









Heart rate variability and quality of life in dogs with mitral valve disease treated with metoprolol

Variabilidade da frequência cardíaca e qualidade de vida em cães com doença valvar mitral tratados com metoprolol

Tamyris Beluque^{1*} , Aparecido Antonio Camacho² , Roberto Navarrete Ampuero² , Jaislane Bastos Braz¹ , Murillo Daparé Kirnew¹ , Raphaela Arantes Marques Canola¹ , Elizabeth Carvalho²  & Marlos Gonçalves Sousa³ 

¹Veterinarian, MSc., Programa de Pós-Graduação em Medicina Veterinária, Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), *campus* Jaboticabal, SP, Brazil.

²Veterinarian, DSc., Programa de Pós-Graduação em Medicina Veterinária, Departamento de Clínica e Cirurgia Veterinária, FCAV, UNESP, *campus* Jaboticabal, SP, Brazil.

³Veterinarian, DSc., Programa de Pós-Graduação em Medicina Veterinária, Departamento de Medicina Veterinária, Universidade Federal do Paraná, Curitiba, PR, Brazil.

Abstract

Mitral valve disease (MVD) is a progressive disease that can reduce cardiac output. Activation of the sympathetic autonomic nervous system is one of the body's first responses in order to maintain cardiac output, but may have deleterious effects on the cardiovascular system. This study investigated the effect of metoprolol on heart rate variability and quality of life in dogs with severe MVD (stage C, according to the guidelines of the American College of Veterinary Internal Medicine). Eight dogs between nine and thirteen years of age were enrolled and screening tests such as complete blood count, serum biochemistry profile, systolic blood pressure, thoracic radiographs, electrocardiogram, echocardiogram and long-term electrocardiography (24 hours) were performed. The patients were treated with enalapril, furosemide, spironolactone, and pimobendan until considered clinically stable, and metoprolol was then added to the therapy. One month later, all animals were re-assessed. Owners responded to a questionnaire about their dog's quality of life before and after beta-blocker therapy. The value for pNN50 (percentage difference between adjacent N-N intervals more than 50 ms) was significantly higher ($P=0.039$) after treatment with metoprolol, indicating higher heart rate variability as indicated by the increased parasympathetic component. Furthermore, quality of life was improved in 30% of patients after metoprolol was initiated. The results suggest that treatment with beta-blockers in dogs with stage C MVD may yield additional benefits in comparison to standard CHF treatment.

Keywords: autonomic nervous system, beta-blockers, holter, pNN50.

Resumo

A doença valvar mitral (DVM) é progressiva e pode levar à redução do débito cardíaco. A ativação do sistema nervoso autônomo simpático é uma das primeiras tentativas para manter o débito cardíaco, no entanto pode culminar em efeitos deletérios para o sistema cardiovascular quando ativada de forma crônica. Este estudo investigou o efeito do metoprolol na variabilidade da frequência cardíaca e qualidade de vida de cães com DVM em estágio C, seguindo a classificação do American College of Veterinary Internal Medicine. Foram avaliados oito cães entre nove e treze anos com DVM de classe C. Foram realizados exames de triagem, tais como hemograma, perfil bioquímico sérico, aferição da pressão arterial sistólica, radiografias torácicas, eletrocardiograma, ecocardiograma e eletrocardiograma de longa duração (24 horas). Os pacientes foram tratados com enalapril, espironolactona, furosemida e pimobendan até a estabilização do quadro e, após, receberam metoprolol durante um mês para então serem submetidos aos mesmos exames. Os tutores responderam um questionário sobre a qualidade de vida de seus cães antes e após a terapia com o betabloqueador. O pNN50 (porcentagem dos intervalos RR adjacentes com diferença de duração maior que 50ms) apresentou aumento significativo ($P=0.039$) após o tratamento com metoprolol, indicando maior variabilidade da frequência cardíaca por permitir a prevalência do sistema nervoso autônomo parassimpático. Além disso, 30% dos pacientes apresentaram melhora na




How to cite: Beluque, T., Camacho, A. A., Ampuero, R. N., Braz, J. B., Kirnew, M. D., Canola, R. A. M., Carvalho, E., & Sousa, M. G. (2021). Heart rate variability and quality of life in dogs with mitral valve disease treated with metoprolol. *Brazilian Journal of Veterinary Medicine*, 43, e003020. <https://doi.org/10.29374/2527-2179.bjvm003020>

Received: November 27, 2020.

Accepted: June 10, 2021.

*Correspondence

Tamyris Beluque
Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias - FCAV, Universidade Estadual Paulista - UNESP
Via de Acesso Professor Paulo Donato Castellane, s/n, Vila Industrial CEP 14884-900 - Jaboticabal, SP, Brasil
E-mail: tamyrisbeluque@gmail.com

 Copyright Beluque et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution and reproduction in any medium provided the original work is properly cited.

qualidade de vida após instituição do metoprolol. Considerando os resultados obtidos é possível inferir que o tratamento com o betabloqueador em cães com doença valvar mitral pode ser considerado uma ferramenta terapêutica adicional na terapia da insuficiência cardíaca congestiva classe C.

Palavras-chave: sistema nervoso autônomo, betabloqueadores, holter, pNN50.

Introduction

Mitral valve disease (MVD) is the most prevalent cardiovascular disease in dogs, affecting up to 42.6% of animals over seven years of age (Santos et al., 2013). This progressive condition often leads to congestive heart failure (CHF), resulting in low cardiac output and hypotension (Triposkiadis et al., 2009).

One of the first responses to reductions in cardiac output and blood pressure is altered autonomic function, which consists of hyperactivity of the sympathetic autonomic nervous system (SANS), to the detriment of the parasympathetic autonomic nervous system (PANS) (Triposkiadis et al., 2009). When cardiac output is impaired, relative hypotension triggers an increase in serum norepinephrine concentration (Jensen et al., 2014), which acts on α 1-adrenergic receptors to augment systemic vascular resistance as well as on β 1-adrenergic receptors, exercising positive inotropic and chronotropic activity (Cunningham, 2004).

Activating the SANS initially benefits the cardiovascular system, but as sympathetic activity becomes chronic several deleterious effects are observed, such as tissue hypoperfusion, left ventricular dysfunction, and greater reduction of cardiac output (Frigerio & Roubina, 2005; Mehta et al., 2003).

In humans, treatment of CHF with beta-blockers has been proven beneficial in combating the harmful effects of chronic SANS activation. The benefits of treatment are decreased global left ventricular remodeling, reduced risk of hospitalization, shorter hospital stays (Klapholz, 2009), improved quality of life, and increased survival (Frigerio & Roubina, 2005; Gheorghide et al., 2003). In contrast, research on the benefits of beta-blockers to treat dogs with endocardiosis is relatively scarce and does not permit definitive conclusions about the effectiveness of this therapy.

The degree of heart rate modulation by the autonomic nervous system (ANS) is most effectively assessed by heart rate variability (HRV). This, in turn, is obtained from long-term electrocardiography (Holter monitoring) (Vanderlei et al., 2009), which is also useful for diagnosing arrhythmias (Rasmussen et al., 2011). More advanced cases of CHF are less influenced by the PANS, with greater interference from the SANS. For this reason, HRV analysis is a very important and accessible tool for understanding the influence of the ANS in the various stages of CHF (Vanderlei et al., 2009).

Due to the consequences of SANS activation, this study was designed to evaluate the effect of metoprolol, a selective β 1 beta-blocker, on HRV and quality of life in dogs with severe MVD.

Material and methods

Samples

Between December 2017 and November 2018, we selected dogs with stage C MVD according to the American College of Veterinary Internal Medicine (ACVIM) consensus guidelines (Atkins et al., 2009). After completion of the study, a new consensus for the diagnosis and treatment of MVD was published (Keene et al., 2019). The patients selected for our study also met the criteria for classification as stage C of the last consensus, since all were symptomatic.

All animals were attended at the Veterinary Hospital of the Faculdade de Ciências Agrárias e Veterinárias (FCAV) - Universidade Estadual Paulista (UNESP), the owners were informed about the study, and after agreeing to include the animals in the experiment signed terms of consent. The study was approved by institutional ethics committee for animal research at FCAV-UNESP three months before research began (protocol number 012225/17).

The echocardiographic inclusion criteria were left atrium/aorta ratio (LA/Ao) \geq 1.6 and left ventricular internal diameter in diastole (LVIDd) above the reference values according to the weight of the animals (Boon, 2011). In the electrocardiographic assessment, animals with sinus arrhythmia, sinus rhythm, or sinus tachycardia without conduction blocks were included. All

animals included in the study presented clinical signs compatible with MVD in the absence of respiratory disease in physical and radiographic evaluations, and systolic blood pressure (SBP) ≥ 120 mmHg and ≤ 159 mmHg (Acierno et al., 2018).

Patients were followed for at least two months. Exclusion factors were concomitant illnesses such as endocrine disorders, liver disease, kidney disease, neoplasms, and infectious diseases. Dogs were evaluated at the beginning of treatment (T0) and again 30 days after the start of CHF therapy and the start of therapy with metoprolol (T1) and 30 days after the start of therapy with the beta-blocker (T2).

Systolic blood pressure assessment

All patients underwent non-invasive systolic blood pressure measurement with the Doppler method (Doppler Flow Detector 812, Oregon, USA) at T0, T1 and T2. Five measurements were taken; the first two were excluded, and the average of the three remaining measurements was calculated. Cuff size was selected to represent 30-40% of the circumference of the patient's thoracic limb (Acierno et al., 2018); either right or left was permissible, but the same limb was used for all measurements (T0, T1, and T3). Patients were kept in the same decubitus position during all measurements. For more restless patients, blood pressure was measured as they sat on the examination table or with the guardian, maintaining the same position during each of the three measuring times (T0, T1, and T3).

Echocardiographic assessment

All patients were evaluated at T0, T1, and T2 using the same echocardiographic device (MyLabTM30 Gold Cardiovascular, Esaote, Italy). Patients were maintained in lateral decubitus to obtain cross-sectional and longitudinal images of the right parasternal window and apical images of the left parasternal window (Boon, 2011). Mitral peak early diastolic filling velocity (E wave) was obtained, as well as early diastolic mitral annular velocity (E') derived from tissue Doppler imaging (TDI), left ventricular internal diameter in diastole (LVIDd) and systole (LVIDs), ejection fraction (EF), fractional shortening (FS) and isovolumetric relaxation time (IVRT), and qualitative assessment of all chambers, myocardium and pericardium.

Long-term electrocardiogram (Holter monitor)

Adhesive electrodes were positioned on the shaved skin surface to capture signals. Two negative electrodes were placed, one on the right chest wall and the other in the manubrium region, and two positive electrodes were also positioned, one on the left chest wall and the other on the xiphoid cartilage. A bandage was applied around the thorax, and the dogs were fitted with a vest to hold the three-channel Cardiolight digital recording device (Cardios Sistemas, São Paulo, Brazil). The animals subsequently wore the device for 24 hours in the owner's home, a familiar environment. This procedure was followed for assessment at T1 and T2; it was not possible to perform this exam at T0 because owners were unwilling to wait for another exam or return over the following days with the Holter monitor.

The cardiac cycles were analyzed using Cardio Manager S540 software (Cardios Sistemas). Rates of HRV assessed over time included standard deviation of all RR intervals (SDNN), standard deviation of the mean RR intervals obtained every five minutes (SDANN), mean standard deviation of the RR intervals obtained every five minutes (SDNNIDX), mean square root of the square of the differences between adjacent RR intervals (rMSSD), percentage of consecutive R-R intervals lasting more than 50 ms (pNN50), and mean duration of RR intervals (mean NN). Normal QRS complexes and arrhythmic events (supraventricular and ventricular) were initially determined by the software, and all morphological patterns and arrhythmias were subsequently corrected by the same person who performed the exams.

Quality of life assessment

The Functional Evaluation of Cardiac Health (FETCH questionnaire) (Freeman et al., 2005) was used to assess quality of life. Owners answered the 17 questions on the questionnaire before the dogs started treatment with metoprolol (T1) and again after one month of treatment with

metoprolol (T2). The responses to the questionnaire use a scale of 0 to 5, with 0 indicating absence of the clinical sign and 5 indicating maximum expression.

Therapeutic protocol

After ruling out concomitant diseases, therapy for CHF was initiated with enalapril maleate (0.5 mg/kg once daily), furosemide (1-2 mg/kg twice daily), and spironolactone (2 mg/kg once daily) at T0. For patients already undergoing treatment with these medications, appropriate dose adjustments were made. The majority (5/8) of the patients were already receiving treatment for CHF, one was treated with only enalapril (1/8), and others received enalapril and furosemide (3/8). The only dog followed at the Veterinary Hospital (FCAV-UNESP) was treated with enalapril, furosemide, spironolactone, and pimobendan for over six months, and the remaining animals were not being treated (3/8). The dog treated with enalapril had received medication for over a year, while the dogs treated with enalapril and furosemide had been receiving medication for a period of 2 (2/8) and 8 (1/8) weeks, respectively. Dogs with systolic dysfunction (5/8) documented by echocardiography also received pimobendan (0.25-0.35 mg/kg twice daily). Patients were reevaluated 30 days after the start of therapy, and metoprolol (0.2 mg/kg twice daily) was then added to the regime.

Statistical analysis

The data obtained were subjected to the Shapiro-Wilk normality test. When Gaussian distribution was found, means and standard deviations were calculated and the paired Student's T test or ANOVA was used for repeated measures, followed by the Tukey multiple comparisons test for comparison between different evaluation times (T1 and T2). The chi-square test was used to analyze the questionnaire. All statistical analyses were performed with R software, and $P < 0.05$ was considered significant.

All evaluations (for example, acquisition of echocardiographic imaging, applying questionnaire to owners, electrocardiography, measurement of systolic blood pressure, and interpreting exams) were performed by the same person (T.B.).

Results

Initially, 18 patients were selected over a period of ten months, but six were lost to follow-up because of the distance to the hospital. Two patients had concomitant illnesses during the experiment, one patient died at the owner's home before starting treatment with metoprolol (unknown cause), and one patient did not continue because the owner was unable to administer the medications properly.

Of the 18 dogs, eight (9-13 years old, 4-12 kg, four males and four females) with stage C MVD according to ACVIM guidelines remained in the study (Keene et al., 2019). All patients had mitral systolic murmur $\geq 3/6$ and LA/Ao ratio ≥ 1.6 . The most frequent clinical signs were cough (7/8; 87.5%) and tiring easily (6/8; 75%). The breeds represented were Poodle (2), Cavalier King Charles Spaniel (1), Brazilian Terrier (1), Maltese (1), Miniature Pinscher (1), Yorkshire (1), and one mixed-breed dog.

Most of the patients were alive for months after the end of the study. Only one patient had acute pulmonary edema 3 months after the end of the study and died at the owner's home a few hours later.

A significant increase was observed in FS ($P=0.02$) at T2 when compared to T0, as well as in EF ($P=0.0008$) (Table 1). There was also a significant decrease in LVIDs ($P=0.002$) and E wave velocity ($P=0.02$) in T2 when compared to T0 (Table 1). There was a statistically significant difference ($P=0.006$) in the LA/Ao ratio in T1 and T2, but there was no statistical difference in the values of LA ($P = 0.41$) and Ao ($P=0.43$) when evaluated individually (Table 1).

The HRV parameter data for dogs before and after treatment with beta-blockers are shown in Table 2. There was a statistically significant increase for pNN50 between T1 and T2 ($P=0.039$). The mean value for NN increased at T2 (Table 2), but the difference was not statistically significant ($P=0.056$). No significant differences were seen in the mean values for minimum HR ($P=0.52$), mean HR ($P=0.06$), or maximum HR ($P=0.55$) at T2.

Supraventricular and ventricular arrhythmias before and after treatment are presented in Table 3. Four patients (50%) had ventricular premature complexes at the first long-term electrocardiogram and four (50%) had supraventricular premature complexes, while only three (37.5%) exhibited both concomitantly. All arrhythmias presented were isolated; no statistically significant change was seen in supraventricular or ventricular arrhythmias at T2 ($P=0.296$ and $P=0.946$, respectively).

Table 1. Echocardiographic parameters and systolic blood pressure obtained in dogs ($n=8$) with stage C mitral valve disease at the beginning of treatment (T0), before (T1) and 30 days after (T2) initiation of therapy with metoprolol.

Parameter	T0	T1	T2	P
SBP (mmHg)	144 ± 28.11	133.57 ± 12.81	138.57 ± 6.26	0.437
FS (%)	47.57 ± 8.10 ^b	51.42 ± 5.41 ^{ab}	54.14 ± 6.14 ^a	0.02
EF (%)	80.38 ± 8.05 ^b	83.38 ± 7.21 ^{ab}	85.37 ± 4.21 ^a	0.0008
IVRT (ms)	31.85 ± 8.07	31.66 ± 9.00	39.50 ± 6.94	0.27
E (m/s)	1.09 ± 0.22 ^a	0.93 ± 0.18 ^b	1.03 ± 0.20 ^{ab}	0.02
E/TRIV	0.03 ± 0.009	0.02 ± 0.012	0.02 ± 0.010	0.12
E' Septal (m/s)	0.07 ± 0.03	0.08 ± 0.04	0.08 ± 0.03	0.826
E'/TRIV Septal	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.435
E' Parietal (m/s)	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.817
E'/IVRT Parietal	0.002 ± 0.0005	0.002 ± 0.0012	0.002 ± 0.0010	0.64
LA (mm)	23.08 ± 5.15	22.82 ± 4.30	23.68 ± 3.84	0.41
Ao (mm)	12.12 ± 1.65	13.03 ± 2.05	11.95 ± 1.12	0.43
LA/Ao	1.87 ± 0.27 ^a	1.71 ± 0.25 ^b	1.87 ± 0.19 ^a	0.006
LVIDd (mm)	33.74 ± 6.55	32.67 ± 6.88	32.80 ± 5.67	0.538
LVIDs (mm)	16.40 ± 3.25 ^a	15.84 ± 3.29 ^{ab}	15.38 ± 3.23 ^b	0.002

SBP: systolic blood pressure; FS: fractional shortening; EF: ejection fraction; IVRT: isovolumetric relaxation time; E: mitral peak early diastolic filling velocity; E': early diastolic mitral annular velocity; LA: left atrium; Ao: aorta; LVIDd: left ventricular internal diameter in diastole; LVIDs: left ventricular internal diameter in systole.

Table 2. Long-term electrocardiography parameters (24-hour Holter) obtained in dogs ($n=8$) with stage C mitral valve disease before (T1) and 30 days after (T2) initiation of therapy with metoprolol.

Parameter	T1	T2	p
Min HR	46.28 ± 10.95	44.42 ± 10.69	0.52
Mean HR	107.57 ± 13.27	96.71 ± 15.39	0.06
Max HR	24.33 ± 8.86	246.66 ± 4.08	0.55
NN	134097.9 ± 29407.27	126858.6 ± 26521.30	0.568
Mean NN (ms)	602.71 ± 90.31	675.42 ± 132.07	0.056
SDNN (ms)	207.42 ± 91.58	258.85 ± 108.71	0.118
SDANN (ms)	139.14 ± 48.27	128.42 ± 37.27	0.51
SDNNIDX (ms)	157.14 ± 88.76	216.57 ± 106.64	0.076
NNN	131305.3 ± 32654.79	126208.4 ± 26555.13	0.718
rMSSD (ms)	104.00 ± 51.90	138.14 ± 39.80	0.308
pNN50 (%)	43.28 ± 20.87 ^b	56.63 ± 14.60 ^a	0.039

T1: stable, before metoprolol; T2: 30 days after starting metoprolol; Min HR: minimum heart rate; Mean HR: mean heart rate; Max HR: maximum heart rate; Mean NN: mean of RR intervals; NN: normal-to-normal RR intervals; NNN: three normal RR intervals; pNN50: percentage of successive RR intervals differing more than 50 ms; SDNN: standard deviation of all RR intervals; SDANN: standard deviation of the means of the RR intervals obtained every five minutes; SDNNIDX: mean of the standard deviations of RR intervals every five minutes; rMSSD: square root of the mean of the square of the differences between adjacent RR intervals. Values accompanied by the same lower-case letters on the same line do not differ from each other.

Table 3. Arrhythmias observed during long-term electrocardiography in dogs (n=8) with stage C mitral valve disease before (T1) and 30 days after (T2) initiation of therapy with metoprolol.

Parameter	T1				T2				P
	Min	Median	Mean	Max	Min	Median	Mean	Max	
SVA	0	1	2.57	11	0	2	6	29	0.296
VA	0	1	182.14	1264	0	0	299.57	1186	0.946

SVA: supraventricular arrhythmias; VA: ventricular arrhythmias.

There was a statistically significant reduction in the FETCH questionnaire score for clinical signs in 37.5% (3/8) of patients when questionnaires were compared individually between T1 and T2 ($P=0.0004$; $P=0.007$; $P=0.006$). Approximately 66% (2/3) of the dogs with reductions in FETCH scores also presented an increase in pNN50 values in T2; however, because there was not sufficient data for testing, it was not possible to assess statistical significance.

Discussion

Prior to therapy with metoprolol, the mean value for pNN50 (shown in Table 2) was similar to the mean value obtained in another study (Oliveira et al., 2012) in dogs with stage C and D MVD. This value was also significantly lower than the mean for healthy patients and patients with MVD without CHF evaluated in this same study, showing that patients with CHF are less influenced by the PANS (Oliveira et al., 2012), which was also corroborated by an additional study (Spier & Meurs, 2004). The mean value for pNN50 increased after therapy with metoprolol and reached the values for healthy animals in other studies (Bogucki & Noszczyk-Nowak, 2015; Oliveira et al., 2012).

HRV parameters in the time domain express the difference between consecutive RR intervals; for example, pNN50 reflects the predominance of vagal action (Bogucki & Noszczyk-Nowak, 2017; Sztajzel, 2004; Zacché et al., 2017), making pNN50 an excellent indicator for assessing the influence of PANS, as in this study. A study in human patients evaluated PANS activity according to pNN50 and found higher values after vagus nerve stimulation (Liu et al., 2017). Consequently, the increase in pNN50 after metoprolol therapy indicates higher HRV, in other words, greater participation of the PANS in the autonomic balance, similar to what occurs in healthy patients (Bogucki & Noszczyk-Nowak, 2015; Oliveira et al., 2012). Although not significant, an increase was seen in the mean NN value after treatment with the beta-blocker, a parameter that also reflects vagal action. It is possible that the short period of treatment with the beta-blocker was not sufficient to permit greater changes in the mean NN between T1 and T2; longer courses of therapy could show more significant changes.

Studies have indicated pNN50 is a good parameter for assessing autonomic balance (Bogucki & Noszczyk-Nowak, 2017; Oliveira et al., 2012), which demonstrates the importance of the statistically significant difference in this variable observed in this present study. Other authors have used this variable to demonstrate more intense performance of the PANS in healthy dogs at rest compared to those that performed physical activity (Blake et al., 2018), which leads us to question whether the effects of metoprolol would have been different in patients with moderate physical activity compared to patients with mild physical activity. Since this was beyond the scope of this present study, activity intensity and duration of the dogs was not evaluated, but could be assessed in future studies. PANS activity was also higher in dogs during various sleep phases in relation to waking periods (Bálint et al., 2019), confirming the need to evaluate electrocardiography over the 24-hour period, because the SANS could have prevailed if the evaluation was performed only when the dogs were most active. Conversely, the PANS could be more dominant if the Holter monitor was in place only when the dogs tended to sleep, altering the real values.

Furthermore, in a comparison between calm dogs and dogs with a history of aggression, more aggressive dogs had lower pNN50 values (Craig et al., 2017); this result is interesting, since two owners reported their dogs had been more irritable for a few months, and that irritability decreased considerably after treatment with metoprolol. For one of these dogs, quality of life according to

the questionnaire improved significantly. Further studies are needed to further investigate the relationship between aggressiveness or irritability and metoprolol in dogs.

Higher pNN50 values were also observed in healthy patients compared to dogs with MVD (Pirintr et al., 2017), in Cavalier King Charles Spaniels without CHF compared to patients already in CHF (Rasmussen et al., 2011), in dogs with advanced MVD without a history of syncope compared to dogs with a history of syncope, and in healthy adolescents compared to those with mitral valve prolapse (Olexova et al., 2020). All these studies cited indicated the lower participation of PANS in patients with MVD, and even lower in cases of CHF. Such findings indicate the importance of studies assessing HRV in dogs with MVD, especially symptomatic animals. Future studies may more concisely define the prognostic role of Holter variables for MVD, and compare them with patients treated with beta-blockers.

In the present study, metoprolol therapy yielded similar values for HRV in the treatment group compared to those of healthy patients included in other studies (Bogucki & Noszczyk-Nowak, 2015; Oliveira et al., 2012), an outcome that was not observed when these patients were only treated with angiotensin-converting enzyme inhibitors, diuretics, and inodilators. Another study (Chompoosan et al., 2014) evaluated pNN50 in patients with MVD before and after 14 days of therapy with enalapril maleate, and found no significant change in this parameter. This finding could corroborate our statement that metoprolol made a major contribution to the increase in HRV, since in the study cited there was no interference with the use of the vasodilator alone. However, the effect of therapy with enalapril associated with a diuretic and inodilator on HRV for a longer period (as in our study) has not been evaluated, and may also have influenced the results observed.

A slight reduction in mean HR was seen after administration of the beta-blocker, although it was not significant. In stabilization of heart disease, the SANS is known to become less stimulated (Rasmussen et al., 2012), and as a result reduced HR is expected.

Interestingly, we found no reduction in arrhythmias one month after starting beta-blocker therapy. The increase in ventricular arrhythmias, although not significant, is believed to result from the evolution of the disease. Valve prolapse can mechanically stimulate the endocardium, and traction exerted by the leaflets on the chordae tendinae and the stretching of the ventricular wall are recognized mechanisms that trigger ventricular arrhythmias (Crosara et al., 2010). This would also explain the greater frequency of ventricular arrhythmias compared to supraventricular arrhythmias. However, the data from this present study contrast with findings from a more recent study (Oliveira et al., 2014) that demonstrated a higher prevalence of supraventricular arrhythmias in dogs with MVD compared to ventricular arrhythmias, and the group of dogs with CHF had a statistically higher number of supraventricular arrhythmias than the group without CHF and the group of healthy dogs.

The quality of life assessment for the patients treated with metoprolol showed significant improvement in more than a third after they started beta-blocker therapy. Two-thirds of the dogs with improved quality of life (according to the FETCH criteria) exhibited changes in HR variability indicative of greater participation by the PANS, represented by an increase in pNN50, although this was not statistically significant. Previous studies in humans have also shown improved quality of life in patients treated with metoprolol (Frigerio & Roubina, 2005; Gheorghiade et al., 2003).

All animals remained stable throughout the study period, and as of their last visit to the veterinary hospital no deterioration was seen in echocardiographic, electrocardiographic, clinical, or SBP parameters. Only one animal died while receiving treatment, 3 months after the end of the study. The dog was a patient of the cardiology service, maintained the routine of return visits, and attended the last appointment a week before death. No systolic dysfunction, hypotension, or any other alteration that could justify this death with the use of the prescribed treatment was seen up to the time of the last visit, nor were there changes indicating CHF progression, since none of the echocardiographic parameters were compatible with the indices of congestion described (Morgan et al., 2020; Schober et al., 2010), and no pattern of congestion was seen in the radiographic evaluation (Oui et al., 2015). A necropsy was not performed since the animal lived in a different city than the study site, but the history of acute dyspnea described by the guardian and the clinical reassessment and recent examinations without worsening of CHF suggest a rupture of the chordae tendinae.

A main concern surrounding beta-blockers is a potential negative effect on hemodynamic balance, since the reduction in HR and inotropism caused by the blockade in β receptors, along with potential blockade in alpha receptors (Plumb, 2011), could further decrease cardiac output (CO). However, the SBP of the dogs in this study remained stable throughout the evaluation period, and no clinical signs compatible with reduced cardiac output were reported by the tutors, nor were there changes in physical examination. This may be due to the selectivity of metoprolol by β receptors (Plumb, 2011), and the safe dose that was used.

Although methods with greater sensitivity for assessing left ventricular systolic function were not utilized, the increase in FS and EF associated with the decrease in LVIDs show that systolic function was not impaired. This was similar to the results obtained in another study (Zacà et al., 2009) that also evaluated the effects of metoprolol in dogs and obtained statistically higher values for EF and lower values for LVID. The minimum dose of metoprolol used may have contributed to these findings in our study, even in patients previously diagnosed with systolic dysfunction as well as patients who did not receive pimobendan.

The present study had some limitations which are common in studies involving clinical patients and owners, but should be considered when interpreting the results. The sample size was small, since over 50% of the patients initially recruited were removed from the study and stage C is not the predominant stage of MVD in our cardiology department. Furthermore, the more advanced age at which patients with MVD usually present clinical signs is also conducive to the onset of other diseases, which excluded many patients. The study period was short, since most owners lived in other cities and were unable to return more often. All patients were selected at the same veterinary hospital, which allows the possibility of bias. Finally, there was no concomitant treatment with a placebo to replace metoprolol, and only one stage of valve disease was used. More evident benefits may be found in other stages of MVD, especially for more extensive therapies and in studies involving larger numbers of patients.

The use of metoprolol should also be evaluated in other classes of MVD in double-blind, placebo-controlled studies while other medications that keep the patient's clinical condition stable are maintained. Assessments involving larger samples and longer treatment periods could demonstrate favorable outcomes from the use of beta-blockers in CHF therapy, especially in patients demonstrating autonomic imbalance during long-term electrocardiography. A longer study period could provide important information on patient survival with and without administration of beta-blockers.

Conclusions

Our study demonstrated that 30 days of metoprolol associated with at least 2 months of classic treatment for severe mitral valve disease improved quality of life and cardiac autonomic function in dogs.

Acknowledgements

We gratefully acknowledge funding from the Brazilian Coordination of Improvement of Higher Level Personnel (CAPES), the work of past and present members of our laboratory, and also the collaboration of the owners who agreed to participate in the research.

Ethics statement

The responsible for the non-human animals formally consented to the study. The study was approved by ethics committee for the use of animals of Faculdade de Ciências Agrárias e Veterinárias (FCAV) of Universidade Estadual Paulista (UNESP), with protocol number 012225/17.

Financial support

TB - received a scholarship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), AAC, RNA, JBB, MDK, RAMC, EC, MGS - none.

Conflict of interests

The authors declare no conflicts of interest.

Authors' contributions

TB - She contributed with design of the work, and performed consultations and exams on patients, prescribed medications, applied the questionnaires to the owners, analyzed and interpreted the data of the work and wrote the manuscript. AAC and MGS - They supervised the conduct of the research, carried out the conception and design of the work, helped the other authors to analyze and interpret the data of the work. RNA, JBB, MDK, RAMC, EC - They participated in the analysis of the exams obtained and interpretation of the questionnaires. They also helped to perform the exams and assisted in the conduct with each patient. All authors reviewed the manuscript before submission, contributed to the writing and approved the final version to submission.

Availability of complementary results

The readers can access any complementary information from the authors on request.

The study was carried out at Veterinary Hospital of Faculdade de Ciências Agrárias e Veterinárias (FCAV) of Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brasil.

References

- Acierno, 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(6), 1803-1822. <http://dx.doi.org/10.1111/jvim.15331>. PMID:30353952.
- Atkins, C., Bonagura, J., Ettinger, S., Fox, P., Gordon, S., Haggstrom, J., Hamlin, R., Keene, B., Luis-Fuentes, V., & Stepien, R. (2009). Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *Journal of Veterinary Internal Medicine*, 23(6), 1142-1150. <http://dx.doi.org/10.1111/j.1939-1676.2009.0392.x>. PMID:19780929.
- Bálint, A., Eleőd, H., Körmendi, J., Bódizs, R., Reicher, V., & Gácsi, M. (2019). Potential physiological parameters to indicate inner states in dogs: the analysis of ecg, and respiratory signal during different sleep phases. *Frontiers in Behavioral Neuroscience*, 13(207), 207. <http://dx.doi.org/10.3389/fnbeh.2019.00207>. PMID:31607871.
- Blake, R. R., Shaw, D. J., Culshaw, G. J., & Martinez-Pereira, Y. (2018). Poincaré plots as a measure of heart rate variability in healthy dogs. *Journal of Veterinary Cardiology*, 20(1), 20-32. <http://dx.doi.org/10.1016/j.jvc.2017.10.006>. PMID:29277470.
- Bogucki, S. Z., & Noszczyk-Nowak, A. (2015). Short-term heart rate variability (HRV) in healthy dogs. *Polish Journal of Veterinary Sciences*, 18(2), 307-312. <http://dx.doi.org/10.1515/pjvs-2015-0040>. PMID:26172180.
- Bogucki, S. Z., & Noszczyk-Nowak, A. (2017). Short-term heart rate variability in dogs with sick sinus syndrome or chronic mitral valve disease as compared to healthy controls. *Polish Journal of Veterinary Sciences*, 20(1), 167-172. <http://dx.doi.org/10.1515/pjvs-2017-0021>. PMID:28525326.
- Boon, J. (2011). *Veterinary echocardiography* (2nd ed.). Chichester: Wiley-Blackwell.
- Chompoosan, C., Buranakarl, C., Chaiyabutr, N., & Chansaisakorn, W. (2014). Decreased sympathetic tone after short-term treatment with enalapril in dogs with mild chronic mitral valve disease. *Research in Veterinary Science*, 96(2), 347-354. <http://dx.doi.org/10.1016/j.rvsc.2014.01.006>. PMID:24559801.
- Craig, L., Meyers-Manor, J. E., Anders, K., Sütterlin, S., & Miller, H. (2017). The relationship between heart rate variability and canine aggression. *Applied Animal Behaviour Science*, 188(1), 59-67. <http://dx.doi.org/10.1016/j.applanim.2016.12.015>.
- Crosara, S., Borgarelli, M., Perego, M., Häggström, J., La Rosa, G., Tarducci, A., Santilli, R. A. (2010). Holter monitoring in 36 dogs with myxomatous mitral valve disease. *Australian Veterinary Journal*, 88(10), 386-392. <http://doi:10.1111/j.1751-0813.2010.00628.x>.
- Cunningham, J. G. (2004). *Tratado de fisiologia veterinária* (3. ed). Rio de Janeiro: Guanabara Koogan.
- Freeman, L. M., Rush, J. E., Farabaugh, A. E., & Must, A. (2005). Development and evaluation of a questionnaire for assessing health-related quality of life in dogs with cardiac disease. *Journal of the American Veterinary Medical Association*, 226(11), 1864-1868. <http://dx.doi.org/10.2460/javma.2005.226.1864>. PMID:15934254.
- Frigerio, M., & Roubina, E. (2005). Drugs for left ventricular remodeling in heart failure. *The American Journal of Cardiology*, 96(12A), 10L-18L. <http://dx.doi.org/10.1016/j.amjcard.2005.09.060>. PMID:16399088.
- Gheorghide, M., Colucci, W. S., & Swedberg, K. (2003). Beta-blockers in chronic heart failure. *Circulation*, 107(12), 1570-1575. <http://dx.doi.org/10.1161/01.CIR.0000065187.80707.18>. PMID:12668487.
- Jensen, B. C., O'Connell, T. D., & Simpson, P. C. (2014). Alpha-1-adrenergic receptors in heart failure: the adaptive arm of the cardiac response to chronic catecholamine stimulation. *Journal of Cardiovascular Pharmacology*, 63(4), 291-301. <http://dx.doi.org/10.1097/FJC.000000000000032>. PMID:24145181.

- Keene, B. W., Atkins, C. E., Bonagura, J. D., Fox, P. R., Häggström, J., Fuentes, V. L., Oyama, M. A., Rush, J. E., Stepien, R., & Uechi, M. (2019). ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. *Journal of Veterinary Internal Medicine*, 33(3), 1127-1140. <http://dx.doi.org/10.1111/jvim.15488>. PMID:30974015.
- Klapholz, M. (2009). Beta-blocker use for the stages of heart failure. *Mayo Clinic Proceedings*, 84(8), 718-729. <http://dx.doi.org/10.4065/84.8.718>. PMID:19648389.
- Liu, H., Yang, Z., Huang, L., Qu, W., Hao, H., & Li, L. (2017). Heart-rate variability indices as predictors of the response to vagus nerve stimulation in patients with drug-resistant epilepsy. *Epilepsia*, 58(6), 1015-1022. <http://dx.doi.org/10.1111/epi.13738>. PMID:28440954.
- Mehta, R. H., Supiano, M. A., Oral, H., Grossman, P. M., Montgomery, D. S., Smith, M. J., & Starling, M. R. (2003). Compared with control subjects, the systemic sympathetic nervous system is activated in patients with mitral regurgitation. *American Heart Journal*, 145(6), 1078-1085. [http://dx.doi.org/10.1016/S0002-8703\(03\)00111-X](http://dx.doi.org/10.1016/S0002-8703(03)00111-X). PMID:12796766.
- Morgan, K. R. S., Monteith, G., Raheb, S., Colpitts, M., & Fonfara, S. (2020). Echocardiographic parameters for the assessment of congestive heart failure in dogs with myxomatous mitral valve disease and moderate to severe mitral regurgitation. *Veterinary Journal*, 263, 105518. <http://dx.doi.org/10.1016/j.tvjl.2020.105518>. PMID:32928487.
- Olexova, L. B., Sekaninova, N., Jurko Junior, A., Visnovcova, Z., Grendar, M., Jurko, T., & Tonhajzerova, I. (2020). Respiratory sinus arrhythmia as an index of cardiac vagal control in mitral valve prolapse. *Physiological Research*, 69(1, Suppl. 1), S163-S169. <http://dx.doi.org/10.33549/physiolres.934402>. PMID:32228022.
- Oliveira, M. S., Muzzi, R. A. L., Araújo, R. B., Muzzi, L. A. L., Ferreira, D. F., Nogueira, R., & Silva, E. F. (2012). Heart rate variability parameters of myxomatous mitral valve disease in dogs with and without heart failure obtained using 24-hour Holter electrocardiography. *The Veterinary Record*, 170(24), 622-625. <http://dx.doi.org/10.1136/vr.100202>. PMID:22645158.
- Oliveira, M. S., Muzzi, R. A. L., Araújo, R. B., Muzzi, L. A. L., Ferreira, D. F., & Silva, E. F. (2014). Heart rate variability and arrhythmias evaluated with Holter in dogs with degenerative mitral valve disease. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 66(2), 425-432. <http://dx.doi.org/10.1590/1678-41626097>.
- Oui, H., Oh, J., Keh, S., Lee, G., Jeon, S., Kim, H., Yoon, J., & Choi, J. (2015). Measurements of the pulmonary vasculature on thoracic radiographs in healthy dogs compared to dogs with mitral regurgitation. *Veterinary Radiology & Ultrasound*, 56(3), 251-256. <http://dx.doi.org/10.1111/vru.12234>. PMID:25545202.
- Pirintr, P., Saengklub, N., Limprasutr, V., Sawangkoon, S., & Kijawornrat, A. (2017). Sildenafil improves heart rate variability in dogs with asymptomatic myxomatous mitral valve degeneration. *The Journal of Veterinary Medical Science*, 79(9), 1480-1488. <http://dx.doi.org/10.1292/jvms.17-0016>. PMID:28717064.
- Plumb, D. C. (2011). *Plumb's veterinary drug handbook* (7th ed.). Hoboken: Wiley-Blackwell.
- Rasmussen, C. E., Falk, T., Zois, N. E., Moesgaard, S. G., Häggström, J., Pedersen, H. D., Ablad, B., Nilsen, H. Y., & Olsen, L. H. (2012). Heart rate, heart rate variability, and arrhythmias in dogs with myxomatous mitral valve disease. *Journal of Veterinary Internal Medicine*, 26(1), 76-84. <http://dx.doi.org/10.1111/j.1939-1676.2011.00842.x>. PMID:22151356.
- Rasmussen, C. E., Vesterholm, S., Ludvigsen, T. P., Haggstrom, J., Pedersen, H. D., Moesgaard, S. G., & Olsen, L. H. (2011). Holter monitoring in clinically healthy Cavalier King Charles Spaniels, Wire-haired Dachshunds, and Cairn Terriers. *Journal of Veterinary Internal Medicine*, 25(3), 460-468. <http://dx.doi.org/10.1111/j.1939-1676.2011.0707.x>. PMID:21418322.
- Santos, W. F., Aben Athar, C. V., Lopes, J. V. R., Santos, I. O. M. G., & Paiva, J. P. (2013). Prevalência da doença valvar degenerativa crônica de mitral e da bronquite crônica diagnosticadas em avaliação pré-operatória em cães (*Canis familiaris*) atendidos no Hospital Veterinário da Universidade Federal Rural do Rio de Janeiro no período de março. *Revista de Educação Continuada em Medicina Veterinária e Zootecnia do CRMV-SP*, 11(2), 87.
- Schober, K. E., Hart, T. M., Stern, J. A., Li, X., Samii, V. F., Zekas, L. J., Scansen, B. A., & Bonagura, J. D. (2010). Detection of congestive heart failure in dogs by doppler echocardiography. *Journal of Veterinary Internal Medicine*, 24(6), 1358-1368. <http://dx.doi.org/10.1111/j.1939-1676.2010.0592.x>. PMID:20840304.
- Spier, A. W., & Meurs, K. M. (2004). Assessment of heart rate variability in Boxers with arrhythmogenic right ventricular cardiomyopathy. *Journal of the American Veterinary Medical Association*, 224(4), 534-537. <http://dx.doi.org/10.2460/javma.2004.224.534>. PMID:14989545.
- Sztajzel, J. (2004). Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. *Swiss Medical Weekly*, 134(35-36), 514-522.
- Tripodiadis, F., Karayannis, G., Giamouzis, G., Skoularigis, J., Louridas, G., & Butler, J. (2009). The sympathetic nervous system in heart failure: physiology, pathophysiology and clinical implications. *Journal of the American College of Cardiology*, 54(19), 1747-1762. <http://dx.doi.org/10.1016/j.jacc.2009.05.015>. PMID:19874988.
- Vanderlei, L. C., Pastre, C. M., Hoshi, R. A., Carvalho, T. D., & Godoy, M. F. (2009). Basic notions of heart rate variability and its clinical applicability. *Revista Brasileira de Cirurgia Cardiovascular*, 24(2), 205-217. <http://dx.doi.org/10.1590/S0102-76382009000200018>. PMID:19768301.
- Zacá, V., Rastogi, S., Mishra, S., Wang, M., Sharov, V. G., Gupta, R. C., Goldstein, S., & Sabbah, H. N. (2009). Atenolol is inferior to metoprolol in improving left ventricular function and preventing ventricular remodeling in dogs with heart failure. *Cardiology*, 112(4), 294-302. <http://dx.doi.org/10.1159/000159123>. PMID:18832825.
- Zacché, E., Assumpção, T. C. A., Corsini, T. B., & Camacho, A. A. (2017). Time domain heart rate variability in Boxer dogs with arrhythmogenic right ventricular cardiomyopathy. *Ciência Rural*, 47(5), 1-6. <http://dx.doi.org/10.1590/0103-8478cr20160740>.