FEMORAL HEAD NECROSIS TREATMENT WITH AUTOLOGOUS STEM CELLS IN SICKLE CELL DISEASE

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SUMMARY

Purpose: To assess the efficacy and safety of autologous bone-marrow mononuclear cells (BMMC) implantation in necrotic lesions of the femoral head in patients with sickle cell disease. Methods: We studied eight patients with stage-I or -II femoral head osteonecrosis according to the system by Ficat and Arlet. BMMCs were harvested and re-infused into the necrotic zone. The primary endpoints studied were safety, clinical symptoms and disease progression, these being assessed according to the Harris hip score (HHS) and to X-ray studies. Results: After eight months, seven of the eight patients reported improvement from symptoms. There were no complications during anesthetic and surgery procedures. There was a significant postoperative increase in the HHS (98.3 +/- 2.5 points) compared to preoperative HHS (78.5 +/- 6.2 points) ($p < 0.001$). X-ray evaluation and cell parameters were found to be favorable. Conclusion: The autologous bone-marrow mononuclear cells implantation seems to be a safe and effective treatment for early stages of femoral head osteonecrosis in patients with sickle cell disease. Although promising, the interpretation of these early results is limited due to the small sample and to the short duration of follow-up. Further studies and advanced cellular assays are required to confirm the results.

Keywords: *Osteonecrosis, Sickle Cell Disease, Autologous transplantation, Bone marrow cells; Stem Cells.*

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INTRODUCTION

Sickle cell hemoglobinopathies include a number of diseases characterized by the presence of hemoglobin S (HbS). Among African descendent patients in the New World, the heterozygous genotype is present in 10% of the U.S. population, over 6% in the south-southern region of Brazil, and as many as 15.7% in the state of Bahia[1]. The homozygosis of the mutation (HbSS) or the association with other hemoglobinopathies, particularly with HbC (HbSC), causes a devastating and chronic disease, associating a severe anemia to the clogging systemic disease, with the progressive blood vessels degeneration. In the bone-joint system, one of the most serious manifestations of sickle cell disease is osteonecrosis, more frequently found on femoral head.

Osteonecrosis of the femoral head affects about 10% - 30% of the sickle cell-ill population[2,3]. If left untreated, the reduced blood flow on femoral head causes a degeneration of the trabecular mesh, collapse of the subchondral bone and secondary arthrosis in up to 70% of the cases[4]. These late complications require surgical intervention such as arthroplasty, which, besides being an invasive procedure showing high morbi-mortality rates, presents poor outcomes in up to 50% of sickle cell-ill patients after 5-10 years[5]. As femoral head osteonecrosis most frequently occurs in young individuals, a treatment preserving femoral head instead of replacing it is preferable whenever possible. However, no fully satisfactory technique is available for treating the early stages of femoral osteonecrosis, a fact that represents a challenge to orthopaedic doctors.

Bone marrow aspirated from the iliac crest contains mesenchymal stem cells with osteogenic and chondrogenic potential, as well as endothelial stem cells able to contribute to vasculogenesis and angiogenesis, providing vascular repair. These attributes have been probed by several groups with the development of experimental and clinical studies using autologous bone-marrow mononuclear cells (BMMC) implants for healing, cell architecture repair, and recovery of local blood flow on injured and ischemic tissues[6].

Recent prospective clinical studies on femoral osteonecrosis

Study conducted at the Department of Surgery, University Hospital Professor Edgard Santos, Federal University of Bahia.
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treatment with the introduction of autologous BMMC and percutaneous decompression have shown effective and safe results. Among the various etiologies contributing to this disease, sickle cell-ill patients present with the best prognosis for therapy, with a failure rate of as low as 5% requiring hip arthroplasty 8 years later[7,8].

The evolution of the overall clinical picture of sickle cell disease and, most specifically of the femoral osteonecrosis, is dependant on the genetic mutation profile causing it and social, diet and sanitary patterns of the involved population[9]. The objective of our study is to assess the feasibility, safety and effectiveness of femoral osteonecrosis treatment by percutaneous decompression and implantation of autologous BMMC in sickle cell-ill patients living in the state of Bahia.

MATERIALS AND METHODS

Patient Selection

In the period between March and September 2006, 8 patients were submitted to a procedure for implanting autologous bone marrow mononuclear cells according to the technique described by Hernigou and Beaujean[8]. Patients with sickle cell disease receiving care at the hematology outpatient facility of the University Hospital Professor Edgard Santos (HUPES, UFBA) and at the Fundação Hemocentro da Bahia (HEMOBA) in Salvador (BA), Brazil, and presenting osteonecrosis of the femoral head at stage I, II or III according to the X-ray classification system proposed by Ficat et Arlet[10] or A, B or C according to the classification proposed by Steinberg[11] (Table 1), and scoring at least 20 points on the pain and daily life activities questionnaire were regarded as eligible to the study[12].

FHO diagnosis was determined by X-ray images at anteroposterior and laterolateral planes or by Nuclear Magnetic Resonance (NMR) images, aiming to determine the following characteristics: presence of femoral head collapse; presence of a sclerosis range on femoral head; crescent sign; double signal at T2 on magnetic resonance image; cyst or sclerosis on femoral head; hip pain at motion; low-intensity focus on T1 (Table 1).

Exclusion criteria included: patients with Ficat's stage-IV and Steinberg's C (or more) osteonecrosis of the femoral head showing bone infection at the limb affected by necrosis; history of previous surgery on the same injured limb; presence of neoplasic disease or any other pathology that could make therapy unfeasible; clinical conditions contraindicating the procedure.

	Pain	X-ray image	Magnetic resonance image
Stage I	+	Normal	+
Stage II	+	Changes on bone trabeculate with sclerosis or osteolysis areas	+
Stage III	+	Osteochondral fracture with sequestrum and flattening of the head	+
Stage IV	+	Advanced lesions	+

Table 1 *– Classification by Ficat et Arlet[10] and Steingberg[11]*

The protocol employed in this study was approved by the Committee of Ethics in Research of the Climério de Oliveira Maternity Hospital (HUPES, UFBA) and by the National Committee of Ethics in Research (CONEP). All patients taking part of the study were required to sign a free and informed consent term, previously approved by the Institution's Committee of Ethics.

Patients' characterization

Table 2 shows the stratification of assessed patients' data distributed for gender, age, weight and diagnosis.

The hematology assays of patients were carried out in an automatic Coulter T 890 device. Disease diagnosis and the hemoglobin expression profile in sickle-cell disease patients were confirmed by the electrophoresis method on cellulose acetate ribbons, pH 8,4 and electrophoresis on agar-citrate, pH 5,3 (Harris Hip Score)[13].

Patient	Diagnosis	Age	Gender	Weight	Date of Procedure	FHO stage -Ficat et Arlet
1	SC	15	F	51	03.31.06	1
2	SS	34	M	68	03.31.06	0
3	SS	45	F	50	03.31.06	2
4	SS	35	F	53	04.12.06	2
5	SC	30	F	70	07.07.06	0
6	SC	42	M	70	07.14.06	1
7	SS	48	F	60	08.25.06	1
8	SS	15	F	66	09.01.06	0

Table 2 *– Epidemiology of operated patients*

Bone marrow cells collection and processing

Approximately 100 ml of bone marrow were collected from puncture and aspiration of patients' posterior iliac crest under general anesthesia at HUPES-UFBA Operating Theater. The filtrated aspirate was processed at the SEPAX cell separator (Biosafe, Switzerland). The mononuclear cells (BMMC) that were isolated and purified in a Ficoll-PaqueTM Plus (Amersham Biosciences) gradient (d=1.077 g/mL) were re-suspended in a final volume (~25 ml) of physiological saline solution with 5% human serum albumin and stored for injection. A small fraction of the cell suspension was reserved for immuno-phenotypíng, cell viability and microbiological assays.

Cell phenotyping and quality control

The viability of infused cells, as determined by Trypan blue exclusion, was above 95%. The study of the presence of potential microbiologic contaminants, monitored by blood culture and traditional microbiologic tests, showed negative results.

The identification and counting of leukocyte populations on the BMMC fraction were assessed by flow cytometry. The characteristic expression of surface marker proteins of cell strains was analyzed by anti-CD34 monoclonal antibodies (multiple potent hematopoietic stem cells) combined with phycoerythrin and anti-CD45 (leukocytes pan-marker) combined with PerCp (Becton Dickison). Murine antibodies of the same isotype were employed as controls. Twenty thousand marked cells were acquired and analyzed on a FACSCalibur Flow Cytometry System (BD Bioscience) an on CellQuest and WinMDI v 2.8 software.

Osteonecrosis decompression and autologous stem cells grafting

Decompression was provided by means of percutaneous and bone transfixation using a trephine needle (3 mm in diameter). The procedure was previously planned using NMR images, and the needle position at the femoral head area was monitored by radioscopy. After decompression, the concentrated BMMC fraction was infused at the osteonecrosis area with the aid of a small trocar. Each patient received a BMMC infusion at the central area of the osteonecrosis usually through a single injection puncture. Leakage of the infused BMMC fraction was avoided by a small local blood tampon.

Clinical and Radiographic Evaluation

Patients were evaluated preoperatively, and three and six months postoperatively, or whenever necessary. Pain, function, joint range of motion, and deformities were rated according to the scores suggested by Harris[12] where a score scale ranging from 0 to 100 is attributed to those parameters. Hip function was rated according to the Harris Hip Score, as excellent (91 - 100 points), good (81 - 90 points), moderate (70 - 80 points) and poor (< 70 points).

X-ray images of the hip were assessed preoperatively, at the early postoperative period and on a quarterly basis in order to examine lesion size, the presence of indicative signs of spinal cord swelling and degenerative changes, according to Table 1.

Statistical Analysis

The paired t-test for variance analysis was employed to determine the significance level of the differences found between pre- and postoperative Harris Hip Score values. P values <0.05 were regarded as significant. Correlation coefficients between clinical assessments results and cell factors were determined by means of the Spearman's correlation test.

RESULTS

No patient showed changes on oxygen saturation, pulse, blood pressure, intertrochanteric fracture or other complications during anesthetic and surgical procedures. The average procedure time was 2.5 h (1.5 – 4h). The injection of BMMC fraction occurred approximately 1.5 h after bone marrow collection. No infection, bruises or chronic pain at the injection site were reported.

Clinical and Radiographic Evaluation

Most patients reported severe pain during preoperative examination, while 7 patients reported absence of pain three months after the procedure. The results of the questionnaire showed a preoperative mean Harris Hip Score of 78.5 +/- 6.2 points. After 3 and 6 months, the average associated score was 98,3 +/- 2,5 points. Questionnaire scores were significantly superior at the postoperative period ($p<0.001$ – paired t-test) (Table 3). This significant difference pointed out to a favorable recovery of hip functions, reduced pain, and higher quality of daily activities on these patients, despite of the relatively limited series.

Results concerning the radiographic aspect were less significant than the clinical evaluation results. In patients whose clinical evolution was favorable, hips have shown to be stable at X-ray, with no patent enlargement or reduction of the lesion size. There was no statistically significant correlation between radiographic staging and the Harris Hip Score, especially due to the sample size and to the relatively short postoperative follow-up period.

Patient	Preoperative	Postoperative	Follow-up Time
1	86	100	6 months
2	72	96	6 months
3	72	96	6 months
4	78	100	6 months
5	71	94	4 months
6	86	100	4 months
7	87	100	4 months
8	76	100	4 months
Average	78.5	98.3	
Std. Deviation	6.2	2.5	

Table 3 *– Pre- and postoperative scores with the Harris Hip questionnaire.*

Patient Satisfaction

In this group, all patients (n=8) presenting good results reported satisfaction with the procedure and that they would be willing to undergo hip surgery again, if necessary, at the contra lateral side. 75% of the patients reported reduced use of analgesic medication after the procedure. 12.5% of the patients reported moderate or minimal daily activities restraints three months after the procedure.

Analysis of the infused BMMC fraction

Two parameters were directly assessed on the purified BMMC fraction: 1) nucleated cells count (number of total leukocytes by 1.0 ml of re-infused bone marrow aspirate), and; 2) prevalence of Cd34+/Cd45low cells by 106 total nucleated cells. The counting of the number of mononuclear cells expressing antigens CD34+/Cd45low was regarded as representative of the number of implanted multiple potent stem cells due to the positive correlation with the number of hemangioblastic stem cells and with the regeneration of treated tissues, according to literature[14]. In addition to these stem cells, the population of nucleated bone marrow cells also contains mesenchymal bone stem cells and other mononucleated cells, some of those may be a source of angiogenic and osteogenic cytokines of clinical relevance. The results with the number of total infused leukocytes and stem cells frequency are presented on Table 4.

An average bone marrow volume of 120 mL was aspirated from the posterior iliac crest of each patient. The number of total leukocytes obtained at the BMMC fraction ranged from 9.4 to 31.9 million cells/mL, at an average of 16.4 +/- 8.8 millions/mL. A perfect linear correlation could not be established between the total number of nucleated cells and gender, weight and pre or postoperative Harris Hip Score. A weak correlation was found between the total number of mononucleated cells and patients' age (Spearman's test, r = 0.30) and the total number of purified CD34+/Cd45low cells (r = 0.69). The mean total number of infused CD34+/Cd45low cells was 0.41 +/- 0.4 million cells, ranging from 0.13 to 1.4 million. No change was found on the prevalence of stem cells with aging (r = 0.10), weight (r = 0.18) and the result of the postoperative Harris Hip Score (r = 0.20). A weak inverse correlation was found between CD34+ stem cells and the preoperative Harris Hip Score values indicating a reduced prevalence of these cells in patients with highly compromised joints (r = –0.40). The general analysis of the correlation coefficients did not show significant differences between clinical evolution and the parameters assessed on the BMMC fraction, probably due to the small sample of patients enrolled in the study and to the brief postoperative follow-up period.

Patient	Total leukocytes x 10^{-6} /mL	Total infused leukocytes x 10^{-6} /kg	$CD34^{+}/CD45^{low}$ concentration (%)	$CD34^{+}/CD45^{low}$ x 10^{-3}/Kg
1	12.8	7.5	2.13	5.3
2	29.4	13.0	4.72	20.4
3	31.9	19.1	1.28	8.1
4	9.4	5.3	2.68	4.7
5	12	5.1	1.90	3.2
6	12	5.1	1.08	1.8
7	11.8	5.9	3.26	6.4
8	11.8	5.4	2.04	3.6

Table 4 – *Total leukocytes concentration and of the sub population of stem cells infused into the FHO.*

DISCUSSION

This was the first clinical assay in Brazil addressing cell therapy on avascular femoral head in sickle cell-ill patients. The main objective was to assess the feasibility and safety of the procedure. As a complement, preliminary data were collected for effectiveness of central decompression combined with infusion of autologous BMMC in the treatment of symptomatic femoral head osteonecrosis in sickle cell-ill patients.

Our results point out to a reduction of pain severity and other joint symptoms associated to early stages of femoral osteonecrosis with this treatment, and, at least for the eight-month follow-up period disease progression remained stable.

Although sickle cell-ill patients usually present a high prevalence of complications related to anesthesia and surgery, additionally to intrinsic complications, we did not find episodes during or after the procedure. There was no report of painful events or superficial infection in the first days following decompression and cell infusion procedures. Intertrochanteric fracture, infection or embolism, all of these previously described in decompression and cell infusion studies[8,15], were not seen in our study.

As described in literature, the prevalence of osteonecrosis in patients with sickle-cell disease is high, reaching up to 50% of these individuals, matching the higher incidence of complications in adolescents[16,17]. The involvement of both hips is seen in 40-91% of the patients. As shown by Hernigou et al[4], the progression to femoral head collapse in sickle cell-ill patients occurs in 90% of the cases within 2 years after stage-I osteonecrosis is first diagnosed. Total hip arthroplasties' ineffectiveness and failure rates in those patients are particularly high, with incidence of pain and significant motion restraints in 75% of the patients with sickle cell disease[18,19]. These studies suggest that the clinical evolution of femoral osteonecrosis in patients with sickle cell disease is more frequent and rapid when compared to reports addressing other conditions associated to non-traumatic osteonecrosis. Our study considers these conclusions and suggests surgical intervention associated to BMMC implant in an attempt to prevent or delay disease progression, and/ or to regenerate injured tissues.

Several procedures target preservation instead of replacing femoral heads of patients with osteonecrosis. Cell infusion and femoral head decompression is an alternative combined technique that has been evaluated by various groups since 2000[4], which was shown to provide satisfactory results, and this was the encouraging factor for us to conduct this study. The central decompression technique employs one or many 8

– 10 mm trephine as a strategy to address a larger portion of osteonecrotic lesion. This is the most frequently used method, but its invasive effects are controversial[15]. Consistently with Heningou e Beaujeanl[8], our protocol employs a 3-mm trephine in a single access port to the necrotic area driven by radiographic monitoring. This technique does not fully compare to central decompression, allowing a less invasive and more appropriate procedure for the extension of the disease.

Osteogenic and angio/vasculogenic properties of the BMMC fraction are well established. The BMMC fraction enhances vascularization and the oxygen flow to ischemic tissues, in addition to accelerate fractures healing in chronic arthritis[20]. A single injection of mononuclear cells into bone's necrotic area is expected to result in a process of bone neoformation and repair, which sometimes occurs spontaneously[19]. In theory, hemangioblastic and osteogenic bone marrow stem cells can repopulate segments of necrotic bone with viable and active cells. Although these topics have not been addressed in our study, future studies are warranted to evaluate such hypotheses.

The most important success factor for cell therapy on femoral osteonecrosis is to eliminate or delay femoral collapse and the need for further surgical procedures, particularly arthroplasty. The results of this study are promising, although its interpretation is limited by the small number of patients and by the brief follow-up time. X-ray evidences of the femoral bone tissue regeneration are expected from the 12th postoperative month on, and the bone structure stabilization must be followed up over many years.

Future assays must be carried out in order to weight the success of this technique with a longer follow-up time. Specific indications and contra-indications to this method will be based on the population being studied. Furthermore, cell parameters should be clearly established in order to properly correlate the BMMC injection with success rates on femoral osteonecrosis treatment.

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