



Stage-related results in treatment of hip osteonecrosis with core-decompression and autologous mesenchymal stem cells

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Our aim is to analyse the clinical outcome of a series of patients affected by avascular necrosis of the femoral head and treated with core-decompression technique and autologous stromal cells of the bone marrow.

We enrolled in our study 29 patients with 31 hips in total affected by avascular necrosis of the femoral head.

The clinical and radiological outcome has been assessed through self-administered questionnaires (HHS, VAS and SF12) X-ray and Magnetic Resonance.

Of all the examined hips, 25 showed a relief of the symptoms and a resolution of the osteonecrosis, 11 of these were at Stage I and 14 at Stage II. The progression of the disease occurred in 6 hips (2 Stage II, 2 Stage III and 2 Stage IV).

Our results show a significant decrease in joint pain level and a success in avoiding or delaying the need of hip replacement in early stages of osteonecrosis.

Keywords : hip osteonecrosis ; stromal cells ; femoral head.

INTRODUCTION

Avascular bone necrosis of the femoral head (AVNFB) mainly affects young adults within their third, fourth or fifth decade of life and it has a large impact on life quality. Its progression can lead to hip joint impairment, and up to the need of pros-

thetic hip replacement. Therapy with glucocorticoids and alcohol abuse are some of the main known causative factors (21). Several pathophysiological mechanisms were formulated about this pathology, including fat emboli, microvascular occlusion of epiphysis vessels and micro fracture of trabecular bone (13,18). An early diagnosis and a continuous monitoring of patients with risk factors (corticosteroids therapy, alcoholism, or irradiated patients) are very important in order to highlight the AVNFB typical alteration as early as possible.

The treatment of osteonecrosis is one of the most controversial subjects in the orthopaedic literature.

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Several parameters need to be examined, such as age, medical history, need for continued cortisone therapy, presence of necrosis of the other hip and size of the necrotic area. In the stage I of the disease, non-operative procedures such as non-weight bearing, pharmacological, hyperbaric, or oxygen therapy, and the use of pulsed electromagnetic fields are all accepted. Operative treatments for stage II include core decompression, tantalum rod, trapdoor procedure, vascularized fibular graft, osteotomy, and non-vascularized bone grafting. Stages III and IV present a wide involvement of the femoral head that often leads to hip resurfacing or total hip arthroplasty.

Core decompression (CD) is the most frequently adopted method for early AVNFB treatment (1,27). It removes the necrotic area and decreases intraosseous pressure, improving venous return and promoting vascularisation of the necrotic area of the femoral head. Nevertheless, various studies about the effectiveness of this technique showed a wide range of results. One of the possible reasons of its failure is that a single CD does not induce an adequate osteogenic activity in the necrotic area (15,16,32). In literature, a large number of studies proved the connection between AVNFB and a decreasing number of bone mesenchymal staminal cells (MSC) with low proliferative activity. The replication ability of the bone progenitor cells decreases in proximal femoral epiphysis of patients affected by AVNFB. Consequently, approach to AVNFB treatment seems to be more appropriate if an autologous bone marrow implantation is performed after core decompression, in order to provide osteogenic precursors to the necrotic area (17).

We studied a series of 29 patients with a diagnosis of AVNFB who were treated with CD technique and bone marrow implantation. Our aim is to investigate, in a retrospective view, the rate and thus the efficiency of the treatment we used according to the gravity of AVNFB stage of our case series.

MATERIALS AND METHODS

We conducted a retrospective clinical study based on a case series of 29 patients affected by avascular osteonecrosis of the femoral head, with 31 hips in total. The

average follow-up time was 37 months (range from 23 to 48 months). The average age of the patients was 34 years (range 26-53 years) at time of surgery. Of the 29 patients, 16 were men and 13 were women. Staging of osteonecrosis was determined according to Steinberg classification (28). At the time of surgery, 11 out of a total of 31 hips were at Stage I, 16 at Stage II, 2 at Stage III, 2 at Stage IV. As for the etiology of the AVNFB, 7 hips were affected by idiopathic osteonecrosis, 2 of which were at Stage I and 5 at Stage II. 18 hips were affected by osteonecrosis due to corticosteroid therapy (14 monolateral and 2 with bilateral involvement), 6 of which at Stage I and 9 at Stage II, 1 at Stage III and 2 at Stage IV. 6 hips were affected by osteonecrosis due to alcoholism, 3 of which at Stage I, 2 at stage II and 1 at stage III (Table I).

All patients were treated from 2006 to 2010 with core decompression and implantation of autologous mesenchymal stromal cells of the bone marrow, harvested from iliac crest. Fibroblast colony forming units (F-CFU) was used to assess the number of grafted mesenchymal stromal cells, calculated on the number of nucleated cells (9). The average F-CFU was 1390/cm³ with a range from 1160 to 1520/cm³.

Our study examined several aspects of the clinical presentation and the pathologic features through : recording of haematological and inflammatory indices, pre-operative and long term radiographic evaluation according to Steinberg classification (28), VAS, as well as Harris Hip Score (24) and SF12 (11) questionnaires to assess hip function, pain and quality of life of our patients. Scorings were registered preoperatively, and every year after the treatment. The radiographic assessment focussed on the progression in radiographic stages, on the presence of bone-neoformation and on the absence or presence of femoral head collapse. Clinical and radiographic follow-up evaluations were made at 1 month, 6 months and every year after surgery. Additional controls with Magnetic Resonance Images were performed at 6 months after surgery and every year afterwards.

Surgical technique followed the P. Hernigou operative technique : aspiration of autologous bone marrow, isolation and concentration of aspirated marrow, core decompression, grafting of the concentrated cells (14). With the patient under general anaesthesia, a skin incision of 5 mm was made at the level of the upper margin of the anterior superior iliac crest, and a needle tip (11 cm, 11 gauge) was introduced for about 6 cm until the spongy bone was reached between outer and inner tables. The needle tip was removed and a sterile syringe of 25 ml (containing 3 ml of heparin) was introduced for the execution of short marrow aspirations of 3-5 ml with the aim

Table I. — Etiology and Stage

No. of patients (hips)	Etiology	Stage I	Stage II	Stage III	Stage IV
7	Idiopathic	2	5	–	–
16 (18)	Corticosteroid use	6	9	1	2
6	Alcohol abuse	3	2	1	–

of optimizing the removal of MSC. After each aspiration in one site, the needle performed a full circle in steps of 45°. At the end of each complete 360° needle revolution, it was moved 1 cm higher or lower in the cancellous bone, and then the whole procedure was repeated until 50 cc of bone marrow were harvested (two syringes). Concentration of the aspirated marrow was performed through the MarrowStim™ centrifuge system, at 3200 RPM for 15 minutes. This system can separate up to 50 cc of bone marrow, concentrating mononucleated and polynucleated cells within a volume of 6 cc of concentrated marrow. The procedure is performed directly in the operating room during the same surgery time. With the patient placed on the operating table with image intensifier C-arm, a 5 cm incision was made at the level of the greater trochanter and, after the subcutaneous diaeresis, the fascia lata and the vastus lateralis were dissected until the lateral cortex of the proximal femur was reached. At this point, it was performed the decompression of the necrotic area with a 3 mm trocar introduced through the greater trochanter into the necrotic lesion, monitoring the trephine tip direction under fluoroscopic control. The tip of the trephine was stopped at a distance of 3 mm from the epiphyseal cartilage. A single hole was made in all the femoral heads treated. The concentrated marrow was injected into the femoral head using a small cannula applied to the syringe. No drainage was used in 31 total hips treated. The last steps were the suture of tissue layers and the surgical wound medication, which usually was about 5 cm.

Patients were hospitalized for an average period of 4 days with a range of 3-6 days. All patients were treated with anticoagulants (Enoxaparin 4000 IU) and proton pump inhibitors (Omeprazole 20 mg). Antibiotics were administered in the operating room (intravenous Cefazoline 2 g), and then repeated three times, every six hours, until 24 hours.

Weight-bearing was forbidden for 5 weeks, after this period it was allowed gradually up to a complete weight-bearing without crutches at 8 weeks. During the rehabilitation period, patients were recommended to do physiotherapy and physical activity in water in order to help the gradual recovery of coxofemoral joint motion, to

strengthen gluteus and quadriceps muscles and to prevent tendon and muscular retractions or harmful postural changes.

Wilcoxon test was performed to assess differences in HHS, VAS and SF12 indices after treatment, according to the different staging groups. A binomial test was used to assess the significance of the success of the treatment. We considered statistically significant values of $p < 0.05$. Data are expressed in the form mean value \pm standard deviation. Data have been analysed in the statistical software SPSS 19.

RESULTS

The results of our study show that there were no complications during anaesthesia. No thrombophlebitis, pulmonary embolism, intertrochanteric fractures were observed postoperatively.

At the end of the follow-up, 25 hips (80.6% of total) had relief of symptoms, 11 of which were at Stage I and 14 at Stage II at the time of treatment; MRI showed no collapse of the femoral head and the restoration of necrotic areas by bone tissue. However, MRI showed an alteration of the signal in T2-weighted sequences due to an inflammatory process and marrow oedema.

Differently, the progression of the disease, assessed by clinic and radiographic evaluations, was registered in 6 cases (19.4% of total hips), all of which needed hip replacement. Of these failures, 2 were at Stage IV at the time of diagnosis, 2 at Stage III and 2 at Stage II.

As for the patients at Stage I, values of Harris Hip Score registered preoperatively and 2 years after the treatment show an increase in all cases, from mean 74.5 ± 1.4 to 96.2 ± 1.2 ($p < 0.01$). Similarly, the mean value of the VAS index decreased from 7.1 ± 0.3 to 2 ± 0.2 ($p < 0.01$), the mean value of the Sf-12 score increased from 82.1 ± 2.2 to 96 ± 0.9 ($p < 0.01$). In patients at Stage II the mean value of

the HHS increased from 73.2 ± 1.3 to 94.2 ± 1.1 ($p < 0.01$), the mean value of the VAS index decreased from 7.4 ± 0.2 to 2.4 ± 0.2 ($p < 0.01$), and the mean value of the Sf-12 index increased from 81.8 ± 1.9 to 95.2 ± 0.8 ($p < 0.01$).

On the contrary, patients at Stage III and IV showed a worsening of scores : the mean value of the HHS decreased from 71.55 ± 1.9 to 69.5 ± 5.86 ($p > 0.05$), the mean value of the VAS index increased from 7.55 ± 0.5 to 7.9 ± 0.6 ($p > 0.05$), and the mean value of the Sf-12 score decreased from 78.3 ± 2.5 to 76.4 ± 1.5 ($p > 0.05$) (Table II).

Thus, clinical and radiographic improvements were observed in all patients at Stage I ($p < 0.001$), in 14 of 16 patients at Stage II ($p = 0.002$), and failed (not significantly) in all patients at Stage III and IV ($p = 6.25$).

DISCUSSION

Treatment of AVNFB with implantation of autologous concentrated bone marrow is based on the evidence that osteogenic cells originate from staminal stromal bone marrow cells. The literature about AVNFB pathogenesis reports an inadequate bone remodelling activity and a lack of recovery in the necrotic area due to a decreasing concentration of MSCs in the femoral head (5,22,30,35).

Although single CD is still one of the most adopted treatment in early stages of AVNFB, as it relieves intraosseous pressure, its results are uncertain and variable. We can only assume that CD fails as it removes necrotic area without stimulating bone and vessel regeneration, leading to an incomplete recovery of the femoral head. Therefore, CD is associated to other treatments in order to avoid depletion of neoangiogenesis. Adult mesenchymal bone marrow

cells are implanted into a necrotic lesion in order to repopulate the area by stimulating and leading bone remodelling with an adequate "creeping replacement" (2,3,17,23).

Thus, CD associated to autologous MSCs grafting appears to be more effective as it combines benefits of necrotic area removal, an immediate reduction of intraosseous pressure and a metabolic stimulation. As a result, CD promotes new bone generation and revascularization of treated area. Furthermore, it is a relatively simple surgical technique, minimally invasive and it rarely causes complications such as subtrochanteric fracture (17).

As described in literature, increased levels of concentration of harvested bone marrow can improve further osteogenesis as a consequence of the increasing number of progenitor cells (5,6,17). A major issue in MSCs grafting treatment is their low concentration in bone marrow in relation to age and pathology (3,4). MSCs average number is estimate to be 1-3.4 MSC cells out of 10^4 mononucleated cells (3). This number is intended to decrease with aging : 1 out of 10^4 in newborns, 0.5 out of 10^6 in 80-year-old adults ; this is a very significant detail for the autologous implantations in elderly patients. Isolated and expanded cells harvested from older patients have a lower replication rate (average replication times are 76.1 ± 23.4 hours in older patients, 44.0 ± 7.7 hours in young patients), resulting in longer cell cycle stages and decreased differentiation potential (34).

Moreover literature shows that the number of bone marrow grafted cells in patients affected by AVNFB is significantly related to necrosis aetiology. In fact, patients with AVNFB caused by corticosteroids therapy or by organ transplants have appreciably lower levels of mononucleated cells,

Table II. — Average of HHS, VAS and SF-12 before treatment and 2 years after

	HHS		VAS		SF-12	
	Pre-op	2 yrs	Pre-op	2 yrs	Pre-op	2 yrs
Stage I	74.5 ($p < 0,01$)	96.2 ($p < 0,01$)	7.1 ($p < 0,01$)	2.0 ($p < 0,01$)	82.1 ($p < 0,01$)	96.0 ($p < 0,01$)
Stage II	73.2 ($p < 0,01$)	94.2 ($p < 0,01$)	7.4 ($p < 0,01$)	2.4 ($p < 0,01$)	81.8 ($p < 0,01$)	95.2 ($p < 0,01$)
Stage III	72.3 ($p > 0,05$)	70.5 ($p > 0,05$)	7.4 ($p > 0,05$)	7.8 ($p > 0,05$)	81.3 ($p > 0,05$)	79.6 ($p > 0,05$)
Stage IV	70.8 ($p > 0,05$)	68.5 ($p > 0,05$)	7.7 ($p > 0,05$)	8.0 ($p > 0,05$)	75.3 ($p > 0,05$)	73.2 ($p > 0,05$)

compared with patients affected by AVN caused by other factors. Analysing the relations between the number of implanted cells and the patients' prognosis, we can observe that new bone formation occurs only when an adequate number of marrow cells is assessed. Clinical effectiveness and prognosis in literature is congruent to the progenitor cells number in grafted bone marrow (12,15,33).

The average F-CFU obtained from harvested marrow of our patients was 1390/cm³, with a range from 1160 to 1520. In each group of patients with different aetiological factors, the number of progenitor cells was different. Hips that received an implantation with a lower number of MSCs were hips affected by necrosis associated to corticosteroids therapy and alcoholism. The 2 patients at Stage II whose treatments failed, both under corticosteroids therapy, received cell grafts with a low F-CFU. A decreased F-CFU was also registered in patients at older age.

The choice of the centrifugation system we adopted (Marrowstim™) is aimed to increase the population of osteogenic cells, by concentrating progenitor cells in aspirated marrow. Data from previous studies demonstrated the effectiveness of this process by concentrating mononucleated cells (progenitor cells, T-cells, macrophages). Comparing the system we used to a standard lab technique, the former provides a larger number of F-CFUs from the same sample of harvested marrow (1506 ± 371) and it takes one-sixth of the same time, with the benefit of "in line tissue engineering" (8,26,29).

The results of our study show that 100% of Stage I hips and 87.5% of Stage II were successfully treated, while failures occurred in 12.5% of femoral heads at Stage II, and in 100% of hips at Stages III and IV. The aetiologies associated with these failures were: corticosteroids therapy for 2 hips at Stage II and 2 hips at Stage IV, alcoholism for 1 hip at Stage III and corticosteroids therapy for 1 Stage III hip. Moreover, best results were registered when therapy was adopted on patients at an early stage of AVNFB, before the collapse of femoral head. This element is in line with the literature (12,13,33).

The chance of a failure in literature are higher for patients at stage III and IV, as is already present an irreversible mechanical subsidence from the begin-

ning (10,12,19,33). According to these evidences, patients at stage III and IV were clearly informed about high chance of failure; they were young people strongly inclined to avoid or delay a hip replacement.

Regarding the surgical technique, during the CD procedure there is a likelihood of mistake in penetrating the joint due to blind zones hid in radiographic images. The use of intraoperative imaging can reduce blind zones, but does not completely remove them, as well as planar images do not allow to discern if the head of the trephine tip is situated inside or outside the femoral head (13). Assessments of femoral head and trochanteric pressure revealed that intraosseous pressure can be adequately relieved even with a little incision (14,20).

Regarding the injection of concentrated marrow, some cells can leak out the device or into femoral circulation, but most of them remain in the necrotic area or in the femoral head. Tests with radionuclide tracers were performed in two patients and confirmed this evidence (13).

Finally, our study has several limitations, as its retrospective design, the absence of a control group, and the fact that it is not randomized; moreover the number of the sample is small and there is still not a complete and deep analysis of costs, which could imply sanitary and economic advantages related to this surgical technique. However, compared to a later hip replacement, CD associated with autologous MSCs graft is surely a minimally invasive operation, with reduced impact on postoperative course and short hospitalization (25).

CONCLUSIONS

The results of our study show that treatment of AVNFB with implantation of autologous concentrated MSCs is indicated for patients at Stage I and Stage II. Our results show a significant reduction of joint pain level, and this could take to a delay, or avoid the need, of hip replacement. Moreover, this study highlight the safety of autologous MSCs graft approach in the treatment of the early stages of AVNFB.

Nevertheless, we cannot completely assess the efficiency of this treatment due to the lack of this

study in correlating the outcome with both aetiology of femoral necrosis and age. A larger chance of failure is indeed registered in cases of AVNFH associated with corticosteroids therapy and in cases of older patients (3,13).

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