Conclusions—This novel PVC animal model demonstrates that frequent PVCs alone can induce a reversible form of cardiomyopathy. Moreover, other observational and nonrandomized studies demonstrated improvement of LV function in otherwise structurally normal hearts. PVC-induced cardiomyopathy lacks gross histopathologic and mitochondrial abnormalities seen in other canine models of cardiomyopathy. (Circ Arrhythm Electrophysiol. 2011;4:543-549.)

Key Words: premature ventricular contractions ■ LV dysfunction ■ cardiomyopathy ■ ventricular bigeminy ■ PVC-induced cardiomyopathy

Premature ventricular contractions (PVCs) are a very common entity associated with cardiomyopathy (CM) and other cardiac diseases, and yet their effects on the cardiovascular system are not well understood. Although the presence of PVCs may carry adverse prognosis, especially in structural heart disease, PVCs in general are thought to be benign or secondary to the cardiomyopathic process. Recently, a few small studies have reported a relationship between high PVC burden and left ventricular (LV) systolic dysfunction.1–4 Moreover, other observational and nonrandomized studies demonstrated improvement of LV function after a successful PVC suppression strategy.1–8 These observations led to the description of an entity called PVC-induced CM.1–6 Consequently, a current clinical conundrum is to recognize when PVCs are responsible for the development of a CM or secondary to a CM.8

Clinical Perspective on p 549

The cardiovascular effects of PVCs have not been prospectively or systematically studied primarily because of the lack of an animal model and the unpredictability and variability of PVCs in the clinical setting.10,11 We describe a novel PVC animal model with a unique pacemaker algorithm to demonstrate the link between frequent PVCs and LV systolic dysfunction.

Methods

Animal Preparation and Pacemaker Implantation

Under general anesthesia, 14 mongrel dogs (2 to 3 years old; weight, 35 to 45 lbs) underwent implantation of an experimental pacemaker (details are in the online-only Data Supplement). Through a left thoracotomy, a bipolar epicardial single-lead (Medtronic model 4968) was sutured in the right ventricular (RV) apex. Appropriate lead position...
The dogs were randomly assigned to either PVC (n=7, enabled premature pacing algorithm) or control groups (n=6, disabled premature pacing algorithm). The control group had a device programmed for sensing only (ODO). After group assignment and initiation of PVC protocol, an echocardiogram was repeated at 2, 4, 8, and 12 weeks to follow changes in LV function in all dogs. Twenty-four-hour, continuous, 3-lead Holter monitoring were obtained before initiation of PVC protocol (outlined below). One animal was excluded from the cohort because of a baseline abnormal LV ejection fraction (EF).

Simulation of Frequent PVCs
A persistent high PVC burden originating from the RV apex was simulated through premature pacing algorithm (described below). Premature pacing algorithm was programmed to deliver PVCs in a bigeminal pattern (1:1 ratio or 50% PVC burden) with a fixed coupling interval of 250 ms (240 bpm) after each intrinsic ventricular sensed event (Figure 1). Pacing threshold was obtained during implantation and on a biweekly basis. Pacing voltage output was programmed at least twice the diastolic threshold to ensure ventricular capture.

Novel Premature Pacing Algorithm
A single-chamber pacemaker (St Jude Medical, Inc, St Paul, MN) with a unique experimental algorithm (patent submitted) was developed to reproduce chronic exposure to frequent PVCs. This pacing algorithm, when enabled in a single-chamber ventricular-lead pacemaker (VVI), can simulate PVCs (Figure 1). PVC burden is programmable from 5% up to 75%, as the algorithm will introduce 1 to 3 pacing stimuli after a chosen number of sensed events (online-only Data Supplement Table 1). For instance, 75% PVC burden is reproduced by introducing 3 pacing stimuli after each sensed events (3:1 ratio), whereas 50%, 25%, and 20% PVC burden is reproduced with 1 pacing stimulus after 1, 3, and 4 sensed events, respectively (Figure 2 A through 2D).

The PVC coupling interval may be programmed as either fixed (programmable between 190 to 375 ms) or adaptive. The adaptive coupling interval is determined as a percentage of average cycle length of prior cardiac sensed events (Figure 2). In contrast to fixed coupling interval, adaptive coupling interval can only be used when premature pacing burden is programmed to <33% (as a minimum of 2 R-R intervals are needed to obtain an average cycle length of cardiac sensed events).

Figure 1. Intracardiac electrograms and markers before and after the premature pacing algorithm is enabled. Initial tracing demonstrates normal sinus rhythm (cycle length close to 500 ms, 120 bpm). After premature pacing algorithm is enabled, a ventricular paced (VP) event occurs 250 ms (240 bpm) after every ventricular sensed (VS) event, reproducing ventricular bigeminy. Furthermore, a compensatory pause can be noted after the paced event.

Figure 2. Examples of the programmability of the premature pacing algorithm in a single canine. A, Premature pacing algorithm programmed to 75% ventricular pacing (VP) burden (3 VP of 4 beats) with 200-ms fixed coupling interval. B, Premature pacing algorithm programmed to 50% VP burden (1 VP of 2 beats, ventricular bigeminy) with 300-ms fixed coupling interval. An adaptive coupling interval (CI) cannot be programmed because of the lack of at least 2 intrinsic R-R intervals. C, Premature pacing algorithm set for 20% PVC burden (1 VP of 5 beats, ventricular pentageminy) and 55% adaptive CI. The initial tracing demonstrates an average cycle length of 2 prior R-R=450 ms, thus VP is delivered at 247 ms (55% of average R-R). Later portion of same panel demonstrates a longer average intrinsic R-R interval (660 ms) with subsequent VP delivered at 55% (363 ms). D, 25% PVC burden and 65% adaptive CI. The initial tracing has an average R-R interval=1080 ms, thus VP is delivered at 702 ms, whereas the last segment has an average R-R interval=650 ms with a VP triggered at 409 ms. R indicates intrinsic ventricular sensed event.
commercially available system (Sequoia c256 Siemens). Animals assigned to the recovery phase had additional echocardiograms at 2 and 4 weeks after discontinuation of PVCs. LVEF, fractional shortening (FS), end-systolic and end-diastolic LV and left atrial dimensions, LV thickness, LV compliance (E/A and E/E’ ratios), and the severity of mitral regurgitation were evaluated using standard criteria by the American Society of Echocardiography12,13 (online-only Data Supplement). Tissue Doppler imaging was used to assess the timing of local contractility (QRS-to-contraction) in 4 different LV locations in reference to the QRS complex (lateral base, septal base, mid lateral wall, and midseptum). LV dysynchrony was assessed by the standard deviation of QRS-to contraction time between these locations.14 PVCs were suspended (algorithm disabled) at least 15 minutes before echocardiography to obtain an accurate calculation of described parameters. The echocardiographic measurements were performed offline by a cardiologist blinded to the randomization arm.

**Ventricular Effective Refractory Period**
Programmed ventricular stimulation (S1S2) with different drive trains (S1; 300 ms, 350 ms, and 400 ms) were performed to determine ventricular effective refractory period (VERP). VERP was defined as the longest S1S2 that did not cause myocardial capture.

**Myocardial Microscopic Evaluation**
Four different LV samples (2 from the LV apex and 2 from the LV anterior wall) in each canine (PVC group, n = 4; control group, n = 3; online-only Data Supplement Figure 1) were obtained and stained with hematoxylin and eosin and Masson trichrome to assess inflammation and fibrosis, respectively. Leukocytic infiltrates, grade of fibrosis (score 0 to 4+), and percentage of fibrosis were assessed in 10 random fields per sample (×10 and ×40 magnification, respectively). Two LV samples (LV apex and anterior wall) in each animal underwent the TUNEL (terminal deoxynucleotide transferase-mediated nick-end labeling assay) technique (DNA fragmentation, Oncor, Gaithersburg, MD) to assess apoptosis as previously described.15 Apoptotic nuclei were counted in 4 to 5 random fields per sample. The apoptotic index was expressed as the number of apoptotic cells of all cardiomyocytes per field16 (online-only Data Supplement).

**Isolation and Analysis of Cardiac Mitochondria**
Cardiac mitochondria were isolated and analyzed according to Palmer et al,17 with minor modifications as previously published.18,19 Oxygen consumption in sarcoplasmal and inter-fibrillar mitochondria were measured using a Clark-type oxygen electrode at 30°C with glutamate (complex I substrate) and succinate+rotenone (complex II substrate).18 Further details are provided in the online-only Data Supplement.

**Statistical Analysis**
Sample size was calculated to reach statistical power of 80% with type I error of 0.05 for LV dysfunction after a 12-week period of ventricular bigeminy (details are in the online-only Data Supplement). All data are expressed in mean±SD. Statistical analysis was performed with the use of SAS/STAT Software (SAS Institute, Inc, Cary, NC). A repeated-measures ANOVA was performed to compare temporal changes in LVEF between study and control groups. We calculated the change score (Δ) from baseline to 12 weeks for all echocardiographic and VERP data for each animal in the PVC and control groups. A 2-sample t test was used to compare the mean change score at 12 weeks between PVC and control groups. The degree of mitral regurgitation at 12 weeks was compared between PVC and control groups by use of the Mann-Whitney U test. A probability value <0.05 was considered significant.

**Results**

**Premature Pacing Algorithm**
Thirteen animals underwent device implantation without surgical complications. Canines were randomized to PVC (n = 7) or control (n = 6, without PVCs) groups. No device or algorithm malfunctions were noted during the study. Twenty-four-hour Holter monitoring showed ventricular bigeminy in the frequent PVC group with an average PVC burden of 49.8±0.01% compared with 0.01±0.001% in the control group (P<0.001). The PVC protocol increased the average heart rate from 81±7 to 130±13 bpm (P<0.001). Figure 3 illustrates ventricular bigeminy with a 2-lead ECG tracing and rate histogram in a single animal before and after premature pacing algorithm is enabled.

**Cardiac Function**
Echocardiographic findings are summarized in the Table. A new LV systolic dysfunction was observed in the PVC group after 12 weeks of ventricular bigeminy with a 34% relative reduction in LVEF (Figure 4), a 45% decrease in FS, and a 39% increase in LV end-systolic dimension (LVESD) (Table). In contrast, no changes in LVEF, FS, or LVESD were noted in the control group. LVEF and FS were significantly lower and LVESD was significantly greater in the PVC group after 12 weeks compared with the control group. Mitral regurgitation (semiquantitative, zero to 3+) reached significant difference between PVC and control groups (control, 0.58±0.2 versus PVC, 1.29±0.7; Mann-Whitney U test; P=0.04; Table). However, no animal had signs of overt heart failure such as lethargy, decreased activity, fluid retention, or tachypnea. Furthermore, no significant difference in LV end-diastolic dimension, LV wall thickness, left atrial size, or left atrial area was found after 3 months between PVC and control groups (Table).

The recovery phase (disabled premature pacing algorithm) in the PVC group (n = 3) demonstrated normalization of LVEF (Figure 4), FS, and LVESD (LVEF, 58±9%; FS, 37±3%; LVESD, 24.3±2.5 mm) after elimination of PVCs for 4 weeks.

**Ventricular Refractoriness**
Ventricular programmed stimulation at 350 ms demonstrated a significant increase of VERP in the PVC group (change score, 22.5; 95% confidence interval [CI], 12.75 to 32.25) when compared with the control group (change score, 8.3; 95% CI, −14.46 to 31.13; P=0.02). In contrast, no significant difference in VERP at 400 ms (PVC mean change, 20; 95% CI, −2.5 to 42.52 versus control mean change, 5; 95% CI, −15.5 to 25.5; P=0.08) and 300 ms (PVC mean change, 15; 95% CI, 3.74 to 26.3 versus control mean change, 5; 95% CI, −15.5 to 25.5; P=0.08) was found between groups.

**Myocardial Microscopic Evaluation**
Despite LV systolic dysfunction, the PVC group (n = 4) did not demonstrate increased inflammation, degree of fibrosis (PVC, 1.75±0.5 versus control, 1.3±0.8), percentage of fibrosis (PVC, 5.4±1.7% versus control, 6.5±3.9%), or apoptotic index (PVC, 2.85±1.77 versus control, 2.59±0.64; online-only Data Supplement Figure 2) when compared with the control group (n = 3). Inflammatory infiltrates were absent in both groups (online-only Data Supplement Figure 3). These findings are purely descriptive, and no statistical results were performed because of small sample size.
Oxidative Phosphorylation of Cardiac Mitochondria

Maximal rates of ADP-stimulated respiration and the coupling of respiration were unchanged in cardiac mitochondria in the PVC group compared with the control group (online-only Data Supplement Table 2).

Discussion

The cardiovascular effects of PVCs have not been systematically studied because of the absence of animal models. In the present study, we have developed a novel animal model, using a unique premature pacing algorithm to reproduce the cardiovascular effects of PVCs, as shown in Figure 3.

Table. Changes in Echocardiographic Parameters in Control and PVC Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (n=6)</th>
<th>12 Weeks</th>
<th>Δ (95% CI)</th>
<th>Baseline (n=7)</th>
<th>12 Weeks</th>
<th>Δ (95% CI)</th>
<th>Control Versus PVC</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF, %</td>
<td>61±6</td>
<td>60.7±4</td>
<td>1.08 (−3 to 5)</td>
<td>59.7±2</td>
<td>39.7±5</td>
<td>−19.21 (−29.3 to −9.1)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>FS, %</td>
<td>31.5±2</td>
<td>32.5±5</td>
<td>0.5 (−8.6 to 9.6)</td>
<td>33±3</td>
<td>18.3±5</td>
<td>−14.7 (−26 to −3.4)</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>33.9±5</td>
<td>34.8±4</td>
<td>0.9 (−6.4 to 8.2)</td>
<td>35.8±3</td>
<td>40.2±2</td>
<td>4.5 (3 to 12)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>23.1±3</td>
<td>23.7±4</td>
<td>0.6 (−3.6 to 4.8)</td>
<td>24±2</td>
<td>33.3±3</td>
<td>9.3 (2.5 to 16.1)</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>PW, mm</td>
<td>7.8±1.1</td>
<td>8.3±0.8</td>
<td>0.5 (−1.1 to 2.1)</td>
<td>8.7±0.5</td>
<td>8.7±0.8</td>
<td>0 (−2 to 2)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>SW, mm</td>
<td>8±0.6</td>
<td>7.8±1</td>
<td>−0.2 (−2.5 to 2.1)</td>
<td>8.7±1.1</td>
<td>8.7±0.8</td>
<td>0 (−1.1 to 1.1)</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>MR, grade 0 to 3+</td>
<td>0.5±0</td>
<td>0.6±0.2</td>
<td>0.1 (−0.3 to 0.5)</td>
<td>0.5±0</td>
<td>1.29±0.7</td>
<td>0.8 (−0.6 to 2)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>LA size, mm</td>
<td>26.7±3</td>
<td>26.2±3</td>
<td>−0.6 (−5 to 4)</td>
<td>29±3</td>
<td>28.7±2</td>
<td>−0.3 (−6.4 to 5.9)</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>LA area, cm²</td>
<td>5.7±2</td>
<td>5.7±1</td>
<td>−0.05 (−1.6 to 1.5)</td>
<td>6.6±2</td>
<td>6.9±1</td>
<td>0.2 (−2 to 2.4)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.7±0.2</td>
<td>1.6±0.1</td>
<td>−0.1 (−0.6 to 0.3)</td>
<td>1.6±0.2</td>
<td>1.3±0.3</td>
<td>−0.3 (−1 to 0.4)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>E/E’ ratio</td>
<td>4.2±0.8</td>
<td>3.8±0.8</td>
<td>−0.4 (−3 to 2)</td>
<td>3.8±0.6</td>
<td>4.3±0.6</td>
<td>0.4 (−1 to 2)</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>SD QRS-to-contraction</td>
<td>4.4±5</td>
<td>7.3±7</td>
<td>3 (−10 to 16)</td>
<td>4.7±2</td>
<td>17.6±11</td>
<td>13 (−9 to 35)</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

LVEDD indicates LV end-diastolic dimension; LVESD, LV end-systolic dimension; PW, posterior wall thickness; SW, septal wall thickness; MR, mitral regurgitation (semiquantitative, graded from 0 to 3+); and SD QRS-to-contraction, standard deviation of the timing of local contractility in reference to the QRS initiation.

P value was determined using a 2-group independent t test comparing the individual change score (Δ) at 12 weeks between groups. Data are expressed as mean±SD.
PVCs and corroborate the clinical entity of PVC-induced CM. The major findings of this model are (1) ventricular bigeminy induced CM characterized by a reduced LVEF and enlarged LV systolic dimension; (2) PVC-induced CM was reversible within 4 weeks after cessation of PVCs; and (3) PVC-induced CM lacks histopathologic abnormalities such as inflammation, fibrosis, increased apoptosis, or abnormal mitochondrial oxidative phosphorylation.

Canines were selected in our PVC model because of their similarity to humans of the cardiac His-Purkinje system and their extensive description in tachycardia-induced CM models. The premature pacing algorithm is capable of reproducing different PVC burdens (Figure 2). Ventricular bigeminy was chosen as a clinically significant PVC burden (50%) that would probably result in PVC-induced CM and demonstrate the concept that frequent PVCs alone can induce LV dysfunction in otherwise normal hearts. Because the heart rate of the dog ranges from 60 to 200 bpm, the pacing stimulus (PVC) was delivered at a fixed coupling interval of 240 ms (250 bpm) after ventricular sensed event to ensure bigeminal pacing even at faster rates of 200 bpm (300 ms).

The echocardiographic findings in our animal model are consistent with previous results of retrospective/observational clinical studies of PVC-induced CM. As our report was finalized, an animal model using 2 RV leads connected to a dual-chamber pacemaker reported similar echocardiographic findings after 4 weeks of ventricular bigeminy. In addition, our model demonstrated that (1) LV dysfunction developed as early as 2 weeks and continued to decline for the following 10 weeks (Figure 4) after initiation of ventricular bigeminy without clinical evidence of heart failure, (2) severity of mitral valve regurgitation increased, and (3) VERP was likely to prolong after chronic bigeminy, whereas (4) CM was reversible as demonstrated by the recovery of LV function and normalization of LV dimensions within 4 weeks after cessation of PVCs.

Importantly, we found a trend of VERP to prolong in this PVC-induced CM model. This was not surprising because VERP prolongation and “electric remodeling” has been previously reported in failing hearts. Electric remodeling has been characterized by alterations in intercellular ion channels, which result in prolongation of action potential duration, VERP, and slowing of conduction. Further investigations are needed to clarify and explain electrophysiological changes and how these relate to the proarrhythmic effects of frequent PVCs reported in patients with and without LV systolic dysfunction.

The histopathologic and metabolic features of PVC-induced CM have never been described. Tissue analysis in this animal model did not show inflammation, fibrosis, or increased apoptosis after 3 months of high PVC burden. The unaltered respiration with complex I and complex II substrates suggests that mitochondrial electron transport was not significantly altered by exposure to PVCs despite the decrease in LV systolic function. On the basis of complete recovery of LV systolic function after cessation of PVCs, it is not surprising that there were no gross structural abnormalities. We believe that this CM is secondary to a functional rather than a structural abnormality due to the lack of gross structural abnormalities in our animal model. For instance, abnormalities in calcium handling could potentially translate in myocardial dysfunction.

The mechanism(s) by which PVCs induce CM are unknown. Two major theories have emerged: (1) a short PVC coupling interval and (2) LV dyssynchrony during PVCs. A short PVC coupling interval in subjects with high PVC burden would result in an overall increase in the average heart rate and “tachycardia,” possibly leading to a pathophysiology similar to a tachycardia-induced CM. We believe that this animal model of CM is clearly distinct from tachycardia-induced CM because the average heart rate with PVCs (130±13 bpm) is significantly lower than described in a tachycardia-induced CM dog model (heart rate >180 bpm). In addition, the absence of fibrosis, increased apoptosis, and mitochondrial dysfunction as well as the normalization of LV diastolic dilatation after cessation of PVCs supports a distinct mechanism from tachycardia-induced CM. Alternatively, frequent PVCs may cause LV dyssynchrony similar to chronic RV pacing, which has been associated with higher mortality rates and a greater incidence of LV dysfunction. The abnormal pattern of electric activation and LV dyssynchrony resulting from these PVCs may cause disruption and further progression of dyssynergic LV wall motion. However, the time course to develop LV dysfunction in this model is quite different from the sole effects of long-term RV pacing. Finally, we propose the chronic effects of “postextrasystolic potentiation” as a third mechanism of PVC-induced CM. This phenomenon was studied extensively in the 1970s when coupled pacing was postulated to be beneficial for the treatment of heart failure. An increase in intracellular Ca concentration and myocardial oxygen consumption was demonstrated with postextrasystolic potentiation, which could also contribute to the development of CM.

To the best of our knowledge, our PVC canine model with chronic ventricular bigeminy describes for the first time the time course of echocardiographic findings, changes in VERP, and the histopathologic and mitochondrial characteristics of PVC-induced CM. Nevertheless, the minimum PVC burden required to induce CM remains unclear. Furthermore, it is uncertain if different sites of PVC origin and coupling intervals would affect the development and/or severity of PVC-induced CM. In contrast to the use of a dual-chamber pacemaker recently reported to reproduce ventricular bigeminy, our novel premature pacing algorithm is able to provide different PVC burdens (from 5% up
to 75%) and coupling intervals that mimic different clinical scenarios (Figure 2).

Limitations
PVCs simulated through a pacemaker in our animal model are not intrinsically, but cardiac bipolar pacing represents local myocardial depolarization similar to a spontaneous ventricular event. RV apical pacing was performed, and it is unclear if these results may be extrapolated to PVCs from other cardiac sites. We cannot exclude that tachycardia plays a lesser role because the average heart rate was increased with premature pacing algorithm. However, this is similar to the clinical scenario investigated in patients with high-burden PVCs. Our histopathologic analysis was limited to anterior and apical segments of the LV. We cannot exclude the presence of regional abnormalities in the remaining LV walls. Similarly, we cannot exclude the possibility that extended exposure to frequent PVCs beyond 12 weeks could result in significant cardiac remodeling with chronic irreversible structural changes.

Novelty and Significance
This study validates the premise that frequent PVCs can result in a reversible LV dysfunction in structurally normal hearts. Even if PVCs are the result of CM, PVCs by themselves may contribute to and further worsen CM and heart failure symptoms.8,9 This findings support further clinical studies in patients with CM associated with frequent PVCs.

Most importantly, this novel premature pacing algorithm and PVC animal model will facilitate further scientific evaluation of the cardiovascular effects of PVCs in structurally normal hearts and other established heart failure models.

Conclusions
In summary, a novel premature pacing algorithm has allowed the study of the clinical entity of PVC-induced CM in structurally normal hearts. The PVC-induced CM canine model demonstrates that frequent PVCs with a bigeminal pattern alone can cause reversible LV dysfunction within 2 weeks, which appears to progress throughout the 3 months of continuous PVCs. Finally, PVC-induced CM lacks histopathologic and mitochondrial abnormalities seen in other canine models of CM.

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Disclosures
Dr Huizara received grant support from St Jude Medical and was a clinical investigator for Biotronik. Dr Kaszala was a clinical investigator for Boston Scientific, St Jude Medical, and Sorin. Dr Ellenbogen received grants and honoraria and was a clinical investigator and consultant for Boston Scientific, Medtronic, St Jude Medical, and Biotronik. Dr Wood was a clinical investigator and speaker for Boston Scientific, Medtronic, and St Jude Medical.

References
LV Dysfunction Induced by Frequent PVCs

Premature ventricular contractions (PVCs) are a common entity associated with cardiomyopathy and other cardiac diseases, and yet their effects on the cardiovascular system are not well understood. This is primarily because of the lack of animal models and the unpredictability and variability of PVCs in the clinical setting. With the use of a novel premature pacing algorithm capable of reproducing different clinical scenarios of ventricular ectopy, the effects of chronic ventricular bigeminy in structurally normal hearts were studied in an animal model. Our canine model validates and describes for the first time the time course of echocardiographic findings, changes in ventricular effective refractory period, and the histopathologic and mitochondrial characteristics of PVC-induced cardiomyopathy. These findings support further clinical studies in patients with cardiomyopathy associated with frequent PVCs because the minimum PVC burden, origin, and coupling interval required to induce cardiomyopathy remains unclear. Finally, this novel premature pacing algorithm and PVC animal model will facilitate further scientific evaluation of the cardiovascular effects of PVCs in structurally normal hearts and other established heart failure models.

CLINICAL PERSPECTIVE


Left Ventricular Systolic Dysfunction Induced by Ventricular Ectopy: A Novel Model for Premature Ventricular Contraction-Induced Cardiomyopathy


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The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/4/4/543

Data Supplement (unedited) at:
http://circep.ahajournals.org/content/suppl/2011/05/16/CIRCEP.111.962381.DC1
SUPPLEMENTAL MATERIAL

Huizar, JF et.al. - LV dysfunction induced by frequent PVCs

Expanded Methods

Animal preparation & pacemaker implantation. Left thoracotomy and pacemaker implantation was performed under general anesthesia. Acepromazine 0.5-2mg/kg SQ was administered as pre-anesthetic. Animals were intubated, ventilated and anesthesia was maintained with isofluorane 2-4% via endotracheal tube. The pacing lead was tunneled to a subcutaneous interscapular pocket and connected to the experimental device. Experimental devices with PVC algorithm were provided by St. Jude Medical (St. Jude Medical, Inc., St. Paul, MN, USA).

Echocardiographic Evaluation. Two-dimensional (2-D) echocardiography was performed with a 5MHz probe using a commercial system (Sequoia c256 Siemens) with tissue Doppler imaging (TDI). Cardiac function was assessed by quantifying the LV ejection fraction (Simpson’s formula), fractional shortening (FS), LV end-systolic and diastolic dimensions (LVESD, LVEDD), LV thickness, left atrial (LA) size, mitral valve (MV) function, and LV compliance (mitral Doppler inflow and tissue Doppler imaging)\(^1\). Premature pacing algorithm was disabled at least 15 minutes prior of assessment of LV function. LV compliance was measured by several parameters including E/A ratio and E/E’ ratio\(^1\).

Parasternal long axis views were used to visualize LV wall thickness, LV end-systolic and diastolic dimensions, LV posterior and septal wall motion, LA size and mitral valve mobility and function with and without color Doppler, \(^2\). M-mode at end-diastole was used to measure LV wall thickness and end-diastolic dimension, whereas LV end-systolic dimension and LA dimension were measured at end-systole. Similarly, Fractional shortening (FS) was calculated using M-mode by the formula \(^1\), $FS = (\text{End-diastolic dimension} - \text{End-systolic dimension}) / \text{End-diastolic dimension} \times 100$. Color Doppler was used in the apical two- and four-chamber views to visually assess degree of MV regurgitation. MV regurgitation was graded as absent, trace, mild,
moderate and severe based on color Doppler. In addition, MV regurgitation was quantified based on the severity (absent=0, trace = 0.5, mild = 1+, moderate = 2+, and severe = 3+). LV end-systolic and diastolic areas were determined in the apical two- and four-chamber views to calculate LV ejection fraction by Simpson formula. The LA area at end-systole and the MV inflow were acquired in the apical four-chamber view. E and A waves (m/s) were obtained with PW-Doppler of the MV inflow. The peak velocity during early filling (E’ wave, m/s) was acquired using PW-tissue Doppler imaging (TDI) positioned at the lateral mitral valve annulus.

Myocardial microscopic evaluation. Leukocytic infiltrates, if present were graded as mild, moderate or severe and the numbers of leukocytes per high power field were counted. Fibrosis was evaluated based on collagen deposits, which were categorized on a 1+ to 4+ scale. Grade 1+ corresponds to minimal collagen fiber staining interspersed between cardiomyocytes (no fibrosis), grade 2+ defined as mild collagen fiber staining interspersed between cardiomyocytes (mild fibrosis); grade 3+ defined as moderate collagen staining with abundant fibrosis around the fascicles (moderate fibrosis) and grade 4+ corresponding to severe fibrosis with obvious disruption of the myocardial architecture (severe fibrosis). In addition, images were acquired to quantify the percentage of fibrosis (surface area of collagen fiber staining / overall surface per field) using ImageJ software 1.43 (rsbweb.nih.gov/ij/).

Ventricular effective refractory period (VERP). Programmed ventricular stimulation was delivered at twice the diastolic pacing threshold. S2 was delivered starting at 120ms with a 10ms increase until ventricular capture was noted.

Myocardial apoptosis. LV samples (LV apex and anterior wall) were stored in a 10% formalin solution until paraffin embedding. Transverse sections were taken and immediately fixed in paraformaldehyde. Apoptosis was assessed using the TUNEL technique (DNA fragmentation - Oncor, Gaithersburg, MD). The detailed protocol was previously published. The apoptotic index was expressed as the number of apoptotic cells of all cardiomyocytes per field. Apoptotic index in anterior wall and apical regions were calculated using 4-5 random fields, which virtually
covered the entire LV. The allocation to different treatments was random, and the pathologist was blinded to the treatment.

*Isolation and analysis of cardiac mitochondria.* Briefly, a portion of the lateral LV free wall was harvested and placed into Chappel-Perry buffer at 4°C. Chappel-Perry buffer contains 100mM KCl, 50mM 3-(N-morpholino) propanesulfonic acid (MOPS), 1mM EGTA, 5mM MgSO4.7 H2O, and 1mM ATP, pH 7.4 at 4°C. Samples were finely minced and homogenized with a polytron tissue processor (Brinkman Instruments, Westbury, NY) for 2.5 seconds at a rheostat setting of 1.5 in Chappel-Perry buffer containing 0.2% bovine serum albumin. Subsarcolemmal mitochondria (SSM) were isolated following differential centrifugation as described and interfibrillar mitochondria (IFM) were isolated by incubation of the remaining skinned myofiber pellet, obtained following polytron treatment, with 5mg/g (wet weight) trypsin for 10 min. at 4°C followed by differential centrifugation. Isolated SSM and IFM were finally suspended in KME (100mM KCl, 50mM MOPS, and 0.5mM EGTA). Mitochondrial protein concentration was determined by the biuret method with bovine serum albumin as the standard. Oxygen consumption in mitochondria was measured using a Clark-type oxygen electrode at 30°C using glutamate (complex I substrate) and succinate + rotenone (complex II substrate).

**Statistical Analysis**

Sample size was based on a continuous measure of LVEF after 12-week period of ventricular bigeminy. Based on our preliminary data, we estimated that the difference in the LV ejection fraction of matched pairs is normally distributed with standard deviation of 11. If the true difference in the mean LV ejection fraction of matched pairs is 15, we will need to study 6 pairs of dogs to be able to reject the null hypothesis that the LV ejection fraction difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.
References


Supplemental Table 1. PVC burden through the premature pacing algorithm is programmable by modifying the ratio between pacing stimulus and cardiac sensed events. VP, ventricular paced event; VS, ventricular sensed event; VT, ventricular tachycardia.

<table>
<thead>
<tr>
<th>PVC burden</th>
<th>Pacing stimulus</th>
<th>Sensed Events</th>
<th>VP/VS Ratio</th>
<th>Clinical scenario</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 %</td>
<td>3</td>
<td>1</td>
<td>3:1</td>
<td>Non-sustained VT or triplets</td>
</tr>
<tr>
<td>66 %</td>
<td>2</td>
<td>1</td>
<td>2:1</td>
<td>Couplets</td>
</tr>
<tr>
<td>50 %</td>
<td>1</td>
<td>1</td>
<td>1:1</td>
<td>Bigeminy</td>
</tr>
<tr>
<td>33 %</td>
<td>1</td>
<td>2</td>
<td>1:2</td>
<td>Trigeminy</td>
</tr>
<tr>
<td>25 %</td>
<td>1</td>
<td>3</td>
<td>1:3</td>
<td>Quadrigeminy</td>
</tr>
<tr>
<td>20 %</td>
<td>1</td>
<td>4</td>
<td>1:4</td>
<td>Pentageminy</td>
</tr>
<tr>
<td>16.6 %</td>
<td>1</td>
<td>5</td>
<td>1:5</td>
<td>Hexageminy</td>
</tr>
<tr>
<td>14.2 %</td>
<td>1</td>
<td>6</td>
<td>1:6</td>
<td>Heptageminy</td>
</tr>
<tr>
<td>12.5 %</td>
<td>1</td>
<td>7</td>
<td>1:7</td>
<td>Octageminy</td>
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<tr>
<td>10 %</td>
<td>1</td>
<td>9</td>
<td>1:9</td>
<td>Decageminy</td>
</tr>
<tr>
<td>7.6 %</td>
<td>1</td>
<td>12</td>
<td>1:12</td>
<td></td>
</tr>
<tr>
<td>5 %</td>
<td>1</td>
<td>19</td>
<td>1:19</td>
<td></td>
</tr>
</tbody>
</table>
### Supplemental Table 2. Oxidative phosphorylation in canine cardiac mitochondria.

<table>
<thead>
<tr>
<th></th>
<th>SSM</th>
<th>IFM</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glutamate</td>
<td>Succinate</td>
<td>Glutamate</td>
<td>Succinate</td>
</tr>
<tr>
<td>Control (n=3)</td>
<td>125 ± 36</td>
<td>131 ± 42</td>
<td>276 ± 61</td>
<td>273 ± 54</td>
</tr>
<tr>
<td>PVC (n=3)</td>
<td>122 ± 46</td>
<td>145 ± 31</td>
<td>273 ± 168</td>
<td>297 ± 102</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± SD.

SSM, Subsarcolemmal mitochondria; IFM, interfibrillar mitochondria.
Supplemental Figure 1. Flow diagram depicts PVC protocol. Thirteen canines were randomly assigned to PVC (enabled premature pacing algorithm) and control (disabled premature pacing algorithm) groups with a 3-month follow-up each. At the end of 3 months, each group underwent a second randomization. Three and four canines in the control and PVC groups, respectively, were euthanized for microscopic cardiac evaluation. The remaining 3 animals in the PVC group were allowed a 1-month recovery phase (disabled premature pacing algorithm).
Supplemental Figure 2. Apoptotic Index in both control and PVC groups. Data expressed in average ± SD.
Supplemental Figure 3. Representative samples of LV anterior wall (x 20 magnification) with hematoxylin-eosin (upper panels) and Masson trichrome staining (lower panels) in PVC and control group. Lack of leukocytic infiltrates and normal fibrin strands are noted in both samples.