Using orthobiologics products in knee osteoarthritis

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Abstract: Objective: comparison of the treatment effectiveness in patients with grades II or III knee osteoarthritis using autologous bone marrow aspirate concentrate (BMAC) and autologous platelet-rich plasma (PRP) infused intraosseously into the area of overload bone marrow edema.

Materials and Methods. The prospective study was conducted on the basis of two medical clinics from 2016 to 2019. It involved 40 patients with grades II–III knee osteoarthritis. The patients of the treatment group underwent a single intraosseous infusion of BMAC, whereas patients in the comparison group were subjected to a single intraosseous PRP infusion. The results were assessed after 1, 3, 6, and 12 months using visual analog scale (VAS), Lequesne index for knee osteoarthritis, WOMAC osteoarthritis index, and verbal rating scale (VRS).

Results. After 12 months, there was a reduction in the VAS index to 3.9±0.3 points in the treatment group and 4.2±0.1 points in the control group. Similar decrease was observed for Lequesne index for knee osteoarthritis (to 5.8±0.7 points in the treatment group and to 6.1±0.8 points in the control group) and WOMAC osteoarthritis index (to 40.6±0.3 points in the treatment group and to 42.5±0.6 points in the control group). The VRS scores after 3 and 6 months were better in the treatment group (subjected to autologous BMAC), while after 12 months, the differences between the groups were not significant.

Conclusion. Use of orthobiologics products for osteoarthritis treatment was effective, with higher efficacy of intraosseous BMAC infusion vs. PRP infusion in terms of pain, knee functionality, physical activity, and patient satisfaction over the entire monitoring period. Both treatment methods were safe.

Keywords: bone marrow aspirate concentrate (BMAC), autologous platelet-rich plasma (PRP), knee osteoarthritis.
Consequently, the development of a technique for osteoarthritis treatment involving the introduction of such orthobiologics products into the focus of bone marrow edema seems quite promising.

Objective – comparison of the treatment effectiveness in patients with grades II or III knee osteoarthrosis with autologous BMAC and PRP administered intrasosseously into the area of overload bone marrow edema.

Materials and Methods
Our randomized prospective study conducted at the clinics of Rostov State Medical University and Volgograd State Medical University from 2016 to 2019 included 40 patients (27 women, 13 men, mean age = 67±7.8 years, body mass index = 32.7±4.8 kg/m2, disease duration = 17.3±3.7 months) with knee osteoarthrosis. Unilateral (n=24) and bilateral (n=16) knee joint lesions, localized mainly in inner parts, were established on the basis of complaints, anamnesis, and radiological examination results (radiography, magnetic resonance imaging).

During our clinical trials, the requirements of the Declaration of Helsinki (2013 revision) were met. Permissions were obtained from the Ethics Committee of Volgograd State Medical University (in 2016) to conduct a clinical trial on the topic, “The Use of Bone Marrow Aspirate Concentrate in Patients with Acute Injuries and Chronic Injuries of the Musculoskeletal System,” as well as from the Ethics Committee of Rostov State Medical University (on January 31, 2018), to carry out the research project on the topic, “The Use of Platelet-Rich Plasma, Autologous Bone Marrow Cells, and Stromal Vascular Fraction in Treating the Diseases and Injuries of the Musculoskeletal System.”

The inclusion criteria for the study were grades II–III primary osteoarthrosis of knee sensu Kellgren–Lawrence classification, the presence of overload bone marrow edema in the area of the medial condyle of the femur and/or tibia, pain syndrome of at least 6 points on the visual analog scale (VAS), and insufficient effectiveness of previously performed conservative treatment.

The exclusion criteria were age under 45 years, a history of hepatitis B or C, HIV infection, blood diseases, chronic diseases of internal organs in the stage of decompensation, oncological diseases, and the absence of a signed informed consent to participate in the study.

Exclusion criteria also encompassed the presence of an inflammatory process in the knee joint area, oral administration of corticosteroids or immunosuppressive pharmaceutical drugs less than 6 weeks before the study, arthroscopy less than 6 months before the examination, and use of PRP or hyaluronic acid preparation less than 90 days before the initial screening.

The inclusion criteria were met by patients of all clinical groups that were comparable in terms of representation, main clinical and morphological parameters, including the duration and manifestations of the disease, and differed solely in the method of osteoarthrosis treatment (Table).

Patients of the treatment group (n=19) underwent a single intrasosseous infusion of BMAC into the area of bone marrow edema. In the comparison group (n=21) an infusion of PRP was performed. The area of overload bone marrow edema and its localization were preliminarily determined via magnetic resonance imaging data, transposing them to fluoroscopic images obtained in the course of manipulations.

The preparation and administration of PRP was performed in a surgical dressing room. PRP was obtained using a special Ycellbio PRP container (Korea). From the patient’s cubital vein, 13 mL of blood were taken and mixed in a container with 2 mL of dextrose citrate solution. The container was placed in a RotoFix 32 centrifuge (Hettich, Germany) with an appropriate counterweight.

The first centrifugation at 3,200 rpm lasted 4 minutes. Then, using a swivel cap on the container, the level of the hematocrit layer was set below its neck.

A second centrifugation at 3,400 rpm for 4 minutes yielded 2 mL of PRP, which was then withdrawn with a syringe.

The concentration of platelets in the resulting PRP was calculated using a hematological analyzer (Erba Elite3, Czech Republic) and coded according to the international PRP classification adopted in 2020 [10].

Sampling and obtaining of BMAC was carried out in the conditions of the operating room or surgical dressing room, subject to the measures of asepsis and antisepsis. The procedure was performed in the position of the patient lying on the back along the medial surface of the proximal tibial metaphysis, using 5.0 mL of a 2% solution of lidocaine, with a local anesthesia of the skin and underlying soft tissues performed until the needle contacted the bone surface. Then, an aspiration trocar (11 G) was inserted with rotational movements through the cortical plate of the tibia until it felt like a dip (by 30–35 mm). After removing the stylet with a 50 mL syringe, bone marrow (50 mL) was sampled. Simultaneously, in order to increase the concentration of cells in the aspirate, the trocar was rotated around its axis during sampling and its depth was changed. The bone marrow thus obtained was mixed with 5 mL of heparin solution and placed in an Ycellbio PRP container (Korea).

Separation of the fraction with a high content of mesenchymal cells from other constituent elements of the bone marrow was carried out by centrifugation at a speed of 2,400 rpm for 20 min. After removing the container from the centrifuge, 2 mL layer of bone marrow concentrate was taken into the syringe with a sterile needle in the isthmus of the container, as described in the method for obtaining PRP.

The number of monocellular cells in BMAC was determined by flow cytometry using antibodies to CD34, CD14, CD73, CD105 and CD90.

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The number of monocellular cells in BMAC was determined by flow cytometry using antibodies to CD34, CD14, CD73, CD105 and CD90.
The introduction of BMAC or PRP was carried out in the operating room under fluoroscopic control in the area of overload bone marrow edema in the condyle of the tibia or femur using an injection needle. The latter penetrated into the cancellous bone to the required depth after local anesthesia of the skin and soft tissues with 5.0 mL of 2% lidocaine solution using the technique of twisting movements. Before the introduction of BMAC or PRP, 1.0 mL of the specified anesthetic solution was slowly infused into the bone.

After manipulations with PRP or BMAC, all patients were recommended cold applications for 2–3 days, walking with a cane for 5–7 days, and taking analgesics for severe pain for 1–2 days.

The results were evaluated after 1 day, 1 week, and 1, 3, 6 and 12 months from the moment of infusion, using the scores of verbal rating scale (VRS), visual analog scale (VAS), Lequesne index for knee osteoarthritis, WOMAC osteoarthritis index. Magnetic resonance imaging of the joints was performed 3, 6 and 12 months after the infusion.

Patient satisfaction with treatment outcomes was assessed using an updated VRS, according to which the result ranged from 0 to 3 points: 0 points = dissatisfied (no improvement), 1 point = fairly satisfied (movements in the joint improved, pain decreased, but functional limitations reducing the quality of life and physical activity persist), 2 points = very satisfied (no restrictions in everyday life, intense exercise, but sports cause pain or discomfort), 3 points = fully satisfied (full recovery, physical activity and sports are possible without significant restrictions).

Objective data on the dynamics of the pathological process in the knee joints of patients under the influence of the treatment were assessed on the basis of magnetic resonance imaging data on the WOMAC scale after 3, 6 and 12 months after infusion.

Statistical data processing was carried out in Excel 2016, Office XP (Microsoft Corp., USA) using the capabilities of the STATISTICA 10.0 software (Statsoft, USA), according to the rules of biomedical research, for which p≤0.05 was a sufficient level of statistical significance of differences. Normality of distributions was confirmed by the Shapiro–Wilk test. The significance of differences was measured using the Student’s t-test, assessing the degree of discrepancy between the arithmetic means, M1 and M2, relative to the variance, σ2. The obtained value was compared with the table value of t at a significance level of p=0.05. Provided that the obtained value of t was greater than the critical value in the table, the difference between the compared values was recognized as statistically significant.

The analysis of nonparametric quantitative traits was carried out using the Mann-Whitney test, which allowed determining whether the overlap area of two distribution series was sufficiently small (a range of parameter values in the first sample and a range of parameter values in the second sample). The obtained value of U criterion was compared with the critical table value of U for a given number of compared samples for selected level of statistical significance (p=0.05). In the case when the value of U was less than the table value, the differences between the parameters in the considered samples were recognized as statistically significant.

Results

The concentration of platelets in the PRP preparation averaged 662±40×10^9/L, which corresponded to the parameters of the indicated orthobiologics product. The content of leukocytes reached (2.7±1.4) ×10^9/L, which allowed classifying the PRP used in the study as plasma with a low content of these cells: this PRP was coded as N3N0-N0N2-N0N0.

Determination of the cellular composition in the bone marrow concentrate after centrifugation revealed an increase in the level of mesenchymal cells up to 0.048% (0.006), and the platelet concentration was (624±30) ×10^9/L.

All patients achieved the end point of the study with the evaluation of treatment results after 1, 3, 6 and 12 months after intraosseous infusion.

VRS-based evaluation in the treatment group given by patients after 1 month was as follows: fairly satisfied – n=4 (20%); very satisfied – n=2 (60%); fully satisfied – n=2 (20%); 3 months after BMAC infusion, 1 patient (5%) did not notice any improvement in the condition of the knee joint, 6 subjects (30%) considered the achieved result fairly satisfactory, 12 participants (60%) were very satisfied, and 1 patient (5%) was fully satisfied. After 6 and 12 months, the numbers of positive feedbacks from patients decreased by 10 and 20%, respectively, redistributing into the segment of fairly satisfied and dissatisfied rating categories (Figure 1).

In the comparison group, the VRS assessment carried out after 1 month was as follows: dissatisfied – n=1 (5%), fairly satisfied – n=5 (25%), very satisfied – n=13 (65%), fully satisfied – n=1 (5%); after 3 months after the PRP infusion, there was absence of any positive changes in 1 patient (5%), 7 respondents (35%) were fairly satisfied with the treatment, while 1 (5%) and 11 (55%) respondents were fully satisfied or very satisfied, respectively, with the achieved treatment results. After 6 and 12 months after the PRP infusion, there was a negative trend in the evaluation of treatment outcomes, similar to that which took place in the treatment group of patients. As a result, the number of positive feedbacks decreased by 20% (Figure 1). Comparison of these two segments in both groups of patients implied that at earlier control time points (3 and 6 months), patient preferences were in favor of treatment with BMAC (65% and 55% of positive responses) vs. PRP (35% and 45% of positive responses), whereas after 12 months, the differences were not statistically significant.

The results of a patient survey in the treatment group in terms of VAS scores after 1 month after the BMAC infusion exhibited an almost twofold reduction in the intensity of pain syndrome from its initial values, 5.9±0.7 to 2.3±0.6 points (U=43; p=0.009); after 3 months, its value was 2.5±0.4 points (U=43; p=0.009). The indicated level gradually decreased to 2.2±0.4 points after 6 months (U=50; p=0.008), and after 12 months, the pain syndrome intensity slightly increased reaching 3.9±0.3 points (U=153; p=0.051) (Figure 2).

In patients from the comparison group, after the introduction of PRP, a 1.5-fold decrease in the mean VAS score was also achieved after 1 month of observation (from 6.4±0.3 to 3.8±0.8 points; U=43; p=0.009). After 3 months, the pain syndrome score increased to 3.9±0.5 (U=43; p=0.009). After 6 months after the onset of treatment, the mean VAS pain index changed only slightly (4.0±0.2 points; U=43; p=0.009), while after 12 months, the intensity of the pain syndrome increased, and the mean VAS score reached 4.2±0.1 points (U=153; p=0.051) (Figure 2).
Comparison of the mean VAS scores in patients of both groups at all control time points demonstrated that despite the pronounced analgesic effect of both treatment methods, the severity of the pain syndrome declined to a greater extent after intraosseous infusion of BMAC vs. PRP. However, after 12 months, the differences were not statistically significant.

Evaluation of the treatment outcomes on the Lequesne scale in patients of the treatment group implied an improvement in the functional state of the knee joint after the infusion of BMAC. The Lequesne index underwent more than a twofold reduction, from 10.3±0.4 to 4.1±0.2 points (U=63; p=0.008), after 1 month of monitoring. In the subsequent periods of observation, the values of the Lequesne index were characterized by a slight negative trend: 5.0±0.6 points after 3 months (U=101; p=0.008), 5.4±0.3 points after 6 months (U=115; p=0.049), 5.8±0.7 points after 12 months (U=190; p=0.051).

In the comparison group, the initial pain severity score was 10.7±0.2. After 1 month after the infusion of PRP, the Lequesne index declined to 5.2±0.2 points (U=63; p=0.008). After 3 months, its value was 5.6±0.3 points (U=63; p=0.008). By 6 months of follow-up, it reached 5.9±0.5 points (U=115; p=0.046). After 12 months, the mean value of the Lequesne index, as after the infusion of BMAC, tended to increase (6.1±0.8 points; U=190; p=0.053) (Figure 2).

Comparison of the dynamics of the Lequesne index means in relation to the baseline values showed a statistically significant decrease in the severity of osteoarthritis after the application of both treatment methods. Comparison of patient groups favored the BMAC method throughout the entire observation period, with differences most pronounced in the first 3 months and exhibiting statistical significance up to 6 months.

The index of the knee joint functional state according to the WOMAC scale declined twofold 1 month after the introduction of BMAC in patients of the treatment group (from 59.3±0.8 to 21.5±0.4 points; U=0; p=0.007). By 3 months of observation, the index value slightly increased to 25.8±0.3 points (U=0; p=0.007), and after 6 months, it was 33.4±0.7 points (U=0; p=0.008). Further on, up to 12 months, the negative dynamics progressed, and the final score reached 40.6±0.3 points (U=37; p=0.009).

In the comparison group, WOMAC scores changed in a similar way. After 1 month after the infusion of PRP, the WOMAC index decreased from the initial value of 61.2±0.3 points to 32.4±0.6 points (U=0; p=0.007), and subsequently gradually increased to 36.7±0.5 points after 3 months (U=0; p=0.007), to 41.3±0.4 points after 6 months (U=0; p=0.007), and to 42.5±0.6 points after 12 months of observation (U=37; p=0.007), indicating a deterioration in the functional state of the knee joint (Figure 3).

Comparison of the index means in both groups of patients with the baseline values of this indicator demonstrated statistically significant differences persisting at all control time points of observation. Despite the negative dynamics of the WOMAC index observed in both groups of patients, especially in the later periods of observation, the increase in means occurred to a lesser extent after the introduction of BMAC vs. PRP.

The objective symptom of the reduction in the pain syndrome severity after the infusion of BMAC or PRP was full disappearance, significant reduction in size, or decrease in the signal intensity in the area of bone marrow edema previously identified via MRI (Figure 5).
After intraosseous infusion of BMAC or PRP, 5 patients (26.3%) of the treatment group and 4 patients (19.04%) of the comparison group required the prescription of painkillers for several days.

Discussion

Osteoarthritis is the second most common cause of disability after cardiovascular disease. According to statistics, 10–12% of the Russian Federation population suffers from osteoarthritis. Its prevalence has increased by 48% in recent years, while its annual primary incidence has increased by over 20%, which is associated with the global aging of the population [1].

The study of this disease pathogenesis and adherence to contemporary principles of evidence-based medicine have created the basis of the formation of clinical guidelines for the treatment of osteoarthritis. An expert opinion on orthobiologics-based therapeutic techniques was presented at the EULAR (The European League Against Rheumatism) congress in 2020. Intra-articular PRP injections constitute an effective treatment for knee osteoarthritis, which should be offered as second-line therapy [5–7].

According to the published sources, PRP promotes the restoration of damaged cartilage tissue through migration, proliferation, and differentiation of progenitor cells. The concentration of platelets 4.6 times higher than the norm allows fully revealing the cellular potential of the orthobiologics product [6, 7].

The anti-inflammatory effect of PRP is due to the active growth factors of platelet α-granules contained in the cell preparation at an increased concentration. However, an excessive increase of platelet density per unit volume of an orthobiologics product, as a rule, leads to the opposite phenomenon. Often, patients report severe pain for 7 days or more, which may be accompanied by reactive synovitis [6].

When creating PRP as an orthobiologics preparation, the researcher is faced with the task of maintaining the balance of biologically active factors and cell concentration per unit volume [6, 10].

The rationale for the clinical use of BMAC is based on two concepts. Mesenchymal cells can contribute to the regenerative activity of the preparation, although it is known that the concentration of mononuclear cells is low, ranging from 0.001 to 0.01%. An inversely proportional correlation of poorly differentiated cells with age was also noted. Despite this finding, it is possible to increase their content by centrifugation up to 0.9% [3, 4]. In BMAC, along with progenitor cells, highly differentiated blood cells are also detected in an increased concentration, which in itself allows classifying such a preparation as PRP. After the formation of the cell product, the level of platelets exceeds the norm by several times, thereby providing an anti-inflammatory effect of BMAC [3, 4].

Analyzing the historical aspect of the cell preparation use, we could classify PRP as a precursor of a more advanced BMAC in the treatment of degenerative dystrophic diseases of the musculoskeletal system [2, 3, 7].

In our study, the platelet content in the PRP preparation was 958±50×10⁹/L, whereas the number of leukocytes did not exceed 2.2±1.7×10⁹/L, which allowed attributing the resulting plasma to the category with a low leukocyte content.

We must bear in mind that in order to obtain a therapeutic effect, it is necessary to increase their concentration by centrifugation above 0.01%, which was done by us in the course of the above procedure.

Most patients, describing the pain syndrome in knee osteoarthritis, point to the region of the medial condyles of the femur and tibia. According to studies, the disorder of vascularization and trophism in the subchondral bone plays an important role in the pathogenesis of osteoarthritis, triggering a cascade of destructive changes in the cartilage tissue and an inflammatory process in the knee joint cavity. Consequently, intraosseous infusion of cell preparations carries a pathogenetic validity. Infusions are performed in an operating room under image intensifier tube control, thereby reducing the risk of para-articular injection of the drug or its penetration under the synovial membrane, which could lead to the development of a pronounced pain syndrome [1, 8].

The dosage and frequency of BMAC and PRP administration in osteoarthritis is based on the identified duration of activation of biological processes initiated by growth factors in cell preparations, as well as on the therapeutic manifestations of the efficacy, amounting to 1 injection of 2 mL [3, 5, 7]. Protocols for the administration of orthobiologics preparations in both clinical groups were standardized, which simplified the comparative study.

According to the published data of systematic reviews and randomized trials, intraosseous infusion of PRP and BMAC preparations helps reducing pain, improving the knee joint function, and, consequently, increasing the quality of life in patients within 6 to 12 months [3]. It is worth noting that the bone marrow preparation has a more complex cellular composition than PRP resulting in a pronounced therapeutic effect for 12 months after infusion. These advantages allow using BMAC more widely in clinical practice [3, 4].

Thus, when assessing the results of satisfaction with treatment, the level of pain syndrome, and the functional state of the knee joint in two compared groups over the period of 12 months, the maximum scores were noted between 3 and 6 months after intraosseous infusion, with a moderate predominance of indicators in case of using BMAC.

References

1. EULAR (2020). Intra-articular PRP injections constitute an effective treatment for knee osteoarthritis, which should be offered as second-line therapy [5–7].

2. BMAC in the treatment of degenerative dystrophic diseases of the musculoskeletal system [2, 3, 7].

3. Intraosseous infusion of BMAC or PRP, 5 patients (26.3%) of the treatment group and 4 patients (19.04%) of the comparison group required the prescription of painkillers for several days.

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Conclusion

Preliminary assessment of the results of infusing cell preparations inside the subchondral bone in the region of the condyles of the tibia and/or femur in grades II and III knee osteoarthritis (sensu 1987 Kellgren–Lawrence classification) therapy yielded pain reduction, improvement in functional parameters and an increase in overall patient satisfaction in treatment outcomes within six months after the onset of therapy. Further monitoring revealed a slight deterioration in all indicators after 12 months from the start of the study.

Intraosseous infusion of BMAC to patients with grades II and III knee osteoarthritis can reduce the intensity of the pain syndrome to a greater extent and contribute to better recovery of the knee joint function after 12 months of observations, compared with the use of PRP.

Further research in the field of orthobiologics would bring the conservative treatment of degenerative dystrophic diseases of the musculoskeletal system to a new level.

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References


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