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Autologous Bone Marrow Aspirate Concentrate in the Treatment of Early-Stage Avascular Necrosis of the Femoral Head

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ABSTRACT

BACKGROUND: The use of bone marrow aspirate concentrate in the treatment of avascular necrosis improves outcomes and may delay or prevent joint replacement. However, the method of preparation of bone marrow aspirate concentrate determines both the cellular composition and treatment outcomes.

AIM: This study aimed to assess the efficacy and safety of a novel method for obtaining bone marrow aspirate concentrate for the treatment of early-stage avascular necrosis of the femoral head (ANFH).

METHODS: The study included 35 patients (64 hip joints) with ARCO stage II–IIIA ANFH. Treatment at the N.N. Priorov National Medical Research Center of Traumatology and Orthopedics involved core decompression combined with administration of bone marrow aspirate concentrate obtained using an original method. The follow-up period was 12 months. Functional outcomes (HHS, WOMAC), pain (VAS), quality of life (SF-36), and ANFH stage and activity (MRI of both joints ⩾1.5 T before and 3, 6, and 12 months after treatment; CT before and 6 and 12 months after treatment) were assessed.

RESULTS: The proposed method significantly increased cell yield in the bone marrow aspirate concentrate after centrifugation compared to native bone marrow. Following treatment, HHS and WOMAC scores improved significantly, as did pain intensity according to VAS. Progression from stage II to IIIA by ARCO was observed in 4 hips (4 patients), and from stage IIIA to IIIB by ARCO in 5 hips (4 patients). After 12 months, the necrotic lesion size remained stable in all joints. Total hip arthroplasty was required in 4 patients (5 hips; 7.7% of all joints).

CONCLUSION: The proposed method for obtaining bone marrow aspirate concentrate enables the injection of the desired amount of cells into the femoral head, restoring normal cellular composition. The efficacy of bone marrow aspirate concentrate has been demonstrated in early-stage avascular necrosis of the femoral head (ARCO stage II), which was observed in the majority of patients in our cohort.

Keywords: avascular necrosis; aseptic necrosis; osteonecrosis; bone marrow aspirate concentrate.

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Применение аутологичного концентрата аспирата костного мозга в терапии ранних стадий асептического некроза головки бедренной кости

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АННОТАЦИЯ

Обоснование. Применение концентрата аспирата костного мозга при асептическом некрозе костей позволяет улучшить результаты лечения пациентов, а также отсрочить или избежать эндопротезирования суставов. Однако клеточный состав концентрата костного мозга и результаты лечения зависят от методики его получения, что в конечном счёте влияет на исход лечения.

Цель. Оценить эффективность и безопасность нового способа получения концентрата аспирата костного мозга для лечения пациентов с ранними стадиями асептического некроза головки бедренной кости (АНГБК).

Материалы и методы. В исследование включены 35 пациентов (64 тазобедренных сустава) с АНГБК II–IIIA стадии по ARCO. Лечение в ФГБУ «НМИЦ ТО им. Н.Н. Приорова» включало туннелизацию с применением концентрата аспирата костного мозга, полученного по оригинальной методике. Период наблюдения — 12 месяцев. Оценивали функциональные результаты (HHS, WOMAC), боль (ВАШ), качество жизни (SF-36), стадию и активность АНГБК (МРТ обоих суставов ≥1,5 Тл до операции и через 3, 6, 12 месяцев после лечения; КТ до операции и через 6, 12 месяцев после лечения).

Результаты. Представленная методика обеспечила значительное увеличение количества клеток в концентрате костного мозга после центрифугирования по сравнению с нативным костным мозгом. Отмечено значимое улучшение показателей HHS и WOMAC, снижение боли по ВАШ после лечения. Прогрессирование со II до IIIA стадии по ARCO наблюдалось в 4 тазобедренных суставах (4 пациента), с IIIA до IIIB стадии по ARCO — в 5 тазобедренных суставах (4 пациента). Размер некротического очага остался неизменным через 12 месяцев во всех суставах. Тотальное эндопротезирование потребовалось 4 пациентам (5 суставов; 7,7% от общего числа суставов).

Заключение. Представленная методика получения концентрата аспирата костного мозга позволяет достичь целевого количества клеток, которое необходимо ввести в головку бедренной кости, чтобы восстановить то же количество клеток, что и в нормальной головке бедренной кости. Эффективность использования концентрата аспирата костного мозга доказана при ранней стадии асептического некроза головки бедренной кости (II стадия по ARCO), которую имели большинство наблюдаемых нами пациентов.

Ключевые слова: асептический некроз костей; аваскулярный некроз; остеонекроз; концентрат аспирата костного мозга.

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BACKGROUND

Avascular necrosis of the femoral head (ANFH) is a severe and rapidly progressing condition that leads to femoral head collapse, secondary osteoarthritis, and, consequently, total hip arthroplasty—even in young patients [1]. At the same time, there is compelling evidence that identifying ANFH at an early stage and timely initiating treatment can delay—and in some cases avoid—radical surgical intervention [2]. Currently, the cornerstone of treatment includes joint unloading and pharmacological therapy combined with subsequent core decompression of the femoral head and neck [3]. The additional local application of orthobiological techniques and cell-based therapies, such as the use of bone marrow aspirate concentrate, increases treatment efficacy [4–7]. The improved outcomes in such cases are attributed to the ability of bone marrow aspirate components to stimulate bone regeneration and enhance local angiogenesis [8]. However, native bone marrow aspirate—as a source of multipotent stromal cells, various growth factors, and cytokines [8]—contains them only in small amounts. The concentration of osteoprogenitor cells per volume unit can be increased by removing the erythrocyte fraction and part of the plasma during centrifugation.

At present, a wide range of devices and systems are used for collecting and processing bone marrow aspirate. However, there is evidence that the cellular composition of the resulting concentrate may vary depending on the specific method used, ultimately affecting treatment outcomes [9, 10].

In this regard, the development of a method capable of maximizing the concentration of nucleated progenitor cells remains relevant.

The work aimed to develop a simple and rapid method for obtaining bone marrow aspirate concentrate to improve the treatment efficacy in patients with early-stage avascular necrosis of the femoral head.

METHODS

Study Design

It was a single-center, prospective, experimental, open-label, non-controlled study.

Eligibility Criteria

Inclusion criteria:

- Avascular necrosis of the femoral head, ARCO stage I–IIIA, confirmed by computed tomography (CT) and magnetic resonance imaging (MRI);
- Age between 18 and 59 years;
- Pain syndrome in the hip joint area;
- Signed informed consent to participate in the study.

Non-inclusion criteria:

- Avascular necrosis of the femoral head, ARCO stage IIIB or IV, confirmed by CT and MRI;
- Age under 18 or over 59 years;
- Severe general condition due to somatic pathology;
- History of fracture of the proximal femur or tumors in the hip joint area;
- Previous surgical interventions in the hip region, including core decompression, bone grafting, titanium implantation, or osteotomy.

Study Setting

The study was conducted among patients receiving treatment at the Scientific Center for Metabolic Osteopathies and Bone Tumors of the Federal State Budgetary Institution N.N. Priorov National Medical Research Center of Traumatology and Orthopedics.

Study Duration

The study was conducted from November 2021 to November 2023.

Intervention

Surgical technique for harvesting bone marrow aspirate and preparing bone marrow concentrate from the iliac crest

The consumables required for the procedure are listed in Table 1 and illustrated in Fig. 1.

Patient positioning and bone marrow aspiration technique

With the patient in the prone position, as shown in Fig. 2, access to the posterior superior iliac crest is obtained under sedation using the opioid analgesic fentanyl (dosage determined by the attending anesthesiologist). The first step involves palpation of anatomical landmarks: the posterior superior iliac spine, iliac crest, and sacroiliac joint (Fig. 2*a*, *b*).

After triple antiseptic preparation of the surgical site, local anesthesia is administered in the projection of the intervention (Fig. 2*b*), targeting the skin, subcutaneous fat, and periosteum of the posterior superior iliac spine.

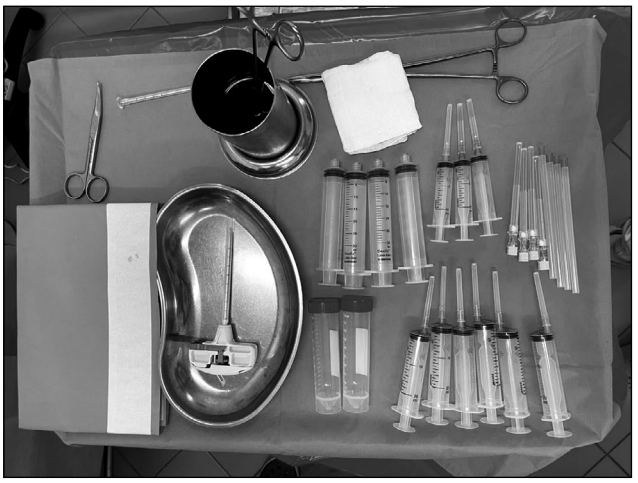

Fig. 1. Surgical table with consumables required for bone marrow aspiration and concentrate preparation.

Table 1. Consumables required for bone marrow aspiration and concentrate preparation

Consumables required for the procedure	Quantity
Trephine biopsy needle, G9, 100 mm	1 pc
Sterile 50 mL tubes (made of ultrapure virgin polypropylene, with high-density polyethylene caps; pyrogen-free, RNase- and DNase-free; single use)	2 pcs
Sterile 50 mL Luer Lock syringes or 30 mL syringes Sterile 20 mL syringes	2 pcs 4 pcs 6 pcs
Sterile 10 mL syringes	3 pcs
Needle, 18G, 1.30 × 88 mm	4 pcs
Needle, 21G, 0.80 × 120 mm	4 pcs
Needle-point scalpel	1 pc
Scissors	1 pc
Straight forceps	1 pc
Sterile gauze pads	10 pcs
Antiseptic solution for surgical site preparation	100 mL
Sterile drape set for surgical field isolation	1 pc
Heparin 5000 IU/mL (5 mL)	5 ampoules

A solution of 20 mL 0.5% ropivacaine combined with 1 mL of 0.01% epinephrine is used (Fig. 2*c*). The syringe intended for marrow aspiration is prefilled with a heparin solution at a concentration of 500 IU per 1 mL of bone marrow to prevent clot formation and coagulation (Fig. 2*d*). The trephine biopsy needle is also flushed with heparin prior to insertion.

A 3–5 mm skin incision is made using a needle-point scalpel (Fig. 2*e*), through which the bone marrow aspiration needle is inserted until it reaches the posterior superior iliac spine (Fig. 2*f*). Then, with the application of controlled force, the needle is advanced through the dense cortical bone into the medullary cavity of the posterior iliac crest to a depth of 2 cm. The trajectory of the needle must be perpendicular to the posterior superior iliac spine. A syringe containing heparin is attached to the aspiration needle, and bone marrow is aspirated in a total volume of 100 mL (Fig. 2*g*), typically using two 50 mL syringes (Fig. 2*h*). Upon completion of the aspiration, a sterile dressing is applied.

Preparation of bone marrow aspirate concentrate

The collected native bone marrow is transferred from the syringes into two sterile 50 mL centrifuge tubes (Fig. 3*a*), and 1 mL is placed into a vacuum hematology tube containing K_3EDTA (tripotassium salt of ethylenediaminetetraacetic acid) for quantitative analysis of the aspirate. Fractional centrifugation is then performed using a CM-6MT centrifuge

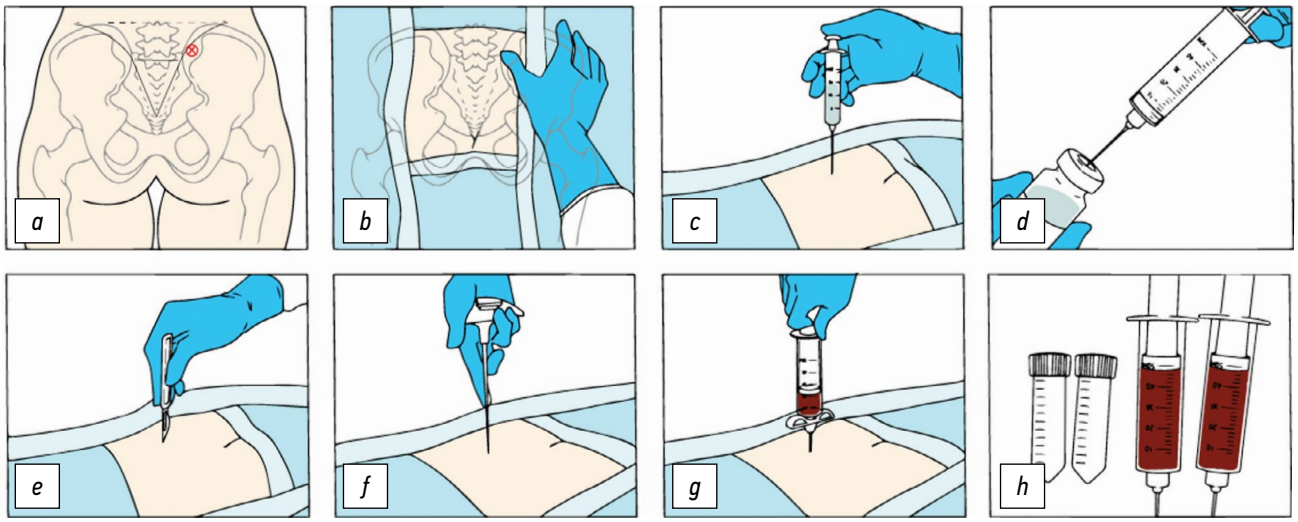

Fig. 2. Bone marrow aspiration technique: *a*, marking the bony landmarks of the posterior iliac crest and sacroiliac joint; *b*, palpation of the intervention site; *c*, local anesthesia; *d*, heparinized syringes; *e*, skin incision using a lancet scalpel; *f*, insertion of trephine biopsy needle into the bone marrow cavity of the posterior iliac crest; *g*, bone marrow aspiration using a 50 mL syringe; *h*, 100 mL of aspirated bone marrow.

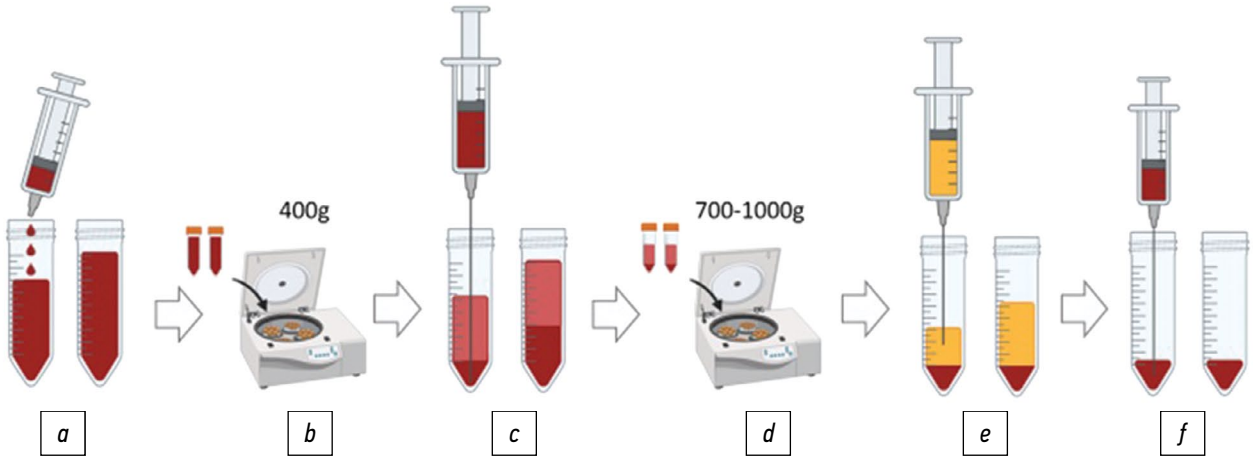

Fig. 3. Bone marrow aspirate concentrate preparation technique: *a*, transferring bone marrow into 50 mL centrifuge tubes; *b*, first centrifugation at 400g for 2 minutes, repeated 2 to 6 times; *c*, removal of erythrocyte sediment (20–25 mL); *d*, centrifugation at 1000g for 15 minutes; *e*, removal of the upper plasma layer from each tube; *f*, collection of the bone marrow aspirate concentrate into two 10 mL syringes

at 400g for 2 minutes, repeated 2 to 6 times under visual control until distinct fraction separation is achieved, with erythrocyte sedimentation reaching the 25–30 mL mark (Fig. 3*b*) [11].

Upon completion of this stage, the erythrocyte sediment in a volume of 20–25 mL is removed from the bottom of each tube using a syringe with a long 18G needle (Fig. 3*c*). The remaining material is subjected to a centrifugation at 1000g for 15 minutes (Fig. 3*d*). Following the second centrifugation, the upper plasma layer is carefully aspirated from each tube using a syringe with a long needle, avoiding disruption of the sediment layer (Fig. 3*e*). The remaining bone marrow aspirate concentrate is collected from both tubes using a long 18G needle and transferred into two 10 mL syringes, each containing 5–7 mL (Fig. 3*f*). Additionally, 1 mL of the final concentrate is transferred into a K_3EDTA vacuum tube for quantitative hematologic analysis and comparison with the native bone marrow sample.

Core decompression of the femoral head and neck with bone marrow aspirate concentrate injection into the osteonecrotic lesion

Under spinal anesthesia, with the patient in the lateral decubitus position on the operating table, core decompression is performed under C-arm guidance using percutaneous drilling with a Kirschner wire and a 3.2 mm drill bit. A fan-shaped pattern is created via multidirectional drilling. Next, a 150 mm trephine biopsy needle is inserted through one of the created channels into the center of the necrotic area, and 3–5 mL of prepared bone marrow aspirate concentrate is slowly injected into the osteonecrotic lesion under C-arm guidance. The wound is then sutured. In cases of bilateral involvement, the same procedure is performed on the contralateral side after repositioning the patient and re-preparing the surgical site. The lateral patient position eliminates the need to manipulate the image intensifier during the procedure, which enables the operation to be performed without an assistant, while maintaining control over the guidewire and drill trajectory in two projections by rotating the hip. However, caution is required during the procedure due to the risk of instrument deformation caused by soft tissue and muscle displacement during improper hip rotation. Additionally, more time is needed to reposition the patient and re-sterilize the surgical field in cases of bilateral ANFH. The procedure can be performed with the patient in the supine position, with the lower limbs secured on a traction table in 20–30 degrees of hip abduction. This positioning allows simultaneous bilateral core decompression without re-sterilization of the surgical field, reduces the risk of drill deformation during hip rotation, but requires an assistant to manage the image intensifier and monitor the guidewire and drill position in two projections.

Study Outcomes and Methods of Their Registration

Hematological analysis

The efficacy of the bone marrow aspirate concentration method was evaluated at the Clinical Laboratory of the Federal State Budgetary Institution N.N. Priorov National Medical Research Center of Traumatology and Orthopedics by analyzing the cellular composition of the bone marrow aspirate in all 35 patients before and after centrifugation.

Aliquots of 1 mL were collected using a fine needle after thorough mixing of the sample and transferred into K_3EDTA tubes, which were then delivered to the laboratory for analysis.

Cell counting was performed using a SYSMEX XT-2000i hematology analyzer (Japan).

The effectiveness of the concentration method was assessed by measuring the total white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), and the number of bone marrow mononuclear cells (MNC), calculated as the sum of lymphocytes (LYMPH) and monocytes (MONO).

Assessment of clinical and radiological outcomes of combined treatment for avascular necrosis of the femoral head

The follow-up period was 12 months. Functional outcomes were evaluated using the Harris Hip Score (HHS), the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), pain severity was assessed using the Visual Analog Scale (VAS), and quality of life was measured using the SF-36 questionnaire. These assessments were performed before surgery and at 3, 6, and 12 months postoperatively. The stage and activity of ANFH were evaluated based on MRI data of both hip joints (field strength ⩾1.5 Tesla) before surgery and at 3, 6, and 12 months postoperatively, as well as on CT scans of the hip joints obtained before surgery and at 6 and 12 months postoperatively.

Ethics Approval

The procedure for harvesting and administering bone marrow aspirate concentrate in patients with ANFH was approved by the Local Ethics Committee of the Federal State Budgetary Institution N.N. Priorov National Medical Research Center of Traumatology and Orthopedics (Protocol No. 1, dated March 3, 2019). Written informed consent was obtained from all individuals who participated in the study prior to surgery.

Statistical Analysis

Statistical analysis was performed using SPSS 2023 software. Normality of distribution was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests.

Depending on the data distribution, quantitative variables were presented as M ± SD (M, mean, SD, standard deviation) or as Me [IQR] (Me, median, IQR, interquartile range). Statistical significance of differences between groups was assessed using the Mann–Whitney U test. A p-value < 0.05 was considered statistically significant.

RESULTS

Participants

Between November 2021 and November 2023, 35 patients (64 hip joints) with early-stage ANFH underwent treatment with bone marrow aspirate concentrate obtained using the proposed method. Patient characteristics are presented in Table 2.

Table 2. Clinical characteristics of patients included in the study

Indicator	Characteristic
Sex (male/female)	29/6
Mean age	35.2 years (25–52 years)
Mean body mass index	28.3 (19–40)
Cause of avascular necrosis	
Primary (idiopathic)	4 patients (8 hip joints)
Secondary:	31 patients (56 hip joints)
• Mild COVID-19	4 patients (8 hip joints)
• Severe COVID-19	26 patients (46 hip joints)
• history of long-term glucocorticoid therapy for the treatment of a chronic disease	1 patient (2 hip joints)
Comorbidities	
Gastrointestinal condition	5 patients (14.3%)
Cardiovascular condition	3 patients (8.6%)
Thyroid disorders	2 patients (5.7%)
Psoriasis	2 patients (5.7%)
Kidney condition	1 patient (2.9%)
Cluster headache	1 patient (2.9%)
Harmful habits	
Smoking	10 patients (28.6%)
Alcohol consumption >70 g per week	12 patients (34.3%)
Stage of avascular necrosis of the femoral head	
Stage II by ARCO	44 hip joints (68.8%)
Stage IIIA by ARCO	20 hip joints (31.2%)
Laterality of the lesion	
Unilateral	6 patients (17.1%)
Bilateral	29 patients (82.9%)

All patients received conservative treatment prior to surgery, which included joint unloading (ambulation with crutches), anticoagulant therapy (rivaroxaban 10 mg), calcium supplementation (calcium carbonate up to 1000 mg/day), active vitamin D metabolites (alfacalcidol 0.75–1.0 μg/day), and antiresorptive therapy (zoledronic acid 5 mg IV, single dose). Surgical treatment in the form of core decompression with bone marrow aspirate concentrate injection was performed in all patients within 2 to 4 weeks from the initiation of therapy.

Hematological analysis

The measurement data are presented in Fig. 4.

After centrifugation, an increase in the number of cells was observed:

- Leukocytes: by 4.79 times (before: 18.93 [12.53; 21.52] × 10^9/L, after: 90.61 [71.87; 126.20] × 10^9/L);
- Neutrophils: by 5.99 times (before: 11.29 [7.32; 14.39] × 10^9/L, after: 58.53 [41.96; 84.06] × 10^9/L);
- Mononuclear cells: by 5.78 times (before: 5.52 [3.34; 5.93] × 10^9/L, after: 31.89 [22.65; 45.41] × 10^9/L);
- Erythrocytes: by 1.36 times (before: 4.05 [3.79; 4.28] × 10^{12}/L, after: 5.49 [4.04; 6.62] × 10^{12}/L);
- Platelets: by 6.43 times (before: 184.00 [147.25; 223.0] × 10^9/L, after: 1102.00 [661.5; 1328.75] × 10^9/L).

In all cases, including erythrocytes, the number of cells after centrifugation was significantly higher (Fig. 5) compared with the native bone marrow aspirate ($p < 0.001$, Mann–Whitney U test).

Evaluation of functional outcomes

Harris Hip Score

The mean Harris Hip Score (HHS) was 62 ± 15 before surgery and 79 ± 14 at 12 months postoperatively. The mean HHS improved significantly following surgical treatment with bone marrow aspirate concentrate ($p = 0.0007$; Fig. 6).

WOMAC Questionnaire

The mean WOMAC score was 86 ± 38 preoperatively and 57 ± 37 at 12 months. The mean WOMAC score showed a significant improvement after surgical treatment with bone marrow aspirate concentrate ($p = 0.02$; Fig. 7).

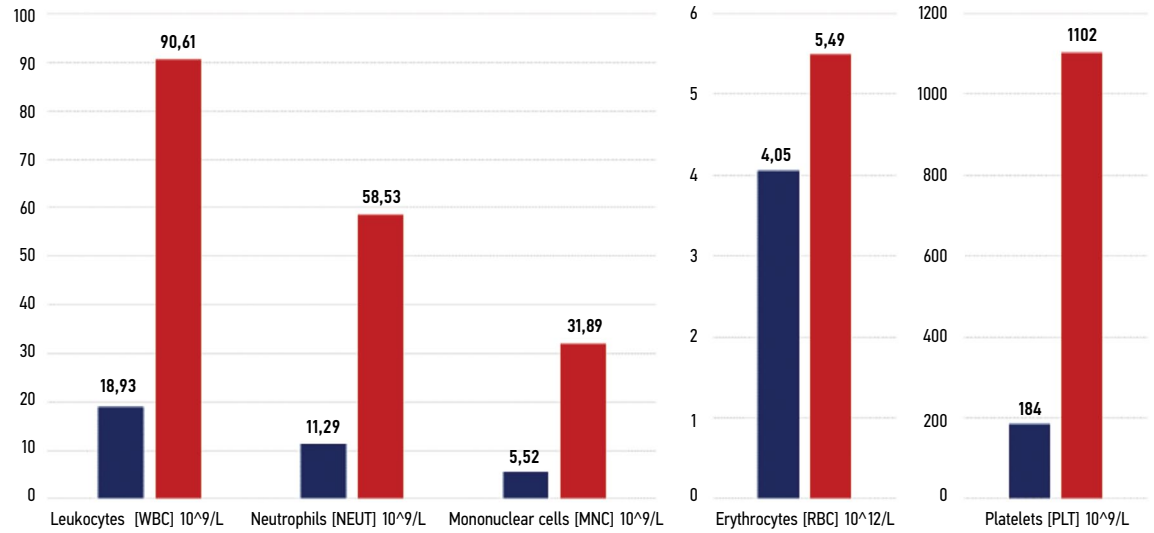

Fig. 4. Median cell count in bone marrow aspirate before and after centrifugation (blue bar: before centrifugation; red bar: after centrifugation).

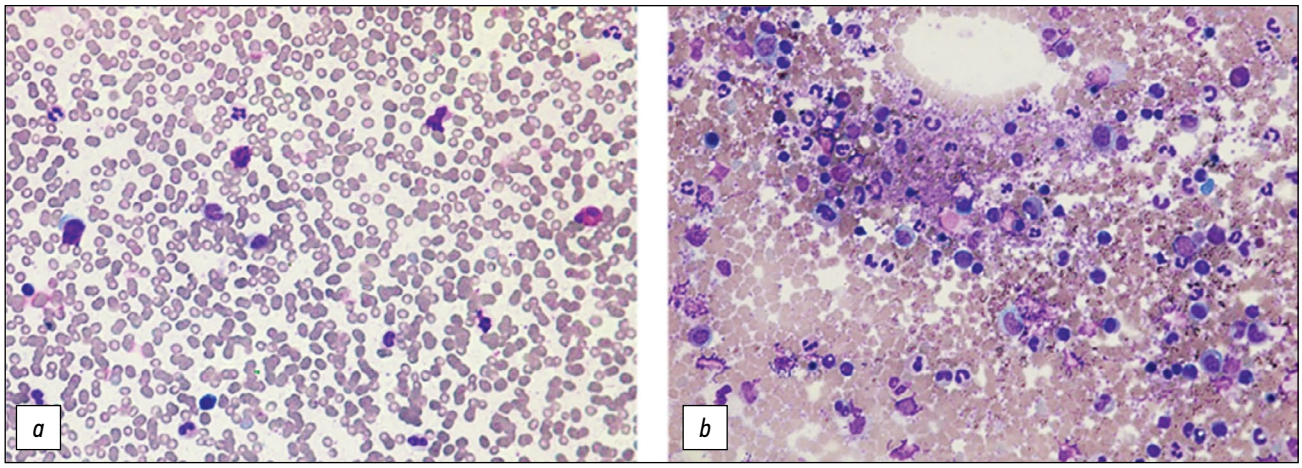

Fig. 5. Bone marrow aspirate samples: *a*, before centrifugation; *b*, after centrifugation. Romanowsky–Giemsa stain, ×200.

Pain assessment

Visual analog scale

Pain levels assessed by the VAS decreased in all patients immediately after the procedure. The mean VAS score was 4.8 ± 1.3 before surgery and 3.2 ± 1.6 at 12 months. The VAS score decreased significantly after surgical treatment with bone marrow aspirate concentrate (p = 0.002; Fig. 8).

Assessment of quality of life

SF-36 Questionnaire

The mean quality of life score on the SF-36 (The Short Form-36) was 79 ± 10 before surgery and 88 ± 13 at 12 months. The mean SF-36 score improved significantly following surgical treatment with bone marrow aspirate concentrate (p = 0.02; Fig. 9).

Radiological progression

Progression from ARCO stage II to IIIA and from stage IIIA to IIIB was observed in 4 hip joints (4 patients) and in 5 hip joints (4 patients), respectively. The size of the necrotic lesion remained unchanged in all hip joints between the preoperative evaluation and 12 months postoperatively (Fig. 10).

Total hip arthroplasty as an outcome of aseptic necrosis of the femoral head

Total hip arthroplasty was required in 4 patients (5 hip joints, accounting for 7.7% of all hip joints in the study population). The indications for this surgical intervention were collapse of the articular surface and persistent pain (VAS score > 6).

In all of these patients, MRI and CT confirmed ARCO stage IIIA (pre-collapse stage) at the time of core decompression with bone marrow aspirate concentrate administration. The mean time to disease progression to ARCO stage IIIB and subsequent conversion to total hip arthroplasty was 10.8 months (8 to 12 months) (Fig. 11).

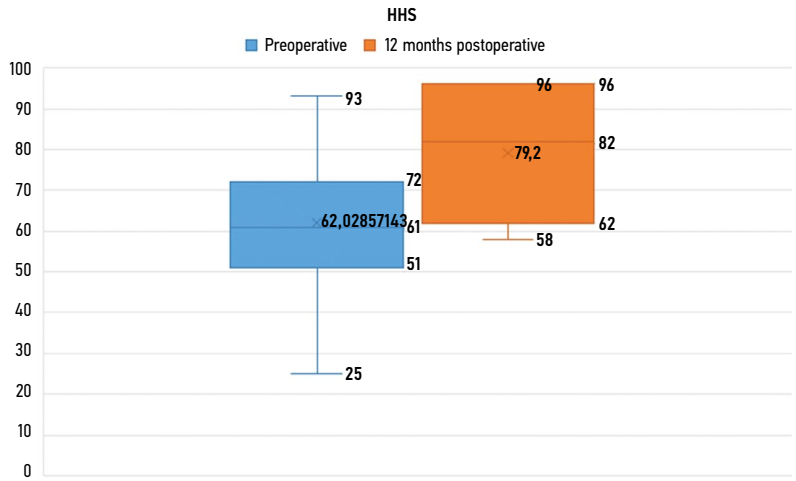

Fig. 6. Hip joint function assessment before and 12 months after core decompression with bone marrow aspirate concentrate according to the Harris Hip Score.

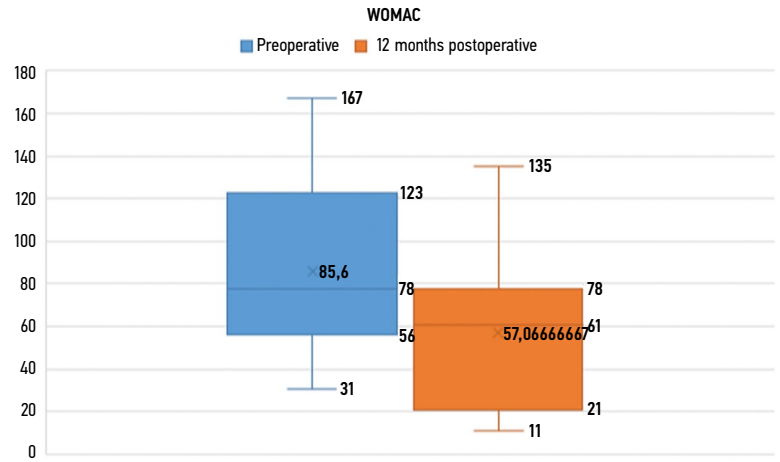

Fig. 7. Hip joint function assessment before and 12 months after core decompression with bone marrow aspirate concentrate according to the WOMAC index.

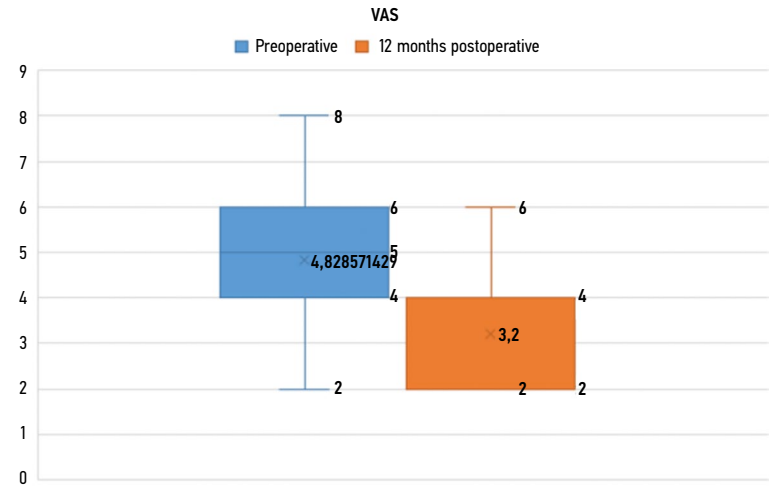

Fig. 8. Pain assessment using a visual analog scale before and 12 months after core decompression with bone marrow aspirate concentrate.

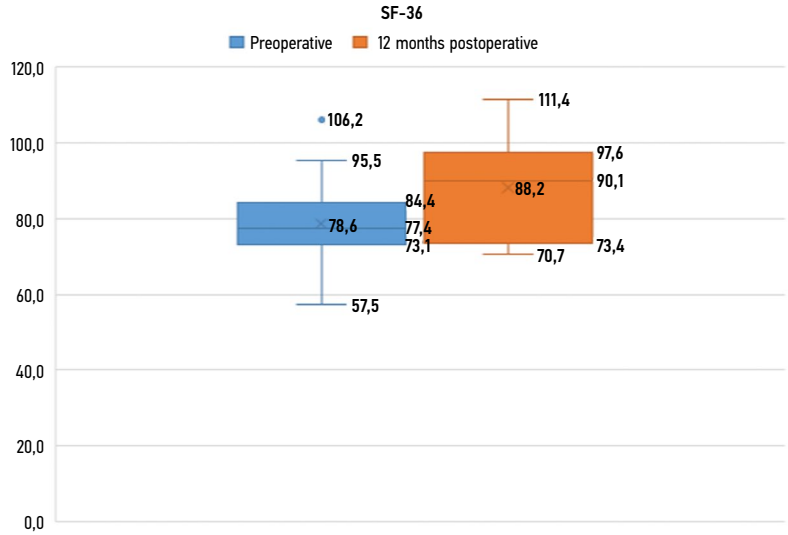

Fig. 9. Quality of life assessment using the SF-36 before and 12 months after core decompression with bone marrow aspirate concentrate.

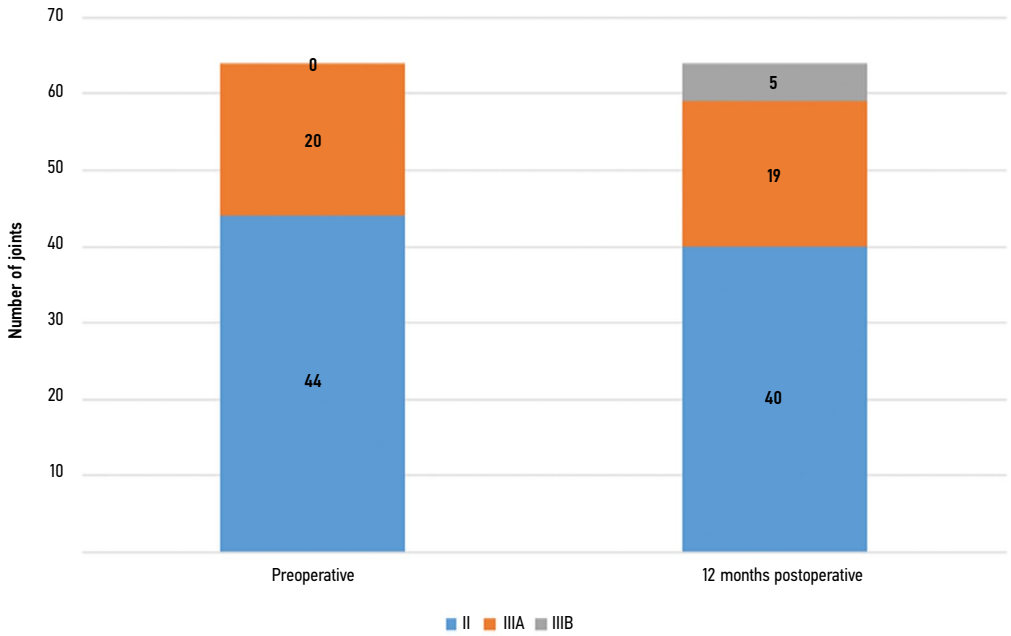

Fig. 10. Radiographic progression of avascular necrosis of the femoral head according to the ARCO classification after treatment.

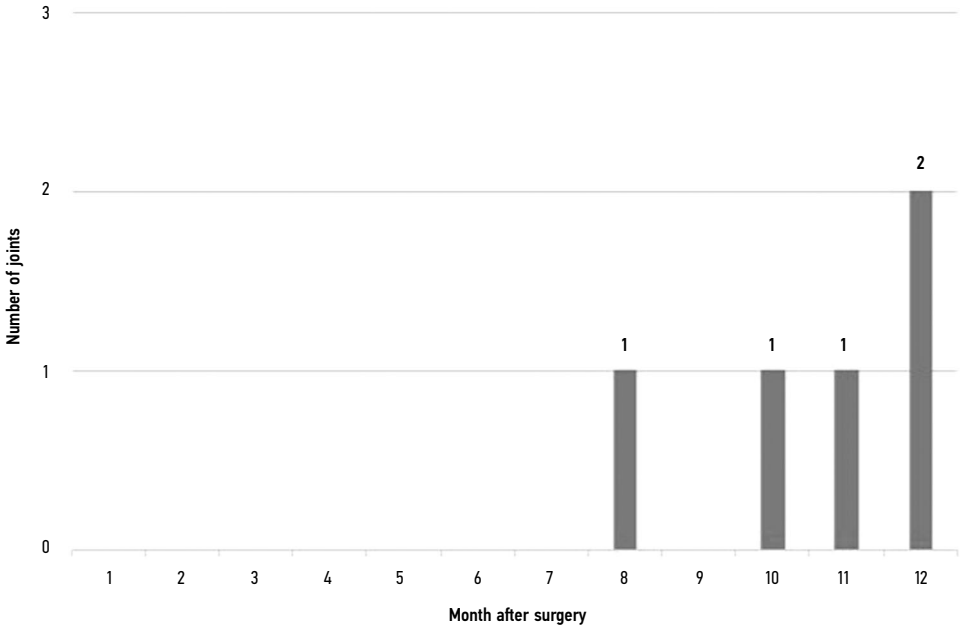

Fig. 11. Total hip arthroplasty as a treatment outcome for avascular necrosis of the femoral head within 12 months after core decompression with bone marrow aspirate concentrate.

DISCUSSION

Despite significant improvement in functional outcomes based on the HHS and WOMAC scores, reduction in pain according to the VAS, and improvement in quality of life, total hip arthroplasty was required in 5 out of 64 hip joints. The need for arthroplasty was due to disease progression to ARCO stage IIIB–IV and the persistence of chronic pain (VAS score > 6) during the 12-month follow-up period. This outcome may be attributed to the fact that core decompression with administration of bone marrow aspirate concentrate was performed at ARCO stage IIIA.

These findings are consistent with other studies reporting low efficacy and a high risk of disease progression at this stage [12].

This study presents the results of a simple, rapid, and cost-effective technique for preparing autologous bone marrow aspirate concentrate, as well as evidence of its effectiveness when used during core decompression of the femoral head and neck as part of a combined treatment strategy for patients with early-stage ANFH.

A critical parameter for achieving optimal outcomes with bone marrow aspirate concentrate is its qualitative and quantitative composition, which depends both on the individual biological characteristics of each patient and on the site of aspiration, the technique used, and the method of concentrate preparation [13].

Regarding the aspiration site, we relied on the published scientific data. Hyer et al., in a comparative study of cell concentrations in bone marrow aspirates from the anterior iliac crest, the distal metaphysis of the tibia, and the calcaneal body, demonstrated that the iliac crest yields the highest cell concentration [14].

Subsequent studies confirmed the superiority of the iliac crest over the femur and tibia in terms of the number of harvested cells [15, 16]. Pierini et al., in a comparison between the anterior and posterior iliac crests, found that aspiration from the posterior superior iliac spine yielded a greater number of cells [17].

Another important determinant of bone marrow aspirate concentrate quality is the method of processing the harvested native bone marrow. Various devices and systems are currently used for bone marrow aspirate collection and processing, including manual, semi-automated, and fully automated centrifugation methods (standard centrifuges, polyester gels, Ficoll solution, the BMCS Arthrex Angel system, the Spectra Optia apheresis system, among others). The quality and quantity of the resulting bone marrow aspirate concentrate vary depending on the system used. Attempts to compare different bone marrow aspirate concentrate preparation systems have not demonstrated scientific evidence in favor of any particular device [18].

Our proposed method enables the production of a fivefold bone marrow aspirate concentrate with minimal time expenditure (compared with Ficoll gradient separation) and lower cost (compared with commercial kits and separation systems).

The total nucleated cell count in our study was derived from the leukocyte count using the SYSMEX XT-2000i hematology analyzer (Japan). The feasibility of using such analyzers for this purpose has been demonstrated previously: the Sysmex XT-1800i was used in the study by Schäfer et al. [19], and the ADVIA 120 was used in the study by Hegde et al. [10]. The observed difference in total cell count (leukocytes) between native bone marrow and the concentrate was 4.79-fold, whereas the difference in the number of neutrophils

and mononuclear cells was 5.99- and 5.78-fold, respectively. These differences are likely due to the hematology analyzer using different methods for counting total and differentiated cells: total cell counts are measured by the aperture impedance method, whereas differential counts are obtained via flow cytometry. We focused on total nucleated cell because this parameter is commonly reported in the scientific data [19, 20], and used neutrophil and mononuclear cell counts as supplementary reference values. Nevertheless, we do not exclude the possibility that the validation of bone marrow aspirate results on hematology analyzers may be addressed in future dedicated studies. The median total nucleated cell (leukocyte count) before centrifugation in our study was 18.93×10^9/L, a value comparable to data reported in other studies [19, 20]. The somewhat lower values compared to some reports [13, 19] may be attributed to the older age of patients in our cohort. The association between age and cell concentration was reported in the study [21], where the authors noted that bone marrow concentrate in patients under 30 years of age contained three times more nucleated cells than in patients over 60. At the same time, sex, race, body mass index, and presence of osteoporosis had no effect on cell concentration. We also do not exclude a possible association between the lower cell concentration observed in our study and the history of severe COVID-19 infection and corticosteroid therapy in the majority of patients.

For an approximate estimation of the concentration of multipotent stromal cells in the bone marrow aspirate concentrate, we relied on previously reported data: multipotent stromal cells in native bone marrow account for 0.01%–0.02% of all nucleated cells [13, 20, 22]. These data were obtained by counting colony-forming unit fibroblasts (CFU-F) in cell culture.

Given that the median leukocyte count in the bone marrow aspirate concentrate was 90.61×10^9/L, the estimated number of multipotent stromal cells in 1 mL of the concentrate was $9.06–18.12 \times 10^3$/mL (0.01%–0.02%), that is, 9060–18,120 cells per mL. In our case, the volume of the fivefold concentrate obtained from 100 mL of bone marrow aspirate was 10 mL, which provided a sufficient volume for bilateral administration of 5 mL of the concentrate into each femoral head.

As each 1 mL of the obtained concentrate contained 9060–18,120 cells, each osteonecrotic lesion (femoral head) received 45,300–90,600 multipotent stromal cells contained in 5 mL of the concentrate.

Previous studies have indicated an association between higher numbers of injected cells and favorable outcomes in the treatment of aseptic necrosis [23]. However, the optimal effective dose of bone marrow-derived multipotent stromal cells remains undefined and is still a subject of ongoing debate [24].

We can only hypothesize a threshold cell dose in the bone marrow aspirate concentrate depending on the bone remodeling rate and the number of mesenchymal cells normally present in the femoral head. It is known that the total number of mesenchymal cells in 1 cm^3 of the femoral head averages 700 ± 264 per cm^3. Given that the average volume of the femoral head is 50 cm^3, the total number of mesenchymal cells is approximately 35,000. This value may be considered a target cell number that needs to be delivered into the femoral head to restore the physiological cell count in a normal femoral head [25]. In a recent large-scale study by Hernigou et al., a significant correlation was found between the number of transplanted cells and the clinical outcome of osteonecrosis treatment. The cell doses delivered to the osteonecrotic lesion were 117,000 ± 42,000 (range 45,000 to 301,000) in stage I osteonecrosis (190 patients) and 127,000 ± 39,000 (range 48,000 to 310,000) in stage II (216 patients) [26]. These values are comparable with the results obtained using our method for producing bone marrow aspirate concentrate. However, it is important to note that the efficacy of bone marrow aspirate concentrate appears to depend not only on the presence and concentration of multipotent stromal cells [27].

Its efficacy is also attributed to the high concentration of platelets, bioactive molecules, growth factors, cytokines, and chemokines, which have been reported to exert both anabolic and anti-inflammatory effects [28, 29]. Outcome analysis demonstrated that the applicability of bone marrow aspirate concentrate for ANFH varies depending on the disease stage. Five out of 64 joints underwent total hip arthroplasty within 12 months after core decompression with bone marrow aspirate concentrate injection; these patients had initially presented with ARCO stage IIIA. The possibility of unfavorable outcomes after core decompression at ARCO stages IIIA and IIIB has also been noted by other researchers [12].

The originality of the study and the feasibility of using bone marrow aspirate concentrate in clinical practice for early-stage ANFH are confirmed by patents [11, 30].

CONCLUSION

Thus, the presented simple, rapid, and cost-effective technique for bone marrow aspiration and preparation of autologous bone marrow aspirate concentrate enables the collection of a target cell count to be delivered into the femoral head in order to restore the cellular content to that of a healthy femoral head. The efficacy of bone marrow aspirate concentrate has been demonstrated in early-stage ANFH (ARCO stage II), which was observed in the majority of patients in our cohort. In cases of ARCO stage IIIA ANFH, core decompression with bone marrow aspirate concentrate injection may be ineffective and often results in total hip arthroplasty within the first year.

ADDITIONAL INFORMATION

Author contributions: A.R. Baikova: data curation, formal analysis, writing—original draft; A.N. Torgashin: investigation, data curation,

writing—review & editing; S.A. Rodionov: investigation, formal analysis, writing—original draft; S.S. Rodionova: formal analysis, writing—review & editing. All the authors approved the final version of the manuscript to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Generative AI: No generative artificial intelligence technologies were used to prepare this article.

Provenance and peer-review: This paper was submitted unsolicited and reviewed following the standard procedure. The peer review process involved two external reviewers, a member of the editorial board, and the in-house scientific editor.

Consent for publication: Written informed consent was obtained from all patients for the publication of their medical data and images.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. А.Р. Байкова — сбор и анализ материала, написание статьи; А.Н. Торгашин — выполнение клинического этапа исследования, сбор и анализ материала, редакция статьи; С.А. Родионов — выполнение лабораторного этапа исследования, анализ материала, написание статьи; С.С. Родионова — анализ полученного материала, редакция текста статьи. Все авторы одобрили финальную версию перед публикацией, а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой её части.

Источники финансирования. Отсутствуют.

Раскрытие интересов. В статье представлены результаты применения запатентованной технологии: https://patents.google.com/patent/RU2827075C1/ru, https://patents.google.com/patent/RU2816790C1/ru. Авторы заявляют об отсутствии других отношений, деятельности и интересов (личных, профессиональных или финансовых), связанных с третьими лицами (коммерческими, некоммерческими, частными), интересы которых могут быть затронуты содержанием статьи, а также иных отношений, деятельности и интересов за последние три года, о которых необходимо сообщить.

Оригинальность. При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, данные).

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