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Concentration of Proinflammatory Cytokines in Blood Serum of Dogs with Myxomatous Degeneration of Mitral Valve

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Abstract.

In the pathogenesis of the formation and progression of chronic heart failure syndrome in dogs with myxomatous degeneration of the mitral valve an important role is played by the chronic increase in the concentration of serum proinflammatory cytokines. The purpose of the work is to study the concentration of proinflammatory cytokines in the blood serum of dogs with myxomatous degeneration depending on the degree of functional class of heart failure, presence of pulmonary edema, left atrial dilatation, and cardiogenic cachexia. The blood serum in 40 dogs with myxomatous degeneration of the mitral valve revealed a higher concentration of interleukin-1α, interleukin-6, interleukin-8 and tumor necrosis factor-α compared to 15 clinically healthy animals. Serum concentration of these proinflammatory cytokines was found to be particularly high in the development of pulmonary edema, left-side atrial remodeling and cardiogenic cachexia syndrome, as well as it positively correlates with the functional class of chronic heart failure and the size of the left atrium. Activation of proinflammatory cytokines plays an important role in the pathogenesis of formation and progression of myxomatous degeneration of the mitral valve in dogs.

Key words: dogs, cytokines, myxomatous degeneration, interleukins, tumor necrosis factor-α.

INTRODUCTION.

Myxomatous degeneration of the mitral valve is the most common nosological entity of the cardiovascular system diseases in dogs, the frequency of its occurrence is approximately 75% of the total number of animals with cardiopathologies [1]. The pathogenesis of myxomatous degeneration in dogs resembles a mechanism of development of primary mitral valve prolapse in humans [2], which is a very common cardiovascular anomaly (population incidence is 3-8 %) [3]. In humans, the occurrence of this pathology is associated with the development of a number of clinically important complications, such as mitral regurgitation, bacterial endocarditis, congestive heart failure, atrial fibrillation and sudden death from cardiac arrest [4]. Therefore, myxomatous degeneration of atrioventricular heart valves in dogs can be used as a model for studying the primary mitral valve prolapse in humans [5].

In dogs myxomatous degeneration is an acquired valvular heart disease, mainly developing after overcoming a 5- to 6-year-old age [6-11]. The pathology is characterized by a chronic progressive degenerative bicuspid valve damage, in which the cusps thicken and deform, their approximation becomes bad and during systole mitral prolapse into the left atrium cavity may occur [5]. The above-mentioned pathological changes in the left atrioventricular valve cusps lead to mitral regurgitation, increased pressure in the left atrium, and remodeling of the left heart chambers. On the whole, most ill dogs have a formed left-side congestive heart failure, which becomes a cause of premature death of the animals [12].

Myxomatous degeneration of the atrioventricular heart valves can develop in dogs of all breeds, but small and medium-sized breeds are the most predisposed ones, especially Cavalier King Charles spaniel, Dackel, Pekingese, Miniature Poodle, Maltese, Yorkshire terrier, Spitz, and Chihuahua [13]. Main pathogenetic mechanisms of the development of myxomatous degeneration of the mitral valve in dogs are associated with the development of chronic heart failure syndrome. The role of systemic inflammation and the concentration of proinflammatory cytokines in blood serum is unclear.

Cytokines are biologically active compounds related to glycoproteins. These biologically active substances are involved in the differentiation and maturation of blood cells. It is believed that these substances can play an important role in the regulation of hypercoagulation processes, participate in the violation of vascular tone regulation, the development and formation of heart failure syndrome, myocardial remodeling, endothelial dysfunction, induction of metabolic processes in skeletal muscles, as well as the progression of muscular dystrophy and cachexia [14, 15].

There are statistically significant differences in the expression of genes of interleukin (IL) type 8 and transforming growth factor-β between clinically healthy dogs and dogs with myxomatous degeneration of the mitral valve [16]. Moreover, it was found that the formation of IL-8 increases with an increase in the functional class of heart failure, and transforming growth factor-β – in asymptomatic dogs with echocardiographic signs of cardiac remodeling.

Another study recorded significant changes in the level of the following cytokines in the blood serum of dogs with myxomatous degeneration of the mitral valve depending on the severity of heart failure syndrome: granulocyte macrophage colony stimulating factor, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, keratinocyte chemokine, interferon-γ and monocyte chemoattractant protein-1 [17].

Proinflammatory cytokines are also involved in the development of cell-mediated immune myocardial damage in dogs with mixomatous degeneration of the mitral valve. This is evidenced by the results of experimental reproduction of chronic heart failure with remodeling of the left ventricular myocardium by prolonged infusion of tumor necrosis factor in laboratory animals [18], as well as by an increase in the content of this cytokine and IL-1 in the blood of dogs with dilatation of the left heart chambers [19]. The same experiments found that IL-1β activation stimulates hypertrophy, fibrosis and systemic inflammation in animals with myxomatous degeneration. The concentration of IL-1β increases both in the myocardium [20] and in the liver [21] in the blood serum of dogs with chronic heart failure. In addition, it has been proved that an increase of transforming growth factor-β in the blood serum of animals with myxomatous degeneration of the mitral valve is not the cause of cardiomyocyte apoptosis [22-25].

Thus, the study of the dynamics of changes in the concentration of proinflammatory cytokines in dogs with myxomatous degeneration depending on different functional classes of heart

failure is relevant and allows to clarify the pathogenesis of this pathology.

The purpose of the work is to study the concentration of proinflammatory cytokines in the blood serum of dogs with myxomatous degeneration depending on the degree of functional class of heart failure, presence of pulmonary edema, left atrial dilatation, and cardiogenic cachexia.

MATERIAL AND METHODS.

The object of the study was dogs with myxomatous degeneration of the mitral valve and clinically healthy dogs of similar age and body weight. The subject of the study is the concentration of anti-inflammatory cytokines in the blood serum of dogs with an established degenerative damage of the mitral valve cusps, as well as the pathogenetic and diagnostic significance of these biologically active substances.

Myxomatous degeneration of the mitral valve was diagnosed according to the criteria of M. Borgarelli and J. W. Buchanan (2012) [7]. The stages of development of this pathology were determined using the classification of the American College of Veterinary Internal Medicine (ACVIM): A (risk group) – clinically healthy animals with genetic determinancy of myxomatous degeneration of the mitral valve (for example, Dackel); B – asymptomatic stage; C – dogs with symptoms of the disease; D – dogs with the developing undruggable (refractory) heart failure. In turn, stage B is divided into 2 sub-stages: B1 – myxomatous degeneration without signs of remodeling the left heart chambers (does not require therapy) and B2 – a disease accompanied by the left atrium expansion and eccentric left ventricular hypertrophy (preclinical stage of pathology requiring preventive therapy).

The functional class of chronic heart failure in ill dogs was evaluated according to the criteria of the New York Heart Association (NYHA) [13]: I – presence of non-intense noise or extrasystoles without weakness, shortness of breath and cough; II – good health in a calm state with light cough and slightly reduced bearableness of physical loads; III – heavy breathing, poor response to physical loads, cough; IV – development of decompensated congestive heart failure with shortness of breath, orthopnea and cough even at rest.

The concentration of interleukins (IL-1α, IL - 6 and IL - 8) and tumor necrosis factor (TNF-α) in the blood serum of clinically healthy dogs and dogs with myxomatous degeneration of the mitral valve was determined by the solid phase enzyme immunoassay of double antibodies using sets of monoclonal antibodies and reagents of LLC "Cytokin" (St. Petersburg, Russian Federation).

Prior to statistical calculations, the normality of the distribution was estimated using the Shapiro-Wilk test. In the normal distribution of quantitative variables, the student t-test was used for independent samples to compare the two groups. When comparing two or more groups whose numerical indices did not correspond to the normal distribution of features, the Mann-Whitney nonparametric U-criterion or the Kruskal-Wallis nonparametric criterion, which is a rank analysis of variations, were used respectively. The difference between the indices of experimental groups of animals was considered to be significant at $p<0.05$. All calculations were done on a personal computer using statistical software STATISTICA 7.0 (StatSoft, USA).

RESULTS AND DISCUSSION.

The experiment included 40 dogs with myxomatous degeneration of the mitral valve, including 13 (32.5 %), 11 (27.5 %), 10 (25.0 %) and 6 (15.0 %) animals at stages B1, B2, C and D of pathology development respectively.

In the blood serum of dogs with myxomatous degeneration of the atrioventricular heart valves, a higher concentration of proinflammatory cytokines was established compared to that in clinically healthy animals (Table 1). Thus, the level of IL-1α, IL-6, IL-8 and TNF-α in animals with myxomatous degeneration of the mitral valve was 3.7; 8.7; 5.8 and 7.5 times higher, respectively, compared to clinically healthy animals (differences were significant, $p<0.001$).

The second stage of research studied the connection of cytokine profile of blood serum of dogs with myxomatous degeneration of the mitral valve with functional class of heart failure (Table 2). The content of serum IL-1α in animas with myxomatous degeneration of the mitral valve from different experimental groups truly ($H=35.4$; $p<0.001$) differed when conducting the ranking Kruskal-Wallis analysis. The concentration of this cytokine in I, II, III and IV functional classes of chronic heart failure was significantly higher than that in clinically healthy animals in 1.6 ($U=29.5$; $p<0.05$); 2.6 ($U=15.0$; $p<0.001$); 3.8 ($U=3.5$; $p<0.001$) and 6.7 ($U=0$; $p<0.001$) times, respectively. The level of IL-1α in the blood serum of ill dogs was significantly correlated with the functional class of chronic heart failure ($r=0.81$; $p<0.001$).

The activity of IL-6 in the blood serum of ill animals significantly increased with the progression of chronic heart failure syndrome ($H=42.0$; $p<0.01$). Thus, in dogs with myxomatous degeneration of the mitral valve with complication of the underlying disease with the I, II, III and IV functional classes of chronic heart failure, the level of this cytokine averaged 8.2 pg/cm^3 ($U=33.0$; $p<0.05$); 18.4 ($U=6.0$; $p<0.001$); 29.9 ($U=0$; $p<0.001$) and 40.8 pg/cm^3 ($U=0$; $p<0.001$), respectively, compared to clinically healthy animals (2.8±1.00 pg/cm^3). There was a positive correlation of IL-6 concentration in blood serum of ill animals with functional class of chronic heart failure ($r=0.88$; $p<0.001$).

IL-8 level in the blood serum of dogs with myxomatous degeneration of the mitral valve and heart failure of different functional classes also significantly changed, as evidenced by the high values of the Kruskal-Wallis rank variations analysis ($H=41.2$; $p<0.001$). IL-8 concentration in ill animals ranged from 1.37 to 25.2 pg/cm^3, with the development of chronic heart failure syndrome of the I, II, III and IV functional classes it averaged 3.1±0.48; 4.9±0.64; 7.6±0.77 and 12.5±2.51 pg/cm^3, respectively, and it was significantly higher ($p<0.05$) than that of clinically healthy animals. The level of this cytokine was positively correlated with the functional class of chronic heart failure ($r=0.87$; $p<0.001$).

Table 1 - Cytokine profile of the blood serum in dogs with myxomatous degeneration of atrioventricular heart valves

Cytokine concentration, pg/cm^3	Clinically healthy, n=15		Myxomatous degeneration of the mitral valve, n=40		Significance of differences
	M±m	Min – max	M±m	Min – max	
IL-1α	1.0±0.07	0.78 – 1.60	3.7±0.45	0.78 – 11,2	p<0.001
IL-6	2.8±1.00	0 – 12.50	24.3±2.45	0 – 60.89	p<0.001
IL-8	1.2±0.11	0.78 – 1.99	7.0±0.87	1.37 – 25.20	p<0.001
TNF-α	1.8±0.62	0 – 6.5	13.5±1.67	2.17 – 44.75	p<0.001

Table 2 - Cytokine profile of the blood serum in dogs with myxomatous degeneration of the mitral valve with different functional classes of chronic heart failure

Cytokine concentration, pg/cm^3	Parameters	Clinically healthy dogs, (n=15)	Functional class of chronic heart failure in ill dogs			
			I, n=10	II, n=10	III, n=10	IV, n=10
IL-1α	M±m	1.0±0.07	1.6±0.19*	2.6±0.50*	3.8±0.73*	6.7±1.02*
	Min	0.78	0.78	0.78	1.27	2.14
	Max	1.60	2.70	5.62	8.20	11.20
IL-6	M±m	2.8±1.00	8.2±1.97*	18.4±2.48*	29.9±3.11*	40.8±4.21*
	Min	0	0	2.78	15.21	18.33
	Max	12.50	16.45	31.12	45.19	60.89
IL-8	M±m	1.2±0.11	3.1±0.48*	4.9± 0.64*	7.6±0.77*	12.5±2.51*
	Min	0.78	1.37	2.85	4.65	3.63
	Max	1.99	5.38	9.87	10.75	25.2
TNF-α	M±m	1.8±0.62	4.1±0.48*	9.0±2.29*	14.5±1.66*	26.3±3.03*
	Min	0	2.17	4.57	6.5	16.41
	Max	6.5	6.5	28.68	24.19	44.75

* Statistically significant differences with clinically healthy dogs.

Table 3 - Influence of pulmonary edema on the cytokine profile of the blood serum in ill dogs with myxomatous degeneration of the mitral valve

Cytokine concentration, pg/cm^3	Dogs with myxomatous degeneration of the mitral valve	
	without pulmonary edema (n=26)	with pulmonary edema (n=14)
IL-1α	2.6 ± 0.31	5.7 ± 0.94*
IL-6	18.6 ± 2.61	35.0 ± 3.68*
IL-8	4.7 ± 0.48	11.2 ± 1.87*
TNF-α	8.9 ± 1.54	21.9 ± 2.58*

* Significant differences with dogs without pulmonary edema.

Table 4 - Influence of left atrial enlargement on the cytokine profile of the blood serum of dogs with myxomatous degeneration of the mitral valve

Cytokine concentration, pg/cm^3	Dogs with myxomatous degeneration of the atrioventricular heart valves	
	without left atrial dilatation (n=13)	with left atrial dilatation (n=27)
IL-1α	1.9±0.30	4.5±0.59*
IL-6	13.1±2.3	29.7±2.94*
IL-8	3.9±0.67	8.5±1.15*
TNF-α	5.2±0.60	17.4±2.05*

* Significant differences with dogs without left atrial dilatation.

Table 5 - Influence of cardiogenic cachexia syndrome on serum cytokine concentration in dogs with myxomatous degeneration of the mitral valve

Cytokine concentration, pg/cm^3	Dogs with myxomatous degeneration of the atrioventricular heart valves	
	without cachexia (n = 29)	with cachexia (n = 11)
IL-1α	2.9±0.45	5.6±0.96*
IL-6	20.2±2.81	35.3±3.21*
IL-8	5.53±0.83	10.9±1.87*
TNF-α	9.9±1.51	22.8±3.21*

* Significant differences with dogs without cardiogenic cachexia.

TNF-α content in the blood serum of animals with myxomatous degeneration of the mitral valve significantly changed with heart failure (H=42.8; p<0.001). So, with the development of chronic heart failure of the I, II, III and IV functional class, the concentration of this cytokine in ill dogs proved to be 2.3 (p<0.05); 5.0 (p<0.001); 8.1 (p<0.001) and 14.6 (p<0.001) times higher than that in clinically healthy animals, respectively. The level of this cytokine in the blood serum of ill animals was logically increasing with an increase in the functional class of chronic heart failure (r=0.89; p<0.001).

14 (35 %) dogs with myxomatous degeneration of the mitral valve revealed pulmonary edema. Data presented in Table 3 show that in the blood serum of animals with pulmonary edema compared with animals without this complication, the concentration of IL-1α, IL-6, IL-8, TNF-α was significantly higher in 2.2 (p<0.01); 1.9 (p<0.01); 2.4 (p<0.001) and 2.5 (p<0.001) times, respectively.

13 (32.5 %) dogs with myxomatous degeneration of the mitral valve showed no left atrium expansion, which corresponded to the disease stage B1. In 27 (67.5 %) cases, the disease was accompanied by left atrial dilatation. The concentration of proinflammatory cytokines IL-1α, IL-6, IL-8, TNF-α was significantly higher in the blood serum of animals with myxomatous degeneration with left atrial enlargement compared to animals without atrial remodeling in 2.4 (p<0.01); 2.3 (p<0.01); 2.2 (p<0.001) and 3.4 (p<0.001) times, respectively (Table 4).

Correlation analysis confirmed the presence of statistically significant dependence (p<0.001) of IL-1α (r=0.80), IL-6 (r=0.82), IL-8 (r=0.77), TNF-α (r=0.82) in the blood serum on the size of the left atrium.

In 11 (27.5 %) dogs, myxomatous degeneration was associated with cardiac cachexia (Table 5). Among them 6 animals registered stage D of myxomatous degeneration of the mitral valve and 5 animals – stage C. The concentration of proinflammatory cytokines IL-1α, IL-6, IL-8, TNF-α in the blood serum of ill animals with myxomatous degeneration of heart valves with cardiogenic depletion syndrome was significantly higher than without such a complication in 1.9 (p<0.01); 1.8 (p<0.01); 2.0 (p<0.01) and 2.3 (p<0.01). 0.001) times, respectively.

An important achievement of cardiology in recent decades has been the formation of an inflammatory hypothesis of the formation and progression of cardiopathology in humans and domestic animals. We and a number of researchers found that myxomatous degeneration of the mitral valve is a widespread pathology, family forms of the disease are often detected. In addition, this nosological entity is manifested among the representatives of dwarf and small breeds of dogs, that is, the etiological prerequisite for the formation and progression of this disease is the activation of pathological genes. It should be added that myxomatous degeneration of the mitral valve is the leading cause of death among dogs, and death can occur due to progression of the congestive heart failure syndrome.

Pathological genes activation leads to the damage of the valve apparatus, deformation and thickening of the atrioventricular heart valves cusps and the development of mitral regurgitation. Myxomatous degeneration of the mitral valve is pathogenetically characterized by the development of volume overload of the myocardium of the left heart chambers, which leads to dilatation of the left atrium and eccentric hypertrophy of the left ventricle.

Our study has found that the concentration of such proinflammatory cytokines as IL-1α, IL-6, IL-8 and TNF-α increased significantly in the blood serum of dogs with myxomatous degeneration of the atrioventricular heart valves. It should also be noted that the serum concentration of these proinflammatory cytokines significantly correlates with the functional class of chronic heart failure syndrome. These data were obtained for the first time.

Thus, systemic inflammation plays an important role in the pathogenesis and progression of congestive heart failure; and the identification of various inflammation biomarkers has been the subject of an intensive study in both human and veterinary medicine. Thus, in the study the dogs with congestive heart failure showed an increase in the concentration of C reactive protein in the blood serum and neutrophilic leukocytosis in the peripheral blood.

In our study 13 (32.5 %) dogs with myxomatous degeneration of the mitral valve showed no left atrium expansion, which corresponded to the disease stage B1, according to ACVIM classification. In 67.5 % of cases, the disease was accompanied by significant left atrial dilatation. The concentration of proinflammatory cytokines IL-1α, IL-6, IL-8, TNF-α was found to be significantly higher in the blood serum of animals with myxomatous degeneration with left atrial enlargement compared with animals without atrial remodeling. The correlation analysis confirmed the presence of a statistically significant dependence of serum concentrations of IL-1α, IL-6, IL-8, TNF-α on the size of the left atrium.

As a result of the volume overload of the myocardium, compensatory mechanisms of cardiohemodynamics regulation are activated: mechanism of Frank-Starling, sympathetic nervous system, adrenal system, antidiuretic hormone system, renin-angiotensin-aldosterone system, endothelin system and vasopressin.

Constant activity of the inflammation system, sympathetic nervous system and hormones against the background of progressive volume overload of the left heart chambers lead to the initiation of left ventricular myocardial remodeling, which is its structural and geometric changes characterized by hypertrophy of the heart muscle, dilatation and sphericity of the heart. This process leads to a change in its geometry and further disruption of diastolic and systolic functions.

Congestive processes in the system of pulmonary veins lead to symptoms of shortness of breath, unbearableness to physical loads and cough. Therefore, the next stage of our research was to assess the influence of pulmonary edema on the concentration of proinflammatory cytokines in the blood serum of dogs with myxomatous degeneration of the heart valves. It was found that the frequency of pulmonary edema in dogs with myxomatous degeneration of the mitral valve was 35 %. In the blood serum of animals with pulmonary edema compared with those without this complication, the concentration of IL-1α, IL-6, IL-8, TNF-α was significantly higher.

Blood congestion in the internal organs induces the processes of their secondary damage. In addition, the deterioration of the systolic function of the left ventricle causes hemodynamic disorders in the large circle of blood circulation and the development of congestive phenomena in the liver, intestines, pancreas, which obviously leads to indigestion and, ultimately, reduces the flow of plastic nutrients into the body of ill animals.

Clinically, this is manifested by stable anorexia and cardiac cachexia (Gentz-Zotz S., Bolger A. P., Anker S. D., 2001). The development of edematous syndrome (ascites, hydrothorax, hydropericardium and peripheral edema) significantly burden hypoproteinemia due to water retention in the blood and tissues of the body. In our study, the progressive stages of myxomatous degeneration of the mitral valve in dogs were associated with the development of cardiac cachexia syndrome. Cachexia in ill dogs was recorded with a frequency of 27.5 %. The concentration of proinflammatory cytokines IL-1α, IL-6, IL-8, TNF-α in the blood serum of ill animals with cardiogenic depletion syndrome was significantly higher than without such a complication.

In the pathogenesis of acquired valvular heart disease in dogs, in addition to the intracardiac pathologic changes, a significant role is played by an activation of systemic inflammation, which significantly contributes to development and progression of chronic heart failure.

CONCLUSION.

In the pathogenesis of the formation and progression of chronic heart failure syndrome in dogs with myxomatous degeneration of the mitral valve an important role is played by the chronic increase in the concentration of serum proinflammatory cytokines. In the blood serum of these animals, compared with clinically healthy individuals, a higher concentration of interleukins (IL-1α, IL-6 and IL-8) and tumor necrosis factor-α was revealed. The concentration of these proinflammatory cytokines in the blood serum of dogs with myxomatous degeneration of the mitral valve was significantly higher in animals with pulmonary edema, atrial remodeling and cardiogenic cachexia, and positively correlated with the functional class of chronic heart failure and the size of the left atrium.

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