Utility of cardiac MRI to diagnose myocardial ischemia and fibrosis in dogs with cardiomegaly secondary to myxomatous mitral valve disease

William A. Clark, DVM1; Randolph L. Winter, DVM, PhD, DACVIM1,2*; Turi K. Aarnes, DVM, MS, DACVAA1; Eric M. Green, DVM, DACVR1; Katarzyna Mikrut, MD3; Patrick Ruz, BS2; Daniel Addison, MD2; Jaylyn D. Rhinehart, DVM, MS, DACVIM1; Karsten E. Schober, DVM, PhD, DECVR1; Harry T. Friel, MS4

1Department of Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH
2Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL
3Division of Cardiovascular Medicine, Department of Medicine, The Ohio State University, Columbus, OH
4Philips Medical Systems, Highland Heights, OH
*Corresponding author: Dr. Winter (rlw0041@auburn.edu)
https://doi.org/10.2460/ajvr.22.05.0076

OBJECTIVE
To assess whether cardiac MRI or various biomarkers can be used to detect myocardial ischemia and fibrosis in dogs with cardiomegaly secondary to myxomatous mitral valve disease (MMVD).

ANIMALS
6 dogs with cardiomegaly secondary to naturally occurring stage B2 MMVD being treated only with pimobendan with or without enalapril and 6 control dogs with no cardiac disease. All dogs were ≥ 5 years old with no systemic illness.

PROCEDURES
Serum cardiac troponin I and concentrations were measured, and dogs were anesthetized for cardiac MRI with ECG-triggered acquisition of native T1- and T2-weighted images. Gadolinium contrast was administered to evaluate myocardial perfusion and late gadolinium enhancement (LGE). Mean T1 and T2 values and regions of LGE were measured with dedicated software. Extracellular volume (ECV) was estimated on the basis of Hct and T1 values of myocardium and surrounding blood. Subjective analysis for myocardial perfusion deficits was performed.

RESULTS
Dogs with MMVD had significantly (P = .013) higher cardiac troponin I concentrations than control dogs, but galectin-3 concentrations did not differ (P = .08) between groups. Myocardial fibrosis was detected in 4 dogs with MMVD and 3 control dogs; no dogs had obvious myocardial perfusion deficits. Native T1 and T2 values, postcontrast T1 values, and ECV values were not significantly different between groups (all P > .3).

CLINICAL RELEVANCE
Results suggest that some dogs with cardiomegaly secondary to MMVD may not have clinically relevant myocardial fibrosis.

Disease progression for dogs with myxomatous mitral valve disease (MMVD) is variable, with some dogs remaining free from clinical signs for their entire lives and others having progressive disease resulting in clinical signs, congestive heart failure, and early death.1,2 Understanding myocardial health, including the presence of ischemia or fibrosis, may be of benefit in more accurately predicting disease prognosis and guiding therapeutic intervention. Some studies1-5 have identified discrete areas of cardiac fibrosis and evidence of myocardial atherosclerosis during histologic evaluation of the hearts from dogs with MMVD. However, these evaluations have, so far, been limited to discrete samples taken from a few locations at necropsy. Myocardial ischemia and infarction are thought to be uncommon complications of MMVD, but have been reported in dogs.6 To our knowledge, however, no in vivo studies have evaluated the extent and geographic distribution of myocardial ischemia and fibrosis, which may alter the progression and prognosis of the disease, in dogs with MMVD.

Recently, advanced diagnostic imaging with cardiac MRI has provided valuable and novel information for human patients with chronic heart diseases similar to MMVD in dogs.7,8 In one study,8 cardiac MRI detected previously undiagnosed myocardial lesions in human patients with heart failure with...
preserved ejection fraction, including patients with myocardial infarction, coronary artery disease, and microvascular dysfunction. Patients with these cardiac MRI changes had standard echocardiographic disease characteristics similar to patients without newly diagnosed cardiac MRI lesions. Another study found that humans with heart failure with preserved ejection fraction had various degrees of focal or diffuse myocardial fibrosis, and that these cardiac MRI findings were independent predictors of poor outcomes. Humans with mitral valve prolapse have been shown to have higher native T1 values and lower postcontrast T1 values, with a subset of patients having evidence of left ventricular late gadolinium enhancement (LGE). In a subset of diseases, myocardial lesions identified with cardiac MRI may represent future therapeutic targets to slow disease progression. As a result of the ease of the procedure and diagnostic information provided, cardiac MRI is performed routinely in humans with many heart diseases.

Most clinical veterinary studies of cardiac MRI in dogs have evaluated cardiac chamber sizes, heart function, and congenital cardiac defects. Cardiac MRI has been used to evaluate myocardial changes in cats with hypertrophic cardiomyopathy and in dogs with pulmonary valve stenosis. However, no studies have evaluated using cardiac MRI to assess myocardial changes in dogs with naturally occurring MMVD. Given the presence of fibrosis and arteriosclerosis identified at necropsy in the hearts of some dogs with MMVD, cardiac MRI may show clinically relevant myocardial changes in dogs with MMVD that are undetectable with standard diagnostic testing. In addition, using cardiac MRI to detect myocardial ischemia and fibrosis in dogs with MMVD may potentially offer insights into prognosis or guide treatment. In dogs with experimentally induced chronic myocardial infarction, both the location and extent of myocardial fibrosis identified with cardiac MRI correlated well with that identified at necropsy. In contrast with histopathologic examination, which relies on discrete sampling of the myocardium, typically at necropsy, cardiac MRI has the additional advantages of allowing imaging in vivo and evaluating the entire heart for pathologic changes.

Serum cardiac biomarkers can be used as noninvasive indicators of the presence and severity of heart disease. Serum concentrations of the N-terminal prohormone of brain natriuretic peptide (NT-proBNP) increase with cardiac stretch and volume overload, whereas increases in serum cardiac troponin I (cTnI) concentrations are associated with cardiomyocyte damage or death. High serum NT-proBNP and cTnI concentrations have both been associated with a poor prognosis in dogs with MMVD, and high cTnI concentrations reflect the degree of cardiac fibrosis and arteriosclerosis identified at necropsy in dogs with MMVD. High NT-proBNP and cTnI concentrations in humans have both been associated with myocardial abnormalities that are unrecognizable without the use of cardiac MRI. Another biomarker that provides information on myocardial health, but is relatively novel to veterinary medicine, is galectin-3. Galectin-3 is a macrophage-derived mediator that stimulates collagen synthesis by myocardial fibroblasts, promoting the formation of myocardial fibrosis. Galectin-3 concentrations in myocardium and serum of dogs with MMVD have been shown to correlate with the percentage of myocardial fibrosis identified on postmortem examination. Left ventricular diastolic dysfunction has been shown to correlate with galectin-3 concentrations in dogs, indicating there may be functional significance to changes in galectin-3 concentrations. In a human study evaluating multiple biomarkers, only galectin-3 concentrations correlated with extent of myocardial fibrosis as assessed by cardiac MRI. Galectin-3 has also been shown to induce atrial fibrosis in humans with atrial fibrillation, suggesting that it may have a role in the progression of disease. Although some of these biomarkers have been associated with histopathologic changes and prognosis in dogs with MMVD, none have been demonstrated to correlate with abnormalities identified on cardiac MRI in dogs.

The objectives of our study were (1) to determine whether cardiac MRI could be used to detect myocardial ischemia and fibrosis in healthy dogs and dogs with cardiomegaly secondary to naturally acquired MMVD and (2) to investigate whether circulating biomarker concentrations could be used as predictors of myocardial changes identified on cardiac MRI. We hypothesized that myocardial ischemia and fibrosis would be present in some dogs with MMVD, that serum cTnI concentrations would be high in dogs with myocardial ischemia, and that serum galectin-3 concentrations would be high in dogs with myocardial fibrosis.

Materials and Methods

Animals

Dogs with cardiomegaly secondary to naturally occurring stage B2 MMVD that had no other clinically relevant systemic or cardiopulmonary illnesses were recruited prospectively from the patient population of the Ohio State University Veterinary Medical Center. Apparently healthy dogs with no evidence of heart disease, no echocardiographic evidence of mitral regurgitation, and no history of other clinically relevant systemic or cardiopulmonary illnesses were recruited prospectively from the pets of staff and students at the Ohio State University (control dogs). Dogs were confirmed to have stage B2 MMVD or structurally normal hearts on the basis of results of echocardiography and thoracic radiography. Dogs were classified as having stage B2 MMVD if the following criteria were met: characteristic mitral valve lesion on echocardiography, mitral valve regurgitation observed with color Doppler echocardiography, heart murmur intensity of at least 3/6, a vertebral heart size > 10.5, a left atrium-to-aortic root ratio > 1.6, and a normalized left ventricular internal dimension in diastole > 1.7. Long-term administration
of pimobendan with or without an angiotensin-converting enzyme inhibitor was permitted in dogs with MMVD but not in control dogs; all other medications known to affect the cardiovascular system were forbidden in all dogs. Dogs were eligible for inclusion if they were ≥ 5 years old and weighed ≥ 3 kg. Dogs were excluded if they had clinical signs consistent with congestive heart failure at the time of the study or historically; if they had evidence of pulmonary hypertension; if they were receiving any forbidden medications; if they had clinically relevant arrhythmias other than sinus arrhythmia, single atrial premature complexes, or single ventricular premature complexes; if they had clinically relevant systemic or cardiopulmonary disease requiring long-term medication; or if they had any metallic foreign material other than surgical implants in the extremities or a microchip in or near the thorax that would interfere with cardiac MRI. Informed owner consent was obtained for each dog before enrollment in the study. The study protocol was approved by the Ohio State University Institutional Animal Care and Use Committee (Protocol No. 2019A00000061).

Clinical evaluation
Each dog was examined on 2 different dates for our study. During the first visit, a complete medical history was obtained, a physical examination (including cardiopulmonary auscultation) was performed, blood pressure was measured noninvasively, thoracic radiography and echocardiography were performed, and blood samples were collected. During the second visit, dogs were anesthetized, and ECG and cardiac MRI were performed. For all dogs, the second visit occurred between 1 and 7 days after the first visit.

Noninvasive indirect blood pressure measurements were obtained with an ultrasonic Doppler flow detector prior to administration of any sedatives. Dogs were restrained gently in lateral recumbency, and 3 to 5 blood pressure measurements were obtained. The mean value of 3 reliable measurements was recorded.

Three-view thoracic radiography was performed in all dogs. Radiographs were assessed for evidence of pulmonary edema, signs of clinically relevant primary pulmonary illness, and metallic foreign material other than microchips. Vertebral heart size was measured in all dogs.24

Blood was obtained from each patient for serum biochemical analyses and a CBC. Plasma samples obtained from fresh blood samples preserved with EDTA were shipped to a reference laboratory (Idexx Reference Laboratories) for measurement of NT-proBNP concentrations. Three to 4 mL of whole blood from each dog was stored in serum tubes at room temperature (22 to 24°C) for 15 minutes to allow for stable clot formation. Samples were then centrifuged for 10 minutes, and the serum was separated and placed in polypropylene tubes that were immediately frozen at −80°C for batch analysis at the end of the study. When enrollment was complete, the frozen serum samples were thawed for analysis. An aliquot of serum was submitted for high-sensitivity analysis of cTnI concentration (Advia Centaur high-sensitivity troponin I assay; Siemens Healthcare Diagnostics). Serum galectin-3 concentrations were measured with an ELISA (canine galectin-3 ELISA kit; RayBiotech) in accordance with the manufacturer’s instructions.

Transthoracic 2-D, M-mode, and Doppler echocardiography was performed by a board-certified veterinary cardiologist (RLW) or a veterinary cardiologist resident (WAC) under the supervision of a board-certified cardiologist. Sedation with butorphanol, trazodone, gabapentin, or acepromazine—alone or in combination—was administered if necessary to maintain dogs in lateral recumbency with light restraint. Dogs were imaged in right and left lateral recumbency with a digital ultrasound system (Vivid E95; GE Medical Systems) and a sector transducer with a nominal frequency of 5 or 6 MHz. Two-dimensional cine loops, M-mode images, and spectral Doppler tracings were recorded with a simultaneous 1-lead ECG as described previously.25,26 Measurements were obtained from high-quality digital still images and were recorded as the mean of 3 to 5 cardiac cycles. All measurements were performed collectively at the end of the enrollment period by the same investigator (WAC) using a dedicated analysis system (EchoPac; GE Medical Ultrasound). The maximum left atrial dimension was measured on a right parasternal 4-chamber view obtained with 2-D echocardiography,27 the left atrium-to-aortic root ratio was calculated from 2-D echocardiographic diameter measurements obtained from a right parasternal short-axis heart base view,28 and the left ventricular internal dimension in diastole was measured on a right parasternal short-axis view at the level of the chordae tendineae obtained with 2-D echocardiography.29 Maximum left atrial dimensions and left ventricular internal dimensions were normalized to body weight with established allometric equations.20 Peak tricuspid regurgitation velocity was measured to assess the echocardiographic probability of pulmonary hypertension.30

Anesthesia
For cardiac MRI, general anesthesia was directed and supervised by a board-certified anesthesiologist (TKA). Hct was measured at the time anesthesia was induced. After food had been withheld for 12 hours, dogs were sedated with butorphanol (0.2 to 0.4 mg/kg IM) and either acepromazine (0.02 to 0.05 mg/kg IM) or midazolam (0.2 mg/kg IM), followed by placement of an IV catheter. Anesthesia was induced with propofol (2 to 6 mg/kg IV) and was maintained with isoflurane after orotracheal intubation. Additional medications such as parasympatholytics and IV fluids were given as needed to maintain blood pressure. Patients received volume-controlled positive-pressure mechanical ventilation (10 to 15 mL/kg), with adjustment of the respiratory rate to maintain an end-tidal partial pressure of carbon dioxide of 35 to 45 mm Hg. Anesthetic monitoring included heart rate, respiratory rate, oxygen saturation, blood
pressure (measured invasively), and end-tidal partial pressure of carbon dioxide.

**ECG**

After dogs were anesthetized, they were positioned in right lateral recumbency, and a 12-lead ECG was recorded at 50 mm/second with optimized sensitivity. Heart rhythm was assessed, and mean heart rate, mean electrical axis, deflection amplitudes, and interval durations were measured. The number of leads with QRS notchting and the specific leads that exhibited QRS notchting were also recorded. Recorded values were the mean of at least 3 separate complexes, and all measurements were performed collectively at the end of the enrollment period by the same investigator (WAC).

**Cardiac MRI**

After the ECG recording was obtained, a brief, full-body fluoroscopic examination was performed to screen for metallic foreign material that would prevent cardiac MRI from being performed safely. Cardiac MRI was performed with a 3.0-T clinical MRI scanner (Ingenia; Philips Healthcare). Dogs were positioned in dorsal recumbency, and cardiac MRI safe electrodes were attached to both sides of the thoracic wall for ECG triggering of image acquisition. Images were acquired at the diastolic phase during an end-expiratory breath hold to limit movement of the heart induced by respiration.

Survey images were obtained to assess cardiac geometry and identify planes of interest, and the following scans were then performed: (1) balanced turbo field echo cine sequence imaging with sensitivity encoding in the 4-chamber, left ventricular outflow tract, short axis, and left ventricular 2-chamber planes; (2) balanced turbo field echo multiple single-slice cine sequence imaging in the 4-chamber, left ventricular outflow tract, and short-axis planes; (3) precontrast native T1 mapping in the short-axis plane at the interventricular septum and left ventricular posterior wall with a modified Look-Locker inversion recovery sequence; and (4) T2 gradient-spin-echo (GraSE) mapping multiecho sequence imaging. Precontrast and postcontrast T1 measurements of the blood pool near the myocardium were also obtained for calculation of extracellular volume (ECV). For these scans, 3 to 5 slices parallel to the short-axis plane and 3 to 5 slices parallel to the 4-chamber plane were obtained, as determined by patient size. The slice thickness was 4.00 mm, the echo time was 1.848 ms, and the repetition time was 7.6968 ms. After completion of these sequences, an IV injection of gadodiamide (0.1 mmol/kg), a gadolinium-based contrast agent, was administered. Seven minutes after contrast administration, the following scans were performed: (1) inversion recovery turbo field echo sequencing in the short-axis plane and (2) turbo field echo sequence imaging with phase-sensitive inversion recovery in the short-axis and 4-chamber planes. Fifteen minutes after contrast administration, T1 mapping was performed. Inversion time was determined individually on the basis of inversion recovery turbo field echo sequencing performed 7 minutes after contrast administration (Look-Locker).

Isoflurane was discontinued when cardiac MRI was complete, and dogs were monitored until they had recovered from general anesthesia. All diagnostic imaging studies were transferred to an offline workstation for processing with dedicated software (CVI42 version 5.13; Circle Cardiovascular Imaging). Measurement of cardiac magnetic resonance images, image analysis, and image interpretation were performed by investigators experienced in cardiac magnetic resonance image interpretation (PR, KM, and DA); these operators were blinded to clinical information. Left ventricular and right ventricular ejection fractions were measured with standard volumetric techniques. Subjective analysis of myocardial perfusion was performed from multiplanar images obtained during the first minute after gadolinium injection. Mean T1 values were obtained from each of the generated T1 maps before and after administration of gadolinium by contouring a region of interest located at the mid-myocardium level in the interventricular septum and the left ventricular posterior wall. Quantification of LGE was performed by comparing data to the reference region of interest and detecting areas with a signal intensity > 5 SD above the reference data. The ECV was calculated as follows:

\[
ECV = \left(1 - \frac{Hct}{100}\right) \times \left(\frac{1}{Postcontrast T1 myocardium - Native T1 myocardium} - \frac{1}{Postcontrast T1 blood - Native T1 blood}\right)
\]

Mean T2 values were obtained from the generated T2 maps at the mid-myocardium level.

**Statistical analysis**

Quantitative data were assessed for normality with the Shapiro-Wilk test. Data are presented as mean ± SD or as median (interquartile range [IQR]) on the basis of distribution. Student t tests or Wilcoxon rank sum tests were performed to compare clinical data, biomarker concentrations, echocardiographic variables, and cardiac MRI data between dogs with MMVD and control dogs, depending on the data distribution. Analyses were performed with commercial software (JMP version 11.0.0; SAS Institute Inc), with values of *P* < .05 considered significant.

**Results**

Six dogs with MMVD and 6 control dogs were enrolled in the study. Dogs with MMVD consisted of 4 Cavalier King Charles Spaniels, 1 mixed-breed dog, and 1 Rat Terrier (1 spayed female and 5 neutered males). Control dogs consisted of 3 mixed-breed dogs, 2 Yorkshire Terriers, and 1 Labrador Retriever (3 spayed females and 3 neutered males). Mean ± SD age of the dogs with MMVD at the time of enrollment (10.6 ± 1.69 years) was not significantly
different \((P = .62)\) from mean age of the control dogs \((8.12 \pm 2.39\) years). Median body weight of the dogs with MMVD \((8.05\) kg; IQR, 6.38 to 8.78 kg) was not significantly different \((P = .26)\) from median body weight of the control dogs \((13.6\) kg; IQR, 4.35 to 27.98 kg). Mean pimobendan dosage of the dogs with MMVD was \(0.58 \pm 0.08\) mg/kg/day. Two dogs in the MMVD group were also receiving enalapril \((mean\ dosage, 1.04\ mg/kg/d)\).

No clinically relevant abnormalities were identified on the basis of CBC or serum biochemical profile results for any dog. Concurrent illnesses identified that were not deemed to be clinically relevant for the purposes of our study included dental disease in 4 dogs, anxiety in 2 dogs, food allergies in 1 dog, hip dysplasia in 1 dog, and an inguinal hernia in 1 dog. Noncardiac medications included gabapentin in 1 dog with MMVD and 1 control dog, fluoxetine in 1 control dog, diphenhydramine in 1 dog with MMVD, and metronidazole in 1 control dog.

Grade 3/6 to 6/6 left apical systolic heart murmurs were identified in all 6 dogs with MMVD. A grade 1/6 left basilar systolic heart murmur was identified in 1 control dog, but echocardiography did not reveal any obvious reason for the murmur in this dog. Seven dogs were sedated to facilitate echocardiography. None of the dogs had a peak tricuspid regurgitation velocity > 3.0 m/second nor any other echocardiographic evidence of pulmonary hypertension. There were no significant differences \((P = .12)\) in blood pressure between dogs with MMVD and control dogs \((Table 1)\). Serum cTnI concentration was significantly different \((P = .013)\) between groups \((Figure 1)\), but there was no significant difference between groups with regard to serum galectin-3 concentrations \((P = .085)\) or plasma NT-proBNP concentrations \((P = .337)\). Dogs with MMVD had a significantly higher left atrium-to-aortic root ratio \((P = .009)\), normalized left atrial diameter \((P = .005)\), normalized left ventricular internal diameter measured in the short axis in diastole \((P = .002)\), and vertebral heart size \((P = .003)\) than control dogs.

The underlying cardiac rhythm was sinus or sinus arrhythmia in all dogs. Arrhythmias noted included single atrial premature complexes in 3 dogs with MMVD and 1 control dog, and single ventricular premature complexes in 1 dog with MMVD and 1 control dog. Notched QRS complexes were noted in 1 dog with MMVD \((lead\ III)\) and 1 control dog \((leads\ I, III,\ and\ aVL)\).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dogs with MMVD</th>
<th>Control dogs</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>130 ± 17.5</td>
<td>112 ± 16.2</td>
<td>.12</td>
</tr>
<tr>
<td>Serum NT-proBNP (pmol/L)</td>
<td>462 (427–2,483)</td>
<td>430 (292–937)</td>
<td>.337</td>
</tr>
<tr>
<td>Vertebral heart size (vertebral units)</td>
<td>11.4 ± 0.8</td>
<td>9.5 ± 0.7</td>
<td>.003</td>
</tr>
<tr>
<td>Left atrium-to-aortic root ratio</td>
<td>1.66 ± 0.25</td>
<td>1.25 ± 0.09</td>
<td>.009</td>
</tr>
<tr>
<td>Normalized left atrial dimension</td>
<td>1.99 ± 0.35</td>
<td>1.32 ± 0.06</td>
<td>.005</td>
</tr>
<tr>
<td>Normalized left ventricular internal dimension in diastole (short axis)</td>
<td>1.92 ± 0.29</td>
<td>1.27 ± 0.12</td>
<td>.002</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD or median (interquartile range). NT-proBNP = N-terminal prohormone of brain natriuretic peptide.
All dogs tolerated anesthesia well, and all standard imaging planes and sequences were achievable in all dogs (Figure 2). Time to complete cardiac MRI was approximately 1 hour, and total anesthesia time was approximately 1.5 to 2 hours. No dogs in either group had obvious myocardial perfusion deficits on cardiac magnetic resonance images. The mean ± SD left ventricular ejection fraction calculated from cardiac magnetic resonance images was 61.9% ± 5.6% for dogs in the MMVD group and 52.1% ± 8.7% for dogs in the control group; these values were significantly different ($P = .046$). Mean right ventricular ejection fraction calculated from cardiac magnetic resonance images was 45.8% ± 8.6% for dogs in the MMVD group and 41.2% ± 12.2% for dogs in the control group; these values were not significantly different ($P = .477$).

Four dogs with MMVD and 3 control dogs were noted to have regions of LGE in the posterior wall and interventricular septum of the left ventricle. No significant differences (all $P > .25$) in native T1 values or postcontrast T1 values were noted between groups at either location (Figure 3). There was also no significant difference ($P = .149$) in native T2 values between groups (Figure 4). Measured ECV was higher in the MMVD group than the control group at the base location (22.1% ± 2.7% vs 20.4% ± 2.6%) and

**Figure 2**—Representative transverse cardiac magnetic resonance image from a dog with stage B2 myxomatous mitral valve disease (balanced turbo field echo cine sequence with sensitivity encoding obtained in the left ventricular 2-chamber plane). Notice the moderate dilation of the left atrium (LA) and left ventricle (LV), and the presence of mitral regurgitation (MR).

**Figure 3**—Pre- (A and B) and postcontrast (C and D) T1 values obtained at the left ventricular posterior wall (base; A and C) and interventricular septum (mid; B and D) in 6 dogs with myxomatous mitral valve disease (MMVD) and 6 healthy control dogs. Individual data points are represented by circles and squares; error bars represent SD.
Native T2 values in 6 dogs with myxomatous mitral valve disease (MMVD) and 6 healthy control dogs. Individual data points are represented by circles and squares; error bars represent SD.

Figure 4—Native T2 values in 6 dogs with myxomatous mitral valve disease (MMVD) and 6 healthy control dogs. Individual data points are represented by circles and squares; error bars represent SD.

Discussion

In our study, we used cardiac MRI to assess for myocardial ischemia and fibrosis in dogs with naturally occurring MMVD, which to our knowledge has not been described previously. The techniques used in this study were feasible and safe to perform in dogs with stage B2 MMVD, and the cardiac MRI protocol was effective in identifying changes consistent with myocardial fibrosis in some dogs. There were no myocardial perfusion deficits identified in any of the dogs with MMVD or in any of the control dogs, and myocardial fibrosis detected as LGE during cardiac MRI was present in some dogs with MMVD and in some control dogs. The ECV and T2 values were not statistically different between groups.

Native T1 mapping, postcontrast T1 mapping, and T2 mapping have been used to identify myocardial abnormalities in animal and human hearts. T1 is a tissue’s longitudinal relaxation time, and T1 mapping refers to the generation of a parametric map with each pixel assigned a T1 value. Native (ie, precontrast) T1 values represent a composite signal from the intracellular and extracellular spaces, and various pathologic changes such as accumulation of edema, protein, lipid, or iron can all cause changes in native T1 values. Administration of gadolinium-based contrast shortens T1 times, and patients with greater degrees of myocardial fibrosis have greater reductions in T1 times. Thus, postcontrast T1 mapping can aid in the assessment of diffuse interstitial myocardial fibrosis. LGE occurs when gadolinium-based contrast is slow to be washed out of regions of myocardium that have undergone scar formation with collagen deposition, so LGE is indicative of regional myocardial fibrosis. T2 is the time constant related to the exponential decay of transverse magnetization. Compared with T1, T2 has a greater fractional increase when water content increases; thus, T2-weighted cardiac MRI can be used to detect myocardial edema and inflammation. The calculation of ECV can help identify expansion of the extracellular space secondary to diffuse myocardial fibrosis or myocardial edema, and ECV is therefore a useful addition to LGE, T1 values, and T2 values.

Native T1 mapping and assessment of postcontrast T1 values and ECV have been performed in cats with preclinical hypertrophic cardiomyopathy, with a significant difference in native T1 values and ECV between clinically normal cats and cats with hypertrophic cardiomyopathy demonstrated. LGE has been used successfully to evaluate myocardial changes in Golden Retrievers with muscular dystrophy, whereas LGE has been shown not to be useful in quantifying myocardial fibrosis in Maine Coon cats with hypertrophic cardiomyopathy. In that study, native T1 imaging was not performed, and the authors speculated that LGE did not identify myocardial fibrosis in those cats because of the inclusion of cats with milder disease that may have been characterized by primarily interstitial fibrosis, which is less likely to be detected by LGE than the replacement fibrosis that might occur with more advanced disease. Evaluation of myocardial edema with T2-weighted cardiac MRI and fibrosis with T1 mapping and LGE in veterinary species has been evaluated most thoroughly in dogs with experimentally induced infarction or reperfusion and diastolic dysfunction. However, the evaluation of these parameters in dogs with naturally occurring MMVD has not been investigated.

The greater left ventricular ejection fraction in the dogs with MMVD compared with the control dogs in our study was expected because of the increased left ventricular preload caused by mitral regurgitation. There was no difference in T2 values between dogs in the MMVD and control groups, and this is consistent with previous histopathologic studies in dogs with MMVD, which suggest myocardial inflammation and edema are not prominent features of this disease. The absence of an identifiable difference between the MMVD and control groups in postcontrast T1 values suggests there was no evidence of greater diffuse fibrosis in dogs with MMVD compared with control dogs. Similarly, the lack of difference in native T1 values between groups suggests that there were no significant myocardial differences between groups. This is in contrast to a study that found significant differences in global native T1 values and LGE between humans with mitral valve prolapse and healthy subjects. To our knowledge, no veterinary clinical trial has thus far demonstrated a significant survival benefit when dogs with preclinical MMVD have been treated with angiotensin-converting enzyme inhibitors.
enzyme inhibitors. The lack of significant differences in myocardial fibrosis between healthy dogs and those with MMVD may explain these historical findings in part. Four dogs with MMVD and 3 control dogs in our study did have evidence of LGE, suggesting that focal areas of myocardial fibrosis were present. The interpretation of this finding is challenging. The fact that both groups were represented similarly suggests that dogs with advanced MMVD may not develop focal areas of myocardial fibrosis significantly more often than what may occur during the normal aging process. However, the number of dogs studied here was relatively low, and further investigation is warranted.

Plasma NT-proBNP concentration is known to correlate with severity of mitral regurgitation and left heart remodeling, so it was somewhat unexpected for there to be no significant difference in NT-proBNP concentrations between the MMVD and control groups in our study. The 2 highest individual NT-proBNP concentrations were in dogs in the MMVD group, but high NT-proBNP concentrations were also seen in dogs in the control group. It is possible that the medications administered to the dogs with MMVD lowered measured NT-proBNP concentrations, in that NT-proBNP concentrations have been documented as being lowered in response to pimobendan administration in dogs and enalapril administration in humans.

The higher cTnI concentrations in dogs with MMVD compared with control dogs in our study was consistent with findings in previous studies. In humans, high cTnI concentrations can be associated with subclinical myocardial infarctions detected by cardiac MRI. In contrast, there were no myocardial perfusion deficits identified in the dogs in our study. Abnormalities identified with cardiac MRI in some humans with diseases that result in subclinical myocarditis and myocardial fibrosis do not always correlate well with cTnI concentrations; instead, cTnI concentrations were associated more commonly with clinically important arrhythmias, echocardiographic changes, and congestive heart failure. A previous postmortem study of dogs with MMVD found correlations between cTnI concentrations and myocardial fibrosis and arteriosclerosis, but it is possible that serum cTnI concentrations increase with early myocardial abnormalities that cannot be identified with cardiac MRI.

The absence of significant differences in native T1 values, postcontrast T1 values, ECV, and serum galectin-3 concentrations between groups in our study was consistent with the conclusion that dogs with advanced MMVD may not develop myocardial fibrosis more than what occurs in healthy older dogs. Dogs with LGE did not have high galectin-3 concentrations compared with dogs without LGE. This contrasts with previous data that plasma galectin-3 concentrations were higher in dogs with MMVD that had myocardial fibrosis identified histologically. The fact that some dogs with MMVD were receiving enalapril may have contributed to the lack of high galectin-3 concentrations in some patients. In hypertensive rats, enalapril resulted in a shift in activation of cardiac fibroblast subpopulations, resulting in reduced expression of genes associated with fibrogenesis. Galectin-3 is also thought to mediate aldosterone-induced vascular fibrosis, indicating that blockade of the renin–angiotensin–aldosterone system may lower galectin-3 concentrations.

Our study had several limitations. The small sample size inherently limits the ability to apply these findings to the general population of dogs with MMVD. Given the need for general anesthesia, we chose to use a small sample size to ensure feasibility of the study and safety of the dogs. All dogs handled the anesthesia and cardiac MRI well, which may be encouraging for additional studies with greater numbers of dogs. Because many geriatric small-breed dogs can have mitral regurgitation even in the absence of a heart murmur, age and breed matching was not feasible. However, we recruited older dogs in the control group to minimize age-related differences, and there was no significant difference in age between groups. We chose not to exclude dogs that had been administered enalapril, because the correlation between angiotensin-converting enzyme inhibitor use and myocardial fibrosis is unknown. However, it is possible that both galectin-3 concentrations and cardiac MRI findings, including LGE, may have been altered by this drug. Long-term follow-up was outside the scope of this study; however, monitoring these patients over time with serial cardiac MRI may be of benefit. It is also possible that dogs with stage B2 MMVD are not the ideal dogs to study, and that dogs with more advanced MMVD may have a greater amount of myocardial fibrosis compared with dogs in our study.

In our study, both dogs with MMVD and healthy control dogs had LGE detected by means of cardiac MRI. However, there were no significant differences in native T1, native T2, ECV, or postcontrast T1 values between groups. Similarly, there was no difference in galectin-3 concentrations between groups. In this small group of dogs, those with MMVD did not have evidence of greater quantities of myocardial fibrosis compared with healthy control dogs. Further studies with larger groups of dogs and longitudinal follow-up with cardiac MRI may provide additional insights into the potential clinical utility of this imaging modality.

Acknowledgments

Funding was provided by the Ohio State University College of Veterinary Medicine and the Cavalier Health Fund. Funding sources did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript. The authors declare there were no conflicts of interest. The authors thank Denise Bailey, Kathleen Bailey, and Olivia Stepp for aiding with data collection, and Dr. Amanda Panfil for assistance with measuring galectin-3 concentrations.

References


50. Garvin AM, de Both MD, Talboom JS, Lindsey ML, Huettelman MJ, Hale TM. Transient ACE (angiotensin-converting enzyme) inhibition suppresses future fibrogenic capacity and heterogeneity of cardiac fibroblast subpopulations. *Hypertension.* 2021;77:904–918. doi:10.1161/HYPERTENSIONAHA.120.16352