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Myocardial collagen deposition and inflammatory cell infiltration in cats with pre-clinical hypertrophic cardiomyopathy

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Highlights

- Cats with mild left ventricular (LV) hypertrophy were identified with early hypertrophic cardiomyopathy (HCM).
- Cats with pre-clinical HCM had inflammatory cell infiltrates and increased collagen content in the myocardium compared to normal cats.
- An inflammatory process might contribute to the pathogenesis of HCM in cats.

Abstract

The histological features of feline hypertrophic cardiomyopathy (HCM) have been well documented, but there are no reports describing the histological features in mild pre-clinical disease, since cats are rarely screened for the disease in the early stages before clinical signs are apparent. Histological changes at the early stage of the disease in pre-clinical cats could contribute to an improved understanding of disease aetiology or progression. The aim of this study was to evaluate the histological features of HCM in the left ventricular (LV) myocardium of cats diagnosed with pre-clinical HCM. Clinically healthy cats with normal (n = 11) and pre-clinical HCM (n = 6) were identified on the basis of echocardiography; LV free wall dimensions (LVFWd) and/or interventricular septal wall (IVSd) dimensions during diastole of 6-7 mm were defined as HCM, while equivalent dimensions < 5.5 mm were defined as normal. LV myocardial sections were assessed and collagen content and inflammatory cell infiltrates were quantified objectively. Multifocal areas of inflammatory cell infiltration, predominantly lymphocytes, were observed frequently in the left myocardium of cats with pre-clinical HCM. Tissue from cats with pre-clinical HCM also has a higher number of neutrophils and a greater collagen content compared with the myocardium from normal cats. The myocardium variably demonstrated other features characteristic of HCM, including arteriolar mural hypertrophy and interstitial fibrosis

and, to a lesser extent, myocardial fibre disarray and cardiomyocyte hypertrophy. These results suggest that an inflammatory process could contribute to increased collagen content and the myocardial fibrosis known to be associated with HCM.

Keywords: Feline; Hypertrophic cardiomyopathy; Myocardial fibre disarray; Inflammation; Collagen

Introduction

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disease characterised by concentric hypertrophy of the left ventricle (LV). In human beings and cats, HCM is caused by mutations in genes that encode for the myofilament sarcomeric proteins, Z-disc proteins, calcium-handling proteins and other protein related to the sarcomere (Ferrantini et al., 2009; Lehrer and Geeves, 2014). To date, 20 genes with over 400 missense mutations have been identified in human beings; some of these mutations have strong evidence for pathogenicity, while others have less evidence (Ferrantini et al., 2009; Tian et al., 2013; Marsiglia and Pereira, 2014).

In cats, two single nucleotide substitutions in the myosin-binding protein C gene have been identified, but the broad genetic spectrum in human beings suggests that many sarcomeric genes could also be implicated for cats (Wess et al., 2010). In both species, there is marked phenotypic heterogeneity and LV hypertrophy can be global or regional. Papillary muscle hypertrophy, systolic anterior motion of the mitral valve and/ or left atrial dilatation have also been identified (Liu et al., 1981; Kittleson et al., 1999; Fox, 2003). In cats, the functional implications of this pathology include diastolic dysfunction that can result in congestive heart failure, systemic thromboembolism and fatal arrhythmias (Fox et al., 1995).

In human beings, the mechanism by which the genetic mutation in the sarcomere translates to the phenotype remains poorly understood. Direct investigation of the effect of the HCM sarcomeric mutation is difficult because human tissue is limited to autopsy samples of patients with terminal disease, small biopsy samples, or myectomised tissue from patients with

LV outflow tract obstruction. For the latter, tissue samples are modified by secondary changes associated with altered haemodynamic and mechanical stress independent of the disease-causing mutation. While transgenic mouse models afford more readily accessible tissue, the HCM phenotype in this species develops without LV outflow tract obstruction and microvascular pathology (Maass and Leinwand, 2000; Shephard and Semsarian, 2009). Studies of the pathogenesis of feline HCM from sarcomeric mutation to phenotypic expression are lacking and histological description is limited to post-mortem investigations of cats with severe and spontaneously terminal disease (Liu et al., 1981, 1993; Fox et al., 1995; Kittleson et al., 1999).

As in human beings, a post-mortem diagnosis of feline HCM is based on the identification of a hypertrophied, non-dilated LV and an increase in absolute and relative heart weight (Liu et al., 1993). Histological changes of LV myocardial tissue stained with haematoxylin and eosin (H&E) include myocardial fibre disarray, intramural coronary arteriosclerosis and myocardial fibrosis (Liu et al., 1981, 1993; Kittleson et al., 1999; Fox, 2003). Cardiomyocytes have been described as hypertrophied, with large, rectangular, hyperchromic nuclei (Fox, 2003), but similar changes have not been identified in cats with HCM (Kershaw et al., 2012).

The aim of the present study was to report the LV histological changes in cats with pre-clinical HCM compared to normal cats. Additional staining techniques were utilised to quantitatively assess the LV myocardium of cats with pre-clinical HCM for collagen content and infiltration by inflammatory cells.

Materials and methods

Animals

Un-owned cats scheduled for euthanasia were obtained from a local animal shelter; their use was approved by the University of Queensland Animal Ethics Committee (approval number SVS/040/09). Cats that appeared overtly healthy and were considered to be normal on clinical examination (n = 28) were sedated SC with 0.1 mg/kg acepromazine (ACP 2, Delvet) and 0.1 mg/kg hydromorphone (Dilaudid, Mundipharma) for echocardiography. Cats without cardiac disease and those with pre-clinical HCM were then recruited for further study.

Normal cats

Cats were identified as normal if the following criteria were met: (1) physical examination was unremarkable, they were well hydrated and had a body condition score of 3-5/5 (Laflamme, 1997); (2) thoracic auscultation identified a regular heart rhythm and no heart murmur; (3) six-lead electrocardiogram identified normal sinus rhythm or sinus tachycardia with a mean electrical axis between -10° and +140° (Harvey et al., 2005); and (4) echocardiography identified LV wall symmetry from the right parasternal short-axis view by continuous base-to-apical sweep with LV free wall (LVFWd) and interventricular septal wall (IVSd) dimensions during diastole of <5.5 mm (Fox et al., 1995), left atrial (LA) to aortic (Ao) root ratio (LA:Ao) < 1.37 (Abbott and MacLean, 2006), subjectively normal right heart with no more than trivial insufficiencies of the pulmonic and tricuspid valves, no insufficiency of the aortic and mitral valves, ventricular outflow velocities determined by pulsed-wave Doppler echocardiography of < 1.5 m/s and pulsed-wave tissue Doppler velocity of the lateral mitral valve annulus determined from the left apical four-chamber view of > 5.8 cm/s (Koffas et al., 2006).

Cats with pre-clinical hypertrophic cardiomyopathy

Cats were identified as having pre-clinical HCM if the following criteria were met: (1) physical examination was unremarkable and body condition score was 3-5 (Laflamme, 1997); (2) thoracic auscultation was unremarkable or identified a systolic heart murmur of grade IV/VI or less; (3) six-lead electrocardiogram identified normal sinus rhythm or sinus tachycardia irrespective of mean electrical axis (Harvey et al., 2005) and; (4) echocardiography identified LVFWd and/ or IVSd dimensions during diastole of 6-7 mm (MacDonald et al., 2006), LA:Ao ratio > 1.37 (Abbott and MacLean, 2006), subjectively normal right heart with no more than trivial insufficiencies of the pulmonic and tricuspid valves, no insufficiency of the aortic valve and right ventricular outflow velocity determined by pulsed-wave Doppler < 2.4 m/s (Rishniw and Thomas, 2002).

Echocardiography

Echocardiographic (Phillips iE33, Phillips Healthcare) examination was performed with the cat lightly restrained in lateral recumbency on a purpose-designed table, which allowed placement of the transducer (12 MHz) on the dependent side of the thorax. Electrodes attached to the skin overlying the stifles and right elbow allowed the simultaneous recording of a lead II electrocardiogram (ECG) that was displayed on the ultrasound monitor. All examinations were performed by the same experienced echocardiographer (FEC).

Dimensional measurements of the LV were made from a right parasternal short axis view (Thomas et al., 1993) at the level of the papillary muscles from two-dimensional short-axis

images using the leading-edge method (Sahn et al., 1978) and included IVSd and LVFWd and the internal diameter of the LV in diastole and systole (LVIDd and LVIDs, respectively). Calipers were positioned at the onset of the QRS complex on the simultaneously recorded ECG for determination of diastolic measurements. Systolic measurements were made from the frame with smallest chamber dimension immediately preceding ventricular expansion.

Using a modification of a previously described technique (Rishniw and Erb, 2000), LA and aortic root (Ao) dimensions were determined from a right parasternal two-dimensional short-axis view at the heart base by directing the calipers in a line along the commissure between the non-coronary and the left coronary aortic valve cusps through the Ao and LA. All LA and Ao measurements were determined immediately preceding atrial systole at the onset of the P wave on the ECG (Abbott and MacLean, 2006).

Echocardiographic examination also included colour-flow Doppler assessment of all valves and pulsed-wave Doppler assessment of both outflow tracts to identify any significant valvular insufficiencies or outflow obstruction suggestive of non-HCM cardiac disease and to quantify peak LV outflow tract velocity when systolic anterior motion of the mitral valve was present with HCM.

Transmitral flow was recorded from the left apical four-chamber view with the 2 mm pulsed-wave sample volume placed between the tips of the open mitral leaflets. Peak early (peak E) and late (peak A) diastolic flow wave velocities were measured. When rapid heart rates produced E and A wave summation, peak velocity of the summed waveform (summed EA) was

recorded. Pulsed-wave tissue Doppler imaging of the LV myocardium was used to determine early (Peak E') and late (Peak A') diastolic velocity of the lateral mitral annulus from the left parasternal four-chamber view. When rapid heart rates produced E' and A' wave summation, peak velocity of the summed waveform (summed E'A') was recorded. For tissue Doppler imaging, the gate of the 12 MHz transducer was placed perpendicular to myocardial movement, the Nyquist limit was set at 10-15 cm/s, sweep speed was 160 cm/s and the filter was set at 50 MHz (Koffas et al., 2006). All measures were made from four to five consecutive cardiac cycles and averaged.

Selection and preparation of tissue sections

Hearts were collected, sectioned and prepared according to a standardised protocol. Cats were humanely euthanased by sodium pentobarbitone (30 mg/kg IV; Lethabarb Euthanasia Injection, Virbac) and heparin was administered (320 IU/kg IV) to prevent thrombosis. Right thoracotomy was immediately performed and the heart was excised from the mediastinum, and peripheral fat and loose connective tissue were removed. The wet heart weight was recorded, then the LV was excised, weighed and fixed in 10% neutral buffered formalin.

Full thickness tissue sections of the LV were taken perpendicular to the long axis of the LV from: (1) the IVS at the point of maximal thickness, and (2) the posterior LVFW about one-half the distance between the mitral valve annulus and the LV apex. These sections were dehydrated in graded ethanol series, embedded in paraffin wax and processed for histopathology. Sections with a thickness of 10 µm for picrosirius red staining, and 6 µm for H&E and Leder (chloroacetate esterase) staining, were mounted onto slides.

183

184 *Routine histopathological examination*

185 LV tissues stained with H&E from normal cats (n = 11) and cats with pre-clinical (n = 6)
186 HCM were evaluated histologically by a single blinded veterinary pathologist (HO). Subjective
187 description of tissue sections included: (1) semi-quantification of myocardial fibre disarray
188 visually estimated as absent (occupying < 5% or 5-15% of the tissue section); (2) presence or
189 absence of myocyte hypertrophy (assessed subjectively as number fold increase in fibre diameter
190 compared to normal healthy tissues), fragmentation and vacuolation; (3) presence or absence,
191 type and degree of infiltrative leucocytes; (4) Subjective description of arteriosclerosis with
192 mural thickening of the small arterioles and the presence or absence of arteriole thrombi; and (5)
193 presence or absence of myocardial fibrosis.

194

195 *Quantification of collagen by picrosirius red stain staining*

196 Dehydrated tissue sections (10 µm thickness) from normal cats and cats with pre-clinical
197 HCM were rehydrated by immersion in lithium carbonate (saturated in distilled water) for 3 min.
198 Slides were rinsed in a water bath (5 min) and washed in distilled water (30 s) before being
199 immersed in phosphomolybdic acid (0.2% in distilled water, 4 min) to reduce non-specific
200 binding of the stain to the section. After rinsing in distilled water, slides were transferred into the
201 picrosirius red stain (0.1% sirius red F3BA in saturated picric acid) and incubated for 45 min.
202 Slides were then placed in 0.01 M hydrochloric acid for 2 min, removed and mounted on Depex
203 with a cover slip and allowed to dry overnight (Allan et al., 2005). Analysis of stained sections
204 was performed using a laser-scanning confocal microscope (Model LSM 510 Meta, Carl Zeiss)

with a HeNel laser. Slides were exposed to a red filter (excitation wavelength of 543 nm, emission wavelength of 560-615 nm).

Since any fibrosis in the normal and pre-clinical cases appeared to be relatively uniform in distribution on the basis of examination of H&E sections, five randomly selected regions of each tissue section were chosen for evaluation. Images were acquired at 40x magnification and analysed for pixel intensity to ascertain the extent of collagen deposition. The data were compiled using Image J software (National Institute of Health). Collagen deposition, given as a percentage of tissue evaluated, was averaged from five images (Fenning et al., 2005).

Identification and quantification of neutrophils

Tissue sections (6 µm thickness) from normal cats and cats with pre-clinical HCM were stained by the Leder method using the 91C-1KT - Naphthol AS-D Chloroacetate (Specific Esterase) Kit (Sigma-Aldrich) to identify and quantify neutrophilic infiltrates (other myeloid cells and mast cells will also take up this stain). The total number of neutrophils per 400x field were counted in 20 randomly selected fields; data is provided as total number of neutrophils per cat.

Statistical analysis

Statistical analysis was performed using SPSS 16 (IBM). Results are reported as medians and interquartile ranges (IQR), since the data were not normally distributed. Wilcoxon's rank sum test was used to identify differences between the two groups. P values < 0.05 were considered to be statistically significant.

Results

Animals

Normal cats - Eleven cats (seven males and four females) were identified as normal for inclusion in the study. Age was unknown, but all appeared to be young adults (n = 4) or middle-aged (n = 7). Breeds represented included Domestic short hair (n = 10) and Domestic longhair (n = 1). The median interventricular septum and left ventricular free wall thicknesses at diastole were 4.1 mm (IQR 3.6-4.75 mm) and 4.1 mm (IQR 3.8-4.9 mm), respectively.

Cats with pre-clinical hypertrophic cardiomyopathy - Pre-clinical HCM was identified by echocardiography in six cats (four males and two females). Age was subjectively assessed as young adult (n = 2) and middle-aged (n = 4). Breeds represented included Domestic short hair (n = 4), Domestic long hair (n = 1) and British blue (n = 1). The median interventricular septum and left ventricular free wall thicknesses at diastole were 6.0 mm (IQR 6.0-6.6 mm) and 6.3 mm (IQR 5.8-6.4 mm), respectively. No murmur was noted in any of the cats on auscultation.

Echocardiographic examination

Cats with pre-clinical HCM had increased IVSd and LVFWd dimensions compared to normal cats. LA dimensions of cats with pre-clinical HCM were within the limits of normal (Abbott and MacLean, 2006) and comparable to the LA of normal cats in this study (Table 1). In comparison with normal cats, cats with pre-clinical HCM had a significantly reduced LVIDs and increased contractility (P = 0.043). There was no difference in the heart rate, LVIDd, Ao, LA or La:Ao ratio (Table 1). Peak E, Peak A, E:A ratio, Peak E' and Peak A' values are shown in

Table 1 but were not analysed due to the small sample size. The summed early and late diastolic velocity (summed E'A') was not significantly reduced ($P = 0.102$) in cats with pre-clinical HCM, compared to normal cats (Table 1).

Gross pathology

Normal cats and cats with pre-clinical HCM had similar bodyweights (BWs), total wet heart weights and LV weights (Table 2). Heart weight:BW ratio and LV:total heart weight ratio did not differ between normal cats and cats with pre-clinical HCM ($P > 0.05$).

Histopathology

Tissue sections from normal cats (Fig. 1) did not demonstrate any evidence of myocardial fibre disarray. Similarly, the myocytes were histologically normal, with no hypertrophy, fragmentation or vacuolation. Rare isolated lymphocytes were identified in LV tissue of all normal cats. Small arterioles appeared to be structurally normal in all samples, with no evidence of intraluminal thrombi.

Tissue sections from 2/6 cats with pre-clinical HCM demonstrated small areas (<5% of sections examined) of mild myocardial fibre disarray and all cats demonstrated mild (up to 1.5 fold) myocyte hypertrophy, but myocyte fragmentation and vacuolation was not apparent. Multifocal regions up to 0.8 mm in diameter containing neutrophils, along with dense populations of lymphocytes, plasma cells and macrophages, were identified in the LV myocardium of 4/6 cats (Fig. 2). The free wall and interventricular septum were both affected in 2/6 cats. All affected tissues had mid-myocardial inflammatory cell aggregates; in cases where

both the free wall and interventricular septum were affected, the free wall also had sub-epicardial aggregates. Up to 10% of the tissue in the section was affected. Mild to moderate mural hypertrophy of small arterioles (up to ~290 µm in diameter) was identified in all cats with HCM but no intraluminal thrombi were identified (Fig. 3). In the septum and free wall of 5/6 cats with pre-clinical disease, there were small multifocal areas where the interstitium was minimally expanded by fibroplasia.

Quantification of collagen

Collagen content was increased in LV myocardial tissue from cats with pre-clinical HCM ($P < 0.001$) compared to myocardial tissue from normal cats (Figs. 4 and 5).

Quantification of neutrophils

Neutrophils were identified in the LV myocardium from 3/11 normal cats and all six cats with pre-clinical HCM (Fig. 6). Tissue from cats with pre-clinical HCM ($P < 0.01$) had comparably increased neutrophil counts relative to tissue from normal cats (Fig. 7).

Discussion

This study demonstrated that the histological features of HCM, including myocardial fibre disarray and cardiomyocyte hypertrophy, arteriolar mural hypertrophy and interstitial fibrosis (Liu et al., 1981, 1993), were present in the LV myocardium of cats with echocardiographic evidence of pre-clinical HCM, thus supporting the echocardiographic diagnosis. In addition, increased collagen deposition and neutrophilic and lymphocytic infiltrates were found in the myocardium of cats in the pre-clinical HCM group.

297

298 The development of cardiomyocyte hypertrophy and gross LV concentric hypertrophy in
299 HCM is poorly understood. The varied sarcomeric defects of HCM generally result in increased
300 myofibrillar calcium sensitivity. Increased rate of calcium binding to troponin C and faster cross-
301 bridge turnover rate suggest that HCM gene mutations result in a hyper-contractile cardiac
302 phenotype (Marston, 2011). The resulting alteration in mechanosensation and
303 mechanotransduction, together with a gross energy deficiency arising from impaired thermal
304 efficiency in HCM may promote compensatory hypertrophy. Diastolic dysfunction can result
305 directly from altered calcium cycling (Fatkin et al., 2000) or, indirectly, as a result of LV
306 hypertrophy, promoting fibrosis and disorganisation of the connective tissue matrix (Factor et al.,
307 1991).

308

309 Intimal hyperplasia and medial hypertrophy of the intramural coronary arteries has been
310 identified to a variable degree in both human beings and cats with HCM, and was identified in
311 the present study in cats with mild pre-clinical HCM. Affected arteries are found in both
312 hypertrophied and non-hypertrophied regions of the LV, but more extensively in human beings
313 with HCM than those with LV hypertrophy due to non-HCM diseases. This finding suggests that
314 arterial pathology represents a primary constituent of the cardiomyopathic process rather than
315 developing secondary to elevated intramyocardial wall tension (Maron et al., 1986; Cecchi et al.,
316 2009). The arterial pathology, particularly when coupled with micro-thrombi, results in luminal
317 narrowing which limits coronary blood flow, with the subsequent potential for myocardial
318 ischaemia, necrosis and replacement fibrosis (Maron et al., 1986; Liu et al., 1993; Cecchi et al.,
319 2009).

The reduced capillary density in the hypertrophic myocardium and the increased oxygen demand of hypertrophic cardiomyocytes might provide an additional mechanism for ischaemia and secondary fibrosis in HCM (Maron et al., 1986; Liu et al., 1993; Cecchi et al., 2009). A relationship between regional ischaemia and fibrosis is supported by the spatial association of affected arteries and fibrotic tissue in the current and previous histological studies (Liu et al., 1981; Maron et al., 1986). Impaired myocardial perfusion has been identified in regions of fibrosis in humans with HCM, further supporting the theory that coronary microvascular dysfunction induces myocardial fibrosis in HCM (Sotgia et al., 2008).

The inflammatory cell infiltration affecting up to 10% of the myocardium in pre-clinical cats in the current study was considered to be significant, since no such inflammatory cell aggregations were noted in control cats. The proximity of lymphocyte infiltrates and fibrosis suggests that myocardial fibrosis in HCM could be an active process that is modified by an inflammatory response. Cytokines released from these inflammatory cells, including interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α might play a role in myocardial remodelling and further fuel the inflammatory process. TNF-α, via regulation of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs, modulates the balance between extracellular matrix synthesis and degradation, and might contribute to the fibrosis identified in cats with HCM (Sivasubramanian et al., 2001). Studies of human patients with mild HCM have identified elevated circulating TNF-alpha, IL-1, IL-6, IL-10 (Hogye et al., 2004; Kuusisto et al., 2012).

342 A correlation between fibrosis and infiltration of the myocardium by T cells and
343 eosinophils has been identified in HCM patients (Kuusisto et al., 2012). Similarly, inflammatory
344 cell infiltrates are identified in the myocardium of human patients with early arrhythmogenic
345 right ventricular cardiomyopathy and are thought to facilitate progressive myocardial necrosis
346 and replacement fibrosis (Fox et al., 2000; Gemayel et al., 2001; Basso et al., 2004).
347
348 Several stimuli for inflammatory cell infiltration of the myocardium in cats with HCM
349 are possible. Altered mechanical stress with HCM could induce inflammatory cytokine
350 expression, as demonstrated in a rat model of hypertensive LV hypertrophy (Shioi et al., 1997).
351 Hypoxia and ischaemia are also potent inducers of inflammatory cytokines, including TNF-α,
352 IL-8 and monocyte chemoattractant peptide (Aukrust et al., 2005). In human patients with
353 arrhythmogenic right ventricular cardiomyopathy, it has been proposed that subclinical viral
354 myocarditis initiates inflammatory infiltrates and is necessary to facilitate gene expression and
355 provoke phenotypic manifestation of this genetic disease (Gemayel et al., 2001).
356
357 In the present study, heart weight lacked sensitivity as an absolute criterion for the post-
358 mortem diagnosis of pre-clinical HCM. An ante mortem diagnosis of HCM in cats is made via
359 echocardiography by identification of diastolic LV wall thickness > 6 mm. At post-mortem
360 examination, hallmark histological findings, and increased absolute and relative heart weight, are
361 consistent with a diagnosis of HCM (Liu et al., 1981; Fox, 2003). However, in the cats in this
362 study with mild pre-clinical HCM, characterised by mild LV hypertrophy and normal left atrial
363 size, the gross heart weight was similar to normal cats. This is likely to reflect the greater
364 sensitivity of two-dimensional and tissue Doppler echocardiography in detecting mild

hypertrophy and diastolic dysfunction. Although the number of cats in our study was small, this finding suggests that HCM cannot be excluded on the basis of normal absolute or relative heart weight at post-mortem examination.

There are several limitations to this study, including the wide age range at which cats with HCM are commonly represented. In our study, normal cats and those with mild HCM were typically young to middle-aged, so it was not possible to determine if there was any degenerative component among histological changes as reported in older cats with HCM. Systemic blood pressure and serum thyroid concentrations were not assessed in cats with pre-clinical HCM; as a consequence, LV hypertrophy secondary to hypertension and hyperthyroidism, rather than primary HCM, cannot be excluded. Cats were allocated to the 'pre-clinical HCM' group on the basis of echocardiographic findings suggestive of HCM (diastolic LVFWd and/or IVSd dimensions of 6-7 mm; Fox et al., 1995; MacDonald et al., 2006). These echocardiographic findings could also occur with systemic hypertension, hyperthyroidism, acromegaly and dehydration (Campbell and Kittleson 2007), which were not excluded in the present study; however, the histological features in the pre-clinical HCM group were consistent with HCM, indicating that our classification was appropriate. Furthermore, the possibility cannot be excluded that the inflammatory infiltrates might represent myocarditis in a hypertrophic left ventricle in cats where the cause of the hypertrophy is not HCM.

Conclusions

This study identified inflammatory cell infiltrates and increased collagen in the myocardium of cats with mild pre-clinical HCM. Cats with pre-clinical HCM had mild LV

hypertrophy with histological findings indicating possible HCM in the early stage of the disease. It would be interesting to determine if this observation is the same in cats with more severe HCM. Further studies that include immunohistochemical myocardial staining and evaluate circulating inflammatory cytokines are indicated to better characterise the role of inflammation in the pathogenesis and early development of fibrosis in feline HCM.

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Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Figure legends

Fig. 1. Representative photomicrographs of left ventricular (LV) myocardium (excluding the junction of the interventricular septum and LV free wall) from (A) a normal cat with normal myofibres and (B) a cat with pre-clinical hypertrophic cardiomyopathy, the latter demonstrating an area of myocardial fibre disarray with disordered arrangement of cardiac muscle cells at oblique angles to each other (haematoxylin and eosin stain). Scale bar = 60 μm.

Fig. 2. Representative photomicrographs of the left ventricular (LV) myocardium from normal cats and cats with pre-clinical hypertrophic cardiomyopathy (HCM) stained with haematoxylin and eosin (A, B and C) and Leder stain (D and E) for inflammatory infiltrates. (A and D) Myocardial tissue from a normal cat. (B, C and E) Myocardium from the interventricular septum and LV free wall from a cat with pre-clinical HCM, demonstrating multifocal inflammatory cell infiltration consisting predominantly of lymphocytes. A, D and E: scale bar = 370 μm; B: scale bar = 550 μm; C: scale bar = 150 μm.

Fig. 3. Representative photomicrographs of (A) normal and (B) hypertrophied arterioles in the left ventricular myocardium from cats with pre-clinical hypertrophic cardiomyopathy stained with haematoxylin and eosin (scale bars = 125 μm). The arteriole in Fig. B has plump reactive endothelium and expansion of the tunica intima.

Fig. 4. Representative photomicrographs of left ventricular (LV) myocardial tissue obtained from normal cats and cats with pre-clinical hypertrophic cardiomyopathy (HCM), demonstrating collagen content (arrows; stained with picrosirius red; magnification 400x). Top row,

582 interventricular septum (IVS; A and B) and LV free wall (LVFW; C and D) from a normal cat;
583 second row, IVS (E and F) and LVFW (G and H) of the LV from a cat with pre-clinical HCM,
584 showing increased deposition of collagen (white arrows). Scale bars = 60 μm.
585
586 Fig. 5. Percentage collagen deposition in the interventricular septum (IVS) and left ventricular
587 free wall (LVFW) myocardium of normal cats and cats with pre-clinical hypertrophic
588 cardiomyopathy (HCM). * $P < 0.05$.
589
590 Fig. 6. Representative image of the left ventricular myocardium of a cat with pre-clinical
591 hypertrophic cardiomyopathy, demonstrating neutrophils (arrows) between myofibres (Leder
592 stain). Scale bar = 125 μm.
593
594 Fig. 7. Number of neutrophils per tissue section in the left ventricular myocardium of normal
595 cats and cats with pre-clinical hypertrophic cardiomyopathy (HCM). * $P < 0.05$.
596
597
598 **Table 1**
599 Median (interquartile range, IQR) of echocardiographic measures for normal cats and cats with
600 pre-clinical hypertrophic cardiomyopathy (HCM).
601

	Normal cats (n = 10)	Cats with pre-clinical HCM (n = 6)	P
Heart rate (bpm)	226 (193-237)	223 (198-232)	0.500
LVIDd (mm)	12.6 (11.1-13.7)	10.7 (10.5-13.0)	0.225
LVIDs (mm)	6.9 (6.2-8.4)	5.1 (1.9-6.0)	0.043
FS (%)	40.7 (37.9-44.6)	55.3 (45.8-84.2)	0.043
Ao (mm)	8.8 (8.2-9.3)	8.9 (7.8-10.2)	0.893
LA (mm)	10.7 (9.6-11.0)	10.7 (9.7-11.8)	0.686

LA:Ao	1.18 (1.13-1.28)	1.18 (1.11-1.26)	0.893
Peak E (m/s)	0.6 (0.57-0.77)	0.49 (0.34-0.59)[a]	- [b]
Peak A (m/s)	0.35 (0.16-0.45)	0.71 (0.68-0.75) [a]	-
E:A	0.98 (0.93-1.14)	0.69 (0.45-0.87) [a]	-
Peak E' (cm/s)	1.25 (0.73-2.52)	0.55 (0.38-0.45) [a]	-
Peak A' (cm/s)	0.7 (0.48-1.43)	0.9 (0.6-0.75) [a]	-
Summed E'A' (cm/s)	1.10 (0.90-1.20)	8.50 (0.70-0.95)	0.102

[a] $n = 2$.

[b] Not analysed.

bpm, beats per min; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; FS, fractional shortening; Ao, aorta; LA, left atrium; LA:Ao, left atrium aortic root ratio, Peak E, peak velocity of early diastolic transmitral flow wave (E-wave); Peak A, peak velocity of late diastolic transmitral flow wave (A-wave); E:A, ratio of Peak E to Peak A; Peak E', peak velocity of early diastolic mitral annulus motion; Peak A', peak velocity of late diastolic mitral annulus motion; Summed E'A', summation of velocity of Peak E' and Peak A'.

Table 2

Median (interquartile range, IQR) of bodyweight (BW) and heart weight of normal cats and cats with pre-clinical HCM.

Weight	Normal (n = 11)	Pre-clinical HCM (n = 6)	*P*
BW (kg)	3.5 (3.2-5.4.3)	3.5 (3.2-4.3)	0.600
Wet heart weight (g)	14.4 (12.0-15.7)	16.2 (14.7-17.5)	0.028
Wet LV weight (g)	10.9 (8.6-11.7)	11.3 (9.9-14.3)	0.345
Heart weight:BW (g/kg)	3.9 (3.8-4.2)	4.2 (3.9-4.5)	0.917
LV weight:heart weight (g/g)	0.66 (0.65-0.75)	0.74 (0.62-0.78)	0.248

LV, Left ventricle.

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