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PATHOMORPHOLOGICAL AND MICROBIOLOGICAL ANALYSIS OF AN EPOXY-TREATED BIOPROSTHETIC HEART VALVE FUNCTIONING FOR 25 YEARS IN A PATIENT WITH RHEUMATIC HEART DISEASE: A CASE REPORT

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Bioprosthetic heart valves (BHVs) rarely last longer than 20 years due to the development of degenerative changes in their leaflets. We present a detailed pathomorphological description of KemCor, an epoxy-treated BHV that was removed from the mitral position 25 years after implantation. Literature review shows that this is the longest recorded lifespan of an epoxy-treated implant.

Keywords: bioprosthetic heart valves, structural valve degeneration, histology, clinical case.

INTRODUCTION

Limited lifespan is a major drawback of bioprosthetic heart valves (BHVs). On average, these medical devices function for 10–15 years before requiring replacement due to structural degeneration (SD) of their biological component [1]. Morphologically and histologically, SD is an irreversible process of fatigue-induced destruction and calcification of the collagen base of the leaflet apparatus of BHVs [1]. Degenerative changes in the leaflets become the cause of hemodynamic dysfunction of BHVs, leading to valve stenosis and/or insufficiency [1].

Several papers have described in detail the histopathological pattern of SD, characteristic of foreign BHVs [2, 3]. It is important to note that the latter differ from some Russian-made models by the method of treatment: in their manufacture they use animal biomaterial treated with glutaraldehyde (GA), whereas in Russia a unique technology of biotissue stabilization with diglycidyl ether of ethylene glycol is widespread [4]. The durability of BHVs treated with diepoxy compounds is similar to that of foreign BHVs [4]; however, the histopathological features of SD are poorly studied for this type of implants [5, 6].

In the present article, we present a morphological and histological description of a KemCor BHV (NeoCor, Russia), which was removed 25 years after implantation due to dysfunction. Our hospital records and literature review show that the studied sample is the longest recorded lifespan of an epoxy-treated BHV.

CLINICAL CASE DESCRIPTION AND CHARACTERISTICS OF THE EXPLANTED BIOPROSTHETIC HEART VALVE

A man born in 1960, suffering from rheumatic heart disease and mitral valve stenosis, was implanted with KemCor BHV (NeoCor, Russia) in 1995. The valve was replaced in 2020 (after 25 years) due to grade IV prosthetic valve dysfunction. No history of hypertension, diabetes mellitus, dyslipidemia or kidney failure was noted in the patient's history.

The BHV retrieved during prosthetic replacement was sent to the laboratory for study. Macroscopic analysis revealed the presence of numerous perforations in the dome of the leaflets (Fig. 1, a). Two leaflets were characterized by extensive intraleaflet hemorrhages, while the leaflet apparatus retained elasticity and had no visible calcifications. There were no vegetations on the surface of the leaflets, the frame struts were covered with connective tissue without signs of calcification. Pannus was present on the BHV on the exit site, but it was poorly developed: connective tissue slightly fixed the leaflets adjacent to each other in the commissure zone on one frame strut.

For microscopic analysis, BHV leaflets were separated from the framework and cryosections were prepared on an HM525 microtome cryostat (Thermo Fisher Scientific, USA) using Neg-50 rapid tissue freezing medium (6502, Thermo Fisher Scientific, USA). Examination of slices stained with hematoxylin (05-06004, Bio-Optica, Italy) and eosin (HK-EV-A500, Biovitrum, Russia) showed pronounced stratification and fragmentation of

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the biomaterial. Due to significant tissue damage, the slices were often not monolithic, but were arranged as separate fragments on a glass slide (Fig. 1, b). Alizarin red S staining (ab142980, Abcam, UK) showed the presence of single small (up to 1 mm) calcifications (Fig. 1, c), while Gram staining (ab150672, Abcam, UK) revealed bacterial colonies at the free edge of one of the leaflets (Fig. 1, d). Histological examination of the microorganisms showed Gram-positive cocci and Gram-negative bacilliform bacteria (Fig. 2, a), however, attempts at taxonomic identification of bacteria by PCR using OneStep test systems (Litech, Russia) did not yield results (we used kits for detection of the main pathogens of infective endocarditis (IE) from the genera: *Enterobacter*, *Enterococcus*, *Escherichia*, *Proteus*, *Serratia*, *Staphylococcus* and *Streptococcus*).

Among other things, the leaflet apparatus of the BHV in question was characterized by moderate cell infiltration. The cells were located singly or formed small clusters (not more than 30 cells) localized on the surface of the leaflets, in the thickness of loosened areas of the biomaterial and near calcifications. Cell typing was performed by immunohistochemical reaction using a commercial kit Novolink Polymer Detection Systems (RE7150-CE, Leica Biosystems, USA) and antibodies to pan-leukocyte marker CD45 (ab10558, Abcam, UK), macrophage marker CD68 (ab955, Abcam, UK), T cell marker CD3 (ab16669, Abcam, UK), B-lymphocyte CD19 (MA5-32544, Invitrogen, USA) and neutrophil marker MPO (ab208670, Abcam, UK). Examination of the cellular infiltrates showed that they consisted exclusively of leukocytes (CD45+), represented predominantly by macrophages (CD68+), and single T cells (CD3+) and neutrophils (MPO+) (Fig. 2, b). Oil Red O staining of slices allowed to reveal accumulations of foam cells and large lipid droplets in the biomaterial (Fig. 2, b, c).

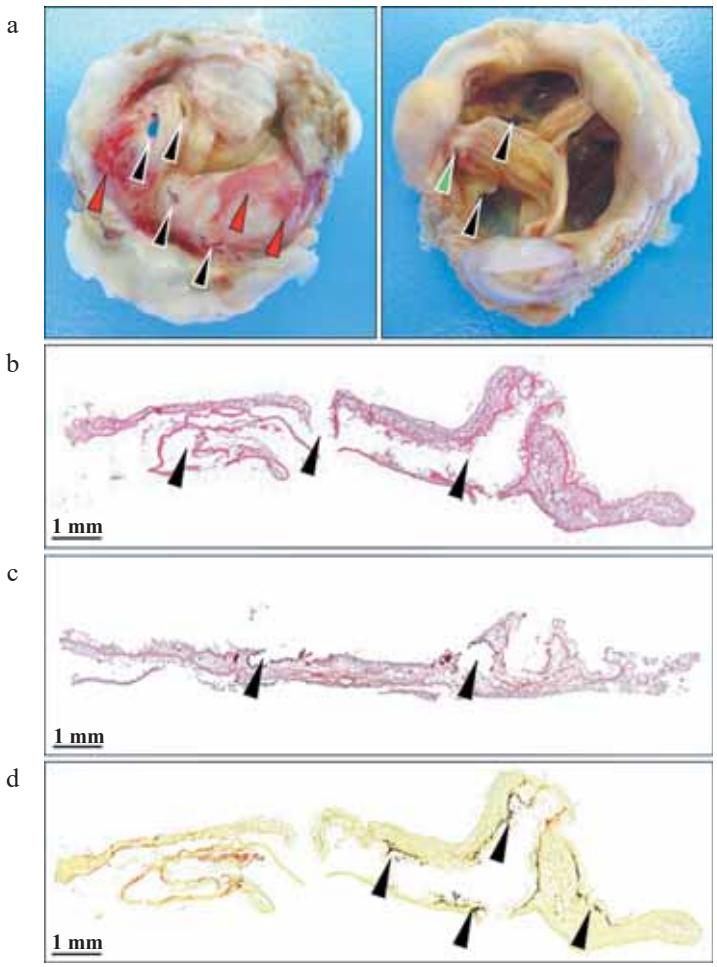

Fig. 1. Results of macro- and microscopic analysis of the studied bioprosthetic heart valve: a, an image of the sample, (left and right – inflow and outflow sections, respectively; black arrows indicate leaflet perforations, red indicate intravalvular hemorrhages, green shows pannus buildup areas); b, histological slice of the leaflet stained with hematoxylin and eosin (arrows indicate areas of delamination and ruptures of extracellular matrix); c, slice stained with alizarin red S (arrows indicate microcalcifications); d, slice stained by Gram staining (arrows indicate colonies of microorganisms)

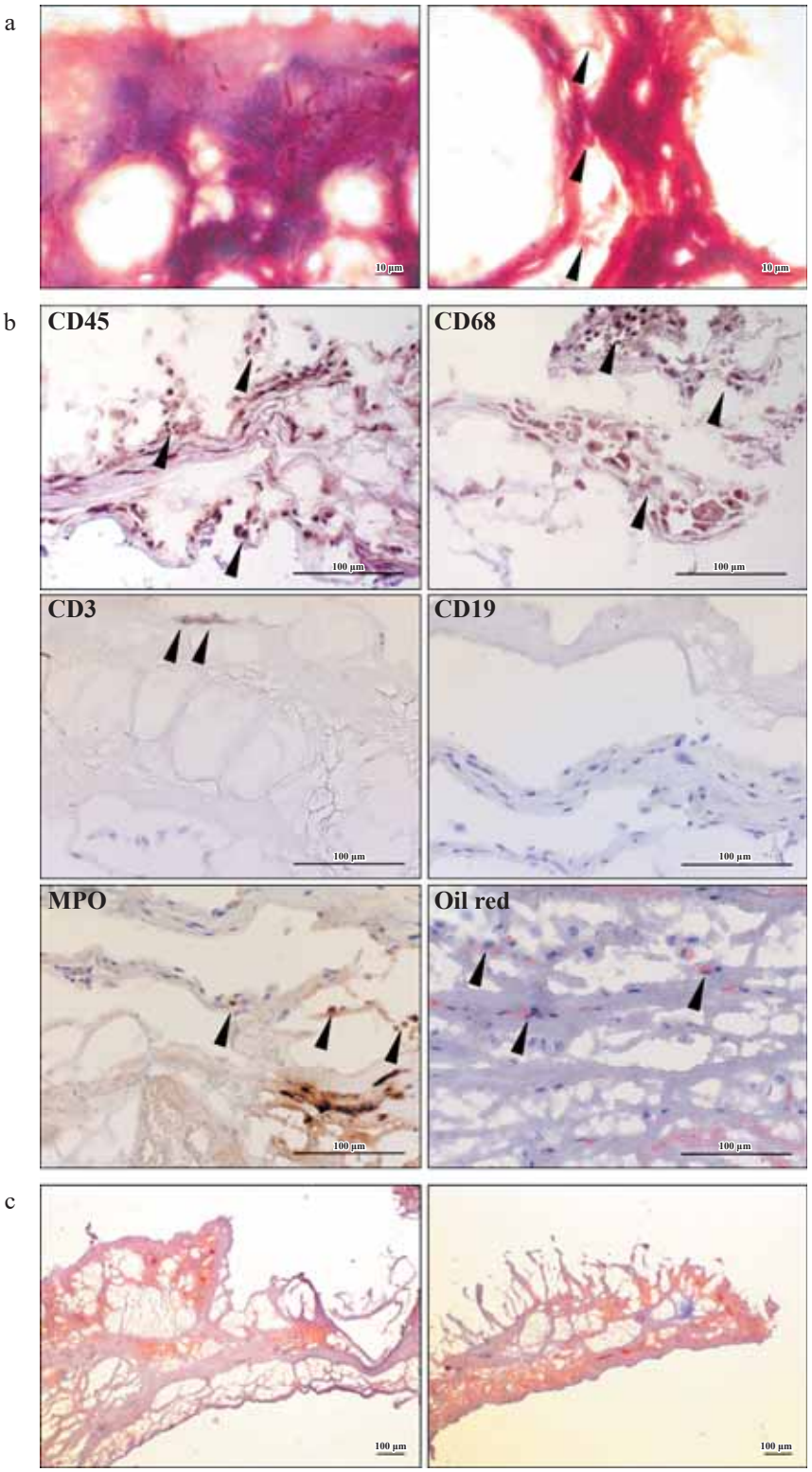

Fig. 2. Results of histological and immunohistochemical staining of the bioprosthetic valve leaflets: a, Gram-stained bacterial colonies composed of Gram-positive cocci and Gram-negative bacilli (arrows indicate recipient cells colocalized with microorganisms); b, cell typing results (arrows indicate positively stained cells; immunohistochemistry and oil red O staining); c, visualization of lipid droplets in the bioprosthetic valve leaflet (on the left is the leaflet dome, on the right is the free edge of the leaflet; oil red O staining)

DISCUSSION

BHVs with a functional lifespan exceeding 20 years are rarely available for histopathological examination. This situation is related to both the relatively short (10–15 years on average) durability of these medical devices and the use of BHVs mainly in patients older than 65 years of age [7]. In the latter case, failed BHVs are usually unavailable for study due to natural death of recipients

or impossibility of retrieval due to transcatheter valve-to-valve replacement method [7]. Consequently, there is little data on BHVs that functioned 20–25 years after implantation [8, 9].

The biomaterial of the leaflets of the BHV in question was characterized by extreme stratification and fragmentation of collagen fibers, which is obviously associated with the duration of exposure of the valve to cyclic loads (over 25 years, the BHV performed about 1 billion cycles of opening and closing). At the same time, calcification of the BHV was negligible. This picture differs from the known cases of long-term (>20 years) functioning of GA-treated BHVs, which are characterized by pronounced calcification of the leaflet apparatus [8, 9]. The obtained data indirectly confirm the opinion of experts, according to which epoxy-treated BHVs are considered to be more resistant to calcification than the GA-treated ones [4]. However, due to lack of a sample, it is still impossible to make a final conclusion about the influence of the tissue preservation method on the nature of degenerative changes in BHVs during their extremely long-term functioning.

Detection of microorganisms in the biomaterial of the BHV was unexpected, although the results of macroscopic analysis, blood cultures and patient intake did not reveal any signs of infective endocarditis (IE) in the patient. It is important to emphasize that the pattern of localization of bacterial agents was not characteristic of classical IE, in the development of which bacteria populate microthrombi on the surface of the flaps with subsequent formation of vegetations. In the case under consideration, bacterial clusters were located in the thickness of the leaflet apparatus in the areas with severe tissue damage. Apparently, the observed picture is due to the entrapment of microorganisms from blood by the network of loosened collagen fibers that form the basis of the biomaterial of the leaflet.

In addition to bacterial agents, the BHV was infiltrated by different types of leukocytes penetrating the thickness of the biomaterial. Apparently, like bacterial contamination, cellular invasion of the flaps is associated with trapping of circulating immune cells from the bloodstream by the loosened tissue. The predominance of macrophages and foam cells in the infiltrates indicates chronic inflammation, which is consistent with the results of previous studies [2, 3, 6]. At the same time, the presence of single T cells and neutrophils may be due to the development of latent IE.

CONCLUSION

In this paper, we reported an epoxy-treated BHV that was removed 25 years after mitral valve replacement. Literature review showed that this is the longest reported lifespan of a BHV treated with ethylene glycol diglycidyl ether. Identification and description of similar cases in the future will help to study the mechanisms of structural degeneration of these medical devices in more detail (in particular, to assess the resistance of an epoxy-treated biomaterial to calcification in a recipient's body compared to a GA-stabilized biomaterial in long-term BHV functioning).

The study was carried out within the framework of the fundamental theme of the Research Institute for Complex Issues of Cardiovascular Diseases, No. 0419-2022-0001 "Molecular, Cellular and Biomechanical Mechanisms of the Pathogenesis of Cardiovascular Diseases in the Development of New Methods of Treatment of Cardiovascular Diseases based on Personalized Pharmacotherapy, Introduction of Minimally Invasive Medical Devices, Biomaterials and Tissue-engineered Implants".

The study was conducted in accordance with the principles of the Good Clinical Practice and Declaration of Helsinki of the World Medical Association, and was approved by the local ethics committee of the Research Institute for Complex Issues of Cardiovascular Diseases (report #19 dated November 6, 2018). The patient signed a written informed consent.

The authors declare no conflict of interest.

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