UNIVERSIDADE FEDERAL DO PARANÁ

M.V. JULIO PEREIRA DOS SANTOS

ASSESSMENT OF HEART RATE TURBULENCE IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **JULIO PEREIRA DOS SANTOS** intitulada: **ASSESSMENT OF HEART RATE TURBULENCE IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE**, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre 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, 28 de Fevereiro de 2019.

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RESUMO

A degeneração mixomatosa da valva mitral é a doença cardíaca mais prevalente na medicina veterinária afetando cães de pequeno porte. Ela reduz o débito cardíaco e uma das respostas é a ativação da porção simpática do sistema nervoso autônomo. Essa ativação leva a redução da variabilidade da frequência cardíaca (HRV). A HRV é uma análise muito precisa derivada de cálculos dos intervalos R-R. Recentemente na medicina humana um novo indicador foi introduzido, conhecido como turbulência da frequência cardíaca (HRT). Esse indicador avalia a resposta do ciclo de batimentos sinusais após uma contração ventricular prematura. A HRT provou ser mais eficiente que a HRV como um indicador independente da chance de óbito. Neste estudo dados de cães de raças pequenas foi coletada, eletrocardiograma, ecocardiografia foram executadas, alguns pacientes também fizeram holter e os dados foram divididos em dois grupos, sintomáticos e assintomáticos. Os indicadores de início da turbulência e de declive da turbulência foram calculados para os grupos e comparados. A diferença entre estágios sintomáticos foi marcante, com correlação moderada com parâmetros ecocardiográficos. Conclusões: A turbulência da frequência cardíaca é um indicador promissor para a degeneração mixomatosa da valva mitral e possivelmente poderia ser utilizado para graduar estágios avançados de acordo com seu reflexo autonômico.

Palavras-chave: eletrofisiologia. baroreflexo. neurocardiologia. ativação simpática. variabilidade da frequência cardíaca.

ABSTRACT

Myxomatous mitral valve degeneration (MMVD) is the most common heart disease affecting small dogs. It reduces cardiac output resulting in compensatory activation of the sympathetic portion of the autonomic nervous system. This activation leads to a reduced heart rate variability (HRV). HRV, from R-R interval calculations, is an accurate marker for sympathetic activation. Recently, in human medicine, a new indicator has been introduced, known as heart rate turbulence (HRT). This indicator evaluates the response of the cardiac cycle after a premature ventricular contraction interrupts sinus rhythm. HRT has also proven to be a more accurate independent odd's death indicator than HRV. Animals and Methods: Data was collected from small breed dogs, electrocardiogram and echocardiography were performed and, in some patients, holter monitoring was performed. Data was divided into two groups, symptomatic and asymptomatic animals. The indicators turbulence onset and turbulence slope were calculated for the groups and compared. Results: There were marked differences between symptomatic stages, with moderate correlation with some echocardiographic parameters. Conclusion: In dogs with MMVD heart rate turbulence is a promising indicator for severity of disease and could possibly be used for advanced staging of patients according to their autonomic reflex.

Key-words: electrophysiology. baroreflex. neurocardiology. sympathetic activation. heart rate variability.

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1 Introduction

In human medicine a recently introduced baroreflex indicator is heart rate turbulence (HRT). This demonstrates the response of the cardiac cycle after a premature ventricular beat interrupts sinus rhythm. This non-invasive method stratifies the risk of death and proved to be a strong and independent indicator in man - with results better than heart rate variability (Schimdt et al, 1999). HRT is considered to be a biphasic indicator, starting with the turbulence onset (TO), defined as the initial or the acceleration phase and the turbulence slope (TS), considered the terminal or deceleration phase. Intrapatient variability may occur depending on coupling interval time, and heart rate. (Bauer et al, 2008)

In people with mitral valve prolapse HRT was statistically different from normal subjects, while heart rate variability (HRV) showed no difference. (Gunduz et al, 2006) When divided by the New York Heart Association scale classes I and II had a significantly higher HRT slope than patients in class III. (Davies et al, 2001)

In dogs myxomatous mitral valve degeneration also known as endocardiosis accounts for 75% of all cardiovascular diseases in dogs under 25 kg and is the heart disease with the highest morbidity and mortality. Clinical signs are generally seen > 7 years of age. The mitral degeneration leads to regurgitation and reduced cardiac pumping ability causing volume overload of the left atrium and ventricle. (Fox, 2012 et al; Chamas et al, 2011: Borgarelli et al, 2008)

The objective of the present study was to assess heart rate turbulence in dogs with myxomatous mitral valve disease at stages B1, B2, C and D and to correlate this measure with commonly used echocardiographic parameters.

2 Materials and Methods

Following consultation and physical evaluation electrocardiograms were recorded and echocardiography performed by veterinary cardiologists. The tricuspid valve was assessed via the left apical 4-chamber view optimized for the right heart left parasternal intercostal window to assess the pulmonary artery pressure through continuous wave doppler. Patients with mild or moderate pulmonary hypertension were included in the study, but the coexistence of other systemic diseases such as renal or endocrine disease and other heart diseases like severe pulmonary hypertension or congenital heart diseases were exclusion criteria for the study. All animals with atrial fibrillation, ventricular tachycardia, or bi or trigemini were excluded.

The main criteria to classify a B2 patient was the finding of a remodeled heart. This was defined as a left atrium to aorta ratio (LA/AO) of 1.6 or the left ventricle internal diameter indexed by the body surface area (LVDNN) > 1.7. The total number of dogs enrolled in groups B1, B2, C and D were 10, 7, 10 and 7 respectively. Dogs in groups C and D were not accepted if they in decompensated failure and showing clinical signs of congestive heart failure eg dyspnea or pulmonary edema. Animals in class C and D were medicated according to guidelines of Atkins (2009) and class C patients were medicated with pimobendam 0.25 mg/kg BID accordingly to the EPIC study (Boswood et al, 2016).

Patients with no VPCs on electrocardiogram or holter recordings were not suitable for the study. The exclusion criteria for VPC analysis were that the VPC needed to be within a sinus rhythm period and when more than one VPC was available the chosen VPC for the calculations was the one with the shortest coupling interval. For the compensatory pause to be accepted the two RR intervals following the VPC had to be longer than the two RR intervals before the coupling interval.

In the electrocardiogram and holter recordings the two R-R intervals before and twenty R-R intervals after the VPC were used for calculation, up to 5 VPCs from Holter recordings were analyzed with times averaged for the calculation while in electrocardiogram studies a single VPC was analyzed.

The turbulence onset (TO) was manually calculated as described by Schmidt et al (1999), as the difference between the mean of the first two sinus RR intervals after a VPC and last two sinus RR intervals before the VPC divided by the mean of the last two sinus RR intervals before the VPC. As described by the following equation:

$$\text{Turbulence Onset} = \frac{(RR1_{af} + RR2_{af}) - (RR1_{bf} + RR2_{bf})}{(RR1_{bf} + RR2_{bf})}$$

The coupling interval and the compensatory pause were considered to be point zero (0) for the calculations. The final TO result is shown as %.

The turbulence slope (TS) was calculated as suggested by Schmidt et al (1999). The R-R intervals after the VPC, were analyzed in groups of five, until the 20th R-R interval using linear regression. Then the steepest regression line found in the results was chosen as TS value. TS was expressed as milliseconds per RR (ms/RR).

For statistical analyses patients were classified according to ACVIM Consensus definitions (Atkins et al, 2009) where stages B1 and B2 were classified as asymptomatic and patients stage C and D were classified as symptomatic.

All data were considered parametric by the Kolmogorov-Smirnov test and the variance comparison between the groups were calculated by ANOVA test followed by Tukey test. The sensitivity and specificity were calculated by the Receiver Operator Characteristic curve (R.O.C.).

3 Results

The data was collected in part retrospectively and in part prospectively from patients from the caseload of the Veterinary Hospital of the Federal University of Paraná A total of 34 subjects, 18 females and 16 males were included. The most common breeds were cross breed dogs (13 or 31%) and poodles (10 or 24%). A total of 19 holter and 15 electrocardiogram examinations were performed.

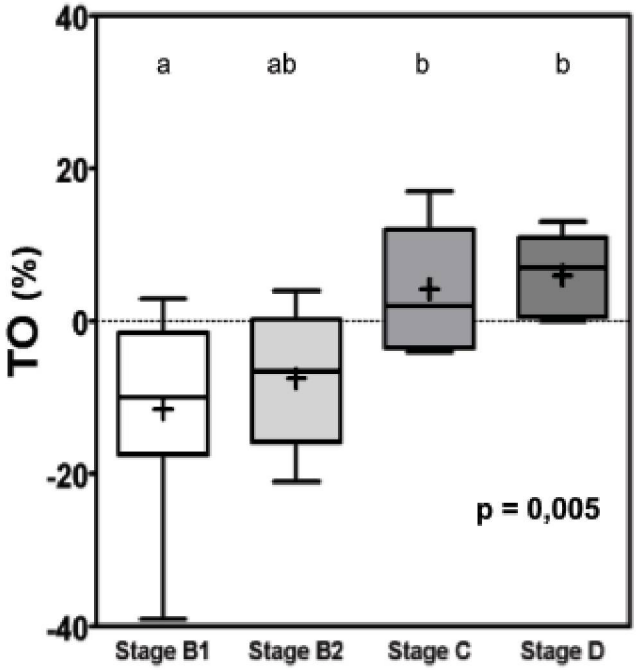
TABLE 1 - AVERAGE, MINIMUM AND MAXIMUM AGE AND WEIGHT OF PATIENTS, MALES AND FEMALES ACCEPTED FOR THE STUDY AND DIVIDED ACCORDING TO THE MMVD GRADE.

	B1		B2		C		D	
	Age	Weight	Age	Weight	Age	Weight	Age	Weight
Female	13.5	12.1	10.5	14.6	13.5	6.2	12	5.6
(min-max)	(12-15)	(1.7-21.7)	(8-14)	(4.8-23)	(9-18)	(2.8-12)	(10-13)	(3-9.5)
Male	12.3	13.5	12.3	6	12.3	10,2	13.7	6.1
(min-max)	(8-15)	(4.5-25.5)	(9-15)	(2.2-9.2)	(10-15)	(7.4-13.8)	(10-15)	(3-9.5)

Groups B1, B2, C and D had average coupling intervals of 307, 280, 292, 302 milliseconds, with an 'all subjects' average time of 295 ms. For the compensatory pause the average time for groups B1, B2, C and D was 646, 503, 549, 575 respectively and the 'all subjects' average was 295 ms.

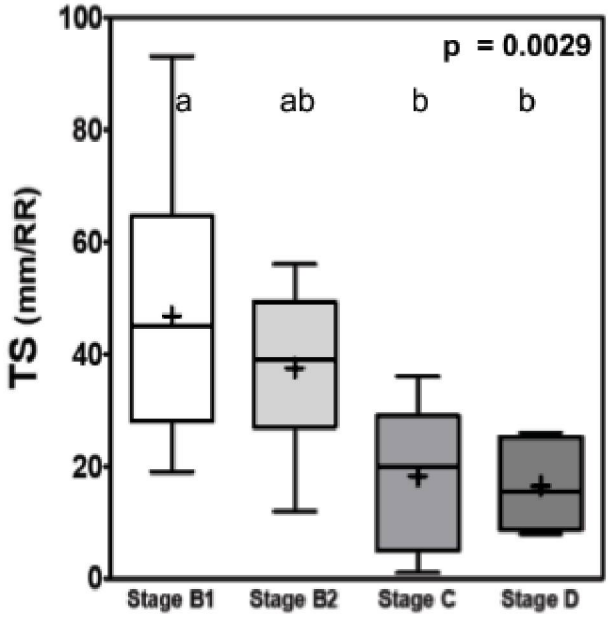
After counting the R-R intervals and calculating the HRT turbulence the means were subjected to variance analyses which showed statistical difference between some groups as illustrated in figures 1 and 2.

FIGURE 1 - BOX PLOT SHOWING THE MEAN, STANDARD DEVIATION AND AMPLITUDE VALUES OF TURBULENCE ONSET FOR DIFFERENT DISEASE STAGES



Note the short interval overlap of the standard deviation asymptomatic (B1/B2) and symptomatic patients (C/D). TO - Turbulence Onset.

FIGURE 2 - BOX PLOT SHOWING THE MEAN, STANDARD DEVIATION AND AMPLITUDE VALUES OF TURBULENCE SLOPE FOR DIFFERENT DISEASE STAGES



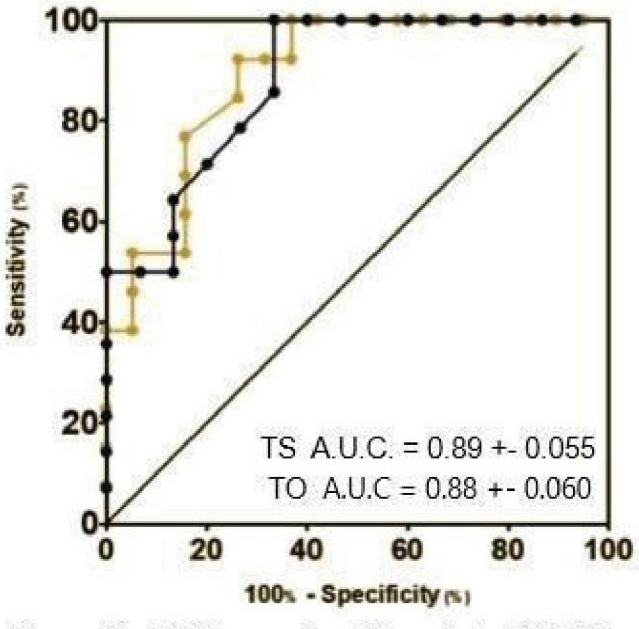
Note the short interval overlap of the standard deviation asymptomatic (B1/B2) and symptomatic patients (C/D). (A)TS - Turbulence Slope.

Both TO and TS tended to change as the disease progresses. In group B1 the TO response had a large variability from negative to positive, but the mean value was negative, as seen in the B2 group. In groups C and D the means were above the zero line. Of note is that the amplitude of standard deviation for both TO and TS tended to be smaller as disease progressed.

The values of TO and TS in the B1 group were considered statistically different from groups C and D, while group B2 had similar values to group B1 and groups C and D, making group B2 impossible to differentiate from the others. Results from groups C and D were also statistically similar.

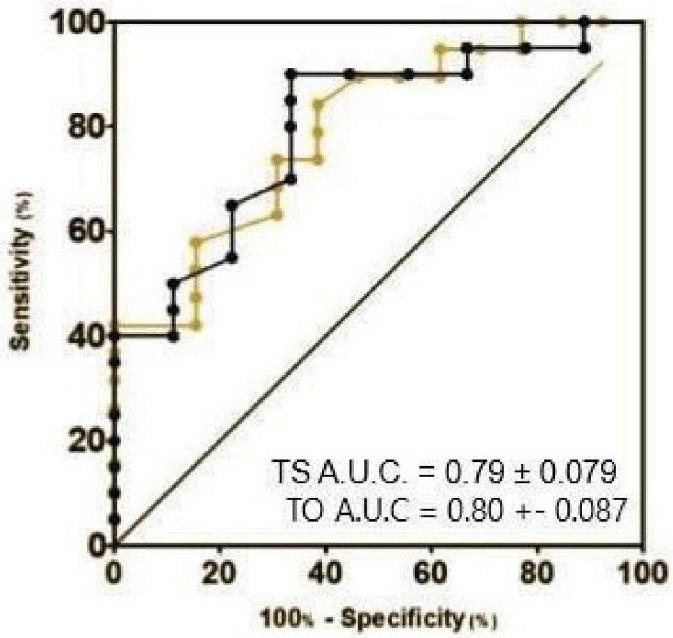
The Receiver Operator Characteristic curve is plotted in figures 3 and 4 as the area under the curve (AUC). In Figure 3 we can evaluate TO and TS ROC Curve showing that asymptomatic and symptomatic dogs can be differentiated with an average area of 0.88 of TO and 0.89 for TS. Figure 4 illustrates the ability to differentiate remodeled hearts (groups B2, C and D) from those unchanged (group B1), for this purpose TO and TS reached the mean value of 0.80 for TO and 0.79 for TS.

FIGURE 3 - R.O.C. FOR DIFFERENTIATE B1/B2 FROM C/D



TS - Turbulence Slope, TS - Turbulence Onset, A.U.C. - Area Under the Curve

FIGURE 4 - R.O.C. FOR DIFFERENTIATE B1 FROM B2/C/D



TS - Turbulence Slope, TS - Turbulence Onset, A.U.C. - Area Under the Curve

Values of TO and TS were also statistically correlated with commonly used echocardiography parameters (statistical significance was set at $P < 0.05$). The results are shown in table 2.

TABLE 2 - CORRELATION BETWEEN HRT PARAMETERS AND ECHOCARDIOGRAPHIC PARAMETERS.

	TO (%)		TS (mm/RR)	
	R	P	R	P
LA/AO	0.4367	0.0178 *	-0.5559	0.0009 *
LVd	0.0290	0.8813	-0.2327	0.1999
LVd-N	0.3410	0.0702	-0.3147	0.0793
LVs	-0.0870	0.6535	-0.1601	0.3814
LVs-N	0.1041	0.5909	-0.2214	0.2233
FS%	0.2513	0.1885	0.0031	0.9864
E	0.4228	0.0223 *	-0.6092	0.0002 *
E/A	0.1269	0.5198	-0.4535	0.0091 *
IVRT	0.0444	0.8191	0.3846	0.0297
E/IVRT	0.1044	0.5900	-0.5443	0.0013 *
E' TDI	0.0689	0.7381	-0.3084	0.0914
E/E'	0.4083	0.0384 *	-0.4068	0.0231 *
TAPSE	-0.0109	0.9596	-0.3463	0.0710

TO - Turbulence Onset. TS - Turbulence Slope. P - Statistical difference. R - Correlation coefficient. LVd-N - Left ventricle diameter in diastole corrected by the body surface area. LVs-N - Left ventricle diameter in systole corrected by the body surface area.* - $p < 0.05$.

Echocardiographic parameters that showed weak correlation to TO were the left atrium/aorta ratio, the left ventricle corrected for body surface area (LVd-N) and the E/E´ ratio. The TS showed a weak negative correlation to the E/A ratio, E/E´ ratio, the maximum velocity of the E´ wave, while moderate negative correlation was found with E/IVRT, maximal E wave velocity and the left atrium/aorta ratio. Weak positive correlation was found between TS and the isovolumetric relaxation time.

Some parameters tended to be correlated, with a p value close to 0.07 eg TO and TS when correlated to the left ventricle in diastole corrected by the

body surface area (LVd-N) and for TAPSE which had a p value of 0.07 when correlated to TS.

4 Discussion

In a study evaluating people with mitral valve prolapse (MVP) both TO and TS were different between normal patients and those with MVP. However, when the prolapse group was split into patients with and without regurgitation there was no difference between the groups. The same study in man also concluded that subjects in MVP and non MVP groups had similar left atrial size. (Gunduz et al, 2006) In contrast with human valve prolapse, dogs have a degenerative disease that tends to worsen with time, this creates large regurgitant jets in the left atrium, which tend to be worse in grades C and D thus increasing left atrium size and LA/AO ratio.

Although not calculated in the present study, it has been proposed in human medicine that HRT parameters should be considered normal when TO < 0% and TS > 25 ms/RR. Patients were further classified as having 0, 1 or 2 HRT parameters affected, and the more changes in HRT the higher the mortality. (Bauer et al, 2008).

Our results suggest that the mean TO value tends to be higher as the patient's condition worsens. The mean values of TO for stages B1 and B2 were <0 while the mean value of stage C and D were >0. These results are similar to those found in dobermann pinschers where the normal group had more negative mean TO values than the group with dilated cardiomyopathy. Those with DCM had TO values similar to those found in DCM+CHF patients. TS results were also significantly different in DCM+CHF group (Harris et al, 2017). In both studies the standard deviation of the HRT parameters reduced as CHF progressed. An interesting observation is that none of the groups with DCM had mean TO value >0, which is in contrast to the patients in this study with grade C and D MMVD. This might be due to the chronicity of MMVD when compared to DCM. Dogs with MMVD have a period of around 766 days after diagnosis before signs of cardiac disease or death. (Boswood, 2016) On other hand Dobermans with DCM usually develop clinical signs around 50 days after diagnosis, while the average survival time in cocker spaniels is 537 days.

Treatment with pimobendam increased survival time of affected dogs.(Luis-Fuentes et al, 2002, Martin et al, 2010)

Mitral regurgitation (MR) results in reduced cardiac output and a consequent increase in sympathetic activity associated with high serum norepinephrine (NE) concentrations (Öztürk et al, 2016), myocardial depletion of NE, abnormal baroreflex and reduced expression of B-adrenergic cell surface receptors. Chronic activation of compensatory mechanism results in a state of unbalanced compensatory response and, once signs of congestive heart failure (CHF) develop, the condition is generally progressive and terminal (Tilley et al, 2008). MR is also responsible for worsening the baroreflex of human patients with untreated MR secondary to left ventricle dysfunction when compared to patients with treated mitral regurgitation. (Öztürk et al, 2016)

When HRT is compared to common anatomic and hemodynamic echocardiographic measurements TO had weak positive correlations while TS had three moderate negative correlations and two negative correlation and a single positive weak correlation.

TO was weakly correlated with the LA/AO ratio and transmitral maximum E wave velocity and the E/E´ ratio showing that the filling pressure influences weakly the TO.

TS was moderately correlated with the LA/Ao and E wave maximal velocity suggesting that diastolic impairment plays a role with atrial dilation and along with high filling pressures. This suggestion is supported by E/IVRT correlation which suggests that the neurohumoral derangement leads to the activation of the sympathetic nervous system and the consequent hypervolemic state has a negative impact on the relation between left ventricular filling time and filling pressure. There was also a weak positive relation between TS and the IVRT.

The TS weak correlation with the E/A ratio and the E/E´ ratio suggest that diastolic function is important in the baroreflex, notably there would be some influence of the myocardial E´ diastolic velocity when we talk about E/E´.

These findings are in agreement with results of other studies that show dogs in CHF have a reduced carotid baroreceptor response to a low pressure situation, ie less HR increase as well as reduced renal, mesenteric and arterial resistance responses. (Higgins et al, 1972)

Two results were close to be considered statistically significant ($p<0.05$) with $p = 0.07$. It is possible that the correlation of TAPSE and TS would reach significance if more patient data were available for statistical analyses. When HRT was correlated with the left ventricular internal diameter in diastole normalized by body surface area (LVd–N), as suggested in EPIC study (Boswood et al, 2016), the results could reach statistically significance with weak correlation if more patient data were available.

The baroreflex response is rapid and influenced by hemodynamic changes and it increases dramatically in human patients after successful percutaneous myocardial intervention. (Bonnemeier et al, 2003) Few electrocardiogram characteristics are able to provide real time strong correlation to the patient's hemodynamic state, with this in mind the HRT indicators could help to evaluate response to treatment in DMVM patients.

This study was not designed to test the influence of coupling interval or compensatory pause on HRT parameters. The role of these two variables is controversial, with some studies showing that they may exert weak influence, while HR rate may have greater effect on HRT results. (Watanabe et al, 2002: Stöckigt et al, 2014)

Considering the prevalence of VPCs in dogs with MMVD, the atrial premature contraction (APC) could be an earlier and more easily identified parameter for detection of SNC activation. Although it may not be as strongly

correlated with SNC activation it has been shown to be useful for stratifying risk after myocardial infarct in human patients. (Wichterle et al, 2004).

The changes in TO and TS could be used for further classification of dogs with MMVD disease. Currently, dogs in stage B2 are distinguished from dogs in stage C by the presence of clinical signs. The signs can be misinterpreted by owners, are highly subjective and some may be seen in association with respiratory disease. (Atkins et al, 2009)

Retrospective collection of data presents some challenges in interpretation. It is difficult to ensure consistent quality of data as the examinations are performed by a number of different operators. Another challenge in data collection is the necessity for VPCs to be present for the HRT calculation, whilst excessive numbers of VPCs can disturb the sinus rhythm. It was hard to acquire sufficient patients in Class D and some suitable patients declined to participate in the study due to the requirement for a 24 hour examination.

It is possible that if more patients had been available for data collection some echocardiographic parameters would have reached statistically significant correlation, as LVd-N was almost significant to TO and TS, while TAPSE showed similar results for TS.

Another limitation of this study was the number of VPCs recorded on the ECG and holter records, unfortunately their number could not be the same for the calculations. HRT could not be calculated on those records without any VPCs, although in human patients the absence of VPCs implies less risk for the patient. (Bauer et al, 2008)

Effect of medication was not evaluated in this study but in man it has been shown that cardiac function may improve 1 year after myocardial infarct and beta-blockade usage. ECGs were recorded 12 months after the infarct

event and only abnormal fractal HR behavior and the slope of HR turbulence still predicted cardiac mortality. (Jokinen, 2003)

5 Conclusions

The behavior of HRT indicators changed over the course of the disease for both parameters evaluated. Although there was statistical overlap between of B2 with B1 and C grades, there were differences between B1 and C and D classes for both the TO and TS. These findings could be used to distinguish the clinical stages after initial B1 stage before remodeling. This was also shown when using the ROC curve area to distinguish class B1 from B2, C and D, with a resulting ROC curve with good AUC. Similar results were found when using TO and TS to distinguish asymptomatic patients in B1 and B2 from symptomatic patients in C and D, but with higher AUC than the last mentioned.

HRT could also be used to distinguish between affected patients in stage C and D from those in stage B1. The ROC curves for TO and TS were similar but TS showed greater and more correlations with echocardiographic indicators. This study shows that HRT is an important indicator of autonomic imbalance in dogs with MMVD especially as severity of cardiac disease increases.

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