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Comparative Assessment of Surgical Treatment Results of Patients with Early-Stage Avascular Necrosis of the Femoral Head

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Abstract

Background. The observed sharp increase in patients with avascular necrosis of the femoral head (ANFH) associated with a new COVID-19 infection determines the need to find some new effective strategies for surgical treatment to achieve long-term positive results.

Aim of the study is to make a comparative assessment of surgical treatment results of patients with early-stage avascular necrosis of the femoral head using different techniques of core decompression and autogenous bone grafting of the femoral head.

Methods. We performed a comparative analysis of the treatment results of patients with early stages of ANFH. The patients were divided by the treatment method into two groups: control and main. Surgical treatment in the control group (n = 19) consisted of an open decompression and autogenous bone grafting of the femoral head using the Rosenwasser's "light bulb" technique. The main group (n = 17) included the patients who had undergone the developed combined impaction autografting of the femoral head. Clinical and functional assessment of the treatment results was performed using the Harris Hip Score (HHS) questionnaire and the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) score. Assessment was performed preoperatively and at 3, 6, and 12 months postoperatively.

Results. The performed comparative analysis showed statistically significant difference in clinical and functional results after operative treatment in patients of the control and the main groups at all follow-ups. Change of the HHS values presented as Me (Q1;Q3) in patients of both groups at 3, 6 and 12 months was 77.0 (68.0;84.0) and 82.0 (75.0;91.0), p = 0.001; 79.0 (69.0;85.0) and 88.0 (79.0;95.0), p<0.001; 81.0 (71.0;86.0) and 90.0 (85.0;92.0), p<0.001, respectively. According to the WOMAC, the following dynamics was revealed for the same values: 30.0 (25.0;35.0) and 25.0 (21.0;32.0), p = 0.002; 27.0 (22.0;33.0) and 20.0 (17.0;27.0), p<0.001; 24.0 (17.0;30.0) and 15.0 (13.0;24.0), p<0.001.

Conclusion. Comparative assessment of efficacy of the open core decompression with autogenous bone grafting of the femoral head defect using the light bulb technique and closed intralesional resection of necrosis focus with combined impaction grafting of the femoral head showed that the minimal damage to para- and intraarticular tissues when performing the approach to the area of the pathological focus and the main stages of the operation allows to achieve the best clinical and functional results and create optimal conditions for bone remodeling in the grafting area.

Keywords: avascular necrosis of the femoral head, ANFH, core decompression, bone grafting.

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Сравнительная оценка результатов оперативного лечения пациентов с асептическим некрозом головки бедренной кости ранних стадий

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Реферат

Актуальность. Наблюдающийся резкий рост пациентов с АНГБК, ассоциированным с перенесенной новой коронавирусной инфекций COVID-19, определяет необходимость поиска новых эффективных стратегий оперативного лечения, позволяющих получить долгосрочные положительные результаты.

Цель — провести сравнительную оценку результатов оперативного лечения пациентов с асептическим некрозом головки бедренной кости ранних стадий с применением разных способов core-декомпрессии и костной аутопластики головки бедренной кости.

Материал и методы. Проведен сравнительный анализ результатов лечения пациентов с ранними стадиями асептического некроза головки бедренной кости. Пациенты были распределены по способу лечения на две группы: контрольную и основную. В контрольной группе (n = 19) оперативное лечение проводили способом открытой декомпрессии и костной аутопластики головки бедренной кости, используя технику M.P. Rosenwasser “light bulb”. В основную группу (n = 17) вошли пациенты, хирургическое лечение которым выполняли с применением разработанного способа комбинированной импакционной аутопластики головки бедренной кости. Клинико-функциональную оценку результатов лечения выполняли с помощью опросника Harris Hip Score (HHS) и шкалы Western Ontario and McMaster University Osteoarthritis Index (WOMAC). Оценку проводили до операции и на сроках 3, 6 и 12 мес. после оперативного вмешательства.

Результаты. Проведенный сравнительный анализ показал статистически значимую разницу клинико-функциональных результатов после оперативного лечения у пациентов контрольной и основной групп на всех сроках наблюдения. Изменение показателей Harris Hip Score в виде Me (Q1;Q3) у пациентов контрольной и основной групп на сроках наблюдения 3, 6 и 12 мес. составило 77,0 (68,0;84,0) и 82,0 (75,0;91,0), p = 0,001; 79,0 (69,0;85,0) и 88,0 (79,0;95,0), p<0,001; 81,0 (71,0;86,0) и 90,0 (85,0;92,0), p<0,001 соответственно. По шкале WOMAC была выявлена следующая динамика показателей на тех же сроках наблюдения: 30,0 (25,0;35,0) и 25,0 (21,0;32,0), p = 0,002; 27,0 (22,0;33,0) и 20,0 (17,0;27,0), p<0,001; 24,0 (17,0;30,0) и 15,0 (13,0;24,0), p<0,001.

Заключение. Сравнительная оценка эффективности открытой core-декомпрессии с костной аутопластикой дефекта головки бедренной кости по способу “light bulb” и закрытой внутриочаговой резекции очага некроза с комбинированной импакционной пластикой головки бедренной кости показала, что минимальное травмирование пара- и интраартикулярных тканей при выполнении доступа к зоне патологического очага и проведения основных этапов операции позволяет достичь лучших клинико-функциональных результатов и создать оптимальные условия для процессов ремоделирования в зоне остеопластики.

Ключевые слова: асептический некроз головки бедренной кости, АНГБК, core-декомпрессия, костная пластика.

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BACKGROUND

Avascular necrosis of the femoral head (ANFH) is an orthopedic pathology with an unclear etiology, the pathogenesis of which lies in the disorder of microcirculation in the femoral head and neck with subsequent death of osteocytes and bone destruction. ANFH affects mainly males, predominantly young and middle-aged, belonging to the most able-bodied group of the population. Within a short period of time from its onset, the disease leads to a collapse of the femoral head, impaired biomechanics of the hip joint, and ultimately to the terminal osteoarthritis [1, 2, 3, 4].

It is worth noting that currently there is a sharp increase in the number of patients with ANFH, associated with the spread of the new COVID-19/long-COVID-19 infection and related to both the pathogenetic mechanisms of its course and the peculiarities of the drug therapy, including the systemic intake of high doses of glucocorticoids [5, 6].

Despite the developed and applied protocol of complex conservative treatment of this pathology, its results are not always satisfactory for patients and physicians. On the one hand, this is associated with the aggressive course of the disease, and on the other hand, with the high demands of patients to the functional state of the joint. That is why organ-preserving (joint-preserving) surgery, which allows not only to preserve the joint, but also to correct the main elements of the pathological processes of avascular osteonecrosis, becomes extremely important in the surgical treatment of patients of this category nowadays.

Among organ-preserving surgeries, combined operations including core decompression of the femoral head and subsequent replacement of the post-resection defect using one or another bone autografting technique are pathogenetically the most reasonable. Combination of these stages and use of autologous bone tissue as a plastic material not only ensures elimination of pathological focus and reduction of intraosseous pressure in the femoral head area, but also creates conditions for bone tissue remodeling and revascularization in the affected area, restoration of the supporting function of the femoral head and prevention of its further collapse [7, 8, 9, 10, 11, 12]. A comparative evaluation of the results of open core decompression with bone autografting of the femoral head defect and closed intralesional resection of the femoral head with bone autografting is an essential problem, the solution of which will allow us to determine the optimal surgical treatment option.

Aim of the study — to make a comparative assessment of surgical treatment results of patients with early-stage avascular necrosis of the femoral head using different techniques of core decompression and autogenous bone grafting of the femoral head.

METHODS

To achieve the objective set in the paper, we performed a comparative analysis of the surgical treatment results of patients with early stages of avascular necrosis of the femoral head who underwent inpatient treatment in the trauma and orthopedic department of the Clinics of FSBEI HE Samara State Medical University of the Ministry of Healthcare of Russia from 2019 to 2021, inclusive.

Study design

An unblinded randomized clinical trial was performed in parallel groups corresponding to the type of surgical treatment. The study design was developed in accordance with the CONSORT 2010 (CONsolidated Standards Of Reporting Trials) guidelines [13, 14].

The study included the analysis of the treatment results of 36 patients.

Inclusion criteria: gender — any; age — under 50 years old; verified ARCO (Association Research Circulation Osseous) stages II and IIIa ANFH [15]; unilateral nature of the lesion.

Exclusion criteria: verified stages IIIb and IV (according to ARCO) of ANFH; bilateral lesions; osteoarthritis of the hip joint; decompensated somatic pathology; pregnancy; alimentary-constitutional obesity above grade II (BMI > 39.9 kg/m²); verified systemic rheumatological pathology; history of proximal femur fractures.

All 36 patients met the aforementioned inclusion criteria and the results of their treatment could be followed up at all the periods according to the study design.

All patients were allocated by stratified randomization into two groups — control and main groups, corresponding to the method of surgical treatment. Stratification was performed by assigning patients to groups taking into account the following factors: gender, age, stage of avascular necrosis and duration of the disease. Stratified randomization of patients into groups is presented in Table 1.

The highlighted groups had no statistically significant differences in the abovementioned parameters.

In the control group (n = 19), the surgical treatment was performed via open decompression and autogenous bone grafting of the femoral head using the M.P. Rosenwasser's light bulb technique [16]. The surgery was performed as follows. An anterolateral approach to the hip joint was made. The anterior wall of the joint capsule was cut in T-shape and fixed with traction sutures. The articular surface of the femoral head was visually assessed. Then, without dislocating the hip, a window was formed at the border of the hyaline cartilage of the femoral head under the control of intraoperative fluoroscopy. Osteonecrosis focus was excised via that window. The bone tissue was harvested in the area of the greater trochanter, which a free structural cancellous autograft of the required shape and size was formed from. At the final stage of operation, we performed autogenous grafting of the femoral head with the formed bone transplant, which was inserted according to the press-fit technique into the area of the postresection defect.

In some cases, when large bone grafts were used, they were additionally fixed with absorbable implants — ActivaPin™ pins (Bioretec Ltd.) (Fig. 1).

The main group (n = 17) included patients who underwent combined impaction autografting of the femoral head. This method was developed at the clinic and at the Department of Traumatology, Orthopaedics, and Extreme Surgery named after acad. RAS A.F. Krasnov of Samara State Medical University (Russian Federation patent for invention No. 2583577 dated 13.04.2016). The idea of the method is to perform the

Table 1

Stratified randomization of patients into groups

Parameter		Control group (n = 19)	Main group (n = 17)	p
Gender	Male	14 (73.7%)	13 (76.4%)	0.531
	Female	5 (26.3%)	4 (23.6%)	
Age, y.o.	18–29	5 (26.3%)	4 (23.6%)	0.668
	30–39	8 (42.1%)	9 (52.9%)	
	40–49	6 (31.6%)	4 (23.5%)	
Disease duration	<12 mos.	11 (57.9%)	8 (47.1%)	0.765
	12–24 mos.	8 (42.1%)	9 (52.9%)	
ANFH stage	II	11 (57.9%)	9 (52.9%)	0.566
	IIIa	8 (42.1%)	8 (47.1%)	

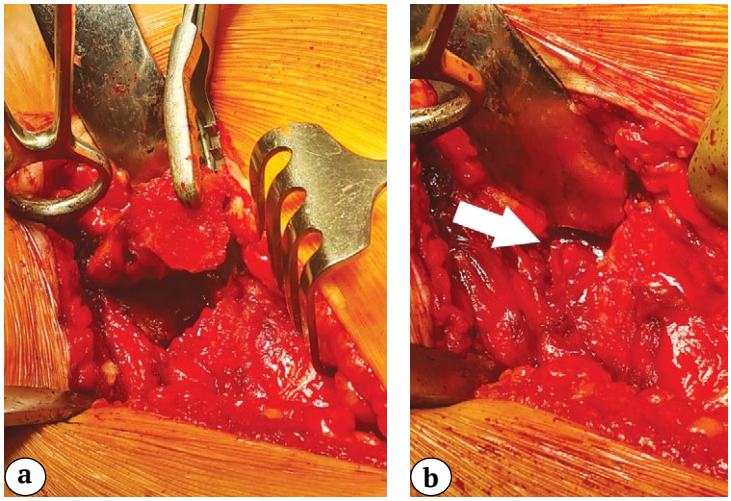

Fig. 1. Stage of the surgery — introduction and fixation of the bone autograft in the area of the postresection defect of the femoral head using bioabsorbable pins:
a — view of the bone autograft before insertion into the defect area;
b — view of the pin fixed in the surgical wound after its insertion (indicated by arrow)

minimally invasive intralesional resection of the femoral head and to use the bone tissue obtained during the formation of the canal to the osteonecrosis zone to replace the defect, and to perform muscle autoplasty with a gluteus medius muscle flap. This makes it possible to avoid additional damage to the bone tissue of the proximal femur (or iliac wing) associated with bone harvesting and to provide optimal conditions for remodeling processes in the area of transplantation.

The surgery was performed as follows. A linear skin incision and dissection of the underlying soft tissues were made to gain access to the region of the greater trochanter of the femur. Then, under fluoroscopy, a 6 mm hollow cutter was used to form a bone canal passing through the intertrochanteric zone, neck and head of the femur directly to the lesion focus in the latter (Fig. 2).

The bone tissue removed from the hollow cutter was preserved, mechanically pulverized and used to form a bone autograft (Fig. 3).

To implement the next stage of surgical intervention, we used a surgical instrument developed by us — a cutter for intralesional bone tissue resection (Russian Federation patent for utility model No. 171951 dated 10.01.2017) (Fig. 4).

Using this instrument under intraoperative fluoroscopy, we performed the sequential resection of the femoral head in the area of the pathological focus, the extent of which was controlled by the angle of opening of the cutting blades (Fig. 5).

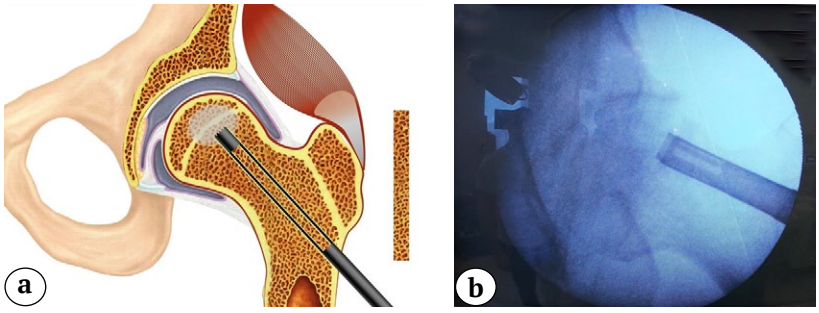

Fig. 2. Stage of the surgery — formation of a bone canal to the osteonecrosis focus in the femoral head:
a — scheme of the stage;
b — intraoperative fluoroscopy control of intraosseous canal being formed

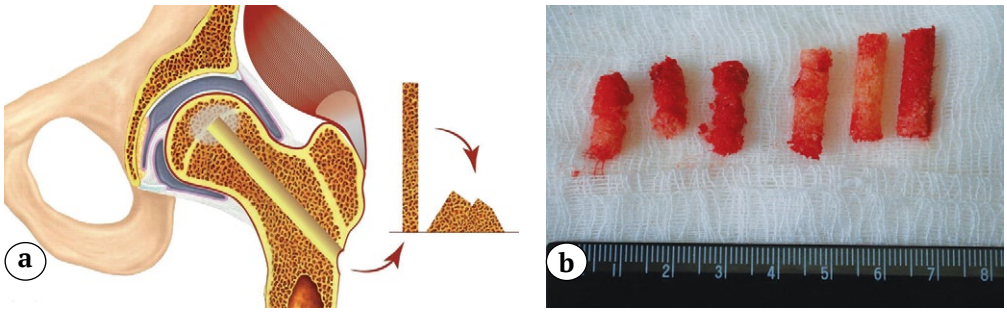

Fig. 3. Stage of the surgery — formation of the bone autograft:
a — scheme of the stage;
b — intraoperative view of the bone tissue obtained as a result of the canal formation

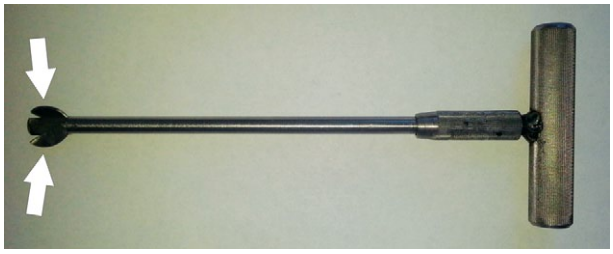

Fig. 4. Cutter for intralesional bone tissue resection with opened cutting blades (indicated by arrows)

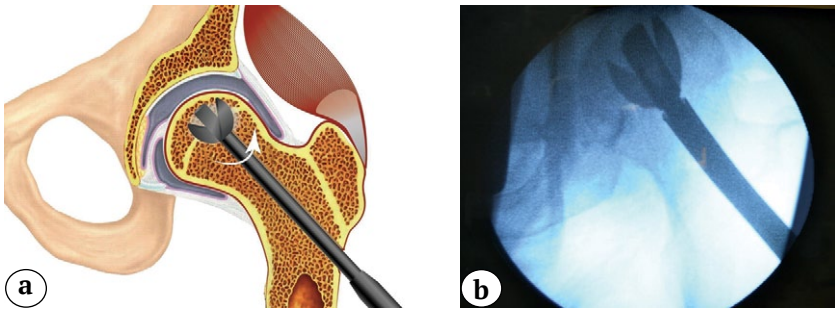

Fig. 5. Stage of the surgery — intralesional resection of the femoral head using the developed cutter:
a — scheme of the stage;
b — intraoperative fluoroscopy control image of the intralesional resection of the femoral head

After necrotic focus removal, we proceeded to the bone autografting stage — the earlier prepared bone autograft was implanted into the area of the femoral head bone defect (Fig. 6).

Then, the isolation of the gluteus medius muscle was performed via the same surgical approach. The fibers of the posterior portion of the muscle were used to form a 5-6 cm long and about 1.5 cm wide muscle flap with preservation of the attachment site to the greater trochanter (Fig. 7).

Myoplasty was performed as the final stage of operation: a muscle autograft was inserted into the distal intraosseous canal of the intertrochanteric region and femoral neck with subsequent transosseous fixation (Fig. 8).

Postoperative management protocol was identical in both clinical groups. The operated lower limb was not immobilized; weight bearing was limited for 12 weeks from the day of surgery, and amplitude movements in the hip were allowed from the first day after surgery. The protocol of perioperative pharmacological therapy was the same for all patients in both groups.

Clinical and functional assessment of treatment results was performed using

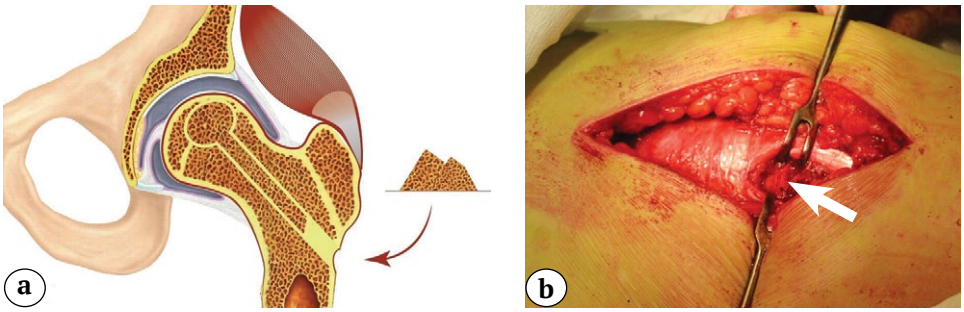

Fig. 6. Stage of the surgery — autogenous bone grafting of the postresection defect of the femoral head:
a — scheme of the stage;
b — intraoperative view of the impacted bone autograft in the canal (indicated by arrow)

Fig. 7. View of the muscle graft from the gluteus medius muscle in the surgical wound

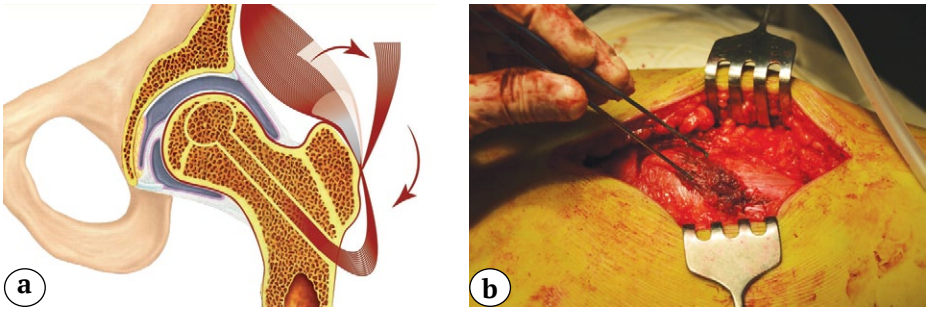

Fig. 8. Stage of the surgery — myoplasty of the distal region of the bone canal:
a — scheme of the stage;
b — view of the muscle autograft after its fixation at the bone canal entry zone

the Harris Hip Score (HHS) and the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) [17]. The assessment was performed before the surgery and at 3, 6, and 12 months after the surgery.

In addition, to analyze the dynamics of the pathological process in the area of the surgery and to objectify the assessment of structural changes of the femoral head in the operated hip, X-rays of the affected joint, CT-scan and MRI were performed in all patients at 6 and 12 months after surgery.

Statistical analysis

The results were compared between the groups using the nonparametric Mann-Whitney U test. Critical values for the level of statistical significance in testing the null hypothesis were taken as $p<0.05$. Correspondence of data distribution to the normal distribution law was tested using the Shapiro-Wilk test. Quantitative data were described using median and quartiles and were presented as Me (Q1;Q3), minimum and maximum (min-max). Qualitative data

were described using absolute and relative frequencies. Statistical processing was performed using the IBM SPSS Statistics 25 PS software.

RESULTS

The values of the WOMAC and Harris Hip Score in patients of both groups at all follow-up periods are presented in Table 2.

Visualization of the dynamics of the WOMAC and Harris Hip Score in patients of control and main groups at all follow-up periods is presented in Figures 9 and 10, respectively.

The performed comparative analysis showed a statistically significant difference in clinical and functional results after surgical treatment in patients of control and main groups. At the same time, the patients of the main group demonstrated better results at all follow-up periods, but the most pronounced difference was observed at 6 months after surgery with further maintenance of this trend up to 12 months after surgery. We believe that this is due to two main factors: firstly, minimally traumatic surgical approach and closed surgery technique at all main stages of operation without damaging

Table 2

Values of the WOMAC and Harris Hip Score

Period	Scale	Control group					Main group					p
		min	Q1	Me	Q3	max	min	Q1	Me	Q3	max	
Before surgery	WOMAC	27.0	31.0	34.0	36.0	38.0	28.0	31.0	32.0	35.0	38.0	0.203
	Harris Hip Score	64.0	71.0	74.0	79.0	81.0	64.0	73.0	75.0	77.5	80.0	0.634
3 mos. after surgery	WOMAC	25.0	27.0	30.0	31.0	35.0	21.0	23.0	25.0	29.0	32.0	0.002
	Harris Hip Score	68.0	72.0	77.0	80.0	84.0	75.0	80.0	82.0	89.5	91.0	0.001
6 mos. after surgery	WOMAC	22.0	23.0	27.0	28.0	33.0	17.0	19.0	20.0	23.0	27.0	<0.001
	Harris Hip Score	69.0	74.0	79.0	82.0	85.0	79.0	84.5	88.0	91.0	95.0	<0.001
12 mos. after surgery	WOMAC	17.0	20.0	24.0	25.0	30.0	13.0	14.0	15.0	16.0	24.0	<0.001
	Harris Hip Score	71.0	75.0	81.0	84.0	86.0	85.0	89.0	90.0	91.0	92.0	<0.001

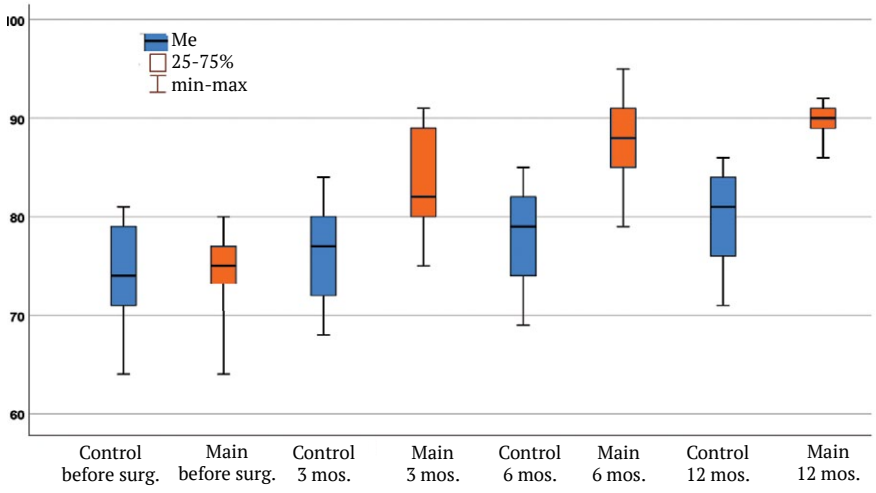

Fig. 9. Dynamics of the WOMAC scale values in patients of study groups

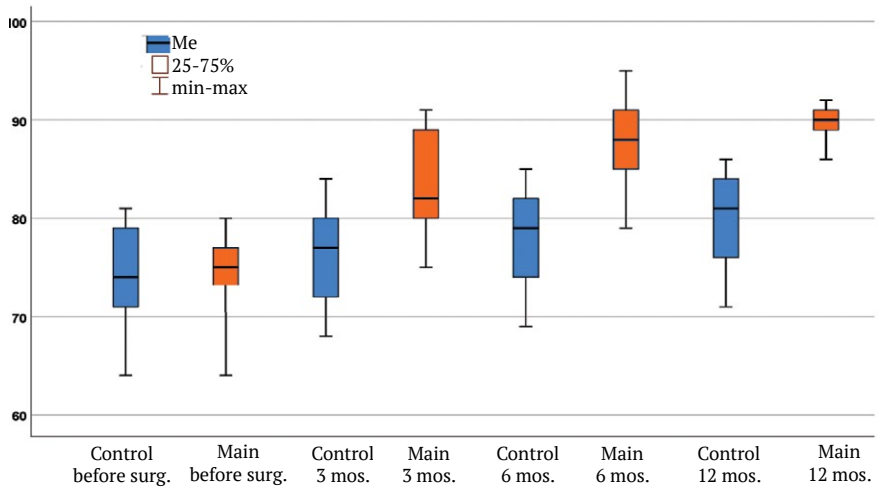

Fig. 10. Dynamics of the HHS questionnaire scores in patients of comparison groups

the peri- and intraarticular tissues of the hip joint; secondly, earlier mobilization of patients and shorter period of their rehabilitation.

When analyzing control X-rays of the hip joint in the AP view, macroscopically visualized changes in the bone structure of the femoral head were assessed, as well as the degree of progression of radiological signs of the destructive-dystrophic process. In 6 months after surgery, we observed the formation of a collapse of the femoral head in one patient of the main group and in three patients of the control group, while radiological signs of extrapolation of the pathological process to the entire joint by developing osteoarthritis were observed only in one patient of the control group.

In 12 months after surgical intervention, a collapse of the femoral head with the progression of stage IIIb ANFH without signs of osteoarthritis was noted in two patients of the control group; in two more patients of this group we observed further progression of the disease with the formation of terminal osteoarthritis of the hip joint. In the main group at this follow-up period, two patients experienced deterioration from stage IIIa to stage IIIb ANFH, but without intensifying of clinical signs in the affected joint.

The changes in CT scans were correlated with the results obtained by X-ray examination at the corresponding follow-up periods. An important substrate of the pathological process, which we verified using CT, was the process of cystic foci formation in the femoral head, which is typical for the ANFH development and indicates its progression. These changes were absent in patients of both groups at 6 months. However, when analyzing the CT scans performed 12 months after operation, we observed an increase in the number of cyst-like lucencies in two patients of the control group, and in one of them the formation of destructive-dystrophic cysts was determined in the area of the acetabular roof.

A typical CT picture in patients of the main group included visualization of a bone canal in the metaepiphysis and bone autograft remodeling at a certain stage (Fig. 11).

Hip MRI was used to assess the severity of trabecular oedema with evaluation of the dynamics of its spread or regression, the size of the pathological focus, the degree of changes in the bone structure in the area of surgery, as well as the presence of synovitis and articular cartilage condition.

MRI evaluation 6 months after surgical intervention showed a similar picture in patients of both groups, which was obviously connected with the homogeneity of developing processes of bone autograft remodeling in the intervention area. However, when analyzing the MRI of the hip joint 12 months after operation, we

observed a marked increase in the spreading zone of bone marrow oedema of the femoral head, as well as the presence of persisting and pronounced perifocal oedema of the transplant area in 5 patients of the control group (Fig. 12).

In the main group, despite the persisting trabecular oedema, integration and pronounced remodeling of the bone autograft at the site of its impaction were observed (Fig. 13).

Fig. 11. CT scans of the hip joints 12 months after the closed combined autografting of the left femoral head: contours of the bone canal and completed remodeling of the femoral head autograft (indicated by arrows) with preservation of its anatomical shape are observed

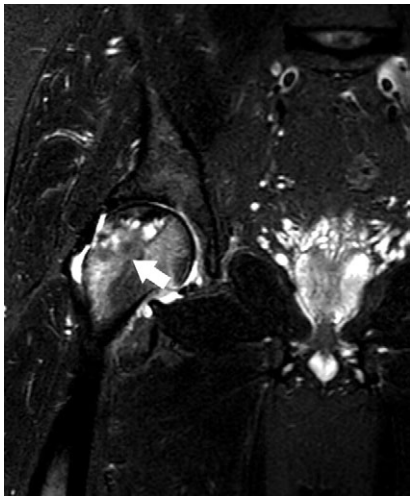

Fig. 12. MRI of the right hip joint 12 months after the core decompression using the light bulb technique: a pronounced trabecular oedema is observed in the area of grafting, spreading to the metaphyseal region (indicated by arrow)

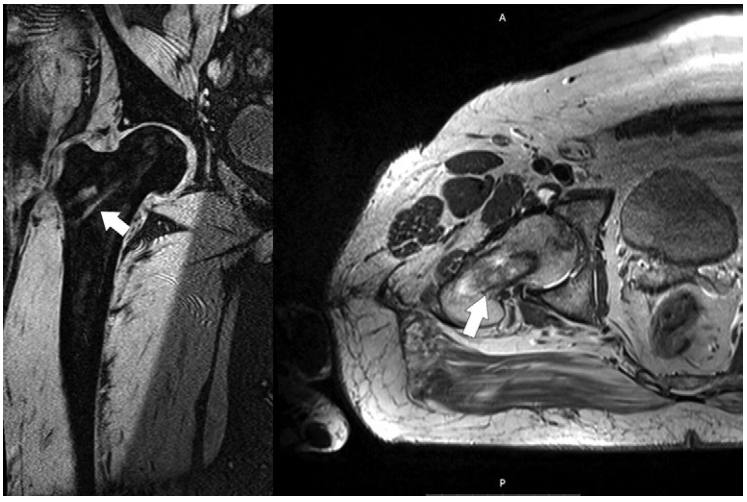

Fig. 13. MRI of the left hip joint 12 months after the closed combined autografting of the femoral head; no pathologic changes in the femoral head, moderate trabecular oedema in the area of the muscle autograft is observed (indicated by arrows)

DISCUSSION

Modern diagnostic methods, such as MRI and CT, make it possible to diagnose ANFH at early stages, which, in turn, enables to start treatment in time to prevent disease progression. However, the choice of treatment method at this stage is a difficult task [18, 19, 20]. The reason for this is that conservative treatment, started even at early stages, in a significant number of cases has an evanescent or indolent clinical effect, forcing soon either to change the treatment tactics or to immediately consider one or another variant of surgical correction [21, 22].

There are two main groups of surgical treatment options for ANFH: the first one includes organ-preserving operations with isolated core decompression of the femoral head, and combined operations including decompression and one-stage femoral head grafting; the second one includes total hip replacement [23, 24, 25, 26].

Total hip replacement in young patients with ANFH is essentially a desperate operation and should be considered as the last option for surgical treatment. In addition to certain functional limitations of the operated joint and risks of prosthesis-associated complications (dislocations, periprosthetic infection, periprosthetic fractures), total hip replacement performed in patients of this age group is associated with a high probability of revision hip replacement in future [1, 22, 27, 28].

Despite the fact that at present the variety of methods of surgical treatment of patients with precollapse stages of ANFH is actively expanding and is represented by a range of options from corrective osteotomies of the proximal femur to intraosseous injection of mesenchymal stem cells, filling of postresection bone defects with metal implants, etc., core decompression of the femoral head with autogenous bone grafting is the most reasonable from the pathogenetic point of view and predictable in terms of results of surgical treatment [29].

There is no agreement on the choice of surgical approach and implementation of the main stages of core decompression and bone grafting of the defect area. Some authors use open techniques to gain access to the lesion area by arthrotomy and subsequent fenestration of the femoral neck or femoral head itself; others favor exclusively closed minimally invasive techniques, which imply acces to the lesion in the femoral head from the infratrochanteric region through the intertrochanteric zone and the femoral neck [30, 31, 32]. At the same time, it should be noted that the choice of surgical approach to the lesion focus in ANFH is an important issue, as it determines the possibility of fulfilment of such important stages of the surgery from the pathogenetic point of view, which, for example, is the muscle autoplasty.

Undoubtedly, one of the most important debatable problems is the choice of technique of implementation of the main stages of this surgery and it is still far from its final resolution. Open techniques (trapdoor and light bulb techniques) have not lost their relevance to date, and their proponents justify their choice by the possibility of exact topical verification of the lesion focus and precision bone autografting *ad oculus* [30, 33].

The proponents of closed methods fairly point out that the damage to the peri- and intraarticular tissues, including the joint capsule and hyaline cartilage, is the most important predictor of inevitable and active progression of destructive and dystrophic processes in the joint in the postoperative period and, consequently, negates the very possibility of obtaining positive long-term treatment results. On the other hand, closed techniques of core decompression require careful planning of the surgical intervention, constant intraoperative objective control of all steps of its implementation, and appropriate surgical instruments [34].

In our opinion, the solution to these problems might be the creation of a

treatment algorithm based on a standardized classification system for ANFH, which would ensure the accuracy of disease progression prognosis and, accordingly, justify the choice of appropriate surgical treatment methods. In addition, it is certainly necessary to conduct further studies, dedicated to comparative analysis of not only mid-term but also long-term results of surgical treatment of this category of patients.

Limitation of the study

Our study, like most similar papers, has a significant limitation in terms of the size of the observation groups, but the factors we have identified should be considered by professionals.

CONCLUSIONS

Comparative assessment of efficacy of the open core decompression with autogenous bone grafting of the femoral head defect using the light bulb technique and closed intralesional resection of necrosis focus with combined impaction grafting of the femoral head in patients with stage II and IIIa ANFH (according to ARCO) showed that the minimal damage to para- and intraarticular tissues when performing approach to the area of the pathological focus and the main stages of operation is a key factor enabling best clinical and functional results and creates optimal conditions for bone remodeling in the grafting area.

DISCLAIMERS

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Ethics approval. Ethical review. The study was performed on the basis of ethical principles of the World Medical Association's Declaration of Helsinki (2013), «Good Clinical Practice in the Russian Federation» approved by the order of the Ministry of Health of the Russian Federation from 19.06.2003 N 266, in compliance with the basics of the tripartite Agreement on Good Clinical Practice (ICH GCP).

The study was approved by the Bioethics Committee under Samara State Medical University (the protocol N 147, 12.11.2014).

Consent for publication. The authors obtained written consent from patients to participate in the study and publish the results.

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