Bone marrow aspirate concentrate in combination with intravenous iloprost increases bone healing in patients with avascular necrosis of the femoral head: a matched pair analysis

Hakan Pilge,[1] Bernd Bittersohl,[1] Johannes Schneppendahl,[1] Tobias Hesper,[1] Christoph Zilkens,[1] Martin Ruppert,[1] Rüdiger Krauspe,[1] Marcus Jäger[2]

[1]Department of Orthopedics, Düsseldorf University Clinic, Düsseldorf, Germany; [2]Department of Orthopedics and Traumatology, University Clinic Essen, Essen, Germany

Abstract

With disease progression, avascular necrosis (AVN) of the femoral head may lead to a collapse of the articular surface. The exact pathophysiology of AVN remains unclear, although several conditions are known that can result in spontaneous cell death, leading to a reduction of trabecular bone and the development of AVN. Hip AVN treatment is stage-dependent in which two main stages of the disease can be distinguished: pre-collapse (ARCO 0-II) and post-collapse stage (ARCO III-IV, crescent sign). In the pre-collapse phase, core decompression (CD), with or without the addition of bone marrow (*e.g.* bone marrow aspirate concentrate, BMAC) or bone graft, is a common treatment alternative. In the post-collapse phase, THA (total hip arthroplasty) must be performed in most of the patients. In addition to surgical treatment, the intravenous application of Iloprost has been shown to have a curative potential and analgesic effect. From October 2009 to October 2014, 49 patients with AVN (stages I-III) were treated with core decompression at our institution. All patients were divided into group A (CD + BMAC) and group B (CD alone). Of these patients, 20 were included in a matched pair analysis. The patients were matched to age, gender, ARCO-stage, Kerboul combined necrotic angle, the cause of AVN, and whether Iloprost-therapy was performed. The Merle d'Aubigné Score and the Kerboul combined necrotic angle in a-p and lateral radiographs were evaluated pre- and postoperatively. The primary endpoint was a total hip arthroplasty. In group A, two patients needed THA while in group B four patients were treated with THA. In group A, the Merle d'Aubigné Score improved from 13.5 (pre-operatively) to 15.3 (postoperatively). In group B there was no difference between the pre- (14.3) and postoperative (14.1) assessment. The mean of the Kerboul angle showed no difference in both groups compared pre- to postoperatively (group A: pre-op 212°, postop 220°, group B: pre-op 213, postop 222°). Regarding radiographic evaluation, the interobserver variability revealed a moderate agreement between two raters regarding the pre- (ICC 0.594) and postoperative analysis (ICC 0.604).This study demonstrates that CD in combination with the application of autologous bone marrow aspirate concentrate into the femoral head seems to be a safe and efficient treatment alternative in the early stages of AVN of the femoral head when compared to CD alone.

Introduction

Avascular necrosis (AVN) of the femoral head refers to a condition in bone metabolism when cells of the trabecular bone spontaneously die. Depending on the amount of femoral head involvement, the articular surface may collapse as the disease advances.[1,2]

The exact pathophysiology of AVN of the femoral head remains unclear, despite various attempts to develop a theoretical model of the disease. Several conditions and environmental factors increasing the patient's risk of developing femoral head AVN have been recognized:[3] alcohol abuse, corticosteroids, idiopathic, smoking, radiation therapy, pregnancy, chronic renal failure, caisson disease, systemic lupus erythematosus, Cushing's disease, organ transplantation, sickle cell disease, chronic pancreatitis, coagulopathy, lipid disorders. In this matter, the most common causes include alcoholism (20-40%) and corticosteroid therapy (35-40%), whereas about 20-40% of cases of femoral head AVN are idiopathic.[4] Many patients may be initially asymptomatic; however, AVN of the femoral head is likely to progress to joint destruction requiring total hip arthroplasty (THA), usually before the fifth decade. Of note, 5-18% of THA surgeries are performed on patients diagnosed with avascular necrosis of the femoral head.[4]

Staging

Based on radiographs of the pelvis, Ficat initially developed a staging system. The most important consideration is the collapse of the femoral head cortex (so-called *crescent sign*). The 1992 ARCO classification (ARCO: Association of Research Circulation Osseous) also uses the crescent sign to separate early- from late-stage AVN.[5] Unlike the Ficat classification, the ARCO classification uses magnetic resonance imaging (MRI) in addition to radiographs to increase sensitivity and specificity, making this the preferred classification at our institution.

Correspondence: Hakan Pilge, University Hospital Düsseldorf, Orthopedic Department" then continuing with Moorenstrasse 5, 40225 Düsseldorf, Germany.
Tel.: +49.211.81.18314 - Fax: +49.211.81.16693.
E-mail: hakan.pilge@med.uni-duesseldorf.de

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Treatment

The treatment of AVN of the femoral head is stage-dependent, whereas two broad categories of the disease can be distinguished: pre-collapse (ARCO 0-II°) and post-collapse (ARCO III°-IV°). Core decompression (CD), with or without the addition of bone marrow or bone graft, is one treatment option for pre-collapse osteonecrosis (ON) of the femoral head. If the disease process is halted in the pre-collapse phase, patients may avoid THA or other salvage-procedures. In addition to surgical treatment, the intravenous application of Iloprost has been proven to have a curative potential and analgesic effect, even though it still represents an off-label treatment option.[6,7] As an insufficient supply of progenitor cells in the area of necrosis is underlying,[8] newer treatment modalities have been developed in the past years to introduce stem cells to these regions in an attempt to prevent a collapse of the femoral head.[1,3,8-10] In such procedures, several thousand bone marrow stem cells, harvested from the iliac crest, are implanted to the femoral head right after core decompression. In cases where the femoral head is collapsed (ARCO III-IV°), the need for THA is common.

Materials and Methods

From October 2009 to October 2014, core decompression was randomly combined with

the application of bone marrow aspirate concentrate (BMAC), or not, at our institution. Altogether, we identified 49 patients with an AVN of the femoral head who underwent core decompression without other surgical procedures in their history. Inclusion criteria were i) the presence of AVN in the femoral head and ii) core decompression performed at our institution. Patients who received surgery other than CD and those who had any other therapy prior to surgery were excluded from this retrospective analysis. In total, 45 patients from both groups underwent intravenous iloprost-therapy for one week after surgery according to a standardized protocol (Table 1).

During that period, ten patients with AVN of the femoral head were treated with both CD and BMAC. These ten patients were matched by age, gender, ARCO-stage, Kerboul combined necrotic angle, the cause of AVN, and whether Iloprost-therapy was performed (Table 2). Patients were categorized into two groups: Group A = treatment with CD plus BMAC and Group B = CD without BMAC. In all 49 patients, the Merle d'Aubigné Score was evaluated, and two authors (HP, MR) evaluated radiographs and MRIs of all patients pre- and postoperatively.

Operating technique: bone marrow harvesting, concentration, and local application

After surgical preparation and draping (BMAC™, Harvest Technologies GmbH), bone marrow aspirate (BMA) was obtained from the ipsilateral iliac crest, according to the manufacturer's instructions, using a size 6-lumen Jamshidi type trocar needle. A total of 60 mL of BMA was extracted with 20 mL syringes, which were pre-flushed with heparin (concentration of 1000 units/mL) (Liquemin, Roche, Grenzach-Wyhlen, Germany). The BMA was then concentrated using the SmartPReP™ 2 centrifuge workstation in the operating suite. Between 7 and 10 mL of the BMAC were then transferred back to the sterile operating field. Samples of BMA and BMAC were sent to our laboratory to determine the concentration of each sample.

A small lateral approach to the femur was performed; a decompression tunnel was made using a trephine through the lateral femur and femoral neck into the necrotic lesion in the subchondral area (Figure 1). Its position in the femoral head and the necrotic segment was monitored using fluoroscopy. The femoral head was rotated in the acetabulum to obtain various radiographic incidences of the head to rule out unrecognized joint penetration. The medial part of the bone core was sent for pathological examination, and the lateral part was incubated in BMAC for 15 minutes. The bone core obtained was then plugged back into the decompression tunnel. Wound was closed in layers.

Inter-observer variability

Two authors evaluated the Kerboul-combined necrotic angle on radiographs (a-p pelvis and lateral view). Both readers (JS, TH) were blinded to the specific therapy of each patient. Readers were not provided with the recorded measurements of the other observer during evaluation of the radiographs. The inter-observer variability was calculated.

Statistical analysis

Statistical analyses were performed using SPSS Statistics 23 (IBM analytics). The student s t-test and the Mann-Whitney test were utilized to identify statistically significant differences between both treatment groups. Statistical power was calculated with the two-sided one-sample t-test with alpha = 0.2 for both groups. Data are given as mean ± standard error of the mean (SEM). Inter-observer agreement was determined by calculating the intra-class correlation coefficient (ICC).

Results

Merle d'Aubigné Score

The Merle d'Aubigné Score evaluates pain, ability to walk and range of motion (ROM). All three categories have 1 to 6 points. A maximum of 18 points means that the patient has no pain, is able to walk without impairment and has a full ROM.

Results of the Merle d Aubigné Score for all patients are given in Table 2. Normality test was passed in both treatment groups (A, BMAC: P=0.234; B, nBMAC: P=0.189). Mean Merle d'Aubigné score for group A increased from 13.5±1.0 to 15.3±0.8 points, whereas the mean score in group B decreased from 14.3±0.8 to 14.1±1.0 points. In both groups, this difference in the Merle d'Aubigné Score was not statistically significant.

Patients in group A had less pain when comparing pre- to postoperative symptoms, had a better ability to walk and had an increased ROM. Patients in group B had only a slight difference concerning pain. Walking ability decreases after the postop evaluation and ROM showed no change from pre- to postoperative evaluation (Table 3).

Notably, power calculation in both groups revealed that the number of patients in this study is too small to predict a difference. With SD=3, power=0.8, and alpha=0.2 a total patient number of 20 patients is recommended statistically.

Kerboul-combined necrotic angle and inter-observer variability of radiographs

The Kerboul-combined necrotic angle was independently evaluated by two blinded observers (JS and TH). Plain radiographs were used to determine the Kerboul-combined necrotic angle. Although we always had excellent quality radiographs in all patients, both authors noted that evaluation of the necrotic angle was not easy in all cases. Figure 2 shows an easily detectable angle and Figure 3 shows a necrotic area which is not as easy to measure. Obtaining an MRI may help in such cases. In this study, a moderate agreement was noted for the preoperative (ICC=0.594) and postoperative (ICC=0.604) Kerboul angle measurement.

Iloprost

No serious adverse reactions due to infusion with Iloprost were recorded. Three patients (15%) had flush symptoms, and two patients (10%) complained of a mild headache during the Iloprost-infusion. In one patient, there was a contraindication for Iloprost and, therefore, Iloprost was not administered (Pat No.15). One patient (Pat No.11) did not consent for Iloprost therapy.

Table 1. Iloprost scheme.

Body weight, kg	Day 1, mL/h (0.5 ng/kg/min)	Day 2, mL/h (0.75 ng/kg/min)	Day 3-5, mL/h (1.0 ng/kg/min)
30	1.10	1.70	2.25
40	1.50	2.25	3.00
50	1.90	2.85	3.75
60	2.20	3.40	4.50
70	2.60	0.40	5.30
80	3.00	4.50	6.00
90	3.40	5.10	6.80

Total hip arthroplasty

In group A, there were two patients with total hip arthroplasty (THA). In group B, there were four patients with THA.

Discussion

In patients with AVN of the femoral head, the blood supply to the head of the femur is interrupted for a number of reasons. As a consequence, the trabecular bone may become necrotic. Spontaneous regression of AVN is rare and AVN progresses to osteoarthritis in most untreated patients, meaning that THA is necessary.[11,12] Several treatment options attempting to halt the progression of AVN, including core decompression, corrective osteotomy and medical treatment alone, have shown disappointing results, with up to 40% of patients needing THA.[13]

To the best of our knowledge, this is the first study comparing the application of BMAC *vs.* non-BMAC in addition to core decompression and Iloprost application in a matched pair analysis. The use of concentrated mononuclear cells after CD and Iloprost therapy seems to show synergistic effects and provides significantly better clinical outcomes than treatment with CD and Iloprost alone.

Although many risks factors have been reported an underlying cause for AVN can often not be determined. Cases without a clear cause for AVN may be due to undetected collagen mutations or clotting abnormalities. Liu *et al.* found that a *COL2A1* gene mutation in all members of three families predisposed to the development of AVN by autosomal dominant inheritance.[14] Jones *et al.* report that 82% of 45 patients with AVN had at least one coagulation factor abnormality *versus* 30% in the healthy control group.[15] Another working hypothesis is that cell death is caused by an increase in pressure in the femoral head, leading to decreased blood flow and cell death caused by a mechanism comparable to a compartment syndrome.[13]

Table 2. Data of n=20 patients included in this matched pair study.

Match	Age at OP	Gender	Iloprost therapy	Cause of AVN	Right/ left	ARCO	BMAC	FU, month	Kerboul pre	Kerboul post	Merle pre	Merle post	THA
1	42	m	y	ankylosing spondylitis, corticoid therapy	r	2	y	69	200	190	9	18	-
1	43	m	y	ankylosing spondylitis, corticoid therapy	l	2	n	57	210	200	17	16	-
2	16	m	y	idiopathic	l	3	y	49	200	200	13	15	-
2	17	m	y	idiopathic	r	3	n	39	190	200	16	13	-
3	35	m	y	idiopathic	l	2	y	4	270	260	16	17	-
3	35	m	y	idiopathic	l	2	n	5	280	280	16	13	THA
4	53	m	y	germ cell tumor, chemoth, radioth	l	2	y	6	200	200	16	16	-
4	44	m	y	anal carcinoma, chemoth	l	2	n	5	200	200	15	12	THA
5	40	m	y	allerg.asthma, corticoid therapy	l	2	y	60	200	200	15	18	-
5	35	m	y	idiopathic, smoker	r	2	n	39	200	200	10	16	-
6	19	f	n	bone tuberculosis, chemoth	l	4	y	42	180	220	9	12	-
6	15	f	y	leukemia, chemoth	l	4	n	20	220	240	15	8	THA
7	43	m	y	idiopathic	r	3	y	37	230	230	15	14	THA
7	54	f	y	idiopathic	l	3	n	35	220	220	14	16	-
8	34	m	n	testicular carcinoma, chemoth	l	2	y	25	180	220	18	17	-
8	41	m	y	testicular carcinoma, chemoth	l	2	n	28	180	180	16	18	-
9	43	m	y	idiopathic	r	2	y	25	240	260	14	16	-
9	46	m	y	idiopathic	l	2	n	35	220	280	15	18	-
10	58	m	y	corticoid therapy, skin disease	l	3	y	24	220	220	10	10	THA
10	54	m	y	corticoid therapy, kidney transplant	r	3	n	7	210	220	9	11	THA

OP, operation; AVN, avascular necrosis; ARCO, Association of Research Circulation Osseous; BMAC, bone marrow aspirate concentrate; FU, follow-up; THA, total hip arthroplasty.

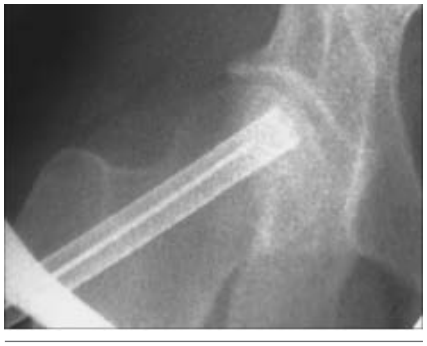

Figure 1. The decompression tunnel was made using a trephine through the lateral femur and femoral neck into the necrotic lesion in the subchondral area.

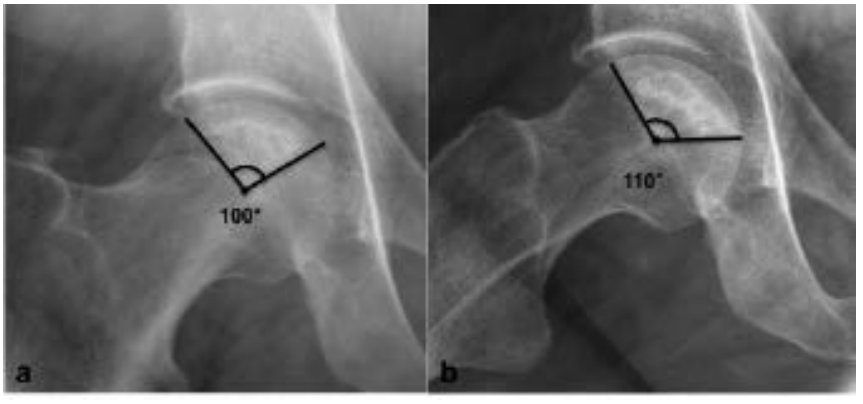

Figure 2. Easy detectable Kerboul angle.

Furthermore, bone regeneration is modulated by progenitor cells and various cellular mediators including angiogenic growth factors,[1,16] bone morphogenetic proteins (BMP),[17,18] interleukins and cytokines,[19] all of which have been reported to treat bone defects. In 2002, Hernigou and Beaujean published a technique to treat osteonecrosis with mesenchymal stem cells via autologous bone marrow grafting.[20] In their study including 116 patients (189 hips), BMAC was applied through a core decompression track to the necrotic area. Patients in the pre-collapse phase (ARCO 0-II°) had excellent results with only 6% (9 of 145) of the hips requiring THA within a five-year clinical follow-up.[20] In the same period, 57% (25 of 44) of the hips that were in a post collapse phase (ARCO III-IV°) preoperatively required THA.

Bone marrow concentrates isolated by the Harvest System (used by Hernigou *et al*. and used in this study) contained identical numbers of myelocytes, granulocytes, lymphocytes, monocytes, proerythroblasts and erythroblasts compared to the initial bone marrow aspirate.[21] It was also observed that the concentrated bone marrow-derived cells preserve their function[21] and that a greater number of progenitor cells transplanted correlated with better outcomes.[8]

Rastogi *et al.* compared patients with AVN (stage I-III) of the femoral head treated either with core decompression and isolated mononuclear cells (group A) or with core decompression and unprocessed bone marrow injection (group B). The follow-up at a minimum of 2 years revealed a considerable improvement in the hip function, as measured by the Harris hip score in group A (78.6) and group B (66.8). On MRI, the size of the lesion significantly decreased in group A, whereas 10% of the patients in group B required total hip replacement. The authors conclude that the better outcome in patients with osteonecrosis of the proximal femur is due to the higher number of progenitor cells and angiogenetic factors in concentrated mononuclear cell transplantation.[22]

CD34-positive cells, which include hematopoietic and endothelial precursor cells, were more commonly detected in the mononuclear cell fraction than in unprocessed bone marrow or peripheral blood. It is postulated that the fraction of endothelial cells stimulates the angiogenesis in osteonecrotic hips and

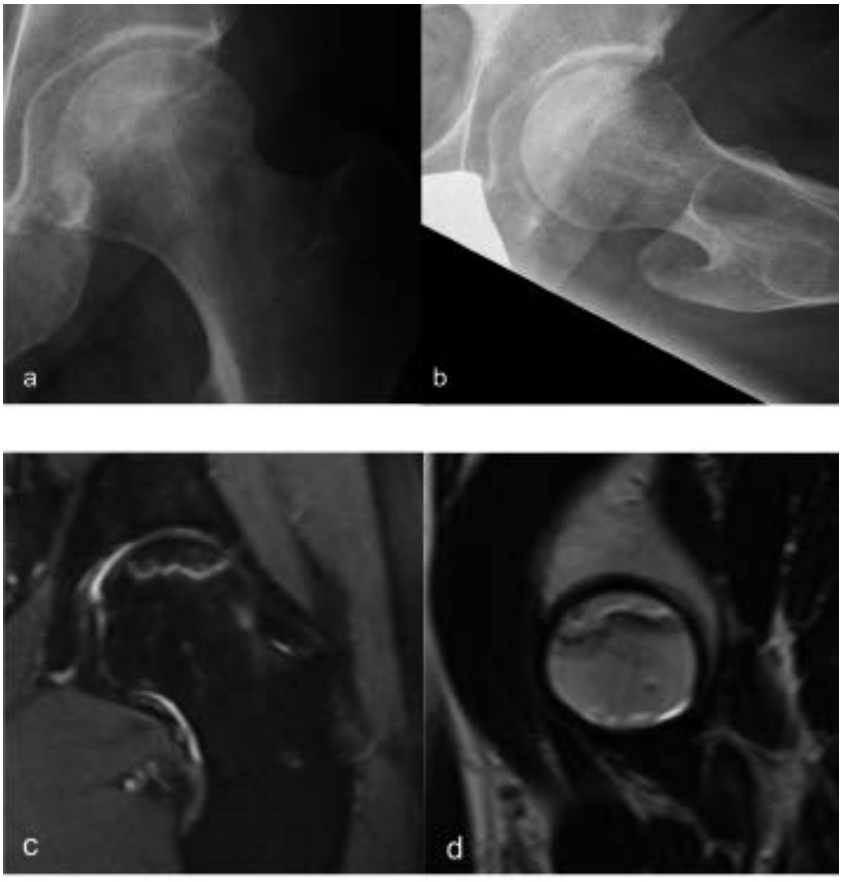

Figure 3. Based upon radiographic assessment, the amount of AVN may be uncertain (a,b). In contrast, MRI clearly depicts the necrotic area (c,d).

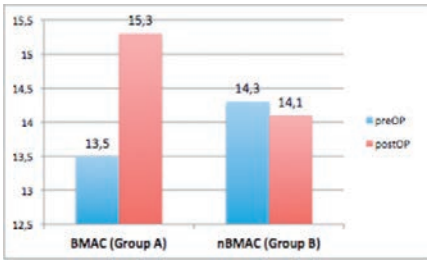

Figure 4. Evaluation of the Merle d'Aubigné Score pre- and postoperative in group A (BMAC) and group B (nBMAC).

Table 3. Merle d'Aubigné Score in detail, showing the scores for pain, walking ability and range of motion (ROM) in group A and group B in comparison of pre- and postoperative evaluation and the statistical differences.

	Pre	Post	P-value
A pain	3.6	4.3	0.308
B pain	3.7	3.9	0.756
A ability to walk	5	5.6	0.744
B ability to walk	5.5	5.1	0.389
A ROM	4.9	5.3	0.167
B ROM	5.1	5.1	0.936

Table 4. Studies with core decompression (CD) alone versus CD+bone marrow aspirate concentrate application.

Author	Year	Hips n.	Type of study
Gangji	2004	18	prospective cohort study
Hernigou	2009	534	retrospective
Gangji	2011	24	double-blind, not randomized
Zhao	2011	97	randomized clinical trial
Sen	2012	51	randomized controlled study
Pilge (data of this study)	2016	49 (20)	matched pair analysis

that these cells have the potential to increase capillary blood supply and form osteoblasts at the necrotic site.

Furthermore, it is known that in patients with a reduced concentration of mononuclear cells in the bone marrow, *e.g.* due to alcohol abuse or steroid use, there is a lack of osteogenesis due to the decreased density of cells at the necrotic site.[20] It is reported that healing time after tibial shaft fracture in patients with alcohol abuse is significantly increased compared to non-abusers.[23] Clinically, patients with osteonecrosis and application of lower stem cell concentration, a history of organ transplantation, corticosteroid exposure, or alcohol abuse show a higher incidence of disease.[20]

Different methods have been described to increase the concentration of mononuclear cells. Simple centrifugation was used by Connolly *et al*. to prepare osteogenic bone marrow concentration.[24] A freezing technique, in which mononuclear cells are separated on a Ficoll gradient using an IBM-Cobe 2991 red cell washer, allows an increase of the concentration of mononuclear cells and stem cells.[25] Others described the use of a porous implantable matrix, which has an appropriate pore size for increasing the concentration of precursor cells by using the matrix as an affinity column for cells. Attached cells within the matrix are then selectively transplanted into the graft site.[26, 27]

In a previous study, we evaluated mononuclear cells before and after processing the bone marrow aspirate. With our technique, we reached a mean of the 7.4-fold concentration of mononuclear cells in 35 patients.

Since the original study of Hernigou was published in 2002, only five studies have been published which prospectively evaluated CD alone *versus* CD+BMAC application (Table 4).[28-31]

In a previous study of our group, we were able to show that application of the prostacyclin analogue Iloprost provided superior clinical results in 95 patients with bone marrow edema and AVN after a mean follow-up of 17.6 months.[7] Our treatment protocol, therefore, includes the standardized application of Iloprost in these patients.

Iloprost is a vasoactive substance which was originally used in the therapy of pulmonary hypertension, vascular occlusion or vasculitis.[6] It has been shown that it also can be used to reduce bone marrow edema and accompanying symptoms in focal osteonecrosis.[6,7,32,33] Its edema-reducing effect is based on a reduction of hydrostatic pressure in the area of the venous branches of the terminal vascular bed. It influences the flow equilibrium towards the absorption and regulation of endothelial function prevents the recurrence of edema by improving the flow characteristics of the blood.[34] In addition, it inhibits platelet aggregation and diminishes the concentration of oxygen free radicals and leukotrienes.[35]

In a prospective observational study, Disch *et al*. evaluated the Harris Hip Score (HHS), the range of movement (ROM) and the extent of edema in 16 patients with isolated edema in comparison to 17 patients, in which edema was associated with focal necrosis of the proximal femur (33 patients, 40 hips). After treatment with Iloprost, the HHS, ROM and the visual analogue scale (VAS) improved significantly in both groups after a follow-up of 25 months.[6]

Several reports indicate that defect size of osteonecrosis significantly correlates with the prognosis and the clinical outcome.[25,29,30] In 1974, Kerboul *et al.* described a method for the classification of osteonecrosis of the proximal femur depending on defect size on two plain radiographs.[36] In our study, two authors independently evaluated the combined necrotic angle on plain radiographs. Although we had a moderate inter-observer variability, we believe that MRI could nowadays be a better modality to evaluate the defect size and to assess the status of the disease exactly to perform the right stage-dependent therapy.

In our study, the Merle d'Aubigné Score increased in group A and decreased in group B (Figure 4). Interestingly, there was no statistical difference between the pre- and postoperative scores in both groups. We recognize that this may be due to the low number of patients, as statistical analysis showed little power of this data.

This study has limitations. Instead of evaluating all of the 49 patients in one cohort, we designed this matched pair study to compare both therapy regimes. Therefore, the number of patients and statistical power decreased significantly. However, we believe that our data contribute to the understanding and prediction of therapy success. In addition, a comparison of subgroups of our patients *e.g.* with/without Iloprost treatment in addition to core decompression with/without BMAC application, would have further helped to understand the power of these different therapies (BMAC, iloprost, core decompression). Nevertheless, due to the limited number of patients, statistical evaluation would not be helpful in such small subgroups.

Conclusions

This is the first study to demonstrate that the application of autologous BMAC in combination with Iloprost application is a safe and efficient treatment in the early stages of AVN of the femoral head. Compared with CD treatment and Iloprost alone, BMAC implantation seems to decrease pain and other joint symptoms, increases the Merle d Aubigné Score, improves range of motion and prevents the progression of disease towards higher stages. Additional BMAC application prevents the collapse of the femoral head and significantly reduces the necessity of further surgery, including THA.

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