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Pathomorphologic evaluation of intra-articular injections of soluble platelet-rich plasma for treatment of experimental osteoarthritis

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Abstract

Introduction Non-surgical treatment of osteoarthritis is aimed at managing joint degeneration and inflammation to prolong the life of the original joint and delay total joint replacement. The objective was to pathomorphologically substantiate preclinical effectiveness of PRP in OA using comparative analysis of depleted plasma and serum.

Material and methods The experiment was performed in 120 Wistar rats, divided into 4 groups. Osteoarthritis was simulated using an original method. Knee joint injection given to the animals after skin dissection under inhalation anesthesia and visual control two weeks later contained 0.05 ml PRP in group 1, 0.05 ml plasma in groups 2 and 0.05 ml blood serum in groups 3. The same volume of physiological saline solution was used for the injections produced for control animals. Injections were administered three times at 2-week intervals. Animals were sacrificed in groups of 10 at 2 weeks of each injection.

Results The median MANKIN value scored 2.0 (1.0; 2.0) in group 1, 6.0 (5.0; 7.0) in group 2 and 7.0 (6.0; 7.0) in group 3 at 6 weeks. The median MANKIN value scored 7.5 (7.0, 8.0) in the control group. Statistically significant differences were determined between the groups at $p < 0.001$.

Discussion Literature data on preclinical evaluation of the effectiveness of PRP therapy in biological models of OA are controversial. An original, low-traumatic functional method was used for simulating knee OA to reproduce major pathogenetic mechanisms in rats.

Conclusion The findings suggested a pronounced therapeutic effect with improved morphofunctional features of the hyaline cartilage and MANKIN score of 2 at 6 days of intra-articular administration of modified PRP as compared with plasma and serum.

Keywords: osteoarthritis, experimental model, laboratory rat, articular cartilage, joint capsule, subchondral bone, treatment of osteoarthritis, platelet-rich plasma, PRP, blood serum

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INTRODUCTION

In recent decades, the orthobiological approach, as one of the principles of regenerative medicine, has been used for the local treatment of OA among specialists developing technology for new or optimized biological materials and among orthopaedic and trauma surgeons for stabilization of degenerative changes in articular cartilage [1-3]. Blood derivatives, including autologous conditioned blood serum (SC) or platelet-rich plasma (PRP) can be used for the treatment of OA [4-12]. Literature data on preclinical evaluation of the PRP therapy in biological models of OA are controversial due to different methods of simulating OA, technologies for obtaining experimental PRP products, the frequency of their intra-articular administration and the intervals between injections [13, 14, 15]. Experimental OA is normally inducted by intra-articular administration of monoiodoacetate or a talc suspension. In our opinion, the effect of chemicals on the hyaline cartilage can cause a chemical injury and affect evaluation of the results of therapy [15, 16]. Therefore, an original, low-traumatic functional method was used in our series for simulating knee OA in rats to reproduce major pathogenetic mechanisms of the condition [14, 17]. The frequency of administration of experimental PRP products varies from one to three intra-articular injections among different authors. Pathomorphological and immunological assessment of the effectiveness of PRP therapy includes increased thickness of the articular cartilage, improved tinctorial properties of the articular cartilage matrix, appearance of fibro-hyaline cartilage and an analgesic anti-inflammatory effect [18-24]. Non-surgical treatment of OA is aimed at managing joint degeneration and inflammation to prolong the life of the original joint and delay total joint replacement. Original technologies for obtaining biologically standardized autologous and donor PRP has been developed at the Republican Scientific and Practical Center for Transfusiology and Medical Biotechnology (Republic of Belarus, Minsk). The effectiveness of the products was evaluated with OA simulated in experimental animals optimizing minimally invasive intra-articular administration of blood derivatives.

The **objective** was to pathomorphologically substantiate preclinical effectiveness of PRP in OA using comparative analysis of depleted plasma and serum.

MATERIAL AND METHODS

Laboratory animals

The experiment involved 120 Wistar rats (60 females and 60 males). The animals were kept in the vivarium of the Gomel State Medical University in cages of 3 individuals, with humidity and temperature controlled. Water and food were provided ad libidum. The day/night cycle was 12 hours. Prior to experiments, the animals got acclimatized for 14 days. Animals were kept and cared for in accordance with the recommendations of Good Laboratory Practice of the Ministry of Health of the Republic of Belarus (TPK 125-2008 (02040)). Animals were sacrificed following the bioethical principles of the Declaration of Helsinki for the Humane Treatment of Laboratory Animals as amended in 2013 [18]. Experimental studies with animals were approved by the ethics committee of Gomel State Medical University (minutes of meeting of the committee No. 4 dated December 23, 2020). Animals were randomized using envelopes/random number generator and divided into 4 equal groups (30 animals each). The first group (study group) included rats that received allogeneic plasma enriched with soluble platelet factors (PRP), the second group (comparison group 1) included rats that were injected with rat plasma, and the third (comparison group 2) received injections with serum blood of rats, the fourth group included controls.

Preparation of plasma, serum, PRP

The protocol for obtaining PRP included several strictly defined stages. Blood sampling was carried out in rats cardially with a syringe under general anesthesia with 2.5 % sodium thiopental administered intraperitoneally at a dose of 45 mg/kg body weight. The method allows you to take approximately 10 ml of blood. The contents of the syringe were transferred into tubes with 3.8 % sodium citrate (9:1 ratio) and centrifuged at room temperature at 1000 rpm within 20 minutes. Plasma and buffy platelets were collected using a Pasteur pipette and centrifuged at 1500 rpm. within 20 minutes. The resulting upper layer was selected and used as plasma with blood components removed, depleted plasma. The platelet content in the depleted and enriched layers was monitored using a Sysmex XP-300 hematology analyzer (Sysmex Europe GmbH, Germany). The lower platelet-rich layer was adjusted with depleted plasma to a platelet concentration of 2.0×10^{12}/ml. To assess the purity of PRP, white blood cells were counted with the count being less than 0.1×10^{3}/μl. The resulting plasma fractions were frozen at -70 °C. The enriched plasma was thawed and centrifuged at 3000 rpm. within 15 minutes after 1 to 3 days. The supernatant was collected, filtered using sterile filters with a pore diameter of 0.2 μm, packaged in 0.25 ml Eppendorf tubes and stored at -70 °C prior to the use [19]. Serum was obtained according to standard methods. The resulting blood in a volume of 5 to 10 ml was left at 4 °C for an hour. The clotted blood was centrifuged for 20 minutes at 2000 rpm. The supernatant, serum without evidence of hemolysis, was transferred into Eppendorf tubes and stored at -70 °C prior to the use.

Surgical simulation of osteoarthritis and dosing protocol

Osteoarthritis of the knee joint was simulated in rats using the original method [25]. The technique included several stages. The skin and fascia of the rodent knee was dissected under inhalation anesthesia in aseptic conditions. A sterile needle was injected into the joint cavity and a mechanical trauma to the cartilaginous structures of the lateral condyles of the femur and tibia was produced with the cutting part of the bevel of needle. The diameter of the needle matched with the overall thickness of the cartilaginous layer of the articular surfaces, and sutures were applied to the dissected tissues. Two days later, rats were subjected to use walking wheel system to create static and dynamic loading on injured joints reproducing major pathogenetic mechanisms of osteoarthritis. Two weeks after simulation of OA under inhalation anesthesia, after skin dissection under visual control, 0.05 ml of PRP was injected into the knee joints of animals in group 1, 0.05 ml of plasma injected in group 2, and 0.05 ml of blood serum administered in group 3. The same volume of saline was injected in control rats. Injections were produced three times at intervals of 2 weeks. Animals were sacrificed two weeks after each injection in groups of 10. Major stages of the experiment are presented in Figure 1.

Preparation of histological preparations

The joint was exposed and placed in the decalcifying liquid Histodecalc (Sigma, Italy) for 48-72 hours immediately after the animal was sacrificed. The joint was sagittally cut and the pieces of tissue fixed in 10 % neutral Lilly-buffered formalin for 24-48 hours. Histological processing was produced using the STP-120 histoprocessor (ThermoScientific, Germany) with the tissues embedded in paraffin blocks. A series of sections 4 μm thick were produced from paraffin blocks using a ThermoScientificMicrom HM 450 microtome (ThermoScientific, Germany). Staining with safranin O was produced after dewaxing as reported by R. Asjid et al. [21].

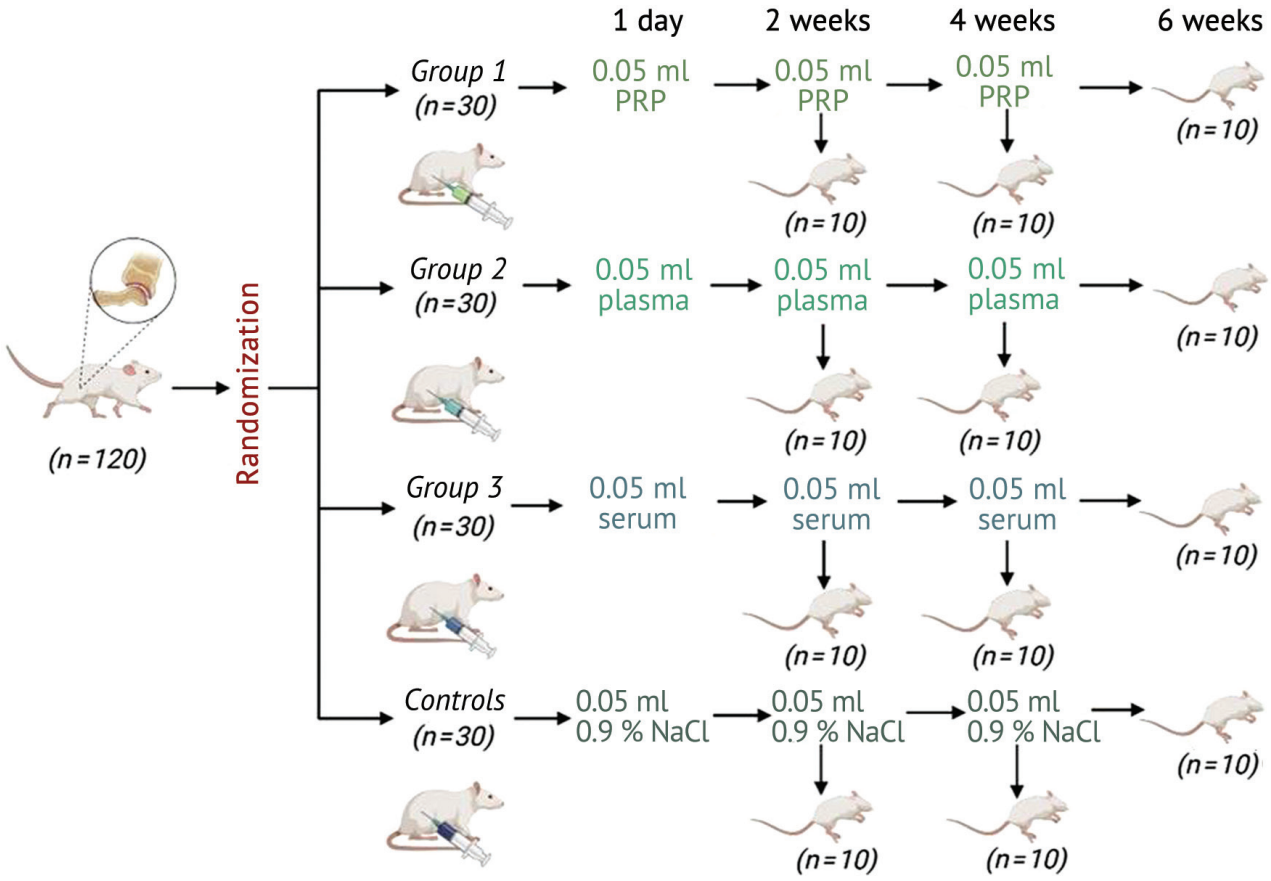

Fig. 1 Diagram of the experiment with therapy of the knee osteoarthritis induced in rats

Morphometric analysis

Microscopic and morphometric analyses were produced using a NikonEclipse 50i light microscope (Nikon, Japan). Morphometric assessment of hyaline cartilage was produced at the site of injury with 3 non-overlapping fields of view. A MANKIN scoring scale modified by F.M.D. Henson et al. was used to evaluate pathological changes in articular cartilage [21] (Table 1).

Table 1

Modified MANKIN score

Structure	Cellularity	Matrix integrity	Integrity of the border line	Score
Smooth surface / normal	Normal location	Normal staining	Normal, not impaired	0
Rough surface/single crack or area of cartilage separation	Clusters of cells in the superficial layer or loss of 10 % of cells	Slight loss of staining	Impaired	1
Multiple cracks/moderate cartilage separation	Disarrangement or loss of 25 % cells	Moderate loss of staining		2
Fragmentation or severe separation of cartilage	Rows of cells are missing or cell loss is up to 50 %	Severe loss of staining		3
Loss of cartilage fragments	Single cells	No staining		4
Erosion does not reach the boundary line				5
Erosion deeper than the boundary line				6

Statistical analysis

The Shapiro – Wilk test did not reveal a normal distribution, and the parameters in our series were presented as the median, 25th and 75th percentiles. Between-the-group comparisons were performed using the Kruskal – Wallis test. Dunn's test was used for post-hoc testing. Results were considered statistically significant at $p < 0.05$. The GraphPadPrism v. 7.04 software package (GraphPadSoftware inc., USA) was used for statistical analysis and graphical presentation of the findings.

RESULTS

All animals tolerated surgical knee manipulations performed under anesthesia and remained alive on to sacrifice. Histological signs of severe osteoarthritis were identified in animals of all groups 2 weeks after the first injection. Destruction of hyaline cartilage with loss of the fragments was observed with areas of cell loss and staining identified, and the border line had areas of discontinuity. The median Mankin score was 10.50 (9.0; 11.0) in group 1, 11.0 (9.0; 12.3) in group 2, 10.5 (9.8; 11.6) in group 3 and 12.0 (10.8; 13.0) in controls. No statistically significant differences were detected between the groups ($p = 0.326$) (Fig. 2a).

Microscopic examination of histological preparations of hyaline cartilage of the rodent joints performed at 4 weeks of the first injection of blood derivatives showed cracks and fragmentation of the cartilage, greater clonality of chondrocytes, areas of lost staining and discontinuity of the border line in group 1. Areas of destruction and loss of cartilage fragments, loss of the cellularity and staining in isolated areas were observed in group 2. Areas of lost cartilage fragments replaced by immature connective tissue, discontinuity of the border line were detected in group 3. Immature connective tissue at the site of the cartilage defect, loss of cellularity and discontinuity of the border line were noted in control animals. The median Mankin score was 3.00 (3.0; 4.3) in group 1, 4.0 (3.0; 5.3) in group 2, 7.0 (6.8; 8.0) in group 3 and 8.0 (7.0; 9.0) in controls at 4 weeks of the experiment. Statistically significant differences were identified between the groups ($p < 0.001$). Between-the-group differences are presented in Figure 2b.

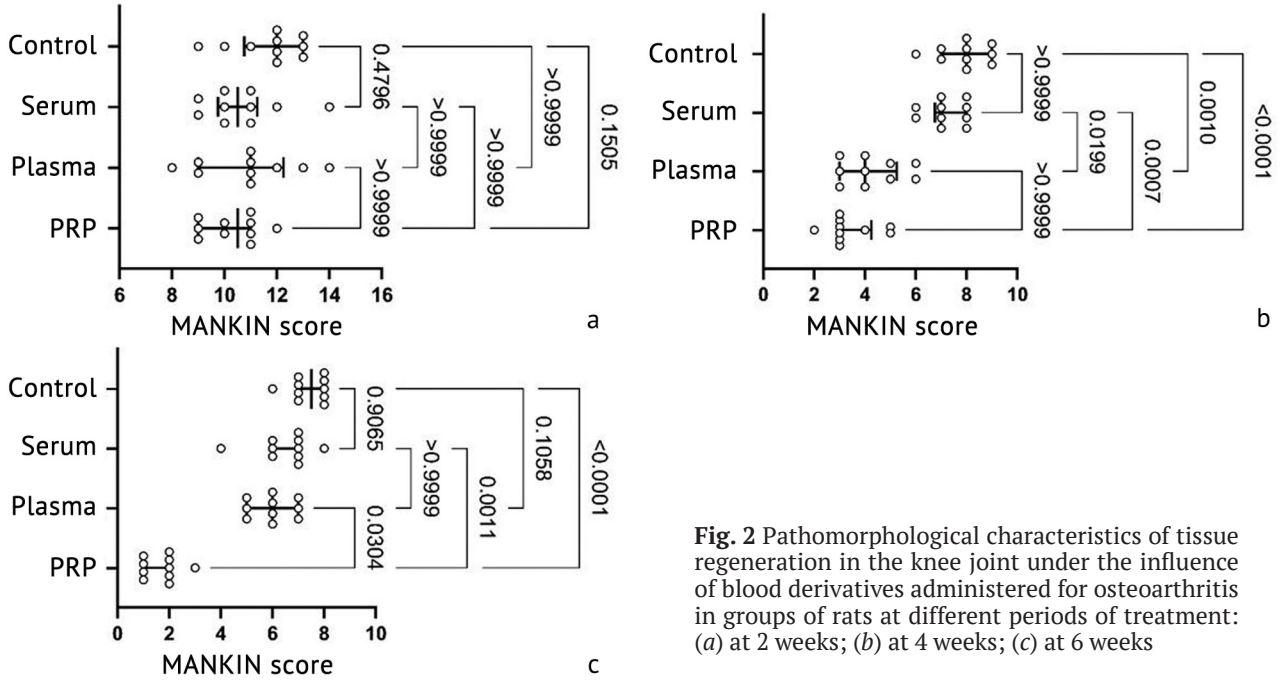

Fig. 2 Pathomorphological characteristics of tissue regeneration in the knee joint under the influence of blood derivatives administered for osteoarthritis in groups of rats at different periods of treatment: (*a*) at 2 weeks; (*b*) at 4 weeks; (*c*) at 6 weeks

Animals in group 1 showed sporadic rugosity of the surface of the hyaline cartilage and small areas of lost staining in the matrix at 6 weeks of the experiment. Sporadic rugosity of the surface of the hyaline cartilage and small areas of cartilage replaced with connective tissue were seen in group 2. Areas of hyaline cartilage replaced with connective tissue and decrease in the cellularity and matrix staining were observed in group 3. Control rats showed areas of cartilage completely replaced with connective tissue to the full depth and a significant decrease in cellularity in these areas. Most representative microphotographs are presented in Figure 3.

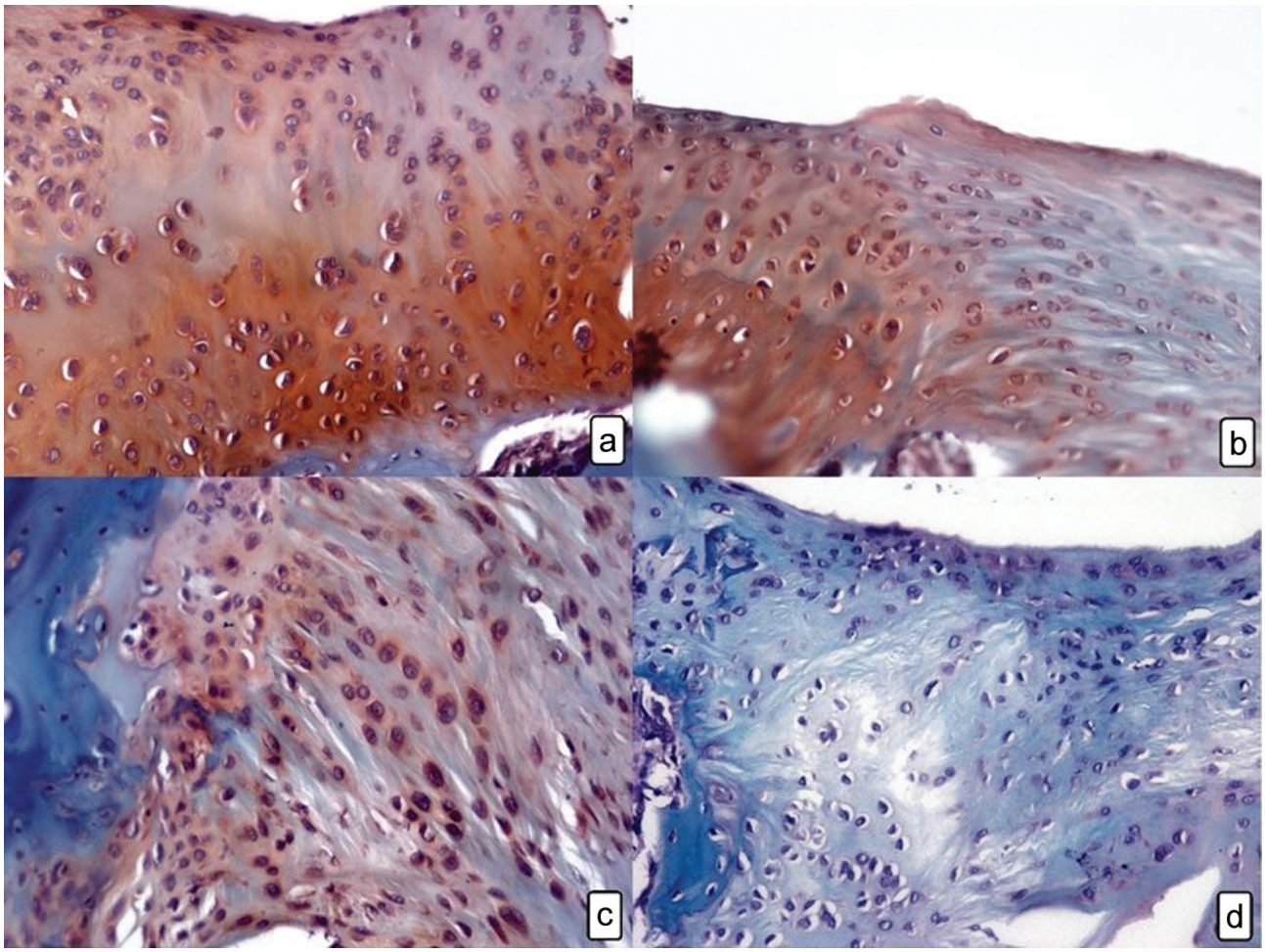

Fig. 3 Pathomorphological picture of the knee cartilage in rats treated for 6 weeks using: (*a*) PRP; (*b*) plasma; (*c*) serum; (*d*) saline solution. Stained with safranin O. Magnification ×200

The median Mankin score was 2.0 (1.0; 2.0) in group 1, 6.0 (5.0; 7.0) in group 2, 7.0 (6.0 ; 7.0) in group 3 and 7.5 (7.0, 8.0) in controls. Statistically significant differences were identified between the groups ($p < 0.001$). Between-the-group differences are presented in Figure 2c.

DISCUSSION

Pathological findings obtained in our series indicated increased cellularity, decreased surface rugosity and accumulation of proteoglycans in the intact hyaline cartilage with a deep cartilage defect being filled with connective tissue [26]. The findings are consistent with those by A. Boffa et al. in a systematic review (2021), showing similar changes in small laboratory animals reported in 28 studies. A group of animals receiving PRP therapy was compared with controls receiving a saline solution in the studies

presented in the systematic review [20]. Our study showed a statistically significant effect of PRP in comparison with control and serum groups [27-28] that can be associated with the pronounced regenerative potential of PRP compared to blood plasma containing no platelets and soluble platelet factors, and the blood serum containing a natural (3-5 times lower) level of soluble platelet factors. Similar data were reported in experimental studies of other authors [26, 29].

CONCLUSION

The findings suggested a pronounced therapeutic effect with improved morphofunctional features of the hyaline cartilage and Mankin score of 2 at 6 days of intra-articular administration of modified PRP as compared with plasma and serum.

Conflict of interest *The authors declare that there are no obvious or potential conflicts of interest related to the publication of this article.*

Financing *The work was produced as part of the project of the Ministry of Health of the Republic of Belarus "Development of a method for obtaining and using autologous plasma-containing soluble platelet factors in the treatment of patients with osteoarthritis" of the subprogram "Transplantation of cells, tissues and organs" of the state scientific and technical program "New methods of providing medical care", state registration No.: 20192357 dated 02.09.2019.*

Ethical review *The studies were approved by the ethical committee of the State Medical University, minutes of meeting No. 4 of December 23, 2020, and were conducted in accordance with the ethical principles of the Declaration of Helsinki.*

Informed consent *Not applicable.*

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Tretyakov A.A. – conceptualization, project management, research, collection and analysis of literature sources, preparation and writing of the manuscript of the article, preparation of the work for publication.

Zinovkin D.A. – analysis of literature sources, methodology, data processing, analysis, preparation of work for publication.

Karpenko F.N., Potapnev M.P. – ideological concept of the work, making adjustments to the original version.

Nikolaev V.I. – conceptualization, project management, control and management of research work; analysis of literature sources, editing of the article.

Pranjol M.Z.I. – data processing, statistical analysis, work with graphic images.