Preliminary Study of Coupling Intervals of Premature Ventricular Complexes in Dogs With Different Cardiac Diseases

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Abstract

Coupling interval (CI), the time (ms) from the onset of a sinus QRS to the onset of the following premature ventricular complex (PVC), and their variability (CIV) might predict mortality and elucidate mechanisms of arrhythmogenesis. There has been limited investigation of CIV in dogs. Therefore, we determined CIV and prematurity index (PI) in three groups of dogs with ventricular arrhythmias that were subject to 24 hour ambulatory electrocardiographic (Holter) monitoring. Dogs in group 1 had presumptive arrhythmogenic right ventricular cardiomyopathy (ARVC), those in group 2 had structural heart disease in which patients with valvular heart disease predominated, and those in group 3 had a dilated cardiomyopathy (DCM) either phenotype or presumed familial cardiomyopathy. In this preliminary study, we did not find significant differences in indices of CIV between groups. Median PI was lower in dogs treated with antiarrhythmic therapy. Severity of cardiac remodeling, except for left atrial to aortic ratio, were not correlated with CIV. It was not possible to determine the mechanism of arrhythmias in ARVC, DCM phenotype or structural heart disease groups and re-entry, triggered activity, and abnormal automaticity are possible etiologies. The effect of antiarrhythmic therapy demonstrated potential drug effect on CIV. Risk for malignant arrhythmias and sudden cardiac death were not examined. A larger study would be needed to determine if differences exist; if present, this would give insight into possible mechanisms and optimal antiarrhythmic therapy.

Introduction

Coupling interval (CI) refers to the duration (ms) from the onset of the sinus QRS complex preceding a premature ventricular complex (PVC) to the onset of the PVC. Sudden cardiac death in humans (SCD), and presumably in animals, occurs after the electrical cascade of ventricular tachycardia degenerates into ventricular fibrillation and then asystole (Murakoshi and Aonuma 2013). Risk stratification of SCD in cardiac disease and decision for an intra-cardiac device (ICD) placement, are the focus of most investigation into human ventricular arrhythmias (VA) (Murakoshi and Aonuma 2013). The causes of SCD in humans, very broadly, are either primary arrhythmias such as those caused by channelopathies, or secondary to structural heart disease or an ischemic event (myocardial infarction). Historically, cardiologists used 'form and frequency' of PVCs in predicting epidemiological risk and formulating therapeutic strategies. Throwing doubt at the significance of R-on-T phenomenon, Thanavaro et al. (1983) and Lowery et al. (1990) examined the association of coupling interval, PVC morphology and repetitive forms. The former examined 10 hour ECG recordings from patients, at least 2-weeks after a confirmed acute myocardial ischemic event (Thanavaro et al. 1983). Early and late PVCs rather than intermediate duration coupling interval PVCs were associated with repetitive ventricular ectopic beats, demonstrating that the entire diastolic cardiac cycle is vulnerable to extrasystolic stimulation. Lowery's study (1990) investigated CI variability (CIV), the measure of the difference between maximum and minimum R-R interval of sequential PVCs, also referred to in other texts as ΔCI , and its association with different QRS morphologies. In addition, whether or not repetitive forms developed and the number of complexes of each repetitive event were also examined (Lowery et al. 1990). The decision making tree of

interpreting PVC morphology and CIV, found that patients with fixed CI, lacked both repetitive forms, and multiformity, unlike patients with variable CI had multiformity and tended to have repetitive forms more frequently. The explanation given, was that the variable QRS morphology was due to temporal dispersion of triggering events; if this occurred in a vulnerable period, a repetitive event could develop. The bimodal distribution of this pattern prompted the authors to propose the role of CIV in risk stratify patients with PVCs and potentially guide treatment recommendations.

The ability to prognosticate using CIV has been subject to contradictory findings. Sosnowski et al. (2004) demonstrated that high CIV, measured by the standard deviation (SD) of normal sinus beat to PVC coupling interval (SDNV), was associated with the probability of cardiac death in a two-year follow-up study of coronary heart disease patients that had undergone a 24-hour ECG for the investigation of a complaint of palpitations. In contrast, Lerma et al. (2013) demonstrated that the presence of a high prevalence of PVCs in 24-hour ECGs in patients, 6 week after myocardial infarction, with low CI variability, was considered a risk marker of fatal or near-fatal arrhythmias. In 2015, Lee et al. (2015) examined the morphologies and CIV of PVCs in two groups of patients categorized according to their cardiac mortality (alive or dead), with either ischemic or dilated cardiomyopathy. The mean and standard deviation of all coupling intervals of VPCs were compared to the time domain variables of the normal sinus beats intervals to determine CIV. This study found measures of CIV, including SD, were higher in the group of patients that did not survive the follow-up period of 63 months due to cardiac related deaths.

Variability of CI has been examined in the setting of categorizing cardiac morbidity associated with PVCs. PVC burden alone cannot predict if left ventricular (LV) dysfunction develops in people. Kawamura et al. (2014) examined the role of CIV, referred to in their paper as coupling interval dispersion, calculated as the difference between measured maximum and minimum CI in ms, in predicting PVC induced cardiomyopathy. Recruited patients had successful radiofrequency ablation (RFA) of apparent PVCs and no apparent cause of cardiomyopathy, presumably idiopathic. The end-point was characterizing the left ventricular ejection fraction (LVEF), $LVEF \geq 50\%$ as normal, and $< 50\%$ as LV dysfunction. Patients with reduced LVEF, not only had higher PVC burden and body mass index, but also significantly longer CI and CI dispersion as compared to those patients with normal LV function. The longer CI was proposed to cause LV dysfunction by reducing the LV filling time for the subsequent sinus beat, creating ventricular dyssynchrony, and increased oxygen consumption. Sosnowski et al. (2004) showed a similar association in the aforementioned study, a higher percent mortality rate was found in the subgroup of patients with reduced LVEF and coronary heart disease that had higher SDNV > 80 ms as compared to similar patients with lower SDNV.

Ambulatory ECG in humans have used measurement of CI and CIV to assign arrhythmia mechanism (Maruyama and Fukata 2015). Two proposed mechanism of high CI variability (CIV) are, reentry, where PVCs exit from multiple reentry circuits with different velocities, exit at different time points and create a wide variation of CI; or due to triggered activity in damaged tissue with less calcium handling or basic tissue responsiveness. Enhanced automaticity as a mechanism for arrhythmias that could also produce PVCs with variable CI can exist if there is unequal counterbalancing of the sodium and calcium current

and the background hyperpolarized potassium current although in general, automaticity is associated with lower CIV or fixed CI. Measuring CIV on electrocardiograms has been proposed to be a non-invasive method of predicting, not only the mechanism, but also anatomical location of arrhythmogenicity (Bradfield et al. 2014; de Vries et al. 2018; Qin et al. 2018). In people, idiopathic outflow tract premature ventricular contractions (IOT-PVCs), a condition that is considered to be benign, is characterized by PVCs originating from the greater arteries and semilunar valves (Bradfield et al. 2014; Qin et al. 2018). In some instances, patients suffer sustained ventricular tachycardia and require RFA in a bid to prevent the development of cardiomyopathy. In the first study, CIV was measured by ΔCI , calculated as the difference in ms between the maximum and minimum CI of the first 12 consecutive VPCs and the origin of the PVCs was determined by electrophysiology (EP) mapping (Bradfield et al. 2014). A marked discrepancy was found in ΔCI , with PVCs of greater vessels and sinus of Valsalva having high CIV, and PVCs of left or right ventricular origin having fixed CI interval (variation does not exceed 40–80 ms). In a second study examining IOT-PVCs, patients that were found to have PVCs originating from the left outflow tract, later confirmed on EP mapping, had higher CI variability (CIV > 100 ms). The importance of these discriminatory findings is that it could reduce time of EP mapping of patients prior to RFA. Another study examined the association of cardiac disease with CIV to determine if CIV predicts the mechanism of arrhythmogenicity (de Vries et al. 2018). The CIV was calculated for each group of patients identified as having either idiopathic ventricular arrhythmias (VA) without structural heart disease, post-myocardial ischemia (MI), non-ischemic dilated cardiomyopathy (NIDCM) and patients classed as familial cardiomyopathy caused by mutation encoding for phospholambin or lamin AC (PLN/LMNA), who were selected from an inherited channelopathy and cardiomyopathy database (de Vries et al. 2018). Despite the mechanism of arrhythmia likely being different between the post-MI, scar-related macro re-entry, and idiopathic VA, likely caused by focal triggered activity, the CIV was identical between these groups. The NIDCM group had similar CIV to the idiopathic VA and post-MI groups from which the authors concluded that despite heterogeneity in etiology of the VA the mechanism of arrhythmogenicity might be similar i.e. either micro- or macro re-entry or triggered activity. The group with familial cardiomyopathy (PLN/LMNA mutation) had higher median CIV, and the authors proposed that a mechanism different from re-entry or focal-triggered that was likely abnormal automaticity. A potential benefit of using CIV to predict the underlying arrhythmia mechanism is that targeted therapy can be selected. In the case of abnormal automaticity, RFA is indicated. In the case of re-entry, interrupting re-entry with a class III antiarrhythmic drug would be more appropriate (AlMahameed and Ziv 2019). In the case of triggered activity, early or delayed afterdepolarization can be suppressed with ranolazine (Murray 2016).

Arrhythmogenic mechanisms and targeted therapy for ventricular arrhythmias in different forms of heart diseases are not well documented in naturally acquired diseases of dogs. One exception is inherited ventricular arrhythmia in the German shepherd, which is proposed to result from triggered activity (early or delayed depolarizations) which manifests during periods of bradycardia (Jesty et al. 2013). The other exception is the English bulldog with ventricular arrhythmias from the right ventricular outflow tract, which is proposed to be a re-entrant mechanism (Santilli et al. 2011).

This study compared CIV, by measuring ΔCI , between dogs diagnosed with either arrhythmogenic right ventricular cardiomyopathy (ARVC), structural heart disease, or dilated cardiomyopathy phenotype. The primary objective of this study was to test the hypothesis that there was significant difference in CIV between the different cardiac diseases groups associated with VA. In addition, mean prematurity index (PI) for PVCs was calculated for each group and compared between groups. A secondary objective was to examine the effect of sotalol on CI variability by comparing indices of variability between boxers treated with sotalol as compared to those dogs not receiving sotalol at the time of Holter recording. Lastly, selected echocardiographic measurements were examined to determine if they correlated to CIV.

Materials And Methods

This was a retrospective study and conducted at one facility.

Animals

Medical records of canine patients presented to the UT-CVM Cardiology service between October 2015 and October 2019 and subject to 24 hour ambulatory electrocardiographic (Holter) monitoring were reviewed. 24-Hour ECG (Holter) examinations were conducted either as part of a screening test for familial cardiomyopathy or for investigation of a clinical sign that could be ascribable to a cardiac disease for example exercise intolerance or syncope. Individual rhythm strips were saved electronically with the help of computer software that generated automatic event summary. Case data were included in analyses if 24-hours of Holter data were available, and if a minimum of 10 PVCs with a distinct QRS morphology in at least two channels were identified in these saved electronic rhythm strips. Cases in which the coupling interval could not be verified by manually counting coupling intervals on the electronic ECG strip on a computer monitor, were excluded. Concurrent arrhythmias, specifically atrial fibrillation would qualify for exclusion due to the inability to calculate sinus R-R interval. Cases with no apparent cause of the arrhythmia were also excluded i.e. presumed non-cardiac etiology. The medical record was reviewed and the signalment of the dog was collected. Antiarrhythmic therapy was recorded, as was the diagnosis at the time of the Holter examination. Based on their diagnosis by the attending clinician from the medical records, the dogs were classified as either group 1 arrhythmogenic right ventricular cardiomyopathy (ARVC), group 2 structural heart disease, and finally group 3 DCM phenotype.

Electrocardiographic analyses

Coupling interval, the duration (milliseconds, ms) from the onset of the sinus QRS complex preceding the premature ventricular complex (PVC) to the onset of the PVC, was determined for the first 10 PVCs of each recording without regard to the actual time the rhythm strip was recorded with the hope that comparable numbers of day and night-time values could be sampled. Each digitally generated CI was verified by measuring the interval on an electronic pdf version of the ECG strip (rate of 25 mm/s) before being recorded in the excel data sheet. This was calculated by counting the small blocks representing millimeters (mm) between a sinus R wave and a PVC R wave (N-V) and then converting this length (mm) into duration (ms). If there was machine error in calculating coupling interval, then a manual count was

substituted. The RR interval preceding the PVC was determined in a similar manner, and the CI was divided by the preceding RR interval, to provide a prematurity index (PI). Coupling interval variability was measured by ΔCI (ms), which was calculated as the difference between the highest and lowest CI duration recorded for each patient's CI data set as described previously (de Vries et al. 2018). The PI for each patient was calculated as per the description by Carvalho et al (2018) with the exception that the median of all 10 VPCs rather than the mean of three of the five VPCs was generated in the calculation of the patient PI. In addition, the correlation between CI and preceding sinus cycle length was examined using the medians of the 10 sinus cycle lengths (CL) and the 10 CI that were included in the calculation of PI. The primary investigator was aware of the diagnosis at the time of measuring the described indices.

Echocardiographic analyses

The data of echocardiographic exams of the animals included in our study were also reviewed and the numerical data were abstracted from the medical record. A complete echocardiogram had to have been performed by boarded cardiologists or cardiology resident supervised by a boarded cardiologist for results to be included. The following indices were included left atrium-to-aorta ratio (LA:Ao), left ventricular end diastolic diameter indexed to body size, and fractional shortening (FS). The LA:Ao was obtained from right parasternal short-axis images as previously described (Rishniw and Erb 2000). End-diastolic left ventricular internal dimension (LVIDd) and end-systolic left ventricular internal dimension (LVIDs) were from M-mode recordings guided by two dimensional right parasternal short axis images. LVIDd was indexed to body size (LVIDDn) using the formula described in the literature (Cornell et al. 2004). Fractional shortening was calculated from LVIDd and LVIDs obtained from M-mode images as described above, using the standard formula: $FS = [(LVIDd - LVIDs) / LVIDd] \times 100\%$.

Statistical analysis

Statistical analyses were performed using commercially available computer software. Distributions of continuous variables were graphically evaluated and determined based on the appearance of histograms and normal quantile plots. Variables for which the distributions were approximately normal are presented as mean ($\pm$ standard deviation). Those that were not normally distributed are presented as median (range). Count data are presented as absolute values and/or proportions expressed as percentages. Continuous variables were compared across categories using the Kruskal-Wallis test or Mann-Whitney Test. Associations between continuous variables were quantified by Spearman's correlation coefficient. Alpha was set to a value 0.05

Results

Twenty-six canine patients met inclusion criteria; the mean $\pm$ SD body weight was 29.8 ± 11.2 kg, and the median age (range) was 9 (3–16) years. Eighteen (69%) were male of which 4 (22.2%) were sexually intact. One (12.5%) of the females was sexually intact. Based on clinical findings, each of the 26 dogs

was assigned to one of three groups: Group 1 (ARVC, n = 14), Group 2 (DCM, n = 6) and Group 3 (Structural Cardiac Disease, n = 6). Zoographic variables and antiarrhythmic therapy administered at the time of Holter recording for each of these groups is presented in Table 1. Most (66.7%) of those in Group 3 had degenerative mitral valve disease, but two (33.3%) had echocardiographically identified peri-aortic masses.

Table 1

Characteristics of 26 dogs assigned to one of three groups based on clinical findings. Group 1 = Arrhythmogenic Right Ventricular Cardiomyopathy, Group 2 = Dilated Cardiomyopathy, Group 3 = Structural Cardiac Disease. Continuous variables are expressed as median (range), count data are expressed by number and percentage.

	Group 1	Group 2	Group 3
Sex (M/F)	9/5	5/1	4/2
Breed (n/%)	Boxer dog (14/100%)	Doberman (4 / 67%) ShihTzu (1 / 16.7%) Standard Poodle (1/16.7%)	Boxer Dog (1/16.7%) English bulldog (1/16.7%) Mix Breed 93/50%)
Body Weight (kg)	32.0 (22.7–40.4)	36 (3.8-55.45)	21.9 (11-28.6)
Antiarrhythmic Therapy	Sotalol (8/57%) Sotalol and Mexiletine (2/14.2%)	None (6/0%)	None (4/66.7%) Sotalol (2/33%)

When values from the entire study sample were included, median PI was 0.65 ± 0.15 and ΔCI was 138.5 (23–805) ms. These variables did not significantly differ when compared across groups (Fig. 1). There was a weak negative correlation between ΔCI and LA:Ao ($\rho = -0.391$, $p = 0.048$) but otherwise, significant relationships between echocardiographic variables and median PI or ΔCI were not identified. There was a strong correlation ($\rho = 0.85$, $p = 0.0001$) between sinus CL and CI. Median PI of patients that received antiarrhythmic therapy were less than median PI of patients that did not receive antiarrhythmic agents (Fig. 2). Sotalol was administered to all patients that received antiarrhythmic therapy, but two of these also received mexiletine; when these two patients were excluded from the analysis, the effect of antiarrhythmic therapy was not significant ($p = 0.08$). Sinus CL of patients that received antiarrhythmic therapy was greater than sinus CL of patients that did not receive antiarrhythmic therapy ($p = 0.0029$).

Discussion

In this population of dogs with PVCs, the calculated median PI and ΔCI , were not significantly different between groups. Coupling interval variability, proposed to offer insight into the mechanism of arrhythmia, and potentially guide selection on the most appropriate class of anti-arrhythmic therapy, was not helpful in differentiating the mechanism of PVCs in three different groups of heart disease in dogs. Due to the overlap of the CIV measurements between the disease categories, it lacks discriminatory power in the

individual patient. Of all echocardiographic measurements, only LA:Ao was significantly (negatively) correlated with CIV. There was also a strong correlation ($\rho = 0.85$, $p = 0.0001$) between sinus CL and CI. Median PI of patients that received antiarrhythmic therapy was less than median PI of patients that did not receive antiarrhythmic agents; and the median sinus CL of patients that received antiarrhythmic therapy was greater than the sinus CL of dogs that did not. The finding of a reduced PI in dogs treated with antiarrhythmic drugs, in this group is likely due to longer preceding CL and slowing of the heart rate caused by administration of sotalol.

The method of measuring CIV by ΔCI in our study was similar to that described by de Vries et al. (2018), Lowery et al. (1990) and Kawamura et al. (2014). Lowery's study calculated the ΔCI from all PVCs on a 24-hour ECG (Lowery et al. 1990). Kawamura et al. (2014) measured CI from all PVCs obtained on a 12 lead ECG from the EP study. Our study methodology was similar to that described by de Vries et al. (2018) with a few notable differences. Our study was measured by one observer, in contrast to two physicians in their study to examine inter-observer variability. Their study demonstrated acceptable inter- and intra-observer variability, tested by two observers, measuring CI from the groups with NIDCM and idiopathic PVCs. Inter- and intra-observer variability was described as very good and good respectively for the NIDCM and idiopathic PVC groups, showing good agreement on Bland-Altman plots for both groups without bias regarding CI measurements (de Vries et al. 2018). This same study showed that hand measurement of CI had an accuracy of 20 ms. In our study, CI were reported by the 24-hour ECG, but all measurements manually checked, as on occasion errors in measurements of the N-V were noted by the ECG software unable to identify an R wave of a PVC, and summation of a sinus R with the R wave of the sinus beat subsequent to the PVC, including the N-V interval, as the recorded measurement, but manual measurements with calipers was not performed. CIV can also be calculated as the SD of the ratio of CI to the root square of R-R interval of normal sinus beat ($CI/\sqrt{R-R}$) as was reported in de Vries' study (de Vries et al. 2018). Computation of heart rate variability is often performed by Poincaré plots, a graphical representation of the correlation between successive R-R intervals as a function of N-N intervals (RRn), but in the case of PVCs, the N-V interval (CI) is displayed as function of the preceding N-N interval. This was software was custom-made for the study by Lee et al. (2015) to allow graphical depiction of the SD of all N-V intervals (SD (ms) for both normal and N-V intervals were expressed as a ratio of SDnv/SDnn. Although, this technique would have likely been a superior technique, if not visually easier to appreciate variability, it was not an option for our 24-hour ambulatory ECG interpretation. We therefore did not consider SD measures and did not account for the influence of heart rate variability except by examining the mean PI. Our study, did attempt to correct the CIs for heart rate variability by examining the median PI although no significant differences were found between diseases groups. PI was decreased in boxers receiving antiarrhythmics and this was likely due to the longer preceding sinus interval rather than a lower CI. PI was described as a novel technique by Carvalho et al. (2018) for distinguishing benign versus malignant PVCs in dogs with degenerative mitral valve disease. In humans, despite suggestions that short CI results in polymorphic malignant ventricular tachycardia, a study by Igarashi et al. (2012) found this not to be the case, and that rather PI was an independent determinant of malignant VA. A small PI is due to a short N-V interval preceded by a relatively long R-R interval, indicating that both heart rate and CI

characteristics are important for initiation of a malignant VA. Their study selected 5 PVCs at random as they were limited by a 3-minute 12 lead ECG for measurements, our study selected the first 10 PVCs on a 24-hour Holter. The reason for our selection was, that some of the dogs only had infrequent PVCs. The criticism of this is that in many dogs with a high PVC burden, the PVCs were likely to be selected very early in the 24 hour ECG, and those with a low burden, this could have been more spread throughout the day. Although the intent was to avoid the effect of sleep on measures such as PI, this could have been the case for those dogs with low PVC burden.

The results of this CI variability in canine PVC study, differed to the findings in a human study comparing ΔCI between patients with different etiologies of PVCs (de Vries et al. 2018). The ΔCI for human patients with familial cardiomyopathy secondary to a lamin A/C or phospholamban (PLN) mutation, a form of arrhythmic cardiomyopathy, was highly variable, unlike patients with post myocardial ischemia, non-ischemic dilated cardiomyopathy and idiopathic right ventricular outflow tract ventricular tachycardia (RVOT-VT), a non-familial benign condition, which all had low ΔCI (de Vries et al. 2018). It is not possible to say if canine ARVC, a familial cardiomyopathy, has a similar mechanism of automaticity as reflected by the CI variability found in our population of dogs. ARVC has an autosomal dominant mode of inheritance in Boxers and English Bulldogs (Meurs 2017). In humans, PLN mutation familial cardiomyopathy, is similar to ARVC in that the founder mutation PLN-R14Del, is identical (van Opbergen et al. 2017). Santilli et al. (2009) described a case of a middle aged English Bulldog with non-sustained monomorphic ventricular tachycardia (VT) of right ventricular outflow tract (RVOT) origin which also had fibrofatty infiltration in the RVOT on necropsy (Santilli et al. 2009). The dog was treated with amiodarone for 8 weeks without success and after an additional 16 weeks, developed congestive heart failure and then ventricular fibrillation. Another study (2011) described a syndrome of segmental ARVC and RVOT VT in the English bulldogs where the changes on the 12 lead ECG, Holter and echocardiogram were compared in 5 dogs. One dog in that study had electrophysiologic (EP) mapping followed by radiofrequency ablation (Santilli et al. 2011). The etiology of the VT in this dog was a segmental arrhythmogenic right ventricular cardiomyopathy (ARVC) phenotypically characterized by a RVOT enlargement on echocardiography. In the EP study, the mechanism was ascertained to be a re-entrant VT. During an isoproterenol infusion, no PVCs nor VT was observed. In humans RVOT VT is idiopathic, but is suspected to be an embryonic remnant, as the heart is structurally normal, but the site of arrhythmia is focal in origin (Kobayashi 2018). The mechanism is either re-entrant, triggered or enhanced automaticity with the re-entrant form being verapamil sensitive. The English Bulldogs with RVOT VT and ARVC described by Santilli et al. (2009 and 2011), failed to respond to class III anti-arrhythmic therapy but successfully treated with RF ablation. The author proposed two diseases, each with a separate substrate of arrhythmia in this breed and therefore the interpretation of re-entrant cannot be extrapolated to the boxer breed with ARVC (Santilli et al. 2011, Santilli et al. 2009). It is likely that ARVC in Boxers shares a similar mechanism of arrhythmia as humans with arrhythmogenic cardiomyopathy and a larger study may be necessary to demonstrate a higher CI variability in this group of dogs.

A negative, albeit weak, correlation was found between LA:Ao and ΔCI ($\rho = -0.391$, $p = 0.048$). There was no apparent association between the size of the ventricle or fractional shortening and PI or CIV of PVCs in

patients with VA. In other studies, more advanced heart failure had negative correlation of cardiac cycle length i.e. faster heart rate, leading to greater PI values and showed a negative correlation of echocardiographic parameters with PI (Carvalho et al. 2018). The likely difference, is the preselection bias of the two studies. Our population of dogs was not categorized according to severity of heart disease but rather likely etiology of VA; their study assigned the category of asymptomatic versus symptomatic. Another potential reason is that our study included many boxer dogs that were screened as healthy dogs in contrast to degenerative valve disease dogs that likely had more advanced disease for VA to develop.

In the current study, most of the boxers with ARVC were treated prior to the recording of the Holter. The use of class III anti-arrhythmic therapy did not significantly influence the CIV, although it was reduced compared to those not treated. As mentioned above, antiarrhythmic therapy was associated with a lower median PI; when sotalol alone was considered i.e. when two dogs receiving combination mexiletine and sotalol were excluded, this finding was not appreciated. The use of sotalol appeared to decrease PI value of PVCs, but this index is influenced by the preceding CL and therefore heart rate, and does not necessarily shorten the instantaneous heart rate of a PCV. Interestingly, Carvalho et al. (2018) study examining PI in dogs with acquired degenerative valve disease and VA, found a greater PI was documented more frequently during periods of paroxysmal ventricular arrhythmias, and repeating pattern of PVCs, in other words malignant arrhythmias. Sotalol is known to increase the risk of Torsades des Pointes in dogs with triggered arrhythmia mechanism of automaticity, such as familial ventricular arrhythmias in German shepherds (Jesty et al. 2013). Although survival and drug toxicity was not examined in this study, it could be argued the smaller PI in boxers treated with sotalol increase the risk for Torsades des Pointes, although this is speculation. Risk of VT and sudden cardiac death has not been definitively determined in humans or dogs with non-ischemic myocardial disease; however, it is generally accepted that considerable arrhythmia burden can influence quality of life; therefore, the treatment of PVCs can alleviate morbidity. In boxers, the presence of ventricular tachycardia, greater than 50 single PVCs/24 hours, or PVC complexity, polymorphic morphology on Holter but not bigeminy, trigeminy, couplet or triplets, was associated with increased risk of cardiac death (Motskula et al. 2013). Outcome measured by survival in boxers diagnosed with ARVC and treated with an anti-arrhythmic is similar between treatment with a class III drug, sotalol and a class Ia drug, mexiletine (Caro-Vadillo et al. 2013). Counterintuitive to thinking that anti-arrhythmic therapy reduces risk of mortality by reducing arrhythmia burden, the Cardiac Arrhythmia Suppression Trial demonstrated flecainide and encainide actually increased the risk of mortality in post-myocardial ischemia patients (Epstein et al. 1993). Mortality was not investigated in this group of dogs. Although the end-point of mortality was what was recorded in the medical record, either from information from the primary veterinarian or another service and details regarding sudden cardiac death or congestive heart failure could not be ascertained.

The main limitation of this study was insufficient number of dogs in groups 2 and 3, as well as their likely lack of homogeneity, with the DCM phenotype and structural heart disease group having half the number of dogs compared to ARVC group. Similarly, the low number of dogs could also account for a potential type II error in comparing the PI between groups, in which no differences were found. The number of PVCs counted per patient was low, and precision would have been superior if 1000 per dog were used.

The timing of PVC measurement could have created bias as mentioned above. The likelihood is that only daytime hours, soon after placement of the Holter and when the dog was active was recorded in most dogs, and although unintentional, may have avoided the circadian effect on variability or the influence of autonomic effect on propagating PVCs. Due to the retrospective nature of this study, the final diagnosis was not standardized and variability in the diagnosis could have influenced the results. Similarly, the arrhythmia diagnosis was not confirmed with electrophysiology studies in any of the patients. Another important limitation is that many of the dogs with clinical signs were already on a variety of anti-arrhythmic drugs as significant ventricular dysrhythmias were diagnosed prior to therapeutic monitoring with a 24-hour Holter, which could influence the coupling interval variability. The 25 mm/s speed on the Holters could also have introduced an element of imprecision in measurement. Electrophysiology studies would have provided information on the mechanism of the arrhythmias and provided a gold-standard for comparison. Examining CIV has its limitations. Reproducibility of this measurement has not been demonstrated. Bias in CI measurement could have crept in as the observer that measured CI was not blinded to the group assigned to each 24-hour ECG, it would have been obvious from the signalment that familial diseases were more likely. Lastly, ventricular premature morphology and the relationship with CI variability was not examined.

Lastly, the categorization of DCM in group 3 in the Doberman group was likely allocated based on examination of the Holter results and at the discretion of the attending veterinarian rather than echocardiographic findings. The reason is that three of the six dogs in this category, and all Doberman pinchers, did not have LV dilation, at least based on the indexed LVIDd; LVIDdn > 1.7 has been used as a criterion for enlargement in mitral valve disease (Boswood et al. 2016). The categorization of left ventricular dilation could have been based on end systolic and diastolic measurements exceeding breed reference intervals for Doberman pinchers (Wess et al. 2017). Two of the boxer dogs in the group ARVC exceeded the upper limit of LVIDDn and might have had a “DCM phenotype” considering their FS%, which calls into question the appropriate allocation of grouping. Of course, LV dilation is part of the syndrome of ARVC and might be the “true” genetic expression of the striatin mutation, but this is rare and this group represents a surprising prevalence (13%) of chamber dilation for a boxer cardiomyopathy group. The reason for the potential overestimation of LV dimensions could be related to the M-mode dimensions over-estimated the LV minor axis because of obliquity of the guiding image. It can be difficult to direct the M-mode beam perpendicular to the septum in short-axis images in deep-chested dogs. Lastly, the English bulldog classed as group 2 could also represent a misclassification, as although the dog was diagnosed with degenerative valve disease as per its medical records with severely enlarged left atrium, ARVC as a cause of arrhythmia could not be excluded without necropsy.

Conclusion

Coupling interval variability (Δ CI) and median PI in dogs with VA, were no different between different groups of cardiac diseases, in this preliminary study conducted at a single center. Neither PI nor Δ CI could aid in elucidating if re-entry, triggered activity or abnormal automaticity was the mechanisms of arrhythmia in ARVC. Except for LA:Ao, which was negatively correlated, echocardiographic changes were

not associated with CIV. Antiarrhythmic therapy, appeared to decrease the PI in treated boxer dogs diagnosed with ARVC.

Declarations

Authorship All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Liza S. Köster, and Jonathan Abbott. The first draft of the manuscript was written by Liza S. Köster and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest/Competing interests The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability (software application or custom code) n/a

Ethics approval This work involved the use of non-experimental animals only (including owned or unowned animals and data from prospective or retrospective studies). Established internationally recognized high standards ('best practice') of individual veterinary clinical patient care were followed. Ethical approval from a committee was therefore not specifically required for publication.

Consent to participate Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken.

Consent for publication No animals or humans are identifiable in this publication, and therefore no written consent was required for publication.

References

AlMahameed ST,Ziv O (2019) Ventricular Arrhythmias. Med Clin North Am 103(5): 881-895. <https://doi.org/10.1016/j.mcna.2019.05.008>

Boswood A, Haggstrom J, Gordon SG et al (2016) Effect of Pimobendan in Dogs with Preclinical Myxomatous Mitral Valve Disease and Cardiomegaly: The EPIC Study-A Randomized Clinical Trial. J Vet Intern Med 30(6): 1765-1779. <https://doi.org/10.1111/jvim.14586>

- Bradfield JS, Homsí M, Shivkumar K et al (2014) Coupling interval variability differentiates ventricular ectopic complexes arising in the aortic sinus of valsalva and great cardiac vein from other sources: mechanistic and arrhythmic risk implications. *J Am Coll Cardiol* 63(20): 2151-2158. <https://doi.org/10.1016/j.jacc.2014.02.551>
- Caro-Vadillo A, Garcia-Guasch L, Carreton E et al (2013) Arrhythmogenic right ventricular cardiomyopathy in boxer dogs: a retrospective study of survival. *Vet Rec* 172(10): 268. <https://doi.org/10.1136/vr.100937>
- Carvalho ER, Ampuero RAN, Tuleski GLR et al (2018) Polymorphism, coupling interval and prematurity index in dogs with degenerative mitral valve disease and ventricular arrhythmias. *Vet Res Commun* 42(2): 153-160. <https://doi.org/10.1007/s11259-018-9718-0>
- Cornell CC, Kittleson MD, Della Torre P et al (2004) Allometric scaling of M-mode cardiac measurements in normal adult dogs. *J Vet Intern Med* 18(3): 311-321. [https://doi.org/10.1892/0891-6640\(2004\)18<311:asomcm>2.0.co;2](https://doi.org/10.1892/0891-6640(2004)18<311:asomcm>2.0.co;2)
- de Vries LJ, Martirosyan M, van Domburg RT et al (2018) Coupling interval variability of premature ventricular contractions in patients with different underlying pathology: an insight into the arrhythmia mechanism. *J Interv Card Electrophysiol* 51(1): 25-33. <https://doi.org/10.1007/s10840-017-0309-8>
- Epstein AE, Hallstrom AP, Rogers WJ et al (1993) Mortality following ventricular arrhythmia suppression by encainide, flecainide, and moricizine after myocardial infarction. The original design concept of the Cardiac Arrhythmia Suppression Trial (CAST). *J Am Med Assoc* 270(20): 2451-2455.
- Igarashi M, Tada H, Kurosaki K et al (2012) Electrocardiographic determinants of the polymorphic QRS morphology in idiopathic right ventricular outflow tract tachycardia. *J Cardiovasc Electrophysiol* 23(5): 521-526. <https://doi.org/10.1111/j.1540-8167.2011.02232.x>
- Jesty SA, Jung SW, Cordeiro JM et al (2013) Cardiomyocyte calcium cycling in a naturally occurring German shepherd dog model of inherited ventricular arrhythmia and sudden cardiac death. *J Vet Cardiol* 15(1): 5-14. <https://doi.org/10.1016/j.jvc.2012.07.005>
- Kawamura M, Badhwar N, Vedantham V et al (2014) Coupling interval dispersion and body mass index are independent predictors of idiopathic premature ventricular complex-induced cardiomyopathy. *J Cardiovasc Electrophysiol* 25(7): 756-762. <https://doi.org/10.1111/jce.12391>
- Kobayashi Y (2018) Idiopathic Ventricular Premature Contraction and Ventricular Tachycardia: Distribution of the Origin, Diagnostic Algorithm, and Catheter Ablation. *J Nippon Med Sch* 85(2): 87-94. https://doi.org/10.1272/jnms.2018_85-14
- Lee CH, Park KH, Nam JH et al (2015) Increased variability of the coupling interval of premature ventricular contractions as a predictor of cardiac mortality in patients with left ventricular dysfunction. *Circ J* 79(11): 2360-2366. <https://doi.org/10.1253/circj.CJ-15-0732>

Lerma C, Gorelick A, Ghanem RN et al (2013) Patterns of ectopy leading to increased risk of fatal or near-fatal cardiac arrhythmia in patients with depressed left ventricular function after an acute myocardial infarction. *Europace* 15(9): 1304-1312. <https://doi.org/10.1093/europace/eus415>

Lowery M, de Marchena EJ, Castellanos A et al (1990) Interrelationship of variable coupling, multiformity and repetitive forms: implications for classification of ventricular arrhythmias. *Am Heart J* 119(2 Pt 1): 301-307. [https://doi.org/10.1016/s0002-8703\(05\)80020-1](https://doi.org/10.1016/s0002-8703(05)80020-1)

Maruyama T, Fukata M (2015) Increased coupling interval variability – mechanistic, diagnostic and prognostic implication of premature ventricular contractions and underlying heart diseases. *Circ J* 79(11): 2317-2319. <https://doi.org/10.1253/circj.CJ-15-0963>

Meurs KM (2017) Arrhythmogenic Right Ventricular Cardiomyopathy in the Boxer Dog: An Update. *Vet Clin North Am Small Anim Pract* 47(5): 1103-1111. <https://doi.org/10.1016/j.cvsm.2017.04.007>

Motskula PF, Linney C, Palermo V et al (2013) Prognostic value of 24-hour ambulatory ECG (Holter) monitoring in Boxer dogs. *J Vet Intern Med* 27(4): 904-912. <https://doi.org/10.1111/jvim.12107>

Murakoshi N, Aonuma K (2013) Epidemiology of arrhythmias and sudden cardiac death in Asia. *Circ J* 77(10): 2419-2431. <https://doi.org/10.1253/circj.cj-13-1129>

Murray GL (2016) Ranolazine is an Effective and Safe Treatment of Adults with Symptomatic Premature Ventricular Contractions due to Triggered Ectopy. *Int J Angiol* 25(4): 247-251. <https://doi.org/10.1055/s-0036-1584880>

Qin F, Zhao Y, Bai F et al (2018) Coupling interval variability: A new diagnostic method for distinguishing left from right ventricular outflow tract origin in idiopathic outflow tract premature ventricular contractions patients with precordial R/S transition at lead V3. *Int J Cardiol* 269: 126-132. <https://doi.org/10.1016/j.ijcard.2018.07.045>

Rishniw M, Erb HN (2000) Evaluation of four 2-dimensional echocardiographic methods of assessing left atrial size in dogs. *J Vet Intern Med* 14(4): 429-435. [https://doi.org/10.1892/0891-6640\(2000\)014<0429:eofemo>2.3.co;2](https://doi.org/10.1892/0891-6640(2000)014<0429:eofemo>2.3.co;2)

Santilli RA, Bontempi LV, Perego M (2011) Ventricular tachycardia in English bulldogs with localised right ventricular outflow tract enlargement. *J Small Anim Pract* 52(11): 574-580. <https://doi.org/10.1111/j.1748-5827.2011.01109.x>

Santilli RA, Bontempi LV, Perego M et al (2009) Outflow tract segmental arrhythmogenic right ventricular cardiomyopathy in an English Bulldog. *J Vet Cardiol* 11(1): 47-51. <https://doi.org/10.1016/j.jvc.2009.03.006>

Sosnowski M, Skrzypek-Wanha J, Korzeniowska B et al (2004) Increased variability of the coupling interval of premature ventricular beats may help to identify high-risk patients with coronary artery

disease. *Int J Cardiol* 94(1): 53-59. <https://doi.org/10.1016/j.ijcard.2003.04.006>

Thanavaro S, Kleiger RE, Miller JP et al (1983) Coupling interval and types of ventricular ectopic activity associated with ventricular runs. *Am Heart J* 106(3): 484-491. [https://doi.org/10.1016/0002-8703\(83\)90690-7](https://doi.org/10.1016/0002-8703(83)90690-7)

van Opbergen CJ, Delmar M, van Veen TA (2017) Potential new mechanisms of pro-arrhythmia in arrhythmogenic cardiomyopathy: focus on calcium sensitive pathways. *Neth Heart J* 25(3): 157-169. <https://doi.org/10.1007/s12471-017-0946-7>

Wess G, Domenech O, Dukes-McEwan J et al (2017) European Society of Veterinary Cardiology screening guidelines for dilated cardiomyopathy in Doberman Pinschers. *J Vet Cardiol* 19(5): 405-415. <https://doi.org/10.1016/j.jvc.2017.08.006>

Figures

Figure 1

a. Median PI obtained from 26 dogs subject to Holter monitoring graphically displayed as box plots. The vertical limits of the boxes represent the 1st and 3rd quartiles, the horizontal line within the box represents the median value. “Whiskers” extend to the outermost value that is within 1.5 times the interquartile range above or below the limits of the boxes. Values that are greater or less than the limits of the whiskers are shown by individual data points. Group 1 = ARVC, Group 2 = Dilated Cardiomyopathy, Group 3 =

Structural Cardiac Disease. Statistically significant differences between groups were not identified by the Kruskal-Wallis test ($p = .57$). b. ΔCI obtained from 26 dogs subject to Holter monitoring graphically displayed as box plots. The vertical limits of the boxes represent the 1st and 3rd quartiles, the horizontal line within the box represents the median value. "Whiskers" extend to the outermost value that is within 1.5 times the interquartile range above or below the limits of the boxes. Values that are greater or less than the limits of the whiskers are shown by individual data points. Group 1 = ARVC, Group 2 = Dilated Cardiomyopathy, Group 3 = Structural Cardiac Disease. Statistically significant differences between groups were not identified by the Kruskal-Wallis test ($p = .52$).

Figure 2

Median PI obtained from 26 dogs subject to Holter monitoring graphically displayed as box plots categorized based on whether antiarrhythmic agents were (1) or were not (0) administered prior to data collection. The vertical limits of the boxes represent the 1st and 3rd quartiles, the horizontal line within the box represents the median value. "Whiskers" extend to the outermost value that is within 1.5 times the

interquartile range above or below the limits of the boxes. Values that are greater or less than the limits of the whiskers are shown by individual data points. Based on the result of a Mann-Whitney U Test, median PI from patients that did not receive anti arrhythmic agents were greater than median PI of patients that did ($p=0.048$).