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Anatomopathological staging of feline hypertrophic cardiomyopathy through quantitative evaluation based on morphometric and histopathological data

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ABSTRACT

Diagnosis of feline hypertrophic cardiomyopathy (HCM) is both clinical and anatomopathological. Since standardized echocardiographic parameters have previously been established for its diagnosis and classification, the aim of the present study is to provide an original, complete and repeatable quantitative anatomopathological evaluation of this myocardial disease. Since ES-HCM is a clearly defined clinicopathological entity of feline HCM, the present study also aims to investigate its temporal evolution. The hearts of 21 cats with previous diagnosis or suspicion of HCM and 6 control animals were submitted for morphometric and histopathological investigations. The proposed quantitative assessment of gross and histopathological features of HCM appears to be original and repeatable. Correlations between morphometric data allow to establish that the progression to the end-stage phenotypes, primarily characterized by increase in left ventricular fibrous tissue deposition, is accompanied by dilation of left ventricular lumen ($P = 0.0004$) and left atrium ($P = 0.0017$) and increase in intramural coronary arteriosclerosis ($P = 0.0293$).

1. Introduction

Feline hypertrophic cardiomyopathy (HCM) is a primary myocardial disease characterized by increased cardiac mass associated with a non-dilated and hypertrophic left ventricle (Fox, 2003). It represents the most common myocardial disease in cats (Ferasin et al., 2003; Riesen et al., 2007). Idiopathic HCM occurs in the absence of other diseases potentially contributing to or producing cardiac abnormalities such as hypertension, hyperthyroidism, mitral dysplasia and acromegaly (Ferasin, 2012). Male and middle-aged cats are the most affected patients (Migliorini, 2012), with a breed preference for Maine Coon, Ragdoll, Persian, British Shorthair (Migliorini, 2012) and Domestic Shorthair (Smith and Dukes-McEwan, 2012). A genetic predisposition has been demonstrated in Maine Coons and Ragdolls only by identification of a causative mutation in the sarcomeric gene for the cardiac myosin binding protein C (MYBPC3) (Meurs et al., 2005; Meurs et al., 2007). A wide phenotypic variability suggests the usefulness of gross examination, since HCM can cause diffuse, asymmetrical or segmental patterns of left ventricular hypertrophy (Fox, 2003). Furthermore, the left ventricular cavity is reduced, papillary muscles are hypertrophied and left atrium is moderately to severely dilated. Pulmonary edema and arterial thromboembolism (ATE) can also occur in a varying percentage of cases. Myofiber disorientation ("disarray") is considered a distinctive histopathological finding in HCM. It appears in the form of peculiar and disorganized cellular architecture. Additional common findings are intramural coronary arteriosclerosis and increased interstitial matrix or myocardial replacement fibrosis (Fox, 2003). End-stage hypertrophic cardiomyopathy (ES-HCM) is the dilated phase of HCM. It is characterized by relative dilation of the ventricular chambers, relative thinning of the ventricular walls, marked left atrial dilation and variable myocardial scarring, with consequent decreased ventricular contractility often closely followed by congestive heart failure (Baty et al., 2001; Cesta et al., 2005).

Echocardiography is the standard method to diagnose and classify feline HCM. In minimal HCM, the thicknesses of the left ventricular free wall and/or the interventricular septum range between 6.0 and 6.5 mm and LA/AO (Left Atrial/Aortic Root ratio) is less than 1.5. In mild patterns, the thicknesses of the left ventricular free wall and/or the interventricular septum range between 6.5 and 7.0 mm and LA/AO is less than 1.8. Finally, in severe HCM the thicknesses of the left ventricular free wall and/or the interventricular septum are greater than 7.0 mm and LA/AO is greater than 1.8 (Migliorini, 2012).

Contrary to the standardized echocardiographic guidelines used for feline HCM, in veterinary pathology the quantitative parameters which allow the characterization of this myocardial disease are limited. Similarly to the quantitative assessment of left ventricular hypertrophy (Kershaw et al., 2012), myofiber disarray (Kershaw et al., 2012) and myocardial fibrosis (Kershaw et al., 2012; Khor et al., 2014) proposed

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for feline HCM, the aim of the present study is to provide an original, complete and repeatable quantitative anatomopathological evaluation of feline HCM features based on morphometric and histopathological data. Since ES-HCM is a clearly defined clinicopathological entity of this myocardial disease (Baty et al., 2001; Cesta et al., 2005), the present study also evaluates the possibility of staging its temporal evolution by a morphological differentiation of the phenotypes in the initial/middle phase of the pathology from the end-stage ones based on anatomopathological findings only.

2. Material and methods

2.1. Animals

Twenty-seven cats were included in the present study. Twenty-one animals showed acute onset or chronic development of cardiac disorders, whereas the remaining six had no heart diseases and were used as controls. After spontaneous death or euthanasia, all cats were referred to the Department of Veterinary Science of University of Turin for postmortem examination between 2010 and 2014. A diagnosis of HCM was made by a combination of anamnesis, clinical signs, radiographic and echocardiographic findings and gross and histological features previously reported by Fox (Fox, 2003). Cases in which clinicopathological investigations indicated an underlying cause of left ventricular hypertrophy, such as systemic hypertension, hyperthyroidism, subaortic stenosis, mitral dysplasia and acromegaly, were excluded (Ferasin, 2012). Since blood pressure measurements were not available, systemic hypertension was ruled out on the basis of post mortem findings. Target organ damages, such as retinopathy/choroidopathy, hypertensive encephalopathy or nephrosclerosis and medial thickening of renal arterioles (Jepsen, 2011), were not detected during gross and histopathological examination. Cats also showed no chronic kidney disease, which is the most common cause of feline systemic hypertension (Jepsen, 2011). Since idiopathic hypertension occurs in the minority of hypertensive cats (Jepsen, 2011), it was considered unlikely and consequently ruled out. Hyperthyroidism was excluded because there was no clinical suspicion and no thyroid lesions were identified by gross and histopathological examination (Peterson, 2012). Finally, acromegaly was ruled out because there was no clinical suspicion and no skeletal abnormalities and pituitary adenomas were detected at post mortem examination (Greco, 2012).

2.2. Gross and histopathological examination

A complete necropsy was performed in all the 27 cats. All thoracic and abdominal organs were collected and fixed in 10% buffered formalin solution. The fixed heart was externally examined and opened following the inflow and outflow tracts after transverse sectioning at the level of the middle third of the ventricles in order to evaluate all the cardiac structures. Heart transverse sectioning was chosen according to previous anatomopathological studies (Liu et al., 1981; Cesta et al., 2005; Kershaw et al., 2012; Khor et al., 2014). Transverse sections of myocardium involving the left ventricular (LV) free wall, the interventricular septum and the right ventricular (RV) free wall and other organs samples were routinely embedded in paraffin wax blocks. Sections were cut at 5 μm thickness, mounted on glass slides, stained with Hematoxylin & Eosin (HE) and Masson's Trichrome (MT) and examined by light microscopy. The transverse myocardial section corresponding to the cut surface was examined with an optical microscope provided with an "Object Marker". Areas of disarray within the LV myocardium were marked at 100× magnification and successively counted. On the same transverse myocardial section, pathological intramural coronary arteries of the entire LV myocardium, characterized by medial and intimal thickening and narrowed lumen, were identified at 200× magnification and manually counted. Both qualitative and quantitative evaluation of disarray and intramural coronary arteriosclerosis were double-blind assessed by two authors (IB and MT). On the transverse myocardial section stained with MT the presence of interstitial, replacement and endocardial fibrosis of the left ventricle was also evaluated.

2.3. Morphometric analysis

Morphometric analysis using the Image®-Pro Plus software was used to evaluate the thicknesses of the LV free wall and the interventricular septum, the surface area of the LV lumen and left auricle and the percentage of LV fibrous tissue. The thickness of the LV free wall and of the interventricular septum were measured by applying the "Measurement" function to the photograph of the transverse section at the level of the papillary muscles. The same photograph was used to measure the surface area of the LV lumen with "Count/Size" function. This function was also applied to evaluate the surface area of the left auricle on the photograph of the auricular face. The use of a marker of known size in every photograph allowed calibration. To obtain the percentage of LV fibrous tissue, the transverse section of the left ventricle stained with MT was photographed and the picture was divided in four quadrants. The "Count/Size" function was applied to each image, exploiting the chromatic difference between fibrous tissue and myocardium highlighted by MT stain. Definitive LV fibrous tissue percentages were calculated as the mean result of the four quadrants.

2.4. Statistical analysis

GraphPad Prism® software was used to perform statistical analysis. Shapiro–Wilk's test was used to establish normality or non-normality of distribution. In order to evaluate the statistical repeatability of the morphometric measurements, ten cases were randomly selected and every gross and histopathological feature evaluated by morphometry was measured three times. Coefficient of Variation (CV) was evaluated in the three series of measurements for every parameter considered. Comparisons between data were tested using Student's *t* test and Mann–Whitney *U* test, while Spearman's rank correlation coefficient was used for correlations. P values <0.05 were considered statistically significant. Normally distributed data were expressed as mean ± SD, while non-normally distributed data were expressed as median and IQR (25%–75%).

3. Results

3.1. Animals

All the 21 cats with cardiac disorders were diagnosed with HCM. In 57% (12/21) of them, HCM was previously diagnosed by echocardiographic examination as indicated by Migliorini (2012). In the remaining 43% (9/21) the diagnosis of HCM was highly suspected on the basis of clinical signs (dyspnea, ATE and/or sudden death) and radiographic findings (cardiomegaly, pulmonary edema and/or pleural effusion). Of all cats with HCM, 57% (12/21) were male, whereas the remaining 43% (9/21) was female. Breeds represented were Domestic Shorthair (13/21, 62%), Persian (3/21, 14%), Ragdoll (2/21, 9%), Maine Coon (1/21, 5%), Devon Rex (1/21, 5%) and British Shorthair (1/21, 5%). Age of cats ranged from 1 to 16 years (7.55 ± 4.16 years). Of all control cats, 67% (4/6) were male, while the remaining 33% (2/6) was female. Breeds represented were Domestic Shorthair (4/6, 66%), Siamese (1/6, 17%) and Sphynx (1/6, 17%). Age of cats ranged from 2 to 14 years (8.00 ± 4.73 years).

3.2. Gross and histopathological examination

Histopathological data related to the evaluation of disarray, intramural coronary arteriosclerosis and LV fibrous tissue deposition are summarized in Table 1. All gross and histopathological features of HCM were identified in the affected cats. All cats presented severe and diffuse myocyte