DE GRUYTER OPEN

Bull Vet Inst Pulawy 59, 135-142, 2015
DOI:10.1515/bvip-2015-0020

Pathomorphological changes of the myocardium in canine dilated cardiomyopathy (DCM)

Izabela Janus, Marcin Nowak, Janusz A. Madej

Divison of Pathomorphology and Veterinary Forensics, Department of Pathology,
Faculty of Veterinary Medicine,
Wroclaw University of Environmental and Life Sciences, 50-375 Wroclaw, Poland
izabela.janus87@gmail.com

Received: July 9, 2014 *Accepted: November 3, 2014*

Abstract

The study was conducted on ventricular and atrial wall preparations from 11 dogs with clinically diagnosed dilated cardiomyopathy. After fixation, the specimens were stained with haematoxylin and eosin and Masson-Goldner trichrome technique. Parenchymal changes (fibrosis and fatty infiltration), vascular changes (congestion and coronary vessel wall hypertrophy), degenerative changes (loss of striation, changes in cardiomycyte and nuclei structure), and presence of inflammatory infiltrates (mononuclear and polynuclear) were estimated. Complex histological changes in both ventricular and atrial muscles were shown. It was not determined whether the processes occurring in the myocardium have a primary character, or are a consequence of developing heart failure. Such issues will be put under further and more detailed examination.

Keywords: dog, heart, dilated cardiomyopathy.

Introduction

Dilated cardiomyopathy (DCM) is a disease of complex aetiology characterised by acquired heart failure combined with the dilation of heart chambers (especially the left ventricle) without other clinically relevant cardiovascular defects (13, 14). It is observed most commonly in large and giant dogs breeds, nonetheless forms of DCM in other dog breeds (*e.g.* cocker spaniels) were also described (1, 2, 5, 13, 14). It is suspected that in both humans and animals, the disease can have a genetic character (13, 14), yet these mechanisms have not been entirely described.

A silent phase of varying duration without clinical signs is observed most commonly in dogs (2, 13, 14, 18). Symptoms of a heart failure are observed in the final stage of the disease, when progression lasts already for months or years. Death occurs most often as a result of chronic heart failure, sudden cardiac death or – in dogs with clinical symptoms occurring in old age – as a consequence of other unrelated fatal diseases (2).

The gross pathology reveals dilation of the left ventricle and left atrium or the enlargement of all heart chambers without other relevant heart defects (1, 2, 5, 13, 14). The histopathological examination shows changes in myocardium structures. These changes are most strongly expressed in the left ventricular free wall, nonetheless, can spread to the right ventricular wall and interventricular septum. Most studies concentrate on changes occurring in the ventricular walls, while changes in atria are not well described (1-3, 13, 14, 18). Changes occurring in the left ventricular myocardium are divided into two types: attenuated wavy fibre type (AWF) and fatty infiltration-degenerative type (FID) (2, 13, 14). The distinction between these two histological types may not be of major clinical importance, yet histological studies extend our knowledge of aetiology and pathogenesis of DCM (2).

The purpose of this study was a histological evaluation of specimens from ventricular walls, atrial walls, and interventricular septum from dogs with clinically diagnosed DCM.

Material and Methods

The study was conducted on samples acquired during autopsy from 11 dogs aged from 5 to 10 years

© 2015 I. Janus et al. This is an open access article distributed under the Creative Commons Atribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

(mean age 7.4 ± 2.5). All dogs had DCM recognised ante-mortem using criteria listed in literature (2, 13, 14). Among the examined dogs, there were nine Doberman Pinschers and two Boxers (seven males and four females).

Intravitally, after the diagnosis (including full clinical examination, ECG, ECHO, and 24-h Holter monitoring), all animals underwent treatment to minimise the effects of developing heart failure and rhythm disturbances. The examined dogs died within 6-24 months after the diagnosis: two due to sudden cardiac death resulting from arrhythmia and nine due to advanced heart failure which resulted in decision of euthanasia.

Directly after animals' death or euthanasia, after obtaining the owners' consent, an autopsy was performed and specimens for histopathological study were collected. The specimens were collected from the left ventricular free wall, right ventricular free wall, interventricular septum, and left and right atria, fixed in 7% buffered formalin, embedded in paraffin blocks, and cut into 6 μm sections. The preparations were stained with standard haematoxylin-eosin (H&E) method and with Masson-Goldner trichrome technique to obtain a better visualisation of connective tissue. The slides were evaluated using light microscopy. Microphotographs of the sections underwent computer-assisted image analysis using Olympus BX53 optical microscope connected to a computer equipped with a ColorView IIIu (Olympus, Japan) digital camera. The morphometric evaluation was performed using cell^A software (Olympus Soft Imaging Solution, Germany).

The sections were assessed in relation to the type of left ventricle wall structural changes (attenuated wavy fibre type – AWF or fatty infiltration-degenerative type – FID). Perivascular and extravascular fibrosis, fat infiltration, presence of inflammatory infiltrates, congestion, hypertrophy of coronary vessels walls, loss of cardiomyocyte striation, changes in the structure of cardiomyocytes, and presence of abnormal cell nuclei were also evaluated. All the features were evaluated in 20 randomly chosen fields from (-) to (+++), as shown in Table 1.

The obtained results were analysed statistically using StatisticaPL® software (StatSoft, Poland), Spearman's correlation analysis, and the Mann-Whitney U test. The level of significance was set at $P \leq 0.05$.

Results

Gross pathology revealed changes in all examined hearts. They showed significant widening of the left ventricle with visible thinning of ventricular wall. Moreover, all hearts showed slight to moderate widening of other heart chambers.

Based on left ventricular specimens stained with H&E and Masson-Goldner trichrome technique, two types of myocardial changes were distinguished: FID (n = 4) and AWF (n = 7). The characteristic changes observed in left ventricles were also present with various intensity in other parts of the heart. Moreover, in four dogs from the AWF group nonspecific changes within the atria, similar to those observed in FID type (increased amount of fibrous tissue, foci of cardiomyocytes degeneration), were seen.

In all dogs histopathological changes were observed in the heart chambers. An increased amount of connective tissue was observed in the atria as compared to the ventricles ($P < 0.02$) with no significant differences ($P > 0.05$) in the intensity of other changes between the atria and ventricles. The intensity of the observed changes is shown on Figs 1-4.

Inflammatory infiltrates and vascular changes. In five dogs, slight diffuse mononuclear inflammatory infiltrates in the atria, and in one dog – additionally in the right ventricular wall were seen. None of the dogs showed inflammatory infiltrates within the left ventricular free wall and interventricular septum. In dogs showing inflammatory infiltrates in the atria, atrial fibrillation was present intravitally, while the single dog with inflammatory cells in the right ventricular wall presented ventricular tachycardia. Moreover, a positive correlation between the inflammatory infiltrates and the amount of perivascular and extravascular fibrous tissue ($P < 0.001$; $r = 0.54$ and 0.58 respectively) and a significantly higher amount of inflammatory infiltrates in the FID group than in AWF group ($P = 0.01$) were noted (Fig. 1).

Congestion of various intensity was observed in the intramural vessels in all heart chambers in ten dogs, while in one dog a slight congestion was noted solely in the left ventricular wall. In four cases, congestion was accompanied by concentric hypertrophy of arterial walls combined with a reduction of their lumen. In six animals, the hypertrophic changes in blood vessels were not observed in each heart preparation (Fig. 5a). The dog indicating congestion only in the left ventricular wall did not display arterial wall hypertrophy. Statistical analysis confirmed a positive correlation between intramural vessel congestion and arterial wall hypertrophy ($P = 0.003$; $r = 0.4$). Moreover, a positive correlation was noted between the vascular changes and cardiomyocyte degenerative changes ($P < 0.02$; $r = 0.32$). Both the congestion and arterial wall hypertrophy was more intense in specimens from the AWF group than from FID group ($P < 0.004$) (Fig. 1).

Parenchymal changes. Perivascular fibrosis of the atria was noted in all dogs, and in ten dogs also in ventricular walls. Out of ten dogs, seven showed various degrees of fibrosis in each heart chamber (Fig. 5b). All dogs displayed extravascular fibrosis in

the atrial walls. In seven dogs fibrosis occurred in ventricular walls as well, and in two dogs it was seen in all heart chambers (Fig. 5c). More intense changes were observed in the atria than in the corresponding ventricles ($P < 0.05$). Furthermore, the statistical analysis revealed a positive correlation between these types of fibrosis (perivascular and extravascular; $P < 0.001$; $r = 0.53$), between extravascular fibrosis and fatty infiltration ($P < 0.001$; $r = 0.48$), and between fibrosis (irrespective to the type) and cardiomyocytes degenerative changes ($P = 0.02$; $r = 0.31$). Both types of fibrosis were more intense in the FID group than in the AWF group ($P < 0.004$) (Fig. 1).

Fatty infiltration of various intensity was observed in atrial walls in nine dogs and in ventricular walls also in nine dogs, with only one dog showing lesions in all heart chambers (Fig. 5d). One dog did not show fatty infiltration in any of heart samples. Simultaneously, positive correlations between fatty infiltration and blood vessel wall hypertrophy ($P = 0.001$; $r = 0.43$), and between fatty infiltration and inflammatory infiltration ($P < 0.001$; $r = 0.43$) were observed.

Degenerative changes. Muscle fibre degeneration was evaluated based on the presence of abnormal cell nuclei, changes in cardiomyocyte structure, and loss of striation (Fig. 5e). The latter was seen in all heart chambers in nine dogs. One dog showed a slight loss of striation only within the right atrium and one dog did not show the loss of striation in any of the heart chambers. Changes in cardiomyocyte structure were observed in nine dogs in all heart chambers, two dogs did not show these changes in samples from the interventricular septum. The presence of abnormal cell nuclei was observed in specimens from all heart chambers in ten dogs. One dog did not show abnormal nuclei in any heart preparations. Statistical analysis showed a strong positive correlation ($P < 0.001$; $r > 0.7$) between individual features of cardiomyocyte degeneration. The presence of abnormal nuclei was more pronounced in the AWF group than in the FID group ($P = 0.002$) (Fig. 1).

Table 1. The criteria of specimens assessment

Evaluated feature	- None or slight changes	+ Weak changes	++ Moderate changes	+++ Severe changes
perivascular fibrosis*	no or slightly expressed in < 25% of blood vessels per slide	present in 25%-50% of blood vessels or severely expressed in <25% of blood vessels per slide	present in 50%-75% of blood vessels or severely expressed in 25%-50% of blood vessels per slide	present in >75% of blood vessels or severely expressed in >50% of blood vessels per slide
extravascular fibrosis*	no or single foci of fibrosis per slide	single foci of fibrosis per field	numerous but not extensive foci of fibrosis per field	numerous extensive foci of fibrosis per field
inflammatory infiltrates (mononuclear or polynuclear)	no or single cells per slide	single cells per field	numerous cells per field without distinct foci of inflammation	numerous foci of cell-rich inflammation per field
congestion	none	present only in biggest vessels (<25% blood vessels per slide)	present in 25%-50% of blood vessels per slide	present in >50% blood vessels per slide
hypertophy of coronary vessels walls	no or slightly expressed in <25% vessels per slide	present in 25%-50% of blood vessels or severely expressed in <25% of blood vessels per slide	present in 50%-75% of blood vessels or severely expressed in 25%-50% of blood vessels per slide	present in >75% of blood vessels or severely expressed in >50% of blood vessels per slide
fatty infiltration*	no or single foci per slide	present in 10-25% of examined fields	present in 25%-50% of examined fields	present in >50% of examined fields
abnormal cell nuclei	no or single per slide	single per field	<50% nuclei per field	>50% nuclei per field
changes in cardiomyocytes structure	no or slightly expressed in <25% cardiomyocytes per slide	present in 25%-50% of cardiomyocytes or severely expressed in <25% of cardiomyocytes per slide	present in 50%-75% of cardiomyocytes or severely expressed in 25%-50% of cardiomyocytes per slide	present in >75% of cardiomyocytes or severely expressed in >50% of cardiomyocytes per slide
loss of striation	no or slightly expressed in <25% cardiomyocytes per slide	present in 25%-50% of cardiomyocytes or severely expressed in <25% of cardiomyocytes per slide	present in 50%-75% of cardiomyocytes or severely expressed in 25%-50% of cardiomyocytes per slide	present in >75% of cardiomyocytes or severely expressed in >50% of cardiomyocytes per slide

* - features evaluated after Masson-Goldner trichrome staining



Fig. 1. The intensity of myocardial changes in the fatty infiltration-degenerative (FID) group and the attenuated wavy fiber (AWF) group; + - weak changes, ++ - moderate changes, +++ - severe changes (see: Table 1)

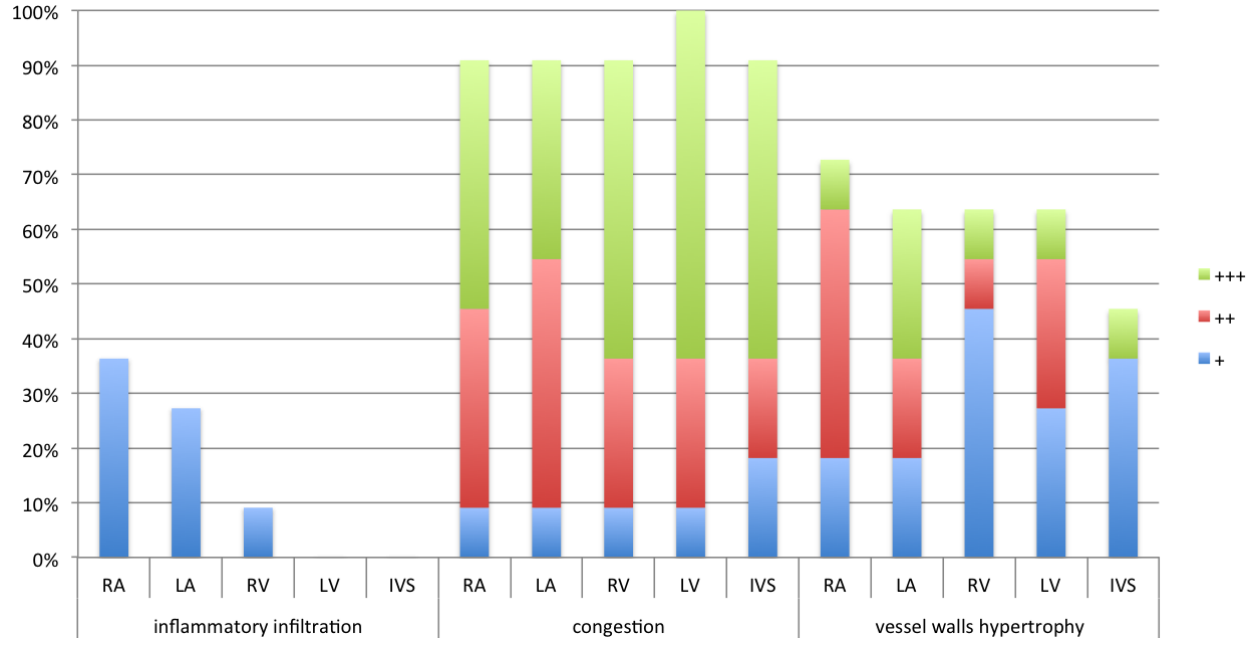


Fig. 2. The intensity of inflammatory infiltrates and vascular changes in individual heart chambers; RA – right atrium, LA – left atrium, RV – right ventricle, LV – left ventricle, IVS – interventricular septum; + - weak changes, ++ - moderate changes, +++ - severe changes (see: Table 1)

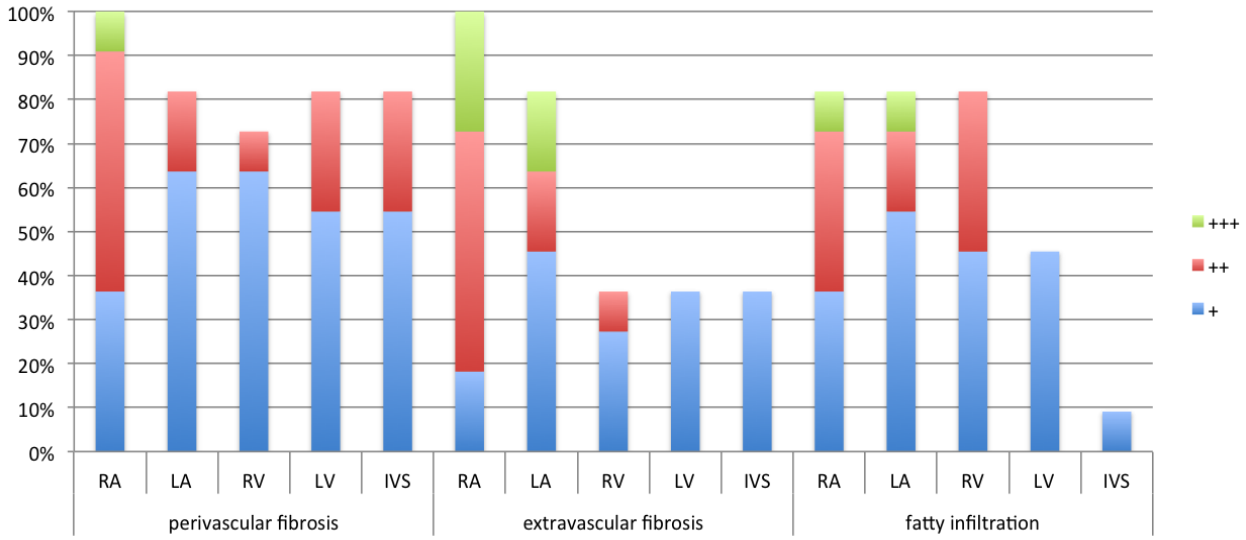


Fig. 3. The intensity of parenchymal changes in individual heart chambers; RA – right atrium, LA – left atrium, RV – right ventricle, LV – left ventricle, IVS – interventricular septum; + - weak changes, ++ - moderate changes, +++ - severe changes (see: Table 1)

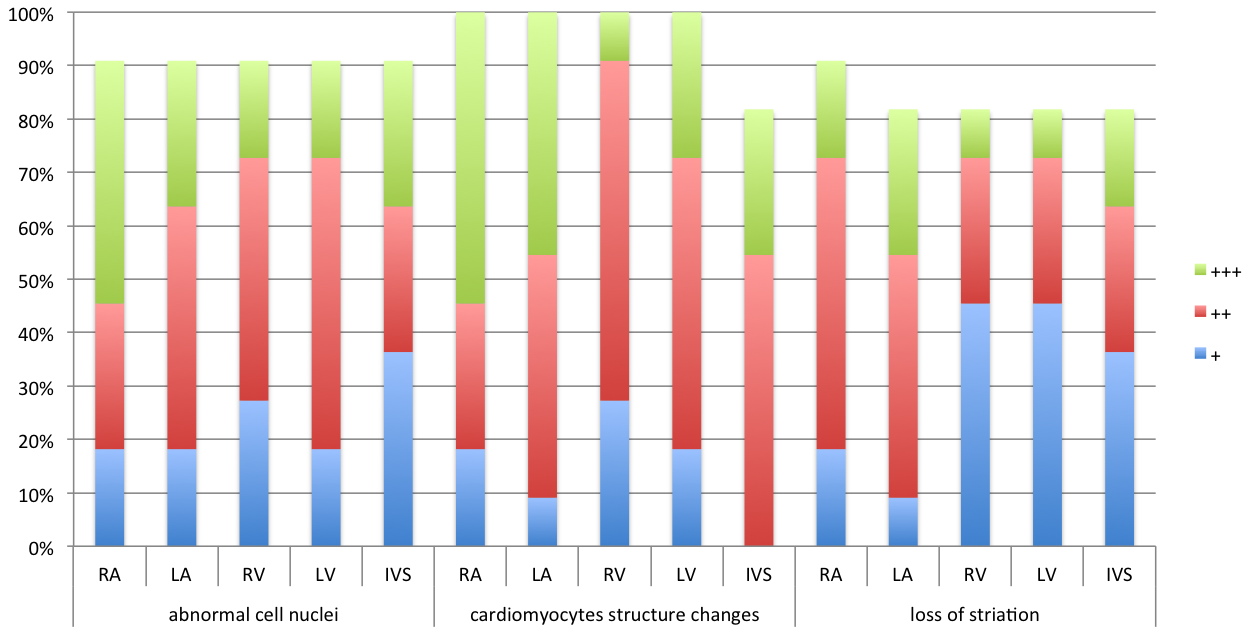


Fig. 4. The intensity of degenerative changes in individual heart chambers; RA – right atrium, LA – left atrium, RV – right ventricle, LV – left ventricle, IVS – interventricular septum; + - weak changes, ++ - moderate changes, +++ - severe changes (see: Table 1)

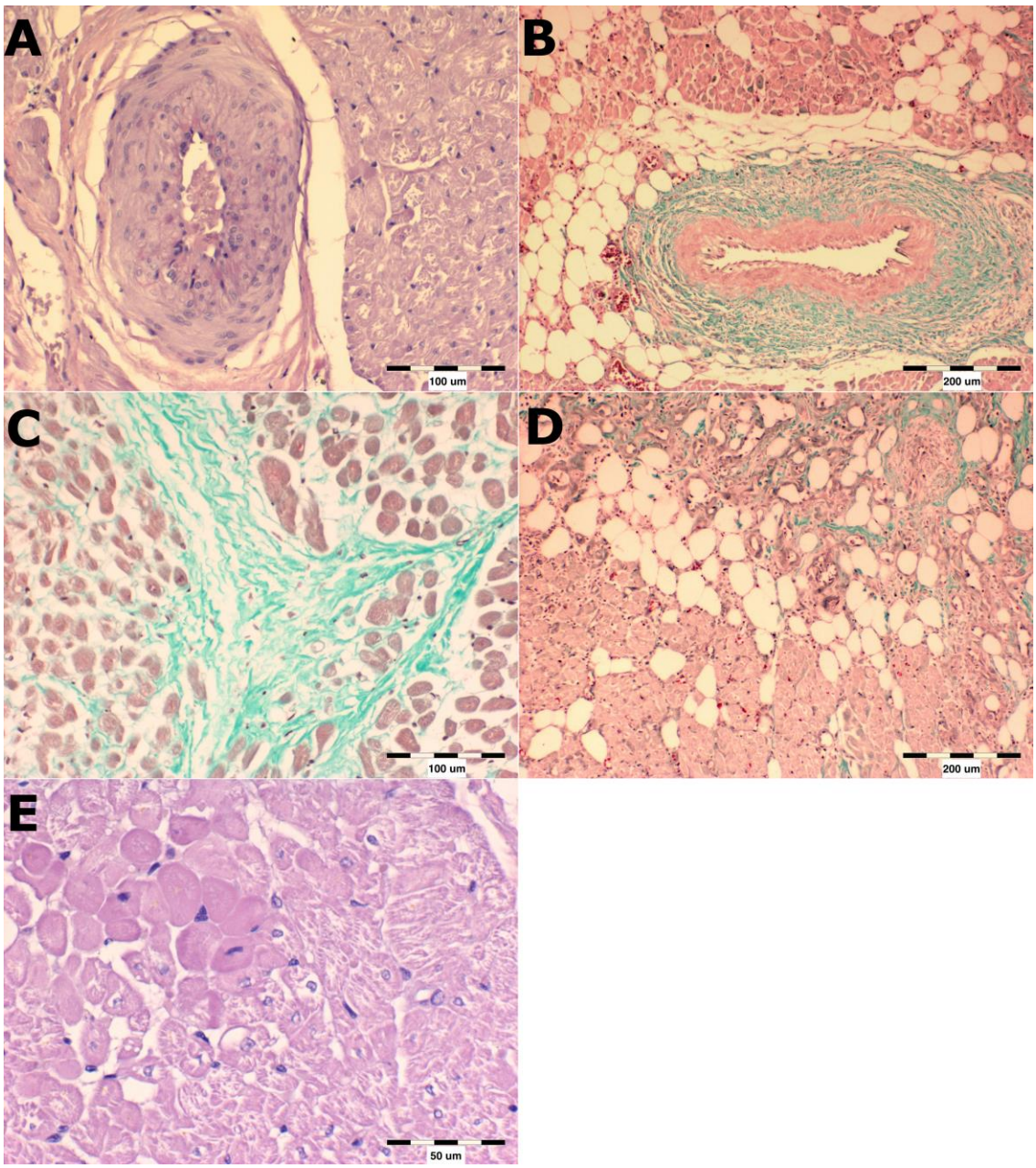


Fig. 5. Histopathological changes of the myocardium; a –concentric hypertrophy of blood vessel wall with secondary reduction of vessel lumen (H&E, 200×); b – increased amount of perivascular fibrous tissue and fatty infiltration (Masson-Goldner trichrome, 100×); c – extravascular fibrosis (Masson-Golnder trichrome, 200×); d – focus of fatty infiltration accompanied by slight amount of extravascular fibrosis (Masson-Goldner trichrome, 100×); e – signs of cardiomyocyte degeneration: abnormal cell nuclei and structure and staining changes (H&E, 400×)

Discussion

DCM affects mainly pure breed dogs, and is characteristic for large breeds (including Doberman Pinschers, Irish Wolfhounds, Great Danes, Boxers, St. Bernards and Newfoundlands) (2, 13, 14). It has been also reported in Estrela Mountain Dogs (5) and Portuguese Water Dogs (1). The study encompasses Doberman Pinschers and Boxers of similar age to that described by other authors (13).

The histological changes are mostly expressed in the left ventricular free wall; nonetheless they can also spread to the right ventricular free wall and interventricular septum (5, 18). Because samples from all heart chambers were evaluated, the distribution of histopathological changes was reported to affect not only ventricular walls but also the atria. Moreover, a more intense fibrosis within the atria compared to the ventricles and no differences in the expression of other changes between atria and ventricles may indicate simultaneous changes in atrial structure as a response to volume overload. These changes, however, may also be a result of a primary predisposition of DCM dogs' heart muscle to remodelling. In fact, changes in histological structure do not seem to be connected with volume overload and secondary stretching of heart walls

because they were not noted in dogs with heart failure due to other causes than DCM. Similar changes are also encountered in dogs diagnosed with the disease but without clinical symptoms of DCM, which may suggest signs of the first stage of the disease before dilation development (2, 12-14).

In our study, the AWF type changes were seen in preparations from seven dogs (including one Boxer and six Doberman Pinschers). More intense congestion, blood vessel walls hypertrophy, and cardiomyocyte degenerative changes as compared to the FID type, and a positive correlation between these changes can indicate a role played by vascular changes in the development of the cardiomyocyte structure disorders.

In patients with DCM and heart failure, a reduced blood flow through the myocardium (8, 17) and disturbances in function of the endothelium of small vessels were noted. These changes resulted in decreased susceptibility of the vessels to dilate during stress (7, 8, 15, 19). The diminished endothelial function of small vessels contributes to the development of the degeneration of cardiomyocytes due to ischaemia (4, 7). It is still not explained whether the endothelial dysfunction is a result of heart failure, or if it emerges during an earlier stage, complicating the course of the disease. The decreased coronary microvascular flow in patients with early DCM stages suggests the second possibility (8).

Though people with DCM do not show hypertrophy of coronary vessel walls, lesions similar to those observed in our study were noted in patients with HCM (6, 15). In the veterinary literature, reports can be found describing hypertrophy of the muscular layer of coronary arteries in dogs with DCM (including dogs showing echocardiographic signs of DCM without clinical signs) (3, 14, 18). These differences suggest that, although alterations in coronary blood flow may play a role in the pathogenesis of DCM in dogs as it does in humans, the mechanism of that process can be different.

In the FID group, more intense inflammatory infiltrates and fibrosis were noted than in AWF group with a simultaneous positive correlation between fibrosis and cardiomyocyte structural changes, and between fatty infiltration and the presence of inflammatory infiltrates. Nonetheless, studies conducted so far do not allow to establish whether the inflammatory infiltration and degeneration of cardiomyocytes are the result of transformation of the myocardium towards fibrous tissue, or whether developing myocardial fibrosis and fatty infiltration is a response to primary degenerative and inflammatory changes within the cardiomyocytes.

The changes in the ventricular myocardium in the FID group and in the atrial myocardium irrespectively of the group are similar to those observed by Sharov *et al.* (11) and Schaper *et al.* (9) in human and canine models of heart failure. Though myocardial fibrosis is combined with changes in structural proteins expression, it is still an open question whether the lesions of myocardium are the cause of worsening of left ventricular contractility or do they result from deteriorating cardiac function (11). A significantly less intense fibrosis in AWF group seen in this study, despite the advanced heart failure, suggests that myocardial remodelling may be the cause of heart failure and not only its result.

The changes in cell nuclei shape in the canine model of heart failure are the determinant of cardiomyocyte degeneration (9, 11). A less intense presence of abnormal nuclei in the FID group than in the AWF group was noted, although the first group is more morphologically similar to heart failure than the second one. This finding can suggest another mechanism of cardiomyocyte degeneration in DCM than due to volume overload. This opinion is also shared by Scholz *et al.* (10), who reported the presence of abnormal cardiomyocyte nuclei in patients with DCM, and suspect that changes in cell nuclei are a primary process causing disturbances in transcription and translation of structural proteins and secondary changes in cardiomyocyte structure.

At this stage of studies, it is impossible to determine whether the changes in the atria (including foci of fibrosis, changes in cardiomyocyte structure, and inflammatory infiltrates) result from the primary changes in the myocardial structure, or these changes are the result of volume overload secondary to ventricular dilation. The presence of supraventricular (mainly atrial fibrillation) and ventricular (mainly ventricular premature complexes) rhythm disturbances in dogs without other symptoms of the disease, and the possibility of sudden cardiac death due to arrhythmia without earlier clinical signs and with slight histopathological changes suggest a primary character of myocardial structural changes. The non-crucial role of volume overload in the development of atrial fibrillation in DCM has been also proved by results obtained by Tuomainen *et al.* (16). These authors have shown significantly lower values of ventricular measurements (left ventricular end-diastolic diameter, left ventricular end-diastolic volume, and left ventricular end-systolic volume) combined with significantly higher values of left atrial diameter in patients with chronic atrial fibrillation and DCM as compared to patients with DCM without the arrhythmia. This shows the primary susceptibility of atrial walls to remodel irrespectively to overload resulting from the left ventricular dysfunction.

The mechanism of myocardial changes is still not well known. It is suspected that defects of structural proteins or insufficient oxygen delivery may play a role (13, 14). The complex changes noted within the myocardium suggest the complexity of pathogenesis of DCM. This topic will be the subject of further and more detailed studies.

Conflict of Interests Statement: The authors declare no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This research was supported by statutory research and development activity funds by Minister of Science and Higher Education assigned to Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences.

References

1. Dambach D.M., Lannon A., Sleeper M.M., Buchanan J.: Familial dilated cardiomyopathy of young Portuguese Water Dogs. J Vet Intern Med 1999, 13, 65–71.
2. Dukes-McEwan J., Borgarelli M., Tidholm A., Vollmar A.C., Häggström J.: Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy. J Vet Cardiol 2003, 11, 7–19.
3. Everett R.M., McGann J., Wimberly H.C., Althoff J.: Dilated cardiomyopathy of Doberman Pinschers: retrospective histomorphologic evaluation of heart from 32 cases. Vet Pathol 1999, 36, 221–227.
4. Liu P.P., Mak S., Steward D.J.: Potential role of the microvasculature in progression of heart failure. Am J Cardiol 1999, 84, 23–26.
5. Lobo L., Carvalheira J., Canada N., Bussadori C., Gomes J.L., Faustino A.M.R.: Histologic characterization of dilated cardiomyopathy in Estrela Mountain Dogs. Vet Pathol 2010, 47, 637–642.
6. Maron B.J., Wolfson J.K., Epstein S.E., Roberts W.C.: Intramural ("small vessel") coronary artery disease in hypertrophic cardiomyopathy. J Am Coll Cardiol 1986, 8, 545–557.
7. Marti V., Aymat R., Ballester M., Garcia J., Carrio I., Auge J.M.: Coronary endothelial dysfunction and myocardial cell damage in chronic stable idiopathic dilated cardiomyopathy. Int J Cardiol 2002, 82, 237–245.
8. Neglia D., L'Abbate A.: Coronary microvascular dysfunction and idiopathic dilated cardiomyopathy. Pharmacol Rep 2005, 57, 151–155.
9. Schaper J., Kostin S., Hein S., Elsässer A., Arnon E., Zimmermann R.: Structural remodelling in heart failure. Exp Clin Cardiol 2002, 7, 64–68.
10. Scholz D., Diener W., Schaper J.: Altered nucleus/cytoplasm relationship and degenerative changes in human dilated cardiomyopathy. Cardioscience 1994, 5, 127–138.
11. Sharov V.G., Kostin S., Todor A., Schaper J., Sabbah H.N.: Expression of cytoskeletal, linkage and extracellular proteins in failing dog myocardium. Heart Fail Rev 2005, 10, 297–303.
12. Tidholm A., Häggström J. Jönsson L.: Detection of attenuated wavy fibers in the myocardium of Newfoundlands without clinical or echocardiographic evidence of heart disease. Am J Vet Res 2000, 61, 238–241.
13. Tidholm A., Häggström J., Borgarelli M., Tarducci A.: Canine idiopathic dilated cardiomyopathy. Part I: aetiology, clinical characteristics, epidemiology and pathology. Vet J 2001, 162, 92–107.
14. Tidholm A., Jönsson L.: Histologic characterization of canine dilated cardiomyopathy. Vet Pathol 2005, 42, 1–8.
15. Treasure C.B., Vita J.A., Cox D.A., Fish R.D., Gordon J.B., Mudge G.H., Colucci W.S., Sutton M.G., Selwyn A.P., Alexander R.W.: Endothelium-dependent dilation of the coronary microvasculature is impaired in dilated cardiomyopathy. Circulation 1990, 81, 772–779.
16. Tuomainen P.O., Magga J., Fedacko J., Kärkkäinen S., Miettinen K., Vanninen E., Kuusisto J, Peuhkurinen K.J.: Idiopathic dilated cardiomyopathy and chronic atrial fibrillation. Clin Physiol Funct Imaging 2014, 34, 133–137.
17. Van den Heuvel A.F.M., Van Veldhuisen D.J., Van der Wall E.E., Blanksma P.K., Siebelink H.M.J., Vaalburg W.M., van Gilst W.H., Crijns H.J.G.M.: Regional myocardial blood flow reserve impairment and metabolic changes suggesting myocardial ischemia in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 2000, 35, 19–28.
18. Vollmar A., Fox P.R., Meurs K.M., Liu S.K.: Dilated cardiomyopathy in juvenile Doberman Pinschers. J Vet Cardiol 2003, 5, 23–27.
19. Zhou Y.L., Li X.L., Zhang H.F., Yao W.M., Tong M., Xu Q., Cao K.J., Huang J.: Decreased small arterial compliance with increased serum vascular endothelial growth factor-A and circulating endothelial progenitor cell in dilated cardiomyopathy. Chin Med J 2008, 121, 316–320.