and by the similar median age of NORMg and DCMg dogs.

The STE variables also showed large intra- and inter-observer measurement variability, which may in part be related to the fact that half of the dogs used in the measurement variability analysis were DCMg (n=3) and half were normal (n=3), which will result in wider standard deviations than using a more homogenous NORMg. The Bland-Altman results should be unaffected by using both NORMg / DCMg individuals in the analysis, since repeatability is assessed by plotting the difference in measurements between observers, against the mean of the observations, and Bland-Altman graphs showed that the mean difference line was parallel to zero (data not shown). Despite the large CV%s, some are sufficiently reliable. The CSt and CSR variables are the most repeatable measurements at the MV and PM level while the Ap seems to have results with the highest variability. However, for those few reliable variables with the lowest CV%, it shows that any change in an individual dog over time (e. g. with progression of disease or response to treatment) has to be over 15 – 20%, to be interpreted as genuine. This, in addition to the overlap between NORMg and DCMg, means that STE variables are not sufficiently sensitive or discriminatory to use in isolation as a screening method. In addition, despite the large number of statistical comparisons reported in this study, P values of <0.05 were accepted as representing statistical significance and therefore risking a type 1 statistical error. Furthermore in the vast majority of these dogs no histopathology analysis was performed for final confirmation of diagnosis.

**CONCLUSION**

An important conclusion of the present study is that there are significant differences in STE variables between NORMg and DCMg dogs especially for SR variables, although the overlap between the two groups indicates these are not discriminatory enough to distinguish NORMg from DCMg.

**Acknowledgements**

The authors BP and HMS cardiology residency training programs at the University of Liverpool were supported in part by Boehringer Ingelheim Vetmedica.

**Conflict of Interest**

The authors JDM and HMS received support from the European Community (LUPA-GA 201270), the Kennel Club Charitable Trust (UK) and the UK Great Dane Breed Council towards this research. JDM has also received previous financial support from Boehringer Ingelheim Vetmedica towards other studies and part-support of cardiology residency training programs (for HMS, BP). This funding did not result in any conflict of interest.

**REFERENCES**

1. Sisson D, O’Grady MR, Calvert CA. Myocardial diseases of dogs. In: Fox PR, Sisson D, Moise NS, editors. Textbook of canine and feline cardiology. 2nd ed. Philadelphia: Saunders; 1999, p.581-620.

2. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups and Council on Epidemiology and Prevention. Circulation 2006;113:1807-16.

3. Meurs KM. Myocardial disease: canine. In: Ettinger SJ, Feldman EC, editors. Textbook of veterinary internal medicine 7th ed. Philadelphia: Saunders; 2010, p.1320-8.

4. Tarducci A, Borgarelli M, Zanatta R, Cagnasso A. Asymptomatic dilated cardiomyopathy in Great Danes: clinical, electrocardiographic, echocardiographic and echo-Doppler features. Vet Res Commun 2003;27:799-802.

5. Stephenson HM, Fonfara S, Lopez-Alvarez J, Cripps P, Dukes-McEwan J. Screening for dilated cardiomyopathy in Great Danes in the United Kingdom. J Vet Intern Med 2012;26:1140-7.

6. Oyama MA, Chittur SV, Reynolds CA. Decreased triadin and increased calstabin2 expression in Great Danes with dilated cardiomyopathy. J Vet Intern Med 2009;23:1014-9.

7. Meurs KM, Miller MW, Wright NA. Clinical features of dilated cardiomyopathy in Great Danes and results of a pedigree analysis: 17 cases (1990-2000). J Am Vet Med Assoc 2001;218:729-32.

8. O'Grady MR, O'Sullivan ML, Minors SL, Horne R. Efficacy of benazepril hydrochloride to delay the progression of occult dilated cardiomyopathy in Doberman Pinschers. J Vet Intern Med 2009;23:977-83.

9. Summerfield NJ, Boswood A, O'Grady MR, Gordon SG, Dukes-McEwan J, Oyama MA, Smith S, Patteson M, French AT, Culshaw GJ, Braz-Ruivo L, Estrada A, O’Sullivan ML, Loureiro J, Willis R, Watson P. Efficacy of pimobendan in the prevention of congestive heart failure or sudden death in Doberman Pinschers with preclinical dilated cardiomyopathy (the PROTECT Study). J Vet Intern Med 2012;26:1337-49.

10. Dukes-McEwan J, Borgarelli M, Tidholm A, Vollmar AC, Häggström J, Cardiomyopathy ETfCD. Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy. J Vet Cardiol 2003;5:7-19.

11. Amundsen BH, Helle-Valle T, Edvardsen T, Torp H, Crosby J, Lyseggen E, Støylen A, Ihlen H, Lima JA, Smiseth OA, Slørdahl SA. Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. J Am Coll Cardiol 2006;47:789-93.

12. Ghio S, Gavazzi A, Campana C, Inserra C, Klersy C, Sebastiani R, Arbustini E, Recusani F, Tavazzi L. Independent and additive prognostic value of right ventricular systolic function and pulmonary artery pressure in patients with chronic heart failure. J Am Coll Cardiol 2001;37:183-8.

13. Voigt JU, Pedrizzetti G, Lysyansky P, Marwick TH, Houle H, Baumann R, Pedri S, Ito Y, Abe Y, Metz S, Song JH, Hamilton J, Sengupta PP, Kolias TJ, d'Hooge J, Aurigemma GP, Thomas JD, Badano LP. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. Eur Heart J Cardiovasc Imaging 2015;16:1-11.

14. Biswas M, Sudhakar S, Nanda NC, Buckberg G, Pradhan M, Roomi AU, Gorissen W, Houle H. Two- and three-dimensional speckle tracking echocardiography: clinical applications and future directions. Echocardiography 2013;30:88-105.

15. Blessberger H, Binder T. NON-invasive imaging: Two dimensional speckle tracking echocardiography: basic principles. Heart 2010;96:716-22.

16. Mondillo S, Galderisi M, Mele D, Cameli M, Lomoriello VS, Zacà V, Ballo P, D'Andrea A, Muraru D, Losi M, Agricola E, D'Errico A, Buralli S, Sciomer S, Nistri S, Badano L; Echocardiography Study Group Of The Italian Society Of Cardiology (Rome, Italy). Speckle-tracking echocardiography: a new technique for assessing myocardial function. J Ultrasound Med 2011;30:71-83.

17. Wen H, Liang Z, Zhao Y, Yang K. Feasibility of detecting early left ventricular systolic dysfunction using global area strain: a novel index derived from three-dimensional speckle-tracking echocardiography. Eur J Echocardiogr 2011;12:910-6.

18. Becker M, Kramann R, Dohmen G, Lückhoff A, Autschbach R, Kelm M, Hoffmann R. Impact of left ventricular loading conditions on myocardial deformation parameters: analysis of early and late changes of myocardial deformation parameters after aortic valve replacement. J Am Soc Echocardiogr 2007;20:681-9.

19. Kalogeropoulos AP, Georgiopoulou VV, Gheorghiade M, Butler J. Echocardiographic evaluation of left ventricular structure and function: new modalities and potential applications in clinical trials. J Card Fail 2012;18: 59-72.

20. Sitia S, Tomasoni L, Turiel M. Speckle tracking echocardiography: A new approach to myocardial function. World J Cardiol 2010;2:1-5.

21. Friedberg MK, Slorach C. Relation between left ventricular regional radial function and radial wall motion abnormalities using two-dimensional speckle tracking in children with idiopathic dilated cardiomyopathy. Am J Cardiol 2008;102:335-9.

22. Zeng S, Zhou QC, Peng QH, Cao DM, Tian LQ, Ao K, Liang X. Assessment of regional myocardial function in patients with dilated cardiomyopathy by velocity vector imaging. Echocardiography 2009;26:163-70.

23. Meluzin J, Spinarova L, Hude P, Krejci J, Poloczkova H, Podrouzkova H, Pesl M, Orban M, Dusek L, Korinek J. Left ventricular mechanics in idiopathic dilated cardiomyopathy: systolic-diastolic coupling and torsion. J Am Soc Echocardiogr 2009;22:486-93.

24. Okada M, Tanaka H, Matsumoto K, Ryo K, Kawai H, Hirata K. Subclinical myocardial dysfunction in patients with reverse-remodeled dilated cardiomyopathy. J Am Soc Echocardiogr 2012;25:726-32.

25. Saha SK, Kiotsekoglou A, Toole RS, Moggridge JC, Nichols KJ, Govind S, Gopal AS. Value of two-dimensional speckle tracking and real time three-dimensional echocardiography for the identification of subclinical left ventricular dysfunction in patients referred for routine echocardiography. Echocardiography 2012;29:588-97.

26. Fallah-Rad N, Walker JR, Wassef A, Lytwyn M, Bohonis S, Fang T, Tian G, Kirkpatrick ID, Singal PK, Krahn M, Grenier D, Jassal DS. The utility of cardiac biomarkers, tissue velocity and strain imaging, and cardiac magnetic resonance imaging in predicting early left ventricular dysfunction in patients with human epidermal growth factor receptor II-positive breast cancer treated with adjuvant trastuzumab therapy. J Am Coll Cardiol 2011;57:2263-70.

27. Suzuki R, Matsumoto H, Teshima T, Koyama H. Clinical assessment of systolic myocardial deformations in dogs with chronic mitral valve insufficiency using two-dimensional speckle-tracking echocardiography. J Vet Cardiol 2013;15:41-9.

28. Suzuki R, Matsumoto H, Teshima T, Koyama H. Noninvasive clinical assessment of systolic torsional motions by two-dimensional speckle-tracking echocardiography in dogs with myxomatous mitral valve disease. J Vet Intern Med 2013;27:69-75.

29. Zois NE, Tidholm A, Nagga KM, Moesgaard SG, Rasmussen CE, Falk T, Häggström J, Pedersen HD, Åblad B, Nilsen HY, Olsen LH. Radial and longitudinal strain and strain rate assessed by speckle-tracking echocardiography in dogs with myxomatous mitral valve disease. J Vet Intern Med 2012;26:1309-19.

30. Zois NE, Olsen NT, Moesgaard SG, Rasmussen CE, Falk T, Häggström J, Pedersen HD, Møller JE, Olsen LH. Left ventricular twist and circumferential strain in dogs with myxomatous mitral valve disease. J Vet Intern Med 2013;27:875-83

31. Takano H, Fujii Y, Yugeta N, Takeda S, Wakao Y. Assessment of left ventricular regional function in affected and carrier dogs with Duchenne muscular dystrophy using speckle tracking echocardiography. BMC cardiovascular disorders 2011;11:23.

32. Saito M, Okayama H, Nishimura K, Ogimoto A, Ohtsuka T, Inoue K, Hiasa G, Sumimoto T, Funada J, Shigematsu Y, Higaki J. Determinants of left ventricular untwisting behaviour in patients with dilated cardiomyopathy: analysis by two-dimensional speckle tracking. Heart 2009;95:290-6.

33. Korinek J, Wang J, Sengupta PP, Miyazaki C, Kjaergaard J, McMahon E, Abraham TP, Belohlavek M. Two-dimensional strain – a Doppler-independent ultrasound method for quantitation of regional deformation: validation in vitro and in vivo. J Am Soc Echocardiogr 2005;18:1247-53.

34. Bogaert J, Rademakers FE. Regional nonuniformity of normal adult human left ventricle. Am J Physiol Heart Circ Physiol 2001;280:610-20.

35. Duan F, Xie M, Wang X, Li Y, He L, Jiang L, Fu Q. Preliminary clinical study of left ventricular myocardial strain in patients with non-ischemic dilated cardiomyopathy by three-dimensional speckle tracking imaging. Cardiovasc ultrasound 2012;10:8.

36. Saito K, Okura H, Watanabe N, Hayashida A, Obase K, Imai K, Maehama T, Kawamoto T, Neishi Y, Yoshida K. Comprehensive evaluation of left ventricular strain using speckle tracking echocardiography in normal adults: comparison of three-dimensional and two-dimensional approaches. J Am Soc Echocardiogr 2009;22:1025-30.

37. Westrup U, McEvoy FJ. Speckle tracking echocardiography in mature Irish Wolfhound dogs: technical feasibility, measurement error and reference intervals. Acta Vet Scand 2013;55:41.

38. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978; 58:1072-83.

39. Thomas WP, Gaber CE, Jacobs GJ, Kaplan PM, Lombard CW, Moise NS, Moses BL. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. J Vet Intern Med 1993; 7:247-52.

40. StataCorp. Oneway - Oneway analysis of variance. In: StataCorpLP. Stata Base Reference Manual Release 14. College Station: Stata Press; 2015, p.1786-97.

41. Dukes-McEwan J, French AT, Corcoran BM. Doppler echocardiography in the dog: measurement variability and reproducibility. Vet Radiol Ultrasound 2002;43:144-52.

42. Atkins C, Bonagura J, Ettinger S, Fox P, Gordon S, Haggstrom J, Hamlin R, Keene B, Luis-Fuentes V, Stepien R. Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. J Vet Intern Med 2009;23:1142-50.

43. Kim WJ, Lee BH, Kim YJ, Kang JH, Jung YJ, Song JM, Kang DH, Song JK. Apical Rotation Assessed by Speckle-Tracking Echocardiography as an Index of Global Left Ventricular Contractility. Circ-Cardiovasc Imag 2009;2:123-31.

44. Opdahl A, Helle-Valle T, Remme EW, Vartdal T, Pettersen E, Lunde K, Edvardsen T, Smiseth OA. Apical rotation by speckle tracking echocardiography: A simplified bedside index of left ventricular twist. J Am Soc Echocardiog 2008;21:1121-8.

45. Buchi M, Hess OM, Murakami T, Krayenbuehl HP. Left ventricular wall stress distribution in chronic pressure and volume overload: effect of normal and depressed contractility on regional stress-velocity relations. Basic Res Cardiol 1990;85:367-83.

46. Esch BT, Warburton DE. Left ventricular torsion and recoil: implications for exercise performance and cardiovascular disease. J Appl Physiol 2009;106:362-9.

47. Fujita N, Duerinekx AJ, Higgins CB. Variation in left ventricular regional wall stress with cine magnetic resonance imaging: normal subjects versus dilated cardiomyopathy. Am Heart J 1993;125:1337-45.

48. Zhong L, Su Y, Yeo SY, Tan RS, Ghista DN, Kassab G. Left ventricular regional wall curvedness and wall stress in patients with ischemic dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 2009; 296: 573-84.

49. Pacileo G, Baldini L, Limongelli G, Di Salvo G, Iacomino M, Capogrosso C, Rea A, D'Andrea A, Russo MG, Calabrò R. Prolonged left ventricular twist in cardiomyopathies: a potential link between systolic and diastolic dysfunction. Eur J Echocardiogr 2011;12:841-9.

**TABLES**

**Table 1: Age and body weight in normal great Danes (NORMg) and Great Danes with dilated cardiomyopathy (DCMg).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Minimum | 25 Percentile | Median | 75 Percentile | Maximum | P |
| Age (months) | NORMg | 48 | 59 | 75 | 92 | 138 | 0.34 |
| DCMg | 48 | 66 | 80 | 93 | 141 |
| Body Weight (Kg) | NORMg | 51 | 58 | 63 | 71 | 88 | 0.24 |
| DCMg | 42 | 60 | 65 | 74 | 90 |

**Table 2: Echocardiographic variables (mean and standard deviation) in normal Great Danes (NORMg) and Great Danes affected by DCM (DCMg).**

|  |  |  |  |
| --- | --- | --- | --- |
|  | NORMg (Mean (SD))(n=40) | DCMg (Mean (SD))(n=37) | P |
| LVIDd (mm) | 50.9 (3.9) | 59.8 (5.3) | <0.001 |
| LVIDs (mm) | 36.61.56 (0.043) | 47.81.68 (0.056) | <0.001 |
| LVIDd(allometric scaling) | 1.480.17 (0.028) | 1.740.24 (0.038) | <0.001 |
| LVIDs(allometric scaling) | 0.99-0.006 (0.040) | 1.280.107 (0.051) | <0.001 |
| EF (%) | 53.9 (6.7) | 40.9 (10.7) | <0.001 |
| FS (%) | 27.8 (5.5) | 19.5 (6.3) | <0.001 |
| ESVI (mL/m2) (Simpson’s method of discs) | 34.41.54 (0.10) | 56.01.75 (0.14) | <0.001 |

Data previously published.5 EF, ejection fraction; ESVI, end-systolic volume index; FS, fractional shortening; LVIDd, left ventricular M-mode internal dimension in diastole; LVIDs, left ventricular M-mode internal dimension in systole.

Table 3: Results of left ventricular speckle tracking echocardiography in healthy Great Danes (NORMg) and in Great Danes with dilated cardiomyopathy (DCMg).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | NORMg(Mean (SD)) | DCMg(Mean (SD)) | P |
| Apical level study | C St Ap (%) | -22.05 (5.77) | -20.07 (5.02) | 0.71 |
| R St Ap (%) | 52.91 (18.28) | 45.41 (16.01) | 0.08 |
| C SR S Ap (sec-1) | -2.50 (0.69) | -2.18 (0.74) | 0.40 |
| C SR E Ap (sec-1) | 2.65 (0.80) | 2.29 (0.82) | 0.36 |
| C SR A Ap (sec-1) | 1.73 (0.72)0.81 (0.18) | 1.60 (1.05)0.88 (0.21) | 0.16 |
| R SR S Ap (sec-1) | 3.05 (1.14)0.46 (0.16) | 2.54 (0.72)0.39 (0.12) | 0.03 |
| R SR E Ap (sec-1) | -2.17 (1.03) | -2.04 (1.01) | 0.67 |
| R SR A Ap (sec-1) | -2.27 (0.98) | -1.85 (1.04) | 0.12 |
| PapillaryMuscleslevelstudy | C St PM (%) | -16.73 (2.58)1 | -14.79 (3.91)1 | 0.18 |
| R St PM (%) | 47.18 (12.00) | 41.26 (12.79) | 0.01  |
| C SR S PM (sec-1) | -2.05 (0.44)1 | -1.71 (0.34)1 | 0.03  |
| C SR E PM (sec-1) | 1.58 (0.35)10.19 (0.10) | 1.42 (0.45)10.13 (0.14) | 0.09 |
| C SR A PM (sec-1) | 1.24 (0.35)10.07(0.16) | 0.98 (0.29)1-0.02 (0.12) | 0.05 |
| R SR S PM (sec-1) | 2.78 (0.82)0.43 (0.12) | 2.37 (0.86)0.35(0.15) | 0.05 |
| R SR E PM (sec-1) | -1.86 (0.73) | -1.95 (0.66) | 0.86 |
| R SR A PM (sec-1) | -1.66 (0.46)1 | -1.72 (0.80) | 0.81 |
| MitralValvelevelstudy | C St MV (%) | -14.66 (2.85)1 | -14.04 (3.44)1 | 0.66 |
| R St MV (%) | 33.09(12.39)1,2 | 31.10 (12.80)1,2 | 0.45 |
| C SR S MV (sec-1) | -2.00 (0.49)1 | -1.81 (0.30) | 0.16 |
| C SR E MV (sec-1) | 1.52 (0.35)1 | 1.27 (0.39)1 | 0.02 |
| C SR A MV (sec-1) | 1.91 (0.50)10.034 (0.20) | 1.04 (0.46)1-0.02 (0.20) | 0.34 |
| R SR S MV (sec-1) | 2.79 (1.26) | 2.36 (1.13) | 0.08 |
| R SR E MV (sec-1) | -1.73 (0.80) | -1.22 (0.75)1,2 | 0.049  |
| R SR A MV (sec-1) | -1.85 (0.80)  | -1.50 (0.65) | 0.12 |

Results of different echocardiographic variables in healthy Great Danes (NORMg) and in Great Danes with dilated cardiomyopathy (DCMg), after correcting for sex, body weight and age. Data that has been logarithmically transformed is shown after the non-transformed data (second line in the same row).

For each echocardiographic variable, numeral superscripts indicate that the variable is significantly different from apical level (Ap)1 and from papillary muscles level (PM)2, respectively.

 Ap, apical; PM, papillary muscles; MV, mitral valve; CSt, circumferential strain; RSt, radial strain; CSRS, circumferential strain rate in systole; CSRE, circumferential strain rate in early diastole; CSRA, circumferential strain rate in late diastole; RSRS, radial strain rate in systole; RSRE, radial strain rate in early diastole; RSRA, radial strain rate in late diastole.

Table 4: Apex-to-Mitral valve (Ap-MV) gradient for all variables in normal (NORMg) and affected groups (DCMg).

|  |  |  |  |
| --- | --- | --- | --- |
|  | Gradient Ap-MV NORMg | Gradient Ap-MV DCMg | P |
| C St (%) | 7.38 | 6.04 | 0.759 |
| R St (%) | 19.82 | 14.31 | 0.034 |
| C SR S (sec-1) | 0.51 | 0.38 | 0.037 |
| C SR E (sec-1) | 1.13 | 1.02 | 0.052 |
| C SR A (sec-1) | 0.18 | 0.56 | 0.252 |
| R SR S (sec-1) | 0.26 | 0.18 | 0.077 |
| R SR E (sec-1) |  0.44 |  0.82 |  0.759 |
| R SR A (sec-1) | 0.41 | 0.35 | 0.083 |

See table 3 for abbreviations.

**Table 5: Mean coefficient of variation to indicate intra and inter-observer measurement variability from 6 randomly selected dogs (3 normal and 3 affected by dilated cardiomyopathy).**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | CV Intra-observer (%) | CV Inter-observer (%) |
| Apical levelstudy | C St Ap | 33.7 | 28.1 |
| R St Ap | 24.1 | 26.8 |
| C SR S Ap | 33.3 | 25.8 |
| C SR E Ap | 22.8 | 19.4 |
| C SR A Ap | 25.5 | 23.4 |
| R SR S Ap | 25.5 | 25.2 |
| R SR E Ap | 23.1 | 18.6 |
| R SR A Ap | 45.4 | 48.6 |
| PapillaryMuscleslevelstudy | C St PM | 13.2 | 13.7 |
| R St PM | 29.3 | 38.0 |
| C SR S PM | 14.9 | 15.8 |
| C SR E PM | 19.3 | 18.8 |
| C SR A PM | 28.0 | 34.4 |
| R SR S PM | 30.0 | 31.6 |
| R SR E PM | 34.8 | 37.9 |
| R SR A PM | 34.3 | 45.3 |
| MitralValvelevelstudy | C St MV | 12.1 | 13.3 |
| R St MV | 30.2 | 41.5 |
| C SR S MV | 12.4 | 15.0 |
| C SR E MV | 17.1 | 21.6 |
| C SR A MV | 26.2 | 23.1 |
| R SR S MV | 24.4 | 28.7 |
| R SR E MV | 34.9 | 24.4 |
| R SR A MV | 24.9 | 23.2 |

See table 3 for abbreviations.

**Table 6: Results of Bland-Altman analysis, showing bias, standard deviation and limits of agreement for intra and inter-observer repeatability (STE variables from 6 randomly selected dogs; 3 normal and 3 affected by dilated cardiomyopathy).**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Bias | Standard deviation | Limits of agreement |
| C St MV inter-observer (%) | 1.97 | 1.94 | -1.84, 5.77 |
| C St MV intra-observer (%) | 0.04 | 2.23 | -4.33, 4.42 |
| C St PM inter-observer (%) | 1.32 | 1.80 | -2.21, 4.86 |
| C St PM intra-observer (%) | 0.39 | 2.12 | -3.75, 4.54 |
| C SR MV S inter-observer (sec-1) | 0.25 | 0.34 | -0.42, 0.92 |
| C SR MV S intra-observer (sec-1) | 0.21 | 0.32 | -0.42, 0.84 |
| C SR MV E inter-observer (sec-1) | 0.10 | 0.35 | -0.59, 0.79 |
| C SR MV E intra-observer (sec-1) | 0.02 | 0.35 | -0.67, 0.71 |
| C SR PM S inter-observer (sec-1) | 0.07 | 0.20 | -0.32, 0.45 |
| C SR PM S intra-observer (sec-1) | -0.01 | 1.4 | -0.27, 0.26 |
| C SR PM E inter-observer (sec-1) | -0.16 | 0.32 | -0.80, 0.47 |
| C SR PM E intra-observer (sec-1) | -0.11 | 0.34 | -0.78, 0.56 |

See table 3 for abbreviations.

**FIGURE CAPTIONS**

Figure 1: Radial Strain curve recorded at the level of the apex of the left ventricle of a normal dog. Left top – 2D image of the apex of the left ventricle with the region of interest divided into six segments identified by six different colours. Left bottom - Color M-mode strain tracing of the apex of the left ventricle; the x-axis represents time and the y-axis represents the wall of the left ventricle divided by segments identified by six different colours. Right - Radial strain curve of the six different segments of the region of interest, identified by six different colours. AVC, Aortic valve closure.

Figure 2: Circumferential strain curve recorded at the level of the apex of the left ventricle of a normal dog. Left top – 2D image of the apex of the left ventricle with the region of interest divided into six segments identified by six different colours. Left bottom - Color M-mode strain tracing of the apex of the left ventricle; the x-axis represents time and the y-axis represents the wall of the left ventricle divided by segments identified by six different colours. Right top – Circumferential strain rate curve of the six different segments of the region of interest, identified by six different colours. Right bottom – Table showing the peak global (peak G) circumferential strain of each one of the six different segments of the region of interest, identified by six different colours; the global circumferential strain rate was calculated by averaging the measurements of the six segments.

**Figure 3:** Boxplots showing significant differences in deformation between NORMg and DCMg dogs recorded at the level of the apex (Ap) (top row), papillary muscles (PM) (middle row) and mitral valve (MV) (bottom row).The boxes show the 25th and 75th percentile, including the median line. The whiskers show the remaining data.

a) RSRApS; Radial strain rate at the level of the apex in systole (p=0.03); b) Log10 RSRApS; transformed Radial strain rate at the level of the apex in systole (p= 0.03);

c) RStPM, radial strain at the level of the papillary muscles (p=0.01); d) CSRPMS, circumferential strain rate at the level of the papillary muscles in systole (p=0.03); e) CSRMVE, Circumferential strain rate at the level of the mitral valve in early diastole (p=0.02); f) RSRMVE, radial strain rate at the level of the mitral valve in early diastole (p=0.049).

**FOOTNOTES**

1. LUPA project, WP2 Cardiovascular disorders. <http://eurolupa.org>
2. Vivid 7, General Electric Medical System, Waukesha, WI
3. EchoPac, General Electric Medical System, Waukesha, WI
4. Microsoft Office Excel 2007, Microsoft corp. USA
5. Minitab 16 for Windows, Minitab Inc, State College, PA
6. StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP.
7. SigmaPlot12.3 Systat Software, San Jose, CA
8. SPSS for Windows, Version 18.0, SPSS Inc, Chicago, IL