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Endothelial monolayer disruption in the bioprosthetic heart valve as a trigger of primary tissue failure

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ABSTRACT

Aim. To study the surface and cellular composition of non-calcified bioprosthetic heart valve (BHV) leaflets with varying degrees of structural deterioration to determine the possible mechanisms of primary tissue failure development.

Materials and methods. An examination of six bioprosthetic heart valves (KemCor and PeriCor) extracted from mitral position due to the structural valve deterioration was performed. The structure of BHV leaflets was studied by hematoxylin – eosin staining and immunohistochemistry assay (with the following indicators – CD3, T lymphocytes; CD20, B lymphocytes; CD31, mature endothelial cells; CD34, endothelial progenitor cells; CD68, monocytes/macrophages; vimentin, mesenchymal cells; α-smooth muscle actin, vascular smooth muscle cells).

Results. The degree of disruption of BHV leaflets in primary tissue failure differed significantly: relatively intact samples with the intact endothelial monolayer, areas with impairment of the surface layers (minimal and moderate damage) and areas with the spread of destruction into the extracellular matrix of the leaflet (expressed degeneration) were determined. Endothelial cells (monolayer with preserved or impaired integrity), macrophages, smooth muscle cells and other mesenchymal lineage cells were identified in BHV. T- and B-lymphocytes were not detected in the BHV leaflets.

Conclusions. A characteristic feature of structurally deteriorated BHVs is impairment of endothelial monolayer integrity in areas of degraded extracellular matrix. In contrast to other types of bioprosthetic dysfunctions, structural valve deterioration was characterized by the absence of lymphocyte infiltration. Therefore, we suppose that endothelial mololayer injury is a trigger of structural BHV deterioration.

Key words: bioprosthetic heart valves, structural valve deterioration, extracellular matrix.

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Нарушение целостности эндотелиального монослоя биопротезов клапанов сердца как триггер развития первичной тканевой несостоятельности

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РЕЗЮМЕ

Цель – морфологическое исследование поверхности и клеточного состава створок некальцинированных биопротезов клапанов сердца (БКС) с различной степенью их повреждения для определения возможных механизмов развития первичной тканевой несостоятельности (ПТН).

Материалы и методы. Исследовано шесть ксеноаортальных клапанов «КемКор» и «ПериКор», извлеченных из митральной позиции по причине развития ПТН. Структуру створок БКС и особенности ее изменения изучали гистологическим (окраска гематоксилин-эозином) и иммуногистохимическим методами. Иммуногистохимическое исследование БКС включало идентификацию маркеров: CD3 (Т-лимфоциты), CD20 (В-лимфоциты), CD31 и CD34 (эндотелиальные клетки), CD68 (моноциты/макрофаги), виментин (клетки мезенхимального ряда), α-гладкомышечный актин (гладкомышечные клетки).

Результаты. Степень нарушения структуры створок БКС при ПТН существенно различалась: определялись относительно сохранные образцы с интактным эндотелиальным монослоем на поверхности створки, образцы с минимальным или умеренным нарушением структуры эндотелиального слоя и образцы с выраженной деструкцией эндотелиального слоя створки БКС. В составе БКС были идентифицированы эндотелиальные клетки (монослой с сохраненной или нарушенной целостностью), макрофаги, гладкие миоциты и прочие клетки мезенхимального происхождения. Следует отметить, что нами не обнаружено Т- и В-лимфоцитов в створках БКС.

Заключение. Характерным признаком структуры БКС, эксплантированных по причине ПТН, является нарушение целостности эндотелиального монослоя в участках дезинтеграции экстрацеллюлярного матрикса. Кроме того, в сравнении с другими типами протезных дисфункций ПТН отличается отсутствием

лимфоцитарной инфильтрации. На основании полученных данных можно сделать вывод о триггерной роли дезинтеграции эндотелиального монослоя в развитии ПТН.

Ключевые слова: биопротезы клапанов сердца, первичная тканевая несостоятельность, экстрацеллюлярный матрикс.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Протокол исследования одобрен локальным этическим комитетом ФГБНУ «Научно-исследовательский институт комплексных проблем сердечно-сосудистых заболеваний» (протокол № 8 от 14.05.2019).

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INTRODUCTION

A generally recognized disadvantage of bioprosthetic heart valves (BHV) is limited duration of their functioning explained by the development of structural insufficiency of the implanted xenomaterial – primary tissue failure (PTF), under the influence of various factors associated with the characteristics of the implants and / or recipient organism [1, 2]. The most common types of BHV dysfunctions requiring surgery to replace the prosthesis are tissue calcification (50%) and prosthetic endocarditis (27%). In a significantly smaller number of cases (15.1%), reoperations are performed due to the development of primary tissue failure [3].

In a number of studies, active participation of recipient cells in the formation of both calcium-associated damage to the implanted xenogenic material and degenerative changes in the structure of BHV caused by exposure to infectious agents was demonstrated [4, 5]. In addition, identification of various types of cells in the functionally preserved BHV [6, 7] suggests permanent remodeling of xenotissue after its implantation in the body, which implies a parallel course of its disintegration and repair processes [7]. On the one hand, this gives grounds to consider calcification as the final stage of the BHV destruction, which inevitably arises over time. On the other hand, it does not exclude the implementation of a fundamentally different scenario for the development of aseptic structural dysfunctions of implanted BHV depending on the type of remodeling.

The aim of this work was to study morphologically the surface and cellular composition of the leaflets of uncalcified BHV with varying degrees of xenotissue damage in order to identify possible mechanisms of development of primary tissue failure.

MATERIALS AND METHODS

Six xeno-aortic BHV models were studied: KemKor (n = 2) and PeriKor (n = 4), (Neokor CJSC, Kemerovo). They were preserved with ethylene glycol diglycidyl ether and removed from the mitral position during repeated surgical interventions. The removal took place due to the development of structural failure of BHV tissues without mineral inclusion deposition as computed tomography showed. Considering the differences in the implanted valve functioning caused by the influence of hemodynamic loads of different strengths [1, 8], only BHVs removed from the mitral position were included in the study. The group of reoperated patients consisted of 5 women and 1 man. The average age of patients at the time of repeated operations was 63.5 ± 4.8 years with an average BHV functioning duration of 7.3 ± 3.1 years.

For histological examination and immunohistochemistry assay (IHC), the whole BHV was placed in a 4% solution of paraformaldehyde for 48 hours. After fixation, fragments of valve leaflets were cut out for subsequent dehydration and embedding into Histomix paraffin mixture (BioVitrum, Russia). Sections (5 μm) were prepared from paraffin blocks on a semi-automatic rotary microtome (MZP 01 – Tekhnom, Russia). The sections were mounted on glass slides with a poly-L-lysine coating (Thermo Scientific, USA). The sections of BHV were stained with hematoxylin – eosin, and an IHG study was also performed. Verification of the absence of calcium in explanted BHV was carried out by staining with alizarin red.

IHC typing of cells was performed using the following markers: CD3 (T-lymphocytes), CD20 (B-lymphocytes), CD31, CD34 (hematopoietic progenitor cells), CD68 (monocytes / macrophages), vimentin (mesenchymal cells), and α- smooth muscle actin (smooth muscle cells). We used monoclonal mouse (CD3, CD20, CD34, CD68, vimentin) and rabbit (CD31, α-smooth muscle actin) antibodies produced by Novocastra Laboratories, Thermo Scientific and Spring Bioscience, which react with human antigens.

To identify the markers described above, high-temperature antigen unmasking was performed in a citrate buffer (0.01 M, pH 6.0) for α-smooth muscle actin, CD68, CD31, CD34, CD3; in a Tris-EDTA buffer (pH 9.0) for vimentin; CD20 – without unmasking. Endogenous peroxidase blocking, dilution of primary antibodies and their exposure time were determined according to the protocols of the primary antibody manufacturers. To detect the results of IHC reactions, the Novolink Polymer Detection System (Novocastra, UK) was used. The enzyme immunoassay was stopped by washing the sections in a phosphate buffer (pH 7.4), after which they were stained with Mayer hematoxylin and enclosed in a mounting medium. In parallel with the detection of antigens at each IHC staining, the positive and negative controls were set up. A negative control was carried out by applying 50 μl of antibody dilution solution to the sections (Ab Diluent, USA). A positive control of CD3, CD20, and CD68 markers was performed on sections of the human palatine tonsil, a positive control of CD31, CD34, vimentin and α-smooth muscle actin was carried out on sections of the human radial artery. The study of drugs and photography was performed using an AXIO Imager A1 microscope (Carl Zeiss, Germany) and a Canon G5 digital camera (Canon, Japan).

RESULTS

The degree of the BHV leaflet structure deterioration was significantly different, which allowed us to conditionally distinguish the following groups: samples with the intact endothelial layer on the surface of the BHV leaflet; samples with minimal or moderate disruption of the endothelial layer structure; and samples with severe endothelial cover destruction of the BHV leaflets, which extended into the leaflet and was accompanied by the destruction of its extracellular matrix.

In samples with the intact endothelial layer, a monolayer of cells morphologically corresponding to endotheliocytes was observed on the atrial and ventricular surfaces of the BHV leaflets. On the ventricular surface (excretory region), the monolayer was represented by flattened cells with elongated nuclei (Fig. 1*a*). On the atrial surface, the cells had rounded nuclei and a more pronounced cytoplasm (Fig. 1*b*). The layer of endotheliocytes on the ventricular surface appeared thinner than on the atrial one. An IHC assay of the BHV leaflets revealed CD31-positive staining of flat cells on both surfaces, confirming their endothelial phenotype (Fig. 1*b*). The absence of positive staining for CD34, in turn, indicated the maturity of endothelial cells (Fig. 1*g*).

BHV stroma was represented by compact, tightly packed bundles of collagen fibers that retained intact tortuosity and tinctorial properties (Fig. 1*a*, *b*). Moreover, a dense arrangement of fibers was observed in the surface layers, and loose arrangement – in deep layers. In some areas, small cavities with transparent contents were present (Fig. 1*a*, *b*).

Violation of the endothelial layer integrity with the presence of sections containing morphologically different cells, which form multilayer or single layer structures, was observed in samples with minimal or moderate damage to the BHV leaflets (Fig. 2). In some of the studied samples, cell infiltration of the underlying connective tissue structures of the BHV leaflet was also noted (Fig. 2*b*). In this case, the stroma of the BHV leaflets was characterized by moderate heterogeneity: in the surface layers – by loosening and thinning of bundles of collagen fibers with expansion of interfibrillar spaces and formation of mesh networks, in the deep layers – by relative intactness of the extracellular matrix (Fig. 2*a*, *b*).

In the zones of fibrous structure disorganization, the presence of CD68-positive cells belonging to the mononuclear phagocyte system was detected (Fig. 2*b*, *d*). Among them, in addition to typical macrophages,

individual multinuclear cells (Pirogov – Langhans cells) were identified. Vimentin-positive cells located singly or in groups were also found in the surface layers of the extracellular matrix, mainly in the areas of endothelial monolayer disturbance, which indicated that they belong to cells of the mechanocyte line.

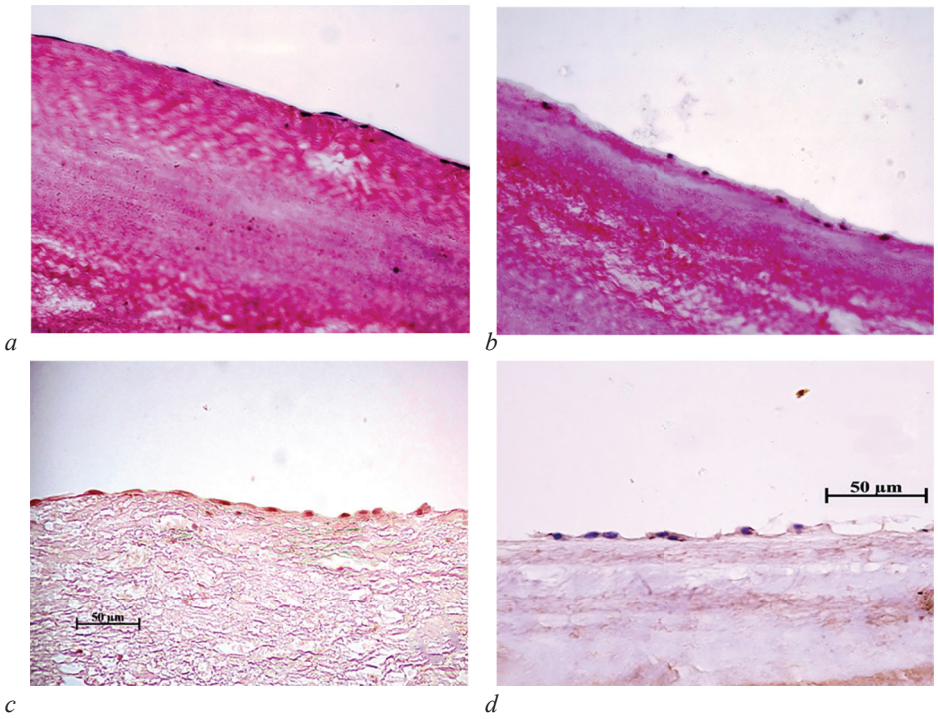


Fig. 1. The structure of the leaflets of bioprosthetic heart valves in areas with minimal damage to their structure, magnification 200. *a* – endothelium of the ventricular surface, *b* – endothelium of the atrial surface (stained with hematoxylin and eosin), *c* – IHC on CD31, *d* – IHC on CD34

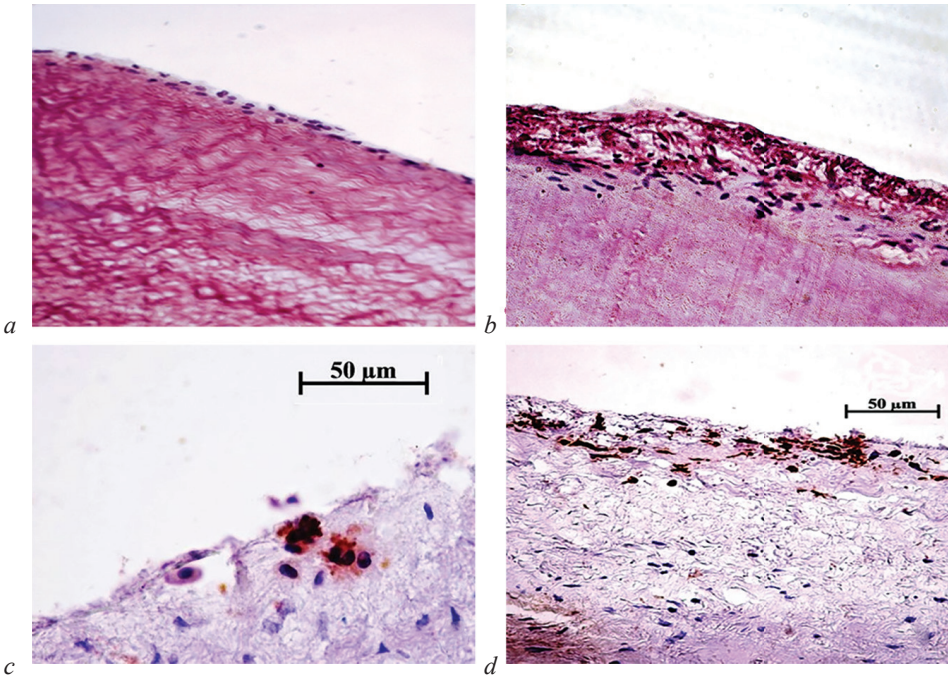
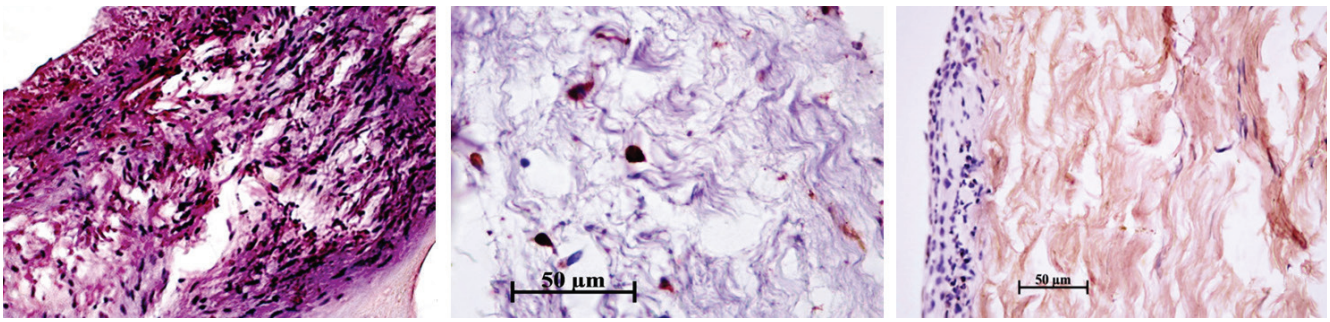


Fig. 2. The structure of the bioprosthetic heart valve leaflets with moderate damage. *a*, *b* – staining with hematoxylin and eosin, magnification 200, *c* – IHC on CD68, magnification 400, *d* – IHC in vimentin, magnification 200

In samples with pronounced destruction of the BHV leaflet surface, the absence of a monolayer of endotheliocytes was noted, which was associated with deep disorganization of their connective tissue base (Fig. 3). Stratification of collagen fiber bundles was combined with their fragmentation and formation of numerous cavities (Fig. 3*a*). Moreover, the entire thickness of the leaflet was infiltrated by cells. In the zones of the greatest destruction, both CD 68-positive and α-smooth muscle actin-positive cells were detected. CD68+ cells were mostly grouped around the cavities, adjacent to the remains of collagen fibers (Fig. 3B, *c*). Also, isolated smooth muscle cells were found in the thickness of the BHV leaflets, among the destroyed connective tissue structures (Fig. 3*c*).

It should be noted that in all three groups of samples, no positive IHC staining for T- and B-lymphocytes was observed.



a *b* *c*

Fig. 3. The structure of bioprosthetic heart valve leaflets with pronounced destruction of their surface and stroma. *a* – stained with hematoxylin and eosin, magnification 200, *b* – IHC on CD68, magnification 400, *c* – IHC on α-smooth muscle actin, magnification 200

DISCUSSION

The value of the presented morphological data for understanding the mechanisms of the degenerative changes in implanted BHVs in the recipient's organism primarily consists in the absence of infection and calcification. Thus, already at the stage of the study group formation, BHV dysfunctions were excluded, the occurrence of which is determined by the features of the immunological and metabolic status of patients leading both to a decrease in the microbial resistance of the biomaterial and its pronounced mineralization [1]. The results of the study suggest that various degrees of damage to the extracellular matrix can be considered as successive stages of destruction of BHV leaflets.

At the initial stage of tissue failure development, that is, in samples with intact structure, both surfaces were covered with a continuous layer of mature endothelium (CD31 +). Moreover, the morphological characteristics of endothelial cells had some differences. From the outflow side, endotheliocytes were characterized by a flattened form, a thin layer of cytoplasm and elongated nuclei with a heterochromatin predominance. On the inflow side, endothelial cells were higher than on the atrial surface, had round nuclei in which euchromatin predominated. Such differences in the endotheliocytes structure probably occur due to the influence of hemodynamic factors and may indicate different metabolic activities of these cells.

High degree of the extracellular matrix preservation suggested a possible protective function of the endothelium in relation to BHV damage by aggressive blood factors [5, 6]. At the same time, the presence of sites with collagen fiber delamination in these samples indicated the onset of destructive processes, presumably caused by prolonged cyclic deformations experienced by implantable BHV [9, 10].

Samples with minimal and moderate leaflet destruction were considered as the next stage in the development of primary tissue failure without mineralization of xenomaterial. It can be assumed that endothelial layer disintegration may trigger the development of hemodynamically significant damage to the structure of BHV. The causes of this process can be mechanical endotheliocyte destruction and exposure of extracellular matrix of valves and expression of endothelial cell adhesion molecules, which contribute to the attraction of monocytes with their subsequent migration deep into the BHV leaflets. The presented endothelial dysfunction can be triggered by various pathological processes [11], as well as by a low shear stress due to turbulent blood flow in the

absence of endothelium [12]. After differentiation of monocytes into macrophages, secretion of matrix metalloproteinases occurred, which led to the progression of destructive processes in the BHV leaflets and the formation of a pathophysiological "vicious circle" [12, 13]. Apparently, the process of cell differentiation was influenced by the microenvironment and the depth of its invasion in the BHV leaflets. For example, fibroblasts were localized mainly near the leaflet surface, and smooth muscle cells, as a rule, were present in the deeper layers of the leaflets.

It should be noted that at this stage of primary tissue failure development, a parallel course of extracellular matrix repair is not ruled out. Thus, the identification of mechanocyte cells, such as fibroblasts (vimentin-positive cells), indicates the possibility of synthesizing the main components of the leaflet extracellular matrix, which is aimed at replacing the degeneratively altered xenotissue [6]. However, progressive destructive processes indicate the predominance of fracture processes over reparation in the xenogenic material of the valves. The low regeneration rate can be caused not only by an insufficient number of fibroblasts, but also by their inability to fully function in atypical microenvironment conditions [14, 15]. In particular, under adverse conditions, a change in the functional properties of smooth muscle cells and fibroblasts and development of their destructive potential can occur [16–18]. This assumption is confirmed by the localization of these cells in the immediate vicinity of large cavities.

The absence of T- and B-lymphocytes in the studied samples, on the one hand, may indicate an insignificant role of inflammation in the development of the described variant of structural dysfunctions in primary tissue failure of BHV. On the other hand, it may suggest that calcification of chemically modified xenotissue in the recipient's body can only be realized under the conditions of immune inflammation activation.

CONCLUSION

The structure of the BHV explanted due to the primary tissue failure is characterized by impaired integrity or complete absence of an endothelial monolayer in the areas of extracellular matrix disintegration, as well as by the absence of lymphocytes. Thus, it can be assumed that it is the disintegration of the endotheliocyte layer that is the trigger for the primary tissue failure development. In other words, the optimal design of BHV should ensure adhesion and viability of endothelial cells on the leaflet surfaces in order to ensure the extracellular matrix integrity.

REFERENCES

1. Barbarash O., Rutkovskaya N., Hryachkova O., Gruzdeva O., Uchasova E., Ponasenko A., Kondyukova N., Odarenko Y., Barbarash L. Impact of recipient-related factors on structural dysfunction of xenoaortic bioprosthetic heart valves. *Patient Prefer Adherence.* 2015; 9: 389–399. DOI: 10.2147/PPA.S76001.
2. Barbarash L.S., Rogulina N.V., Rutkovskaya N.V., Ovcharenko E.A. Mechanisms underlying bioprosthetic heart valve dysfunctions. *Complex Issues of Cardiovascular Diseases*. 2018; 7(2): 10–24 (in Russ.). DOI: 10.17802/2306-1278-2018-7-2-10-24.
3. Rutkovskaia N.V., Stasev A.N., Odarenko Iu.N. Biological prostheses of heart valves: realities, problems and solutions. *Cardiology and Cardiovascular Surgery*. 2013; 6: 70–77 (in Russ.).
4. Nair V., Law K.B., Li A.Y., Phillips K.R., David T.E., Butany J. Characterizing the inflammatory reaction in explanted Medtronic Freestyle stentless porcine aortic bioprosthesis over a 6-year period. *Cardiovasc Pathol.* 2012; 21(3): 158–168. DOI: 10.1016/j.carpath.2011.05.003.
5. Mukhamadiyarov R.A., Rutkovskaya N.V., Sidorova O.D., Barbarash L.S. Cellular Composition of Calcified Bioprosthetic Heart Valves. *Annals of the Russian Academy of Medical Sciences*. 2015; 70(6): 662–668 (in Russ.). DOI: 10.15690/vramn560.
6. Mukhamadiyarov R.A., Rutkovskaya N.V., Mil'to I.V., Sidopova O.D., Kudryavceva Yu.A., Barbarash L.S. Research of the structure functionaly saved xenopericardial bioprosthesis in long-term implantation. *Pathology Archives*. 2017; 79(5): 25–33 (in Russ.). DOI: 10.17116/patol201779525-33.
7. Mukhamadiyarov R.A., Rutkovskaya N.V., Mil'to I.V., Barbarash L.S.. Pathogenetic parallels between the development of calcification of native aortic valves and xenogenic bioprostheses of heart valves. *Genes and Cells*. 2016; 11(3): 83–91 (in Russ.).
8. Tillquist M.N., Maddox T.M. Cardiac crossroads: deciding between mechanical or bioprosthetic heart valve replacement. *Patient Prefer Adherence.* 2011; 5: 91–99. DOI: 10.2147/PPA.S16420.
9. Soares J.S., Feaver K.R., Zhang W., Kamensky D., Aggarwal A., Sacks M.S. Biomechanical Behavior of Bioprosthetic Heart Valve Heterograft Tissues: Characterization, Simulation, and Performance. *Cardiovasc. Eng. Technol.* 2016; 7(4): 309–351. DOI: 10.1007/s13239-016-0276-8
10. Ovcharenko E.A., Klyshnikov K.U., Savrasov G.V., Glushkova T.V., Barbarash L.S. Investigation of the hydrodynamic performance of the minimally invasive aortic valve prosthesis. *Complex Issues of Cardiovascular Diseases*. 2016; 5(2): 39–45 (in Russ.). DOI: 10.17802/2306-1278-2016-2-39-45.
11. Brown B.A., Williams H., George S.J. Evidence for the involvement of matrix-degrading metalloproteinases (mmps)

in atherosclerosis. *Prog. Mol. Biol. Transl. Sci*. 2017; 147: 197–237. DOI: 10.1016/bs.pmbts.2017.01.004.
12. Heo K.S., Fujiwara K., Abe J. Shear stress and atherosclerosis. *Mol. Cells*. 2014; 37 (6): 435–440. DOI: 10.14348/molcells.2014.0078.
13. Manji R.A., Hara H., Cooper D.K. Characterization of the cellular infiltrate in bioprosthetic heart valves explanted from patients with structural valve deterioration. *Xenotransplantation.* 2015; 22 (5): 406–7. DOI: 10.1111/xen.12187.
14. Beziere N., Fuchs K., Maurer A., Reischl G., Brück J., Ghoreschi K., Fehrenbacher B., Berrio D.C., Schenke-Layland K., Kohlhofer U., Quintanilla-Martinez L., Gawaz M., Kneilling M., Pichler B. Imaging fibrosis in inflammatory diseases: targeting the exposed extracellular matrix. *Theranostics.* 2019;9 (10):2868–2881. DOI: 10.7150/thno.28892
15. Wu Y., Grande-Allen K.J., West J.L. Adhesive peptide sequences regulate valve interstitial cell adhesion, phenotype and extracellular matrix deposition. *Cell Mol. Bioeng*. 2016; 9(4): 479–495. DOI: 10.1007/s12195-016-0451-x.
16. Amin M., Pushpakumar S., Muradashvili N., Kundu S., Tyagi S.C., Sen U. Regulation and involvement of matrix metalloproteinases in vascular diseases. Front Biosci (Landmark Ed). 2016; 1; 21: 89–118.
17. Ohukainen P, Ruskoaho H, Rysac J. Cellular mechanisms of valvular thickening in early and intermediate calcific aortic valve disease. *Curr. Cardiol. Rev.* 2018; 14 (4): 264–271. DOI: 10.2174/1573403X14666180820151325.
18. Yang L., Gao L., Nickel T., Yang J., Zhou J., Gilbertsen A., Geng Z., Johnson C., Young B., Henke C., Gourley G.R., Zhang J. Lactate promotes synthetic phenotype in vascular smooth muscle cells. *Circ. Res.* 2017; 121(11): 1251–1262. DOI: 10.1161/CIRCRESAHA.117.311819.

Authors contribution

Mukhamadiyarov R.A. – conception and design of the study, analysis of the data, drafting of the manuscript. Rutkovskaya N.V. – analysis of the data, drafting of the manuscript. Kutikhin A.G. – drafting of the manuscript. Milto I.V. – carrying out of the immunohistochemistry assay, analysis of the data, drafting of the manuscript. Sidorova O.D. – analysis of the data. Barbarash L.S. – drafting of the manuscript.

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