УДК 617.3-089.816:[611.018.1"712.4":611.013.395]](045)

DOI: http://dx.doi.org/10.15674/0030-59872025156-64

Quality criteria for minimally manipulated biotechnological products based on autologous bone marrow aspirate for use in traumatology and orthopedics

S. S. Strafun [1], Ye. L. Holiuk [1], D. O. Zubov [1], O. M. Magomedov [1], H. S. Starodub [2], H. K. Malova [3]

[1] SI «National Institute of Traumatology and Orthopedics of the NAMS of Ukraine», Kyiv
[2] SU «National Scientific Center of Radiation Medicine, Hematology and Oncology of the NAMS of Ukraine», Kyiv
[3] Kyiv City Clinical Hospital No. 9. Ukraine

Autologous biotechnological products based on bone marrow (BM) are a source of stem cells, in particular, hematopoietic and multipotent mesenchymal stromal cells, and act as one of the alternative therapeutic agents that can slow cartilage degeneration, improve its repair and, ultimately, prevent joint replacement. Objective. To develop quality and safety criteria of minimally manipulated biotechnological products from autologous bone marrow for use in patients with pathology of the musculoskeletal system. Methods. The material for the development of quality and safety criteria for the manufacture and use of biotechnological products from autologous red bone marrow aspirate was 85 patients with osteoarthritis and aseptic necrosis of the hip and knee joints, who used biotechnological products made from it. Cell counts in the myelogram and CFU-analysis of MMSC-BM were performed in all patients. Results. As a result of the development of quality and safety criteria for biotechnological products from BM aspirate, 3 types of it were established: 1 — polymorphic (with the presence of progenitor cells of all types within the normal range), 2 — moderately cellular (with the presence of all types of progenitor cells, but some of them below the norm), 3 — hypocellular (with the presence of most types of progenitor cells, some of them below the norm). Functional quality criteria of biotechnological products from BM aspirate were developed on the basis of CFU-analysis and calculation of seeding efficiency coefficient (KEP) of MMSK-BM. Conclusions. Quality and safety criteria of biotechnological products based on autologous bone marrow aspirate based on the results of myelograms and CFU analysis have been established. According to the results of myelograms, type 1 BM aspirate was evaluated as excellent for the further manufacture of a biotechnological product, type 2 — good, type 3 — satisfactory. According to the results of the KUOf analysis, the KEP indicator < 0.001 % was evaluated as unsatisfactory, the KEP indicator within the range of 0.001–0.003 % — satisfactory, the KEP indicator > 0.003 % — good. The KEP < 0.001 % — unsuitable for use.

Аутологічні біотехнологічні продукти на основі кісткового мозку (КМ) є джерелом стовбурових клітин, зокрема гемопоетичних і мультипотентних мезенхімальних стромальних клітин. Вони являють собою один із альтернативних терапевтичних засобів, який може сповільнити дегенерацію хряща, покращити його репарацію та запобігти ендопротезуванню суглоба. Мета. Розробити критерії якості та безпеки маломаніпульованих біотехнологічних продуктів з аутологічного КМ для застосування в пацієнтів із патологією опорно-рухового апарата. Методи. Для отримання біотехнологічних продуктів використано аспірат червоного кісткового мозку 85 пацієнтів з остеоартрозом та асептичним некрозом кульшового та колінного суглобів. В усіх зразках проводили підрахунок клітин у мієлограмі та КУОф-аналіз мультипотентних мезенхімальних стовбурових/стромальних клітин кісткового мозку (ММСК-КМ). Результати. Під час розробки зазначених якостей біотехнологічних продуктів з аспірата КМ встановлено 3 його типи: 1 — поліморфний (клітини-попередники усіх типів у межах норми); 2 — помірноклітинний (присутні всі типи клітин-попередників, але деякі з них нижче норми); 3 — гіпоклітинний (із наявністю більшості типів клітин-попередників, деякі з них нижче норми). Розроблено функціональні критерії якості біотехнологічних продуктів з аспірата КМ на підставі КУОф-аналізу й обрахунку коефіцієнта ефективності посіву (КЕП) ММСК-КМ. Висновки. Виявлено критерії якості та безпеки біотехнологічних продуктів на основі аутологічного аспірата КМ за результатами мієлограм та КУОф-аналізу. За результатами мієлограм тип 1 аспірата КМ оцінювали, як відмінний для подальшого виготовлення біотехнологічного продукту, тип 2 — добрий, тип 3 — задовільний. За КУОф-аналізом показник КЕП < 0,001 % визначали як незадовільний, у межах 0,001–0,003 % — задовільний, у разі > 0,003 % — хороший. Аспірат КМ із КЕП < 0,001 % розцінювали непридатним для застосування. Ключові слова. Регенеративна ортопедія, регенеративна ін'єкційна терапія, остеоартроз, асептичний некроз, кульшовий суглоб, колінний суглоб.

Key words. Regenerative orthopedics, regenerative injection therapy, osteoarthritis, avascular necrosis, hip, knee

Introduction

Recent advances in biotechnology and regenerative medicine have significantly expanded the range of applications of biotechnology products, in particular in traumatology and orthopedics. Currently, the source of cell and tissue products for the treatment of diseases and injuries of the musculoskeletal system is red bone marrow (BM) [1, 2], as it is considered to be one of the most accessible sources of multipotent mesenchymal stem/stromal cells (MMSCs) in the adult body [3, 4]. Therefore, discussing the prospects and effectiveness of using biotechnology products obtained from these tissue sources for the treatment of osteoarthritis and aseptic necrosis of the hip and knee joints is a rather relevant issue. Most often, BM concentrates containing MMSCs are used in clinical practice today [5, 6].

Autologous biotechnological products based on BM are a source of stem cells, in particular HSCs and MMSCs, and are one of the alternative therapeutic agents that can slow down cartilage degeneration, improve its repair and, ultimately, prevent joint replacement [7, 8]. MMSCs are able not only to directly differentiate into chondrocytes, but also produce many biologically active substances that have immunomodulatory and anti-inflammatory effects, stimulate angiogenesis and are inducers of chemotaxis for endogenous progenitors. Due to their high proliferative potential *in vitro*, paracrine effects and ability to restore damaged cartilage and bone tissue *in vivo*, MMSCs are considered an effective tool for cell therapy of musculoskeletal pathologies. Among the studied sources of stem and progenitor cells, red bone marrow MSCs (BM-MMSCs) can be considered the most promising in terms of availability, safety, and expected therapeutic efficacy [7]. Key prognostic characteristics of the therapeutic efficacy of BM-based biotechnology products for regenerative orthopedics are cellularity indicators and the ratio of cell types in the myelogram (hemopoietic cells) and during the CFU analysis of stromal cells (BM-MMSCs).

Purpose: to develop criteria for the quality and safety of minimally manipulated biotechnological products from autologous bone marrow for use in patients with musculoskeletal disorders.

Material and methods

The study was conducted at the Department of Tissue and Cell Therapy of the State Institution "ITO NAMS of Ukraine" in the period from 2021 to 2024. Informed consent was obtained from all patients before the study and treatment. The study was performed in compliance with the principles of bioethics (protocol No. 1 of the meeting of the Bioethics Committee of the State Institution "Institute of Traumatology and Orthopedics of the NAMS of Ukraine" dated 11 January 2021).

During the study, red bone marrow aspirate from 85 patients with osteoarthritis and aseptic necrosis of the hip and knee joints was used, from which biotechnological products were subsequently manufactured. BM aspirate from 71 patients was used for cell counting in the myelogram, and from 14 patients for the CFU analysis of MMSC-BM. We isolated red bone marrow from the iliac crest. According to literature sources, this localization prevails over other possible ones in terms of the number of progenitor cells obtained compared to the tibia and calcaneus [9].

We used three approaches for BM aspiration (Fig. 1): anterior parallel (through the iliac wing) (Fig. 1, a), posterior parallel (parallel to the posterior superior iliac spine) (Fig. 1, b, c) and posterior perpendicular (perpendicular to the posterior superior axis of the iliac bone) (Fig. 1, d). Aspiration was performed with an 11G trocar 100 or 150 mm long, after flushing its lumen with a small amount of heparin. After local anesthesia, by manually placing the cannula in the iliac crest, rotating it clockwise and counterclockwise, we simultaneously applied axial force or pressed the trocar to the bone, pushing the cannula through the cortical layer. After its passage, we felt some relief in the movement of the trocar, then we deepened it into the spongy layer of the iliac bone by another 0.5 cm. After making sure that the trocar was in a stable position in the thickness of the bone, we connected a Luer-Lock syringe, then carefully pulled its piston towards us, without much effort, and made sure that there was bone marrow aspiration in its lumen. Having determined its presence, further sampling should be performed slowly, since significant efforts during aspiration will increase the patient's pain syndrome.

Bone marrow sampling from one area leads to a decrease in the number of mesenchymal stem cells due to dilution with peripheral blood, so after filling 2–3 syringes, we changed the depth of sampling. We obtained 100 ml of aspirate to isolate one dose of the mononuclear fraction. To obtain several doses, sampling was performed from several accesses. If necessary, ultrasound or a C-curve was used for navigation.

After completing the BM aspirate sampling procedure, the patients were recommended to remain in a horizontal position for 30 minutes.

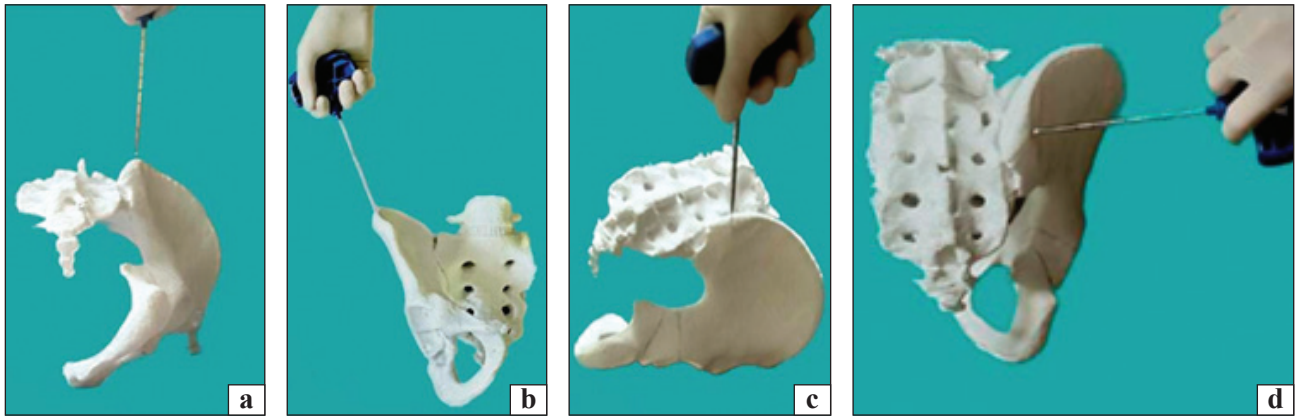

Figure. Accesses to the pelvic bone for collecting BM aspirate: a) anterior parallel; b), c) posterior parallel; d) posterior perpendicular

Table 1

Example of a myelogram of patient G., whose aspirate is unsuitable for further manufacturing of a biotechnological product

Cellular element		Result	Norm (in % according to A. I. Vorobyov)
Reticular cells		—	0.1–1.6
Blasts that do not undergo morphological differentiation		—	0.1–1.1
Myeloblasts		0	0.2–1.7
Neutrophilic granulocytes	Promyelocytes	0.2	1.0–4.1
	Myelocytes	1.4	7.0–12.2
	Metamyelocytes	0.8	8.0–15.0
	Rods	4.0	12.8–23.7
	Segmented	52.0	13.1–24.1
Eosinophilic granulocytes	Promyelocytes	—	0.5–5.8
	Myelocytes	—	0.5–5.8
	Metamyelocytes	0.6	0.5–5.8
	Rods	—	0.5–5.8
	Segmented	—	0.5–5.8
Basophilic granulocytes	Promyelocytes	—	0.0–0.5
	Myelocytes	—	0.0–0.5
	Metamyelocytes	0.2	0.0–0.5
	Rods	—	0.0–0.5
	Segmented	—	0.0–0.5
Elements of erythropoiesis: – erythroblasts		0	0.2–1.1
– pronormocytes		0.2	0.1–1.2
– basophilic normocytes		2.2	1.4–4.6
Normocytes polychromatophilic		7.2	8.9–16.9
Normocytes oxyphilic		0	0.8–5.6
Promegaloblasts		—	—
Megaloblasts basophilic		—	—
Megaloblasts polychromatophilic		—	—
Megaloblasts oxyphilic		—	—
Elements of lymphopoiesis: – lymphocytes		22.4	4.3–13.7
– plasma cells		0.2	0.1–1.8
Elements of monocytopoiesis: – monocytes		8.6	0.7–3.1
– mitoses of white germ elements		—	2 : 500
– mitoses of red germ elements		—	3 : 500
Bone marrow indices: – leuko:erythro		9.4 : 1.0	3.5–4 : 1.0
– neutrophil maturation		0.04	0.6–0.8
– erythrokaryocyte maturation		0.8	0.8–0.9

Preparation of a bone marrow aspirate smear for counting the cellular composition

Having taken the slide by its long edges, we touched its surface (stepping back 0.5–1 cm from the narrow edge) to a drop of aspirate (but not to the skin). The drop should be small in size, and it should be placed so that the entire smear fits on the glass, not reaching 1–1.5 cm to its edge. Its fixation was carried out according to May-Grunwald [10]. The smear of the BM aspirate was stained according to Romanovsky: the cell elements were stained in different colors and shades with a mixture

Table 2

Example of a patient C's type 1 myelogram

Cellular element		Result	Norm (in % according to A. I. Vorobyov)
Reticular cells		—	0.1–1.6
Blasts that do not undergo morphological differentiation		—	0.1–1.1
Myeloblasts		0	0.2–1.7
Neutrophilic granulocytes	Promyelocytes	1.2	1.0–4.1
	Myelocytes	10.4	7.0–12.2
	Metamyelocytes	6.0	8.0–15.0
	Rods	15.2	12.8–23.7
	Segmented	26.4	13.1–24.1
Eosinophilic granulocytes	Promyelocytes	—	0.5–5.8
	Myelocytes	—	0.5–5.8
	Metamyelocytes	0.4	0.5–5.8
	Rods	—	0.5–5.8
	Segmented	—	0.5–5.8
Basophilic granulocytes	Promyelocytes	—	0.0–0.5
	Myelocytes	—	0.0–0.5
	Metamyelocytes	0.4	0.0–0.5
	Rods	—	0.0–0.5
	Segmented	—	0.0–0.5
Elements of erythropoiesis: – erythroblasts		0.4	0.2–1.1
– pronormocytes		0.4	0.1–1.2
– basophilic normocytes		3.2	1.4–4.6
Normocytes polychromatophilic		9.6	8.9–16.9
Normocytes oxyphilic		6.4	0.8–5.6
Promegaloblasts		—	—
Megaloblasts basophilic		—	—
Megaloblasts polychromatophilic		—	—
Megaloblasts oxyphilic		—	—
Elements of lymphopoiesis: – lymphocytes		15.6	4.3–13.7
– plasma cells		0.4	0.1–1.8
Elements of monocytopoiesis: – monocytes		4.0	0.7–3.1
– mitoses of white germ elements		—	2 : 500
– mitoses of red germ elements		—	3 : 500
Bone marrow indices: – leuko:erythro		4.0 : 1.0	3.5–4 : 1.0
– neutrophil maturation		0.4	0.6–0.8
– erythrokaryocyte maturation		0.8	0.8–0.9

of basic (azure II) and acidic (water-soluble yellow eosin) dyes. Staining was carried out with a ready-made solution of Romanovsky dye for 40 minutes. After complete drying, the smear is ready for counting, which was carried out in the clinical diagnostic laboratory of the municipal non-profit enterprise "Kyiv City Clinical Hospital No. 9". Based on the results of the smear counting, a myelogram of the corresponding BM aspirate sample was formed.

MMSC-CM CFU analysis

Heparinized (2 units/ml heparin sodium) red bone marrow aspirate, taken from the iliac crest, was seeded in complete growth medium containing MEM alpha modified (BioWest), 10 % ETC (Sigma-Aldrich), 1 ng/ml bFGF (Sigma-Aldrich), antibiotic-antimycotic solution (BioWest), 2 units/ml heparin sodium at the rate of 5.7 million nucleated cells of red bone marrow aspirate per large Petri dish with a diameter of 100 mm (3 dishes with 10 ml of complete growth medium per each bone marrow sample) and cultured for 14 days in a CO_2 incubator at 37 ºC and in a 5 % carbon dioxide atmosphere and 96 % humidity. The growth medium in the dishes was changed every third day. After 14 days of cultivation, Petri dishes with colonies (colony-forming units of fibroblasts, or CFUs) were washed with phosphate-buffered saline and fixed for 20 min at room temperature with buffered formalin solution and stained with hematoxylin-eosin solution. Stained colonies were counted.

Results

Morphological criteria for the quality and safety of BM aspirate and biotechnological products made from it

According to the results of the analysis of myelograms of patients who underwent sampling for the manufacture of a biotechnological product, all variants of the aspirated fluid were divided into 2 types: aspirate of BM and without signs of the presence of BM. The results of determining the type of BM aspirate in 16 samples obtained were polymorphic, in 26 samples — moderately cellular, in 29 — hypocellular.

Table 3

Example of a patient K's type 2 myelogram

Cellular element		Result	Norm (in % according to A. I. Vorobyov)
Reticular cells		—	0.1–1.6
Blasts that do not undergo morphological differentiation		—	0.1–1.1
Myeloblasts		0.2	0.2–1.7
Neutrophilic granulocytes	Promyelocytes	0.6	1.0–4.1
	Myelocytes	12.8	7.0–12.2
	Metamyelocytes	16.0	8.0–15.0
	Паличкоядерні	8.8	12.8–23.7
	Сегментоядерні	27.2	13.1–24.1
Eosinophilic granulocytes	Promyelocytes	—	0.5–5.8
	Myelocytes	—	0.5–5.8
	Metamyelocytes	0.6	0.5–5.8
	Паличкоядерні	—	0.5–5.8
	Сегментоядерні	—	0.5–5.8
Basophilic granulocytes	Promyelocytes	—	0.0–0.5
	Myelocytes	—	0.0–0.5
	Metamyelocytes	0.2	0.0–0.5
	Паличкоядерні	—	0.0–0.5
	Сегментоядерні	—	0.0–0.5
Elements of erythropoiesis: – erythroblasts		0.2	0.2–1.1
– pronormocytes		0.6	0.1–1.2
– basophilic normocytes		5.6	1.4–4.6
Normocytes polychromatophilic		9.6	8.9–16.9
Normocytes oxyphilic		0	0.8–5.6
Promegaloblasts		—	—
Megaloblasts basophilic		—	—
Megaloblasts polychromatophilic		—	—
Megaloblasts oxyphilic		—	—
Elements of lymphopoiesis: – lymphocytes		13.6	4.3–13.7
– plasma cells		0.2	0.1–1.8
Elements of monocytopoiesis: – monocytes		3.8	0.7–3.1
– mitoses of white germ elements		—	2 : 500
– mitoses of red germ elements		—	3 : 500
Bone marrow indices: – leuko:erythro		5.3 : 1.0	3.5–4 : 1.0
– neutrophil maturation		0.8	0.6–0.8
– erythrokaryocyte maturation		0.6	0.8–0.9

When examining the aspirated fluid, two criteria were considered: megakaryocytes in the myelogram as a marker for bone marrow, and the leuko-erythrocyte index. In the absence of megakaryocytes in the myelogram and the leuko-erythrocyte index of more than 20:1, the aspirated fluid is not suitable for further manufacture of a biotechnological product (Table 1).

Conclusion: bone marrow punctate is hypocellular. No megakaryocytes were observed in the preparation. Considering the approximation of the cellular composition of the bone marrow to the cellular composition of the peripheral blood, the absence of megakaryocytes and fragments of the bone marrow reticulum, a significant admixture of blood to the aspirate cannot be ruled out.

In turn, the BM aspirate obtained during collection was divided into 3 types according to the results of myelogram analysis. The first is polymorphic, with the presence of precursor cells of all types within normal limits. This type of aspirate is characterized by the presence of megakaryocytes in the myelogram, the leuko-erythrocyte index does not exceed 4:1. An example of a myelogram of this type of BM aspirate is given in Table 2.

The bone marrow punctate is moderately cellular, polymorphic. The dimensions of the erythron are preserved (20.0 %), with normal maturation. The granulocytic series is preserved (59.2 %), mature forms of granulocytes prevail. Megakaryocytes are single in the preparation, freely located platelets in sufficient quantity.

Type 2 is moderately cellular (with the presence of all types of precursor cells, but some of them below normal). This variant of the aspirate is characterized by the presence of megakaryocytes in the myelogram, the leuko-erythrocyte index is (5:1)–(10:1).

An example of a myelogram of this type of BM aspirate is given in Table 3.

Therefore, the bone marrow punctate is moderately cellular. The dimensions of the erythron are preserved, closer to the lower limit of the norm, with delayed maturation in young forms. The gran-

Table 4

Example of a patient C's type 3 myelogram

Cellular element		Result	Norm (in % according to A. I. Vorobyov)
Reticular cells		—	0.1–1.6
Blasts that do not undergo morphological differentiation		—	0.1–1.1
Myeloblasts		0	0.2–1.7
Neutrophilic granulocytes	Promyelocytes	0	1.0–4.1
	Myelocytes	2.8	7.0–12.2
	Metamyelocytes	1.6	8.0–15.0
	Паличкоядерні	3.6	12.8–23.7
	Сегментоядерні	42.0	13.1–24.1
Eosinophilic granulocytes	Promyelocytes	—	0.5–5.8
	Myelocytes	—	0.5–5.8
	Metamyelocytes	0.8	0.5–5.8
	Паличкоядерні	—	0.5–5.8
	Сегментоядерні	—	0.5–5.8
Basophilic granulocytes	Promyelocytes	—	0.0–0.5
	Myelocytes	—	0.0–0.5
	Metamyelocytes	0.4	0.0–0.5
	Паличкоядерні	—	0.0–0.5
	Сегментоядерні	—	0.0–0.5
Elements of erythropoiesis: – erythroblasts		0	0.2–1.1
– pronormocytes		0	0.1–1.2
– basophilic normocytes		2.4	1.4–4.6
Normocytes polychromatophilic		6.4	8.9–16.9
Normocytes oxyphilic		1.2	0.8–5.6
Promegaloblasts		—	—
Megaloblasts basophilic		—	—
Megaloblasts polychromatophilic		—	—
Megaloblasts oxyphilic		—	—
Elements of lymphopoiesis: – lymphocytes		32.0	4.3–13.7
– plasma cells		0	0.1–1.8
Elements of monocytopoiesis: – monocytes		6.4	0.7–3.1
– mitoses of white germ elements		—	2 : 500
– mitoses of red germ elements		—	3 : 500
Bone marrow indices: – leuko:erythro		9.0 : 1.0	3.5–4 : 1.0
– neutrophil maturation		0.1	0.6–0.8
– erythrokaryocyte maturation		0.8	0.8–0.9

ulocytic series is preserved, with normal maturation. Megakaryocytes are single in the preparation.

Type 3 is hypocellular, with the presence of most types of precursor cells, some of them below the norm. This variant of the aspirate is characterized by the presence of megakaryocytes in the myelogram, the leuko-erythrocytic index is (10:1)–(20:1).

An example of a myelogram of this type of BM aspirate is given in Table 4.

The cellularity of the bone marrow punctate was found to be reduced. There are cells of all hematopoietic germs at different stages of maturation.

Type 1 of the BM aspirate was assessed as excellent for further production of a biotechnological product, type 2 — good, type 3 — satisfactory.

Functional criteria for the quality of BM aspirate and biotechnological products made from it

Today, the CFU analysis is considered one of the "gold standards" for determining the frequency of clonogenic MMSC-BM. According to the results of the analysis of the cultivation of nucleated cells of the BM aspirate, the number of colonies in three samples was counted and the average indicator was determined for each patient. The seeding efficiency coefficient (the percentage of MMSC among all nucleated cells) was calculated using the following formula: SEC (seeding efficiency coefficient) = the average number of colonies in three samples of each patient * 100% / 5.7 million (the number of nucleated cells per 1 large Petri dish). The results of determining the seeding efficiency are given in Table 5.

The SEC index < 0.001% was considered unsatisfactory, within 0.001–0.003% — satisfactory, in the case of > 0.003% — good. Thus, the BM aspirate with SEC < 0.001% was considered unsuitable for use as a biotechnological product. The optimal for use is the BM aspirate with SEC > 0.003 % (i. e. more than 3 clonal colonies of MMSC per 1×105 nucleated cells of the BM aspirate).

Discussion

Today, autologous bone marrow aspirate is increasingly used for the treatment of orthopedic and trauma patients, in particular in conditions

Table 5

Determination of the efficiency of seeding nucleated cells from BM aspirate

Patient No.	Number of nucleated cells in 5 ml of BM aspirate (million)	Number of colonies (CFU) of nucleated cells (NC) in three samples	Average number of nucleated cells colonies	SEC (%)
1	145.0	246	199.00	0.0035
		163		
		188		
2	230.0	203	195.00	0.0034
		157		
		225		
3	79.5	345	336.70	0.0059
		298		
		367		
4	247.0	203	205.33	0.0036
		211		
		202		
5	83.0	139	127.67	0.0022
		126		
		118		
6	242.0	263	209.67	0.0037
		235		
		131		
7	150.0	104	94.66	0.0017
		117		
		63		
8	281.0	289	264.00	0.0046
		271		
		232		
9	77.0	55	78.33	0.0013
		77		
		103		
10	191.0	96	106.33	0.0019
		122		
		101		
11	316.0	88	85.00	0.0015
		87		
		80		
12	86.0	38	24.33	0.004
		24		
		11		
13	106.0	99	121.67	0.0020
		117		
		149		
14	167.0	129	165.67	0.0030
		158		
		210		

of osteoarthritis and aseptic necrosis [11, 12]. Literary sources describe treatment with autologous concentrated BM aspirate mostly as "stem cell therapy" [13, 14]. However, it should be noted that it contains different types of cells, most of which belong to the hematopoietic lineage, not mesenchymal. Our study also confirms this fact. We, like other researchers, found only a small percentage of mesenchymal stem cells in bone marrow aspirate [15, 16]. This fact indicates the feasibility of a more detailed approach to the terminology of biotechnology products. We can only talk about treatment with mesenchymal stem cells if they have been isolated from bone marrow aspirate and cultured *in vitro*.

In the case of using BM aspirate and its derivatives (concentrated BM aspirate, mononuclear fraction of BM aspirate), it is more appropriate to use the term "regenerative therapy", since the best explanation of the positive effect of biotechnological products from bone marrow aspirate is the paracrine effect of the obtained cell concentrate due to the growth factors it contains [17].

Conclusions

As a result of the development of quality and safety criteria for biotechnological products derived from bone marrow aspirate, three classifications have been established: 1) Polymorphic, which includes the presence of progenitor cells of all types within the normal range; 2) Moderately cellular, where all types of progenitor cells are present, albeit with some below the normal range; and 3) Hypocellular, characterized by the presence of most types of progenitor cells, with several below the normal range. Bone marrow aspirate type 1 was evaluated as excellent for subsequent biotechnological product manufacturing. Type 2 was deemed good, while type 3 was considered satisfactory.

To determine the functional criteria of quality of biotechnological products from BM aspirate, we used CFU analysis and MMSC-BM seeding efficiency coefficient. If the SEC indicator is less than 0.001 %, it is considered unsatisfactory; between 0.001 % and 0.003 %, it is regarded as satisfactory; and greater than 0.003 %, it is deemed good. BM aspirate with SEC < 0.001 % is unsuitable for use.

It has been established that mesenchymal stem cells constitute a minor population in bone marrow aspirate, and the clinical impact of biotechnological products made from bone marrow aspirate is likely to occur at the expense of hematopoietic progenitor cells.

Conflict of interest. The authors declare that there is no conflict of interest.

Prospects for further research. Research on cultured autologous and allogeneic biotechnological products of bone marrow, development of their quality and safety criteria, development of a differentiated and personalized approach to the use of minimally manipulated and cultured biotechnological products in patients with orthopedic and traumatological profile

Information on funding. The research, the results of which are published in the article, was funded within the framework of the research project "Development and experimental investigation of regenerative technologies for osteoarthritis and aseptic necrosis of the hip and knee joints" (state registration number 0122U000200).

Authors' contribution. Shtrafun S. S. — definition of research directions, formulation of conclusions; Golyuk E. L. — analysis of research results, writing of the article; Zubov D. O. — study of functional criteria of quality of bone marrow aspirate and biotechnological products made from it, CFU analysis of multipotent mesenchymal stem/stromal cells; Magomedov S. — processing and preparation of bone marrow aspirate smears for counting the cellular composition; Starodub G. S. — analysis of patients' myelograms; Malova H. K. — counting cells in patients' myelograms.

References

1. Eder, C., Schmidt-Bleek, K., Geissler, S., Sass, F. A., Maleitzke, T., Pumberger, M., Perka, C., Duda, G. N., & Winkler, T. (2020). Mesenchymal stromal cell and bone marrow concentrate therapies for musculoskeletal indications: A concise review of current literature. *Molecular Biology Reports, 47*(6), 4789–4814. https://doi.org/10.1007/s11033-020-05428-0
2. Deng, Z., Luo, F., Lin, Y., Luo, J., Ke, D., Song, C., & Xu, J. (2022). Research trends of mesenchymal stem cells application in orthopedics: A bibliometric analysis of the past 2 decades. *Frontiers in Public Health, 10*. https://doi.org/10.3389/fpubh.2022.1021818
3. Wagner, W., Wein, F., Seckinger, A., Frankhauser, M., Wirkner, U., Krause, U., Blake, J., Schwager, C., Eckstein, V., Ansorge, W., & Ho, A. D. (2005). Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Experimental Hematology, 33*(11), 1402–1416. https://doi.org/10.1016/j.exphem.2005.07.003.
4. Xia, P., Wang, X., Lin, Q., & Li, X. (2015). Efficacy of mesenchymal stem cells injection for the management of knee osteoarthritis: A systematic review and meta-analysis. International *Orthopaedics, 39*(12), 2363–2372. https://doi.org/10.1007/s00264-015-2785-8
5. Puissant, B., Barreau, C., Bourin, P., Clavel, C., Corre, J., Bousquet, C., Taureau, C., Cousin, B., Abbal, M., Laharrague, P., Penicaud, L., Casteilla, L., & Blancher, A. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: Comparison with bone marrow mesenchymal stem cells. *British Journal of Haematology, 129*(1), 118–129. https://doi.org/10.1111/j.1365-2141.2005.05409.x
6. Pak, J. (2011). Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: A case series. *Journal of Medical Case Reports, 5*(1). https://doi.org/10.1186/1752-1947-5-296
7. Linetsky, F. S., Alfredson, H., Crane, D., & Centeno, C. J. (2014). Treatment of chronic painful musculoskeletal injuries and diseases with regenerative injection therapy (RIT): Regenerative injection therapy principles and practice. *Treatment of Chronic Pain by Integrative Approache*s, 145–168. https://doi.org/10.1007/978-1-4939-1821-8_12
8. Centeno, C. J., Kisiday, J., Freeman, M., & John, R. S. (2006).

Partial regeneration of the human hip via autologous bone marrow nucleated cell transfer: A case study. *Pain Physician, 9*, 253–256.
9. Bowen, J. E. (2015). Technical issues in harvesting and concentrating stem cells (Bone marrow and adipose). *PM&R, 7*(4S). https://doi.org/10.1016/j.pmrj.2015.01.025
10. Vareniuk, I. M., & Dzerzhynskyi, M. Ye. (2019). *The methods of cytohistologic diagnostic*. Kyiv, Interservice.
11. Kim, G. B., Seo, M., Park, W. T., & Lee, G. W. (2020). Bone marrow aspirate concentrate: Its uses in osteoarthritis. *International journal of molecular sciences, 21*(9), 3224. https://doi.org/10.3390/ijms21093224
12. Jindal, K., Aggarwal, S., Kumar, P., & Rathod, P. (2021). Core decompression with bone marrow aspirate concentrate in post collapse avascular necrosis of hip: A systematic review and meta-analysis. *Journal of clinical orthopaedics and trauma, 17*, 78–87. https://doi.org/10.1016/j.jcot.2021.02.010
13. Pithadia, P. (2021). BMAC and adipose-derived MSCs treatment for knee osteoarthritis: A systematic review. *International journal of clinical case reports and reviews, 7*(04), 01-11. https://doi.org/10.31579/2690-4861/150
14. Brozovich, A., Sinicrope, B. J., Bauza, G., Niclot, F. B., Lintner, D., Taraballi, F., & McCulloch, P. C. (2021). High variability of Mesenchymal stem cells obtained via bone marrow aspirate concentrate compared with traditional bone marrow aspiration technique. *Orthopaedic Journal of Sports Medicine, 9*(12). https://doi.org/10.1177/23259671211058459
15. Goodman, S. B., & Zwingenberger, S. (2021). Concentrated autologous bone marrow aspirate is not "stem cell" therapy in the repair of nonunions and bone defects. *Biomaterials and Biosystems, 2*, 100017. https://doi.org/10.1016/j.bbiosy.2021.100017
16. Schäfer, R., DeBaun, M. R., Fleck, E., Centeno, C. J., Kraft, D., Leibacher, J., Bieback, K., Seifried, E., & Dragoo, J. L. (2019). Quantitation of progenitor cell populations and growth factors after bone marrow aspirate concentration. *Journal of translational medicine, 17*(1). https://doi.org/10.1186/s12967-019-1866-7
17. Sugaya, H., Yoshioka, T., Kato, T., Taniguchi, Y., Kumagai, H., Hyodo, K., Ohneda, O., Yamazaki, M., & Mishima, H. (2018). Comparative analysis of cellular and growth factor composition in bone marrow aspirate concentrate and platelet-rich plasma. *Bone marrow research,* 2018, 1–9. https://doi.org/10.1155/2018/1549826

The article has been sent to the editors	Received after review	Accepted for printing
01.08.2024	10.01.2025	16.01.2025

QUALITY CRITERIA FOR MINIMALLY MANIPULATED BIOTECHNOLOGICAL PRODUCTS BASED ON AUTOLOGOUS BONE MARROW ASPIRATE FOR USE IN TRAUMATOLOGY AND ORTHOPEDICS

S. S. Strafun [1], Ye. L. Holiuk [1], D. O. Zubov [1], O. M. Magomedov [1], H. S. Starodub [2], H. K. Malova [3]

[1] SI «National Institute of Traumatology and Orthopedics of the NAMS of Ukraine», Kyiv
[2] SU «National Scientific Center of Radiation Medicine, Hematology and Oncology of the NAMS of Ukraine», Kyiv
[3] Kyiv City Clinical Hospital No. 9. Ukraine

✉ Sergiy Strafun, MD, Prof.: strafun-s@ukr.net; https://orcid.org/0000-0003-2485-5487
✉ Yevhen Holiuk, MD, PhD in Orthopaedics and Traumatology: holyuk@yahoo.com; https://orcid.org/0000-0001-8940-8536
✉ Dmytro Zubov, PhD in Biol.Sci: Zoubov77@yahoo.com; https://orcid.org/0000-0002-3134-2594
✉ Sadrudin Magomedov, Dr in Biol. Sci, Prof.: alexandr@magomedov.kiev.ua; https://orcid.org/0000-1234-5678-9101
✉ Halyna Starodub, MD, PhD: gal.starodub@gmail.com; https://orcid.org/0000-0002-8402-2156
✉ Hrystyna Malova, MD: kristyna.malova@gmail.com; https://orcid.org/0009-0000-6534-948X