Core decompression and autologous bone marrow concentrate for treatment of femoral head osteonecrosis: a randomized prospective study

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Abstract

The aim of this study was to investigate the safety of injection of bone marrow aspirate concentrate during core decompression and to study its clinical (visual analogue scale; Harris-Hip-score) and radiological outcomes (magnetic resonance imaging). In this prospective and randomized clinical trial we evaluated 24 consecutive patients with non-traumatic femoral head necrosis (FHN) during a period of two years after intervention. *In vitro* analysis of mesenchymal stem cells was performed by evaluating the fibroblast colony forming units (CFU-Fs). Postoperatively, significant decrease in pain associated with a functional benefit lasting was observed. However, there was no difference in the clinical outcome between the two study groups. Over the period of two years there was no significant difference between the head survival rate between both groups. In contrast to that, we could not perceive any significant change in the volume of FHN in both treatment groups related to the longitudinal course after treating. The number of CFU showed a significant increase after centrifugation. This trial could not detect a benefit from the additional injection of bone marrow concentrate with regard to bone regeneration and clinical outcome in the short term.

Introduction

Aseptic non-traumatic avascular osteonecrosis of the femoral head is a multifactorial disease, the etiology of which is not entirely clear. Femoral head necrosis (FHN) most commonly affects young patients, often leading to femoral head collapse and resulting secondary osteoarthritis.[1] Around 10% of all THA are performed due to FHN.[2] In the early stages of the disease [Association Research Circulation Osseous (ARCO) stage I - II] the treatment aims to preserve the joint and to prevent the collapse of the femoral head. One of the most widely used treatment options is the core decompression by retrograde drilling into the necrotic zone. Systematic reviews verified the significantly better head survival rates compared to non-operative treatment options.[2] Based on the data by Mont *et al.,* core decompression leads to clinical success in 53-71% of treated patients leaving room for further therapy improvement. Mesenchymal stem cells and osteoblasts that could potentially induce bone formation have been shown to be decreased in both number and activity in afflicted bone. Therefore the local application of autologous mesenchymal stem cells (MSC) into the necrotic region could stimulate the regeneration of the affected bone. MSCs can be isolated from the mononuclear cell fraction of bone marrow and expanded *in-vitro* to high-cell numbers using laboratory equipment and then injected into the necrotic zone.[3] Bone marrow expansion is subject to restricted regulatory laws in many countries.Introduction of a single-use closed system would allow the surgical team to carry out the concentration procedure in the operating room.[4] Hernigou *et al.* introduced the technique of intra-osseous injection of autologous concentrated bone marrow for the treatment of femoral osteonecrosis and pseudarthrosis.[5] They reported on the results of core decompression with additional bone marrow grafting in 145 hips with early stage osteonecrosis (ARCO I - II). At 5 years after surgery, the head survival rate in their study was 93%, which would appear to be a considerable improvement over previous studies. Although this prospective study included an impressive number of patients, a control group and a randomized study protocol were missing. The aim of this study was i) to investigate the safety of additional injection of bone marrow aspirate concentrate (BMAC) during core decompression and ii) to study the clinical (visual analogue scale, VAS; Harris-Hip-Score, HHS) and radiological outcome (magnetic resonance tomography, MRI) in comparison to core decompression only. In addition, in vitro analysis of MSCs was performed by evaluating the fibroblast colony forming units (CFU-F's).

Materials and Methods

Study design

In this prospective and randomized clinical trial we evaluated 24 consecutive patients (25 hips) with non-traumatic FHN during a period of two years after intervention. All patients signed the informed consent, the institutional review board of the hospital approved the study; all procedures were performed in accordance with the Declaration of Helsinki. The inclusion criteria for this study were age over 18 years and the presence of stage II femoral head osteonecrosis according to the Association of Research Circulation Osseous (ARCO) classification. Before inclusion into the study all patients were evaluated with two plane radiographs and an MRI of the affected hip. At the day of the procedure patients were randomized in two groups using a simple randomization method based upon sequential patient allocation: core decompression only *vs.* core decompression with the application of BMAC. For clinical outcome the VAS and HHS were measured. For radiological outcome the volume of the necrotic zone and the stage according to ARCO classification was evaluat-

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Key words: Femoral head necrosis; core decompression; mesenchymal stem cells; autologous bone marrow concentrate.

Contributions: WP has made substantial contributions in carrying out the experiments including cell centrifugation during the operations, carrying our data evaluation and has drafted and revised the manuscript; PK has made substantial contributions in the design of the study; NAB has made substantial contributions in assisting in data evaluation and helping to draft and revise the manuscript; PJ has made substantial contributions in carrying out in vitro analysis of MSC assisting in data evaluation; ME has made substantial contributions in carrying out the design of the study, performing the core decompression and BMAC instillation, assisting in data evaluation and helped to draft and revise the manuscript.

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ed with an MRI. The subsequent course of osteonecrosis was evaluated at 12 and 24 months postoperatively. This study was approved by the ethics committee of University of Heidelberg, Germany.

Core decompression procedure

In all patients a core decompression procedure was performed under general anesthesia. Under the guidance of an image intensifier in two planes, three 2.0 mm K-wires (Kirschner wires) were drilled through the major trochanter and along the femoral neck axis into the femoral head, reaching the subchondral necrotic area (2-3 mm from the articular cartilage). The most centrally placed K-wire in the necrotic zone was over drilled using 5 mm trephine as has been previously described in the literature.

Bone marrow aspiration, processing and instillation

The harvesting of autologous bone marrow was performed by percutaneous aspiration from the ventral iliac crest (both sides) using a bone marrow biopsy device. The initial volume of harvested marrow was 200 to 220 mL. Intra-operative processing and concentration of stem cells was performed using a Sepax centrifugation device from Biosafe SA (Eysins, Switzerland) with a volume reduction protocol that isolates the nucleated cell (NC) fraction in the buffy coat. After cell segregation, the erythrocytes, nucleated cells and plasma were automatically decanted. 12 mL of bone marrow concentrate suspension was isolated. 2 mL of the final cell concentrate volume (BMAC) was collected for further experimental investigations. 10 mL of BMAC was instilled into the necrotic zone through the canulated trephine drill after removal of the central k-wire.

In vitro analysis of mesenchymal stem cells

The number of MSCs within the native bone marrow and the bone marrow concentrate was analyzed using the CFU-F's. Assuming that each adherent MSC is able to form a fibroblast colony, the number of MSCs within a cell culture could be estimated. Per donor, 2×10^6 mononucleated cells (MNC) out of the native bone marrow and the BMAC were seeded into three T25 tissue culture flasks each and cultured in standard medium consisting of high glucose DMEM, 20% fetal calf serum (FCS), 1% penicillin/streptomycin, 10 ng/mL recombinant human epidermal growth factor (EGF) and 10 ng/mL recombinant human platelet-derived growth factor (PDGF). After seven days of culturing, cells were washed with phosphate buffered saline (PBS), fixed with 70 % ethanol stained with toluidin blue. Fibroblast colonies were counted manually using a microscope at 25th magnification. (Modified protocol from Castro-Malaspina *et al*; 1980).[6]

Statistics

Data were expressed as mean or mean ± SEM. The nominal data of patients' characteristics were tested using the test and χ^2 Fisher exact test. The distribution of continuous data was determined using the Kolmogorov-Smirnov test and the appropriate parametric or non-parametric test was selected. Differences within the groups for radiological and clinical outcome data were compared with the Wilcoxon test and between the groups – with Mann-Whitney test. A $P<0.05$ was considered statistically significant. For radiological evaluation MRI scans of 25 hips before surgery, 20 hips at 1 year and 15 hips at 2 years after operation were available due to the presence of endoprosthesis and other reasons. For longitudinal and transverse comparison of the volume of the osteonecrosis, only data of patients with full radiological observation was analyzed. Calculation of FHN volume was performed using GE Healthcare RIS/PACS software (Milan, Italy). SPSS (SPSS Inc., Chicago, IL, USA) and Graph Pad Prism software (Graph Pad, La Jolla, CA, USA) were used for statistical analysis.

Results

Twenty-five hips of 24 patients with femoral head osteonecrosis were included into this study between 2008 and 2010 and assigned by random to the treatment method (14 in control group, 11 in verum group). All 25 hips were fully evaluated clinically (VAS: preoperatively, 3 months postoperatively, 1 and 2 years after treatment; HHP: preoperatively, 3 months, 1 and 2 years after treatment) and if feasible evaluated radiographically (MRI preoperatively, 1 and 2 years after treatment). Both study populations did not significantly differ in age, gender, side of FHN and risks factors for

Table 1. Relevant clinical characteristics of patients with femoral head necrosis before operative procedure.

Characteristics	CD (n=14)	CD with BMAC (n=11)	P-value
Age (years)	44.5±3.3	44.3±3.4	n.s.
Gender (m/f)	12/2	10/1	n.s.
Side (left/right)	8/6	6/5	n.s.
Chemotherapy	2	0	n.s.
Immunosuppressive therapy	3	1	n.s.

CD, core decompression; BMAC, bone marrow aspiration concentration; n.s., not significant.

Table 2. Number of nucleated cells and CFU within the bone marrow of verum group patients before and after processing using SEPAX centrifugation device.

Parameters	Native	SEPAX	Concentration	P-value
Nuclear cells (10^6 cells/mL)	18.9±3.1	118.9±15.1	6,3-fold	<00001
f-CFU (20%FCS/2Mio)	33.0±9.5	50.0±15.9	1,5-fold	<0.0178

f-CFU, colony forming units.

Table 3. A comparison of head survival rates at two to five years after core decompression and BMAC (bone marrow aspiration and concentration) application or similar bone marrow treatment options in femoral head necrosis in recently published studies.

Literature (year)	ARCO	Survival rate verum	Survival rate control	P-value
Hernigou *et al.*[33] (2002)	I-III*	155/189 (82%)	No control	-
Gangji *et al.*[40] (2004)	I-II	9/10 (90%)	3/8 (37.5%)	0.016
Gangji *et al.*[41] (2011)	I-II	10/13 (77%)	3/11 (27%)	<0.05
Zhao *et al.*[10] (2012)	I-II	51/53 (96%)	34/44 (77%)	<0.05
This study (2015)	II	7/11 (64%)	8/14 (57%)	>0.05

ARCO, Association Research Circulation Osseus. *Hernigou *et al.* used Steinberg classification for recruiting patients in his study (Steinberg I-IV).

osteonecrosis (steroid therapy in the patients' history) as shown in Table 1. According to the inclusion criteria all patients suffered from an ARCO II FHN and had no prior trauma. No side effects (hematoma, infection, nerve injury and others) either from the bone marrow aspiration from the pelvic rim or from the injection into the femoral head were observed during the entire study period.

Clinical and functional outcome

The clinical and functional outcome was measured using standardized scores (VAS; HHS). At the time of inclusion into the study there was no significant difference between the two groups regarding pain and function. Postoperatively, significant decrease in pain associated with a functional benefit lasting the entire observation period was observed. However, there was no difference in the clinical outcome between the two study groups (Figures 1 and 2).

Radiological outcome

Before treatment there was no significant difference between the mean volumes of the osteonecrosis in both groups. In contrast to the clinical outcome, we could not perceive any significant change in the volume of FHN in both treatment groups related to the longitudinal course after treating (Figure 3). Again no statistically significant difference was detected between the groups. FHN progressing to ARCO III or IV was defined as failure of the treatment. This includes all patients with joint replacement (total hip arthroplasty) due to the FHN within two years in both groups. Over the period of two years there was no significant difference between the head survival rate of 8/14 (57%) in the control group, and 7/11 (64%) in the verum group. There was no difference between the two groups with regard to the interval between core decompression with or without BMAC application and THA.

Bone marrow

The bone marrow was analyzed before and after the centrifugation procedure using the Sepax centrifugation device. Table 2 shows the significant increase in the number of nucleated cells due to the centrifugation step. Additionally, the number of CFU that best represents the number of MSC shows a significant increase (Table 2).

Discussion

Femoral head necrosis is a painful disease usually afflicting young male patients and the natural course of this disease tends to be progressive and ends in secondary osteoarthritis.[7] In late stage osteonecrosis (ARCO III and IV), total hip arthroplasty (THA) currently seems to be the best treatment with good functional restoration. However, the young patient age and subsequent expected life span is substantially longer than the currently expected longevity of primary THA. Early recognition of FHN and lastly established less invasive treatment modalities open new possibilities to treat this disease for preservation of the spherical femoral head shape indicating a better outcome and finally,[8] avoiding THA. One of these operations is the core decompression procedure[9-11] and the literature indicates that it is superior to non-operative therapy.[12,13] It has been shown that core decompression leads to significant post-operative pain reduction in early stage FHN.[2,14] Nevertheless, in postoperative MRI and patho-morphologic trials it could be shown that no significant repair process in the necrotic area of FHN could be achieved with core decompression alone,[15] making it a controversial procedure and calling for additional alternative treatment options. The long term results of this procedure are mainly influenced from the initial stage of the osteonecrosis with best prognosis for ARCO I and worst for ARCO III and IV. The outcome of the non-reversible early stage ARCO II is still under discussion. Therefore we included ARCO II hips in this study only.

The injection of mesenchymal progenitor cells into the osteonecrosis seems to improve the outcome of FHN as shown by Hernigou *et al.*[16] The application of *ex vivo* cultivated stem cells for treatment of diseases of the musculoskeletal system is possible, but currently requires advanced laboratory and technical effort. In addition, this two-step procedure is associated with a higher risk of complications and higher costs. On the other hand, cell therapy systems using centrifugation steps were recently developed which permit intra-operative enrichment of mesenchymal progenitor cells from BMAC simultaneously at the time of core decompression followed by application of the BMAC into the necrotic area of FHN at the end of the same procedure. Simultaneous application of BMAC is praised as an encouraging approach. Hernigou *et al.* (2002, 2005)

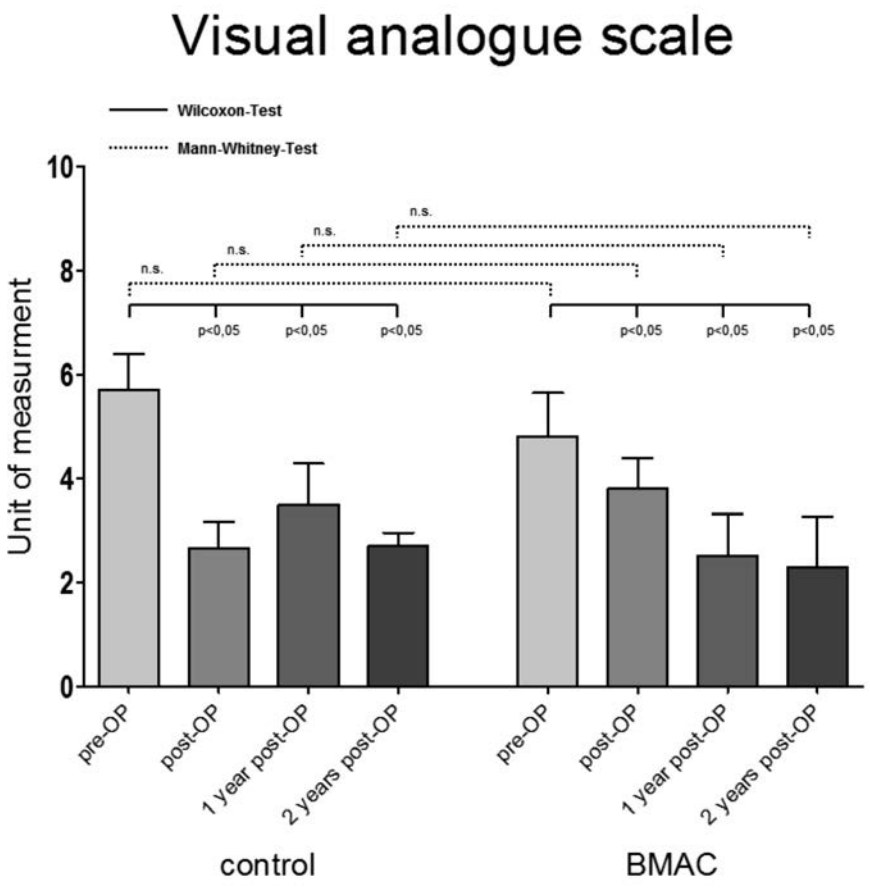

Figure 1. Pre- and postoperative outcome of patients referring to visual analogue scale after core decompression vs. core decompression with bone marrow aspirated and concentrated application (BMAC). Significance value P<0.05; n.s., not significant.

inaugurated this treatment option and published a large patient series, that demonstrates an obvious improvement in the head survival rate compared to other literature data with core decompression only.[5,16] Lastly, Civinini *et al.* showed good results of treating early stage femoral head necrosis using injectable calcium and sulphate/calcium bioceramic with bone marrow concentrate.[17]

The first aim of our investigation was to evaluate the safety of the additional aspiration and injection of autologous bone marrow. In our study we did not encounter any complications resulting from this method of bone marrow transplantation. In particular, there were no infections, excessive new bone formation or tumor induction and there were no local complications at the harvesting side. These findings are in line with published data showing the very low risk of this one step procedure.[18]

The second aim of our study is the effect of the additional injection of BMAC into the femoral head on the clinical and radiological outcome. We found that significant pain relief and functional improvement could be achieved in both groups at the 2 year follow up independent of the operative procedure (core decompression or core decompression with BMAC application). There are a limited number of studies evaluating pain and functional gain after core decompression, and a direct comparison with these studies is difficult. Rajagopa *et al.* performed a systematic review of four studies evaluating core decompression of femoral head necrosis and its effect on pain and function of the hip over a minimum of two years postoperatively.[19] The results of their analysis showed a variable gain of function after core decompression procedure. One of the articles quoted demonstrated a significant improvement in hip function,[20] a second one just moderate improvement[21] and two other studies only minor, or no improvement.[22,23] Our study supports the latter. We did not note any significant differences relating to pain or functional state of patients between both groups, supporting the hypothesis, that additional BMAC application after core decompression provides no benefit to patients with FHN at this time. This finding seems to be in contrast to the studies of Hernigou *et al*. However, the study presented here was set up as a randomized controlled trial with restrictive inclusion criteria (ARCO II only). The rejection of ARCO I patients with a better prognosis might, at least in part, explain differences to the results of Hernigou *et al.* and other studies. Table 3 shows a comparison of head survival rate of this study and recently published studies using BMAC or similar bone marrow treatment options in femoral head necrosis. Because of different study protocols, meaningful comparison of these research findings is limited.

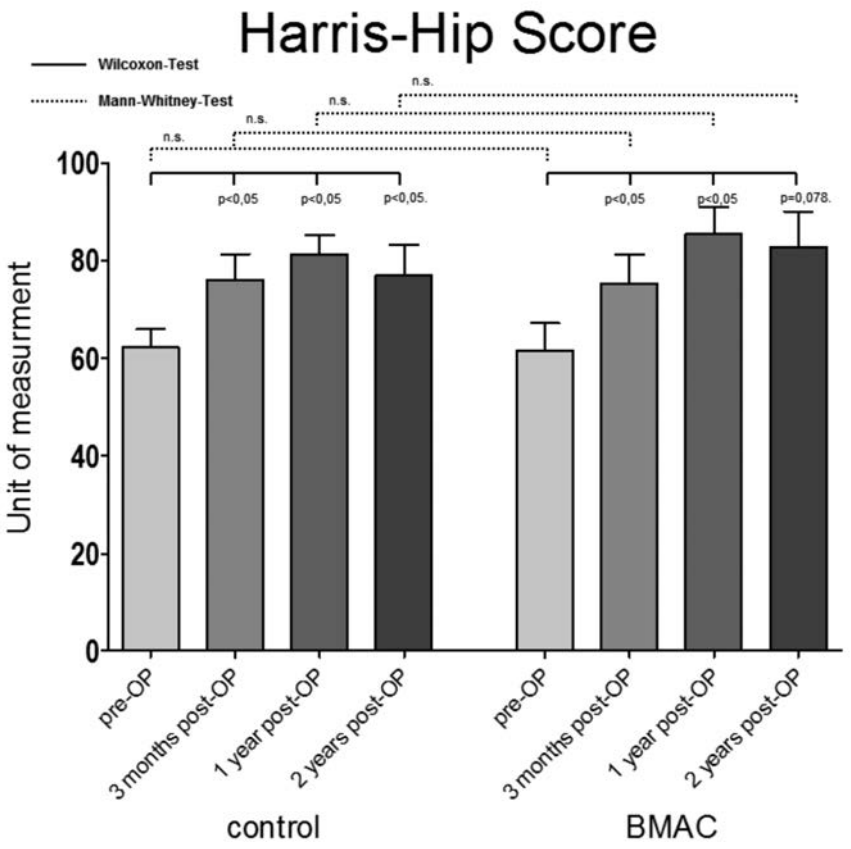

Figure 2. Pre- and postoperative functional outcome of patients measured with Harris-Hip Score after core decompression vs. core decompression with bone marrow aspirated and concentrated application (BMAC). Significance value P<0.05; n.s., not significant.

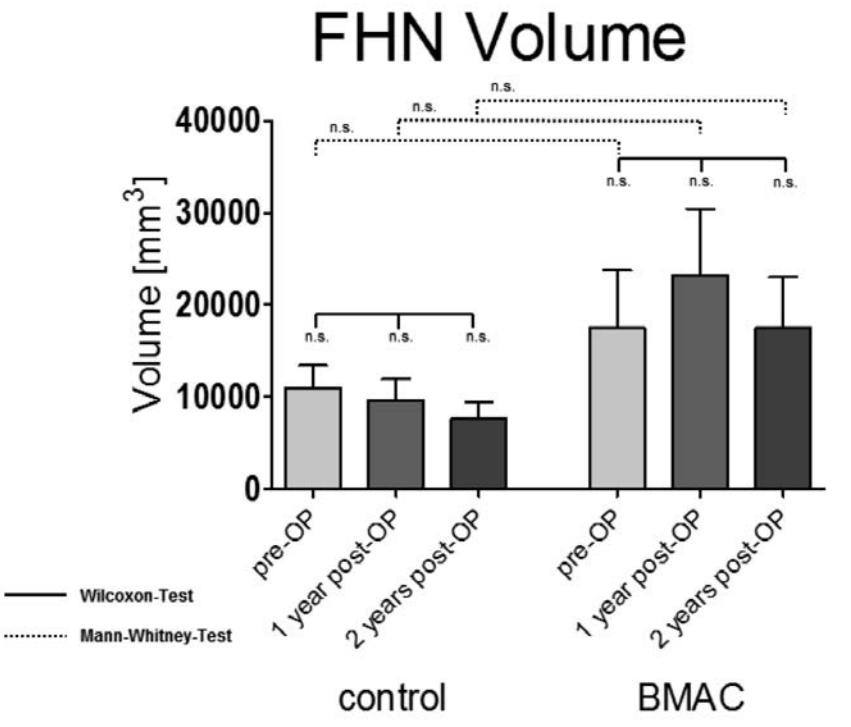

Figure 3. Pre- and postoperative femoral head necrosis (FHN) volume of patients after core decompression vs. core decompression with bone marrow aspirated and concentrated application (BMAC). Significance value P<0.05; n.s., not significant.

A study with a protocol similar to ours was published by Gangji *et al.*[24] They presented the results of a randomized and prospective clinical trial of ARCO I-II patients treated with core decompression only vs. core decompression with autologous bone marrow application. Femoral head survival rate of this study is also mentioned in Table 3. Gangji *et al.* also describe an increased head survival rate in the BMAC group. Although this is a randomized controlled prospective trial, there are some principal limitations to this study: there is a short follow-up period (two years) and there are a small number of patients in both study groups. The small study population is a result of the prospective design and very restrictive inclusion criteria of FHN patients (ARCO II only). In comparison to the study presented by Hernigou *et al.*,[16] the major advantage of this study is the comparison of a BMAC group with a control group, thereby increasing explanatory power.

Conclusions

Femoral head necrosis with a spherical head and irreversible necrosis of the bone (ARCO II) profits from core decompression. In contrast to previous series the current study excluded ARCO I stages of FHN which can regenerate on its own, and inclusion of ARCO I makes interpretation of drilling of this stage difficult. This trial of 25 hips could not detect a benefit from the additional injection of bone marrow concentrate with regard to bone regeneration and clinical outcome in the short term. Further studies of BMAC properties with a larger sample size and longer follow-up are needed to better validate our results and possibly modify our procedure.

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