Safety of autologous bone marrow aspiration concentrate transplantation: initial experiences in 101 patients

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Abstract

The clinical application of cellular based therapies with *ex vivo* cultivation for the treatment of diseases of the musculoskeletal system has until now been limited. In particular, the advanced laboratory and technical effort necessary, regulatory issues as well as high costs are major obstacles. On the other hand, newly developed cell therapy systems permit intra-operative enrichment and application of mesenchymal and progenitor stem cells from bone marrow aspirate concentrate (BMAC) in one single operative session. The objective of the present clinical surveillance study was to evaluate new bone formation after the application of BMAC as well as to record any possible therapy-specific complications

For this purpose, the clinical-radiological progress of a total of 101 patients with various bone healing disturbances was documented (surveillance study). The study included 37 necrosis of the head of the femur, 32 avascular necroses/bone marrow edema of other localization, 12 non-unions, 20 other defects. The application of BMAC was performed in the presence of osteonecrosis via a local injection as part of a core decompression (n=72) or by the local adsorption of intra-operative cellular bone substitution material (scaffold) incubated with BMAC during osteosynthesis (n=17) or in further surgery (n=12).

After an average of 14 months (2-24 months), the patients were re-examined clinically and radiologically and interviewed. Further surgery was necessary in 2 patients within the follow-up period. These were due to a progression of a collapsed head of the femur with initial necrosis in ARCO Stage III, as well as inadequate new bone formation with secondary loss of correction after periprosthetic femoral fracture. The latter healed after repeated osteosynthesis plus BMAC application without any consequences. Other than these 2 patients, no further complications were observed. In particular, no infections, no excessive new bone formation, no induction of tumor formation, as well as no morbidity due to the bone marrow aspiration from the iliac crest were seen.

There were no specific complications within the short follow-up period and a simple intra-operative use of the system for different forms of bone loss could be demonstrated. In the authors' opinion, the on-site preparation of the bone marrow cells within the operating theater eliminates the specific risk of *ex vivo* cell proliferation and has a safety advantage in the use of autologous cell therapy for bone regeneration. Additional studies should be completed to determine efficacy.

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Key words: bone marrow aspirate concentrate application.

Received for publication: 25 September 2009
Revision received: 9 December 2009
Accepted for publication: 9 December 2009

Orthopedic Reviews 2009; 1:e32
doi:10.4081/or.2009.e32

Introduction

The treatment of bony defects and bone healing disorders represents one of the biggest challenges in orthopedics and trauma surgery. In order to gain sufficient bone material, previously established operative procedures involved the drilling of neighbouring surrounding bone, the transplantation of autogenous or allogenic bone and callus distraction, and the additional use of bone substitution materials.[1] In contrast to the osteoconductive bone substitution materials (allogenic bone, different synthetic/natural biomaterials), recombinant growth factors from the group of bone morphogenic proteins (BMPs) induce the formation of new bone[2], also in ectopic sites. Their possible mechanism of action is via local activation of mesenchymal precursor cells. In animal experiments, the direct transplantation of mesenchymal precursor cells leads to osteoinduction.[3] Clinical results of transplantations of precursor cells in patients with necrosis of the femoral head, non-unions or other bone healing disturbances have shown the first promising results.[4-12] So far cell-based therapies for bone regeneration have only propagated to a limited extent due to the considerable logistical time and effort needed, and the complicated legal constraints in many countries with correspondingly high logistical requirements and exorbitant costs.

In the meantime, however, approved autologous cellular preparation systems are available for human use which allow quantitatively relevant purification and concentration of mononuclear cells from bone marrow aspirate directly in the operating theater.[13] The objective of the present study is to record complications and evaluate short-term clinical results in 101 patients in whom intra-operative autologous BMAC with mesenchymal and hematopoietic precursor cells isolated by means of a density centrifugation procedure were used.

Materials and Methods

Patients

A total of 101 patients (female/male: 48/53, mean age: 51 years) with bone healing disorders or osteonecrosis were surveyed in a prospective clinical surveillance study with additive application by BMAC. The indication for supportive therapy with BMAC was carried out in 37 cases due to necrosis of the head of the femur, and in 32 patients because of avascular osteonecrosis/bone marrow edema of another localization. BMAC was also used in 12 cases of non-unions and 20 times in bone healing disorders of another origin or for bone induction (arthrodesis of the upper ankle joint, humeral four-fragment fractures, and others)(Figure 1).

All patients received information about the planned operation with all general and typical risks and complications. In addition, extensive clarification of and documentation concerning the practical procedure of the intended cell therapy was provided, including the novelty of the method of BMAC, the insufficient long-term experience, as well as the potential risk of therapeutic failure, an excess of new bone formation, the possible activation of existing infections (e.g. malaria) or cancer. Attention was also drawn in particular to the so-called unknown 'surgical risks'. Patient consent to participate in the clinical surveillance study was obtained.

In all patients, X-rays of the affected body region were performed pre-operatively as well as post-operatively in 2 planes. In several cases, MRI studies were also performed. There were no additional X-rays performed due to the study.

Bone marrow aspirate concentrate application

The harvesting of autologous bone marrow

was performed under standardized conditions by Yamshidi vacuum aspiration from the dorsal or ventral iliac crest using a stab incision. Intra-operative processing and concentration of the mononuclear cells took place under standardized conditions in the operating theater by density gradient centrifugation using an automatic, micro-processor controlled centrifuge system (SmartPReP Bone Marrow Aspirate Concentrate System BMAC™, Harvest Technologies GmbH, Munich, Germany) consisting of a sterile two-chamber centrifuge process kit as described previously.[12] The initial volume of harvested bone marrow was 60 mL or 120 mL with anticoagulation (heparin/ACD-A solution 8 mL or 16 mL, respectively), according to the area of application and indication. After centrifugation for 15 min and segregation of the erythrocyte portion of the cell suspension, the nucleated cells and plasma were automatically decanted into the second chamber of the process kit. After process completion, the plasma was removed leaving a predetermined BMAC volume of 7-10 mL for the surgical application which was handed on to the surgeon via a sterile adapter.

In case of osteonecrosis, a core decompression was performed and BMAC was delivered via a decompression drill channel as an additive injection (n=72). In 17 cases of osteosynthesis and in 12 cases of other operations due to disturbed bone healing or for the indication of new bone formation in the defective osseous zone, BMAC was applied locally as biomaterial-cell-composite.

Subsequently, the bone substitute material (CopiOs® Bone Void Filler, Zimmer, Freiburg, Germany) was used as a bio-absorbable calcium phosphate (dibasic) compound with osteoconductive properties[14,15] while the BMAC was mixed with the carrier material immediately before the implantation.

In the event of a transcutaneous puncture in femoral head necrosis, a target wire was positioned through the neck of the femur to the subchondral necrosis zone via a stab incision. After image intensifier controls in 2 planes, the transcutaneous, fan-shaped counter-bore in the sense of a core decompression was performed with a long canulated 4.5 mm drill. Subsequently, the drill was pulled back a few millimeters creating an osseous canal, which was checked by introducing a Kirschner's wire in order to remove any drill dust immediately before the BMAC suspension was injected via the drill using a short redon tube.

The post-operative treatment consisted of adequate medication for pain treatment, physiotherapeutic measures with relief of the lower extremities for four weeks as well as the partial loading with 20 kg for a further four weeks. In the case of avascular necrosis, post-operative MRI controls were carried out after four and eight weeks (Figure 2).

Follow-up

Demographic parameters and diagnosis were determined pre-operatively. Post-operative complications were recorded as part of the clinical progress examinations and also taken from the patients' files. Patients were questioned about the incidence of complications, further surgical interventions and their subjective satisfaction. In particular, pain or complications at the removal site and subjective problems from the bone marrow aspiration were registered.

Results

The mean post-examination period for all patients was 14 months (2-24 months). With regard to subjective satisfaction, 84 patients were satisfied or very satisfied with the result of the operation, 7 patients reported moderate satisfaction and one patient, for whom the indication of a total hip replacement was made during the further course of recovery, evaluated the procedure as non-satisfactory.

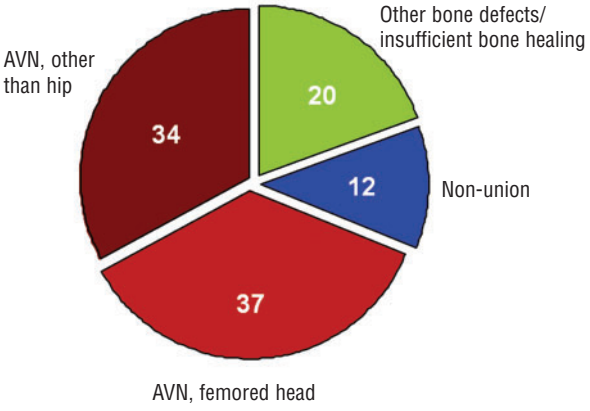

Figure 1. Indications in 101 autologous mesenchymal stem cell transplantations. AVN: avascular necrosis.

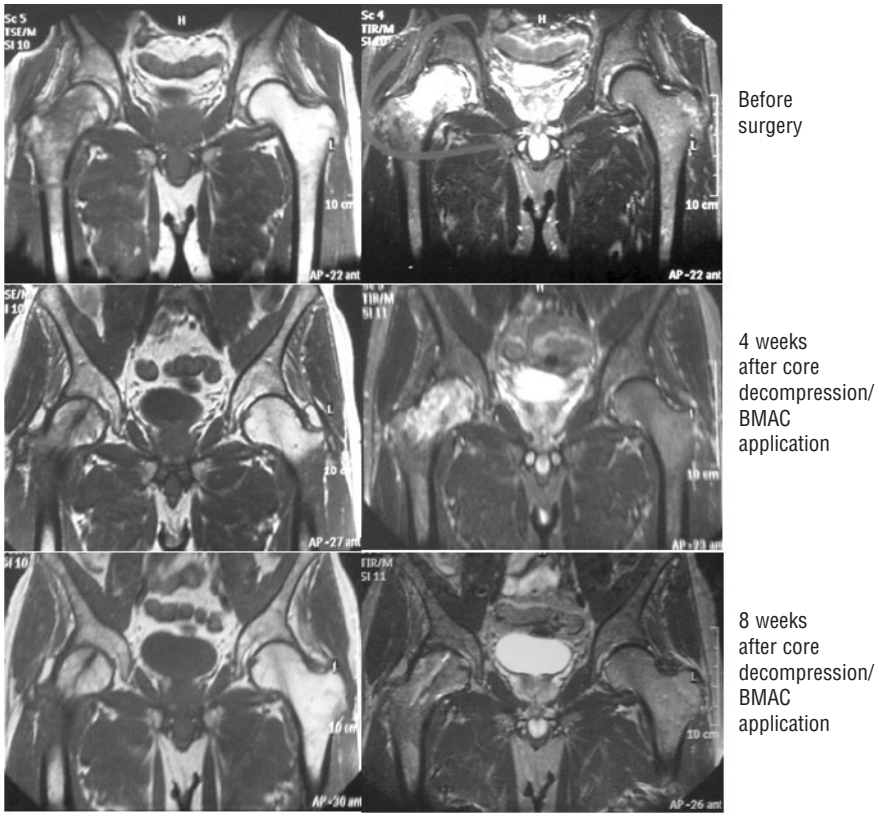

Figure 2. A 53-year old patient with necrosis of the head of the femur with accompanying large bone marrow edema on the right side. Transcutaneous core decompression and BMAC transplantation. MRI controls after four and eight weeks. In addition to the clearly distinguishable drill channels, an almost complete normalization of the bone marrow signal can be seen.

Independent of the total level of satisfaction, pain reduction was achieved in all patients.

Further surgery was needed in 2 patients. These were the only complications which were noted during this follow-up period. In particular, no complications in the form of infections, excessive new bone formation or renewed increase of complaints were noted. Also, there were no cases of complications or morbidity with respect to the bone marrow removal site. Subjectively, the bone marrow aspiration was not considered negatively by any of the patients.

Case 1

A male patient (age 36 years) had suffered a lateral fracture of the neck of the femur two years previously while playing football which had been treated using a dynamic hip screw. A radiologically confirmed necrosis of the femoral head with incipient subchondral fracture according to Association Research Circulation Osseous (ARCO) stage III was shown. The patient was offered a trial of BMAC cell therapy after clarification about the unfavourable mid-term prognosis of a joint-preserving therapy by means of core decompression. While the post-operative period was uneventful, within five months a further collapse of the femoral head occurred which required total joint replacement (Figure 3).

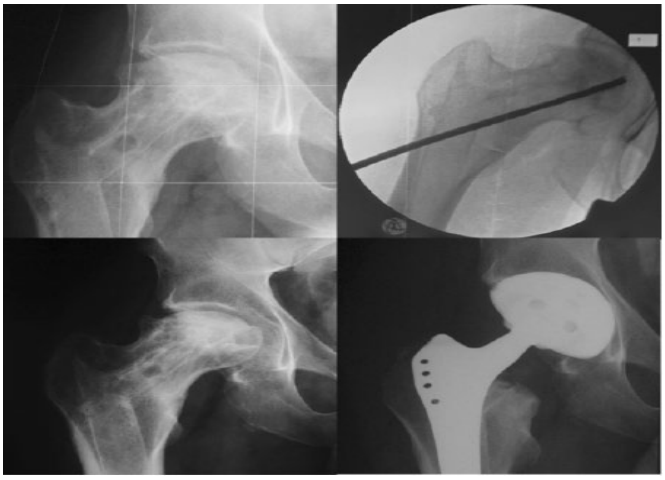

Figure 3. A 36-year old patient with necrosis of the head of the femur after DHS. BMAC transplantation with incipient subchondral fracture. During the further course of treatment, there was collapse of the femoral head followed by a total joint replacement.

Case 2

A female patient (age 86 years) had suffered a periprosthetic femur fracture caused by a fall after total hip replacement. The fracture had been treated by means of a less invasive stabilization system (LISS) plate. After two months, plate breakage occurred due to inadequate bone healing and was treated by re-osteosynthesis with bone substitute material and BMAC. After a further three months, an incipient axial deviation could be seen in the area around the operation site despite distinct new bone formation. An additional femur plate osteosynthesis from the anterior was inserted with additional bone substitute material and BMAC. Histological examination of tissue material from the former transplantation displayed a distinct formation of new woven bone. The fracture then healed uneventfully after a further three months (Figure 4).

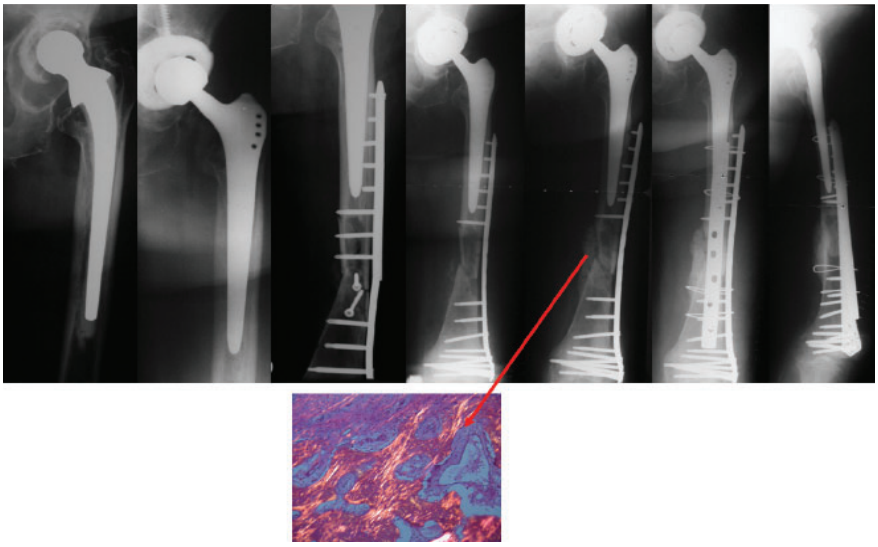

Figure 4. An 86-year old female patient with periprosthetic fracture after total hip revision surgery showed a failure of LISS osteosynthesis. Re-osteosynthesis was combined with application of bone substitute material (CopiOs®) augmented by autologous BMAC. Despite good new bone formation, increasing axial deviation was noted after two months. Additional internal fixation by plate osteosynthesis from the anterior combined with a second CopiOs/BMAC transplantation was performed. Here, some tissue from the initial transplantation site was taken for histology. The patient showed a solid fusion of the fracture after a further three months post-operatively. The histological analysis of the transplantation site showed a significant new formation of woven bone (polarization optics, magnification x 200).

Discussion

The objective of our investigation was to show whether the intra-operative concentration of mesenchymal progenitor cells is to be considered a low risk procedure for patients. Specific complications from the method of stem cell transplantation did not occur in any of the 101 patients. In particular, no complications were observed concerning excessive new bone formation, infections, tumor induction or morbidity at the removal site on the iliac crest. However, further surgery was needed in 2 patients within the follow-up period. In one case, there was a further fracture of the femoral head after stem cell transplantation following post-traumatic necrosis of the head of the femur in Stage III which led to implantation of a total hip replacement. Here the therapy carried out was considered to be a rescue attempt and the patient was informed accordingly. During plate osteosynthesis in a periprosthetic fracture of an 86-year old female patient, secondary loss of correction occurred with loosening of the LISS plate proximal to the fracture. This is a typical complication, particularly of elderly patients,[16] even without additive application of BMAC. Marked bone formation in the area of transplanted BMACs was confirmed histologically at re-osteosynthesis. After additional internal fixation and BMAC application, the fracture healed uneventfully within three months. In all other patients, the clinical situation improved without further surgery.

The treatment of bone healing disorders with purified bone marrow is not a new therapy. Attempts at direct bone marrow inoculation

fail due to the volumes necessary for successful therapy. For this reason, the concentration of mononuclear cells with the help of density gradient centrifugation was developed. With this procedure a mixed population of different cells is produced including mesenchymal precursor cells: colony forming units-fibroblastic (CFU-F) and hematogenic stem cells ($CD34^+$-cells).[10,11] In a prospective study in patients with avascular necrosis of the femoral head treated with this method, it could be shown that no total joint had to be implanted after an average of seven years in 94 of 116 patients. Local or systemic side-effects were not observed.[4] Also, in 53 of 60 patients with non-infected atrophic non-unions, healing of the fracture could be achieved by the injection of precursor cells from autologous bone marrow which had been purified in the laboratory. Based on the analysis of the failures, it was shown that the number of injected progenitor cells plays a critical role. As a rule of thumb, it has been suggested that 1 ml bone marrow contains sufficient numbers of cells to form 1 ccm of bone.[7,9]

In a controlled study with 13 patients (18 hips) with necrosis of the femoral head a clear difference could be determined between the active treatment and the control group. In addition to a significant reduction of pain, disease progression was also positively influenced by the treatment with stem cell concentrate.[17-19]

Ultimately, cell therapy by density gradient centrifugation outside the operating theater according to the current European legislation requires a good manufacturing practice (GMP)-certified hematologic laboratory with a manufacturing permission for medicines [Advanced Therapy Medicinal Products (ATMPs) and associated national amendments for tissue engineering products and cell therapeutics].[20] This is currently clearly hindered not least by the legal and bureaucratic obstacles, in particular for the therapy of non-vital threatening diseases, a category which includes bone healing disorders.[21]

Therefore, so far there are only individual data on the efficiency of *ex vivo* expanded mesenchymal progenitor cells with the objective of a clinical application to the musculoskeletal system.[22] The first studies have shown the feasibility in principle on a small patient cohort with non-union (n=13) and necrosis of the femoral head (n=3; n=6).[23-25] In addition to the increased laboratory effort, it is, however, not yet clear to what extent cell propagation in the laboratory leads to a change in the biological properties of the subsequently transplanted progenitor cells (*in vitro* ageing, polyploidization of the genetic material, transdifferentiation) and the related risks for patient safety.[26] The closed centrifugation process used in the present study avoids or minimizes these risks, as has been shown in the data of other working groups.[13,27] Furthermore, the described technique as a minimum invasive procedure reduces the amount of bone to be transplanted and thus contributes to a shorter operating time as well as to a reduction in the co-morbidity associated with excessive bone removal.

In a study with osseous substance defects, bone healing was achieved in connection with bone substitution material in all 24 patients. Infections or wound healing disorders did not occur.[16] Also, in jaw surgery the successful use of BMAC transplantation in a case of jaw pseudoosteoarthritis has been reported.[28] In addition to these first positive results, the present study shows that no significant specific risks and complications are connected with the intra-operative removal and purification of progenitor cells in a relevant number of patients. The weakness of the present study is that it is not a prospective randomized study and there was only a relatively short follow-up period. Accordingly, long-term post-surgical examinations and studies are necessary on statistically relevant patient numbers. The good results in femoral head necroses and pseudoosteoarthritis which were achieved with progenitor cells purified in the laboratory makes this simple procedure a valuable addition to the previous therapy options.

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