Autologous bone marrow cell implantation in the treatment of non-traumatic osteonecrosis of the femoral head: Five year follow-up of a prospective controlled study

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Abstract

Objective: To determine the efficacy of bone marrow cell implantation into the necrotic lesion of the femoral head on clinical symptoms and the progression of osteonecrosis of the femoral head in comparison with core decompression.

Methods: We studied nineteen patients and twenty four hips with early stage osteonecrosis of the femoral head. The hips were allocated to either core decompression only or core decompression and implantation of bone marrow cells. Both patients and assessors were blind with respect to treatment group assignment. The primary outcomes were clinical symptoms and disease progression.

Results: Bone marrow implantation afforded a significant reduction in pain and in joint symptoms and reduced the incidence of fractural stages. At 60 months, eight of the eleven hips in the control group had progressed to that stage. Survival analysis showed a significant difference in the time to failure between the two groups at 60 months. Patients had only minor side-effects after the treatments.

Conclusions: This long term follow-up study confirmed that implantation of autologous bone marrow cells in the necrotic lesion might be an effective treatment for patients with early stages of osteonecrosis of the femoral head.

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Introduction

Non-traumatic osteonecrosis of the femoral head is a painful disorder of the hip that affects young patients with risk factors such as glucocorticoids or alcohol abuse [1]. The factors influencing the progression of the disease from the appearance of the necrotic lesion to subchondral fracture and femoral head collapse are not yet fully understood, but size and stage of the osteonecrosis have been shown to be predictive of the clinical outcome [2]. Because of the young age of many of these patients, hip replacement cannot be expected to last the patient’s lifetime and therefore attempts should be made to save the femoral head prior to collapse with the use of less invasive procedures. So far, the efficacy of joint preserving surgeries like core decompression for early stage osteonecrosis has been variable and is still controversial [3–5].

The pathogenesis of osteonecrosis is still unclear but it can be seen as a vascular and bone disease. On one hand, the function of the capillaries serving as a conduit for the stem cells and bone cells needed in the bone remodelling unit and providing blood supply could be altered by emboli or thrombosis [6,7]. On the other hand, mesenchymal stem cells and osteoblasts that could potentially induce bone formation have been shown to be decreased in number and activity [8,9]. Moreover, osteocytes and bone lining cells in the necrotic lesion and the proximal femur undergo apoptosis [10,11]. This altered bone remodelling can be responsible for three different events in the pathogenesis of osteonecrosis; the appearance of osteonecrosis itself, the insufficient bone repair that occurs after osteonecrosis and its evolution to the subchondral fracture. These findings raised interest for a pathophysiological approach of osteonecrosis treatment by implantation in the necrotic lesion of concentrated bone marrow containing stem cells for mesenchymal tissues including bone [12,13]. The two year results of the prospective double blind controlled pilot study on the efficacy of bone marrow implantation suggested that cellular based therapy could improve joint symptoms and delay disease progression [12]. However, these results needed to be confirmed by the five year follow-up to emphasise their clinical relevance. We will present here the five-year results of this study on the effect of implantation of autologous bone marrow cells in the necrotic lesion of femoral heads with early stages of osteonecrosis.
Methods

Study protocol

This controlled double blind pilot study was initiated at Erasme Hospital, Université Libre de Bruxelles. Twenty three patients were recruited. Four patients were excluded and 19 patients were able to complete the evaluations at 24 months and 60 months. The study was conducted in accordance with the ethical standards of the institutional committee and with the Helsinki Declaration of 1975, as revised in 2008. We obtained informed written consent from patients after explaining the procedure and the risks. Patients were considered eligible for the study if they suffered from ARCO stage 1 or 2 osteonecrosis of the femoral head [14]. Patients’ hips were allocated to either a core decompression procedure (control group) or to core decompression plus autologous bone marrow cell implantation (bone marrow graft group). Both procedures were performed alternately. In accordance with the recommendation of the ethical committee, the hips were not randomised in order to analyse patients who had bilateral osteonecrosis and had undergone bone marrow implantation on one hip and core decompression on the other hip. For bilateral hips, the first hip to treat was alternately the right and then the left. The physician was the only person to know the group assignment. All patients were blind to treatment assignment. Investigators who assessed the outcomes were blind to group assignment. Primary outcomes sought were clinical symptoms and disease progression. Clinical symptoms included pain and joint symptoms. Disease progression from ARCO stage 1 or 2 osteonecrosis to ARCO stage 3 was assessed by radiographs and magnetic resonance imaging (MRI). The secondary outcome was reduction of the volume of the necrotic lesion measured on MRI [12].

Participants

Twenty three patients were recruited. Four patients were excluded (did not want to participate anymore because of MRI evaluation) and 19 patients (10 women and 9 men) including 24 hips with ARCO stage 1 or 2 osteonecrosis, were able to complete the study. Baseline characteristics of patients and osteonecrosis are similar at baseline as listed in Table 1. Osteonecrosis was diagnosed by MRI [15]. All patients had hip pain due to osteonecrosis of the femoral head. Five patients suffered from bilateral hip involvement. Four hip joints were identified with ARCO stage 1 and 20 hips with ARCO stage 2 osteonecrosis. Seventeen patients (20 hips) suffered from osteonecrosis as a result of corticosteroid therapy (none of the patients were still on corticotherapy during the study); one patient (2 hips) had alcohol-induced osteonecrosis. For one patient (2 hips), no etiological factors could be detected.

Exclusion criteria were: malignant disease during the past 5 years, serious current infection, age older than 65 years and ARCO stage 3 or 4 osteonecrosis of the femoral head.

Procedures

Clinical evaluation

Two investigators, who were unaware of treatment group assignments, performed all postoperative outcome assessments. Patients were assessed at baseline, 3, 6, 12, 24, 36, 48 and 60 months. Patients’ assessments of pain were marked on a visual analogue scale (VAS) from 0 mm (no pain) to 100 mm (severe pain) [16]. The severity of hip disease was gauged using Lequesne algofunctional index, the higher the index, the worse the severity of symptoms [17]. Symptoms of osteonecrosis were also assessed by the Western Ontario and McMaster universities (WOMAC) score [18]. The higher the WOMAC score, the worse the severity of symptoms, with 96 being the highest possible total score. In case of hip bilateral involvement, patients were asked to fulfill the questionnaires for each hip separately.

At each visit, patients were assessed for side-effects.

Radiological evaluation

Anteroposterior and frog-leg lateral radiographs and MRI of the affected hip were taken at the time of each clinical assessment. All radiographs were analysed by a single reader who was unaware of treatment assignments. Radiological progression of osteonecrosis was measured by reference to ARCO-defined stages [14]. For the MRI, the measurements were prepared on 3 mm coronal T1-weighted scans. The contours of the necrotic lesion and of the femoral head were drawn on each slice and the volumes were then calculated using a computer work station. The relative volume of the necrotic lesion was calculated as a percentage of the entire femoral head. This method of volume measurement was proven to be reliable if measured by a single reader as shown in the 24 month evaluation study [12].

Table 1

<table>
<thead>
<tr>
<th>Characteristics of patients and osteonecrosis. Osteonecrosis of the femoral head was defined by reference to ARCO staging. Data are mean ± standard error of the mean. The p values indicate that the baseline characteristics are not statistically different in the two groups.</th>
<th>Control group</th>
<th>Bone marrow graft group</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics of patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45.7 ± 2.6</td>
<td>44.2 ± 2.6</td>
<td>0.369</td>
</tr>
<tr>
<td>Time to diagnosis (months)</td>
<td>4.9 ± 0.6</td>
<td>5.1 ± 0.7</td>
<td>0.603</td>
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<tr>
<td><strong>Characteristics of osteonecrosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of hips</td>
<td>11</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>ARCO stage 1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ARCO stage 2</td>
<td>9</td>
<td>11</td>
<td></td>
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<tr>
<td>Location—central (B)</td>
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<td>5</td>
<td>0.801</td>
</tr>
<tr>
<td>Location—lateral (C)</td>
<td>7</td>
<td>8</td>
<td>0.709</td>
</tr>
<tr>
<td>Volume of lesion/volume of femoral head (%)</td>
<td>19.2 ± 3.9</td>
<td>16.0 ± 2.2</td>
<td>0.605</td>
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<td><strong>Etiological factors</strong></td>
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<tr>
<td>Corticosteroids</td>
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<tr>
<td>Alcohol abuse</td>
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<td>1</td>
<td></td>
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<td>Idiopathic</td>
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<td>1</td>
<td></td>
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<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
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<tr>
<td>Visual analogue scale (mm)</td>
<td>46.2 ± 7.2</td>
<td>32.8 ± 7.1</td>
<td>0.186</td>
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<tr>
<td>Lequesne index</td>
<td>8.6 ± 1.4</td>
<td>7.2 ± 1.2</td>
<td>0.392</td>
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<tr>
<td>WOMAC score</td>
<td>30.5 ± 5.5</td>
<td>25.5 ± 4.5</td>
<td>0.531</td>
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</table>

Statistical analysis

Continuous data are presented as mean ± SEM.
Analyses for efficacy were based on the per-protocol principle (24 hips).

We analysed the primary clinical variables (visual analogue scale, Lequesne index, WOMAC score) and the secondary variable (volume of the necrotic lesion) with an ANOVA at two factors: one repeated-measurements factor (time) and one between factor (group), to test any difference in time or group. The interaction between group and time was also inspected to assess whether changes over time followed the same pattern in the two groups. If statistically significant, the ANOVA was followed by Wilcoxon paired samples in order to compare the baseline data with the values obtained at 3, 6, 12, 24, 36, 48 and 60 months.

A Kaplan–Meier survivorship analysis was used to compare the progression from ARCO stage 1 or 2 to ARCO stage 3 and the need for total hip replacement. The rates of survival of the femoral head for the two treatment groups that is, the duration between the time of enrolment in the study and the endpoints were compared with a log-rank test. The rates of progression to the fractural stage (ARCO stage 3) were compared between the two groups with the Fisher’s exact test. Missing data due to any reason as well as total hip replacement was replaced using last observation carried forward from previous visit.
of CD34+ cells which are precursors of hematopoietic cells and 92.6±22.4×10^7/cells of femoral head 24 h after implantation.

Results

Characteristics of patients and of osteonecrosis are shown in Table 1. The two groups of patients had similar baseline demographic and osteonecrosis characteristics. The hips displayed non-fractional (ARCO stage 1 or 2) osteonecrosis in terms of location, volume of lesion and symptoms at enrolment.

The bone marrow harvest from the iliac crest was sorted and concentrated to a final volume of 49.7±2.3 ml [12]. The sorted bone marrow contained 1.9±0.2×10^8 mononuclear cells including 1.0±0.1% of CD34+ cells which are precursors of hematopoietic cells and 92.6±22.4×10^7/cells of fibroblast colony-forming units, an indicator of stromal cell activity. A homing study was performed to determine the proportion of bone marrow cells that remained in the femoral head after implantation. For three hips in three different patients, the concentrated bone marrow was mixed with Indium-111-oxine labelled leukocytes in order to have a semi-quantitative measurement of the amount of bone marrow that remained in the femoral head. Static images of the hips were obtained 24 h after implantation on a Sopha DST gamma camera showing that 56±6.1% of the labelled leukocytes remained in the femoral head 24 h after implantation (Fig. 1).

Bone marrow implantation effected a reduction with time in the number of hips that progressed to the fractural stage of osteonecrosis (ARCO stage 3). The rate of progression to the stage 3 was statistically different between the two groups at 60 months in favour of the bone marrow graft group (p=0.038). At 60 months, eight of the eleven hips in the control group and three of the thirteen hips in the bone marrow graft group had progressed to ARCO stage 3. At 60 months compared to 24 months of follow-up, there was no further progression to the stage 3 in the control group whereas one hip progressed to that stage in the bone marrow graft group. In order to compare the treatment groups analysing patients instead of hips, for bilateral hips (randomly selected). At 60 months, seven out of nine patients in the control group and three out of ten patients in the bone marrow graft group progressed to ARCO stage 3 at 60 months (p=0.069) (Fig. 2). The mean survival time was 57.2 months (53.48–60.97 95% CI) for the bone marrow graft group and 50.2 months for the control group (40.24–60.13 95% CI).

Bone marrow implantation did not delay the need for total hip replacement. Three patients in the control group underwent unilateral total hip replacement at 14, 27 and 28 months respectively whereas total hip replacement was performed for two patients in the bone marrow graft group at 26 and 52 months of follow-up. Survival analysis did not show any significant difference in the time to arthroplasty between the two groups at 60 months (log-rank test; p=0.42). The mean survival time was 57.2 months (53.48–60.97 95% CI) for the bone marrow graft group and 50.2 months for the control group (40.24–60.13 95% CI).

Bone marrow grafting afforded some reduction in pain and joint symptoms of the osteonecrotic hip. Overall a significant decrease in the level of pain was observed in the bone marrow graft group compared to the control group after 60 months (p=0.009). VAS decreased in the bone marrow graft group from 32.8±7.1 at baseline to 18.8±6.9 at 24 months (p=0.055), 18.2±6.4 at 36 months (p=0.039) and 20.8±7.7 at 60 months (p=0.129) (Fig. 3). Overall patients treated with bone marrow implantation also demonstrated a marked decreased in joint symptoms after 60 months of follow-up, according to the scores on the Lequesne index (p=0.030). In the bone marrow graft group, the Lequesne index decreased from 7.2±1.2 at baseline to 3.3±1.5 at 24 months (p=0.025), 3.5±1.6 at 36 months (p=0.041) and 4.8±1.8 at 60 months (p=0.081) (Fig. 3). The bone marrow graft patients did not improve their joint symptoms with respect to the total WOMAC pain subscore compared to the control group (p=0.091). However, the results of the WOMAC pain subscore in the bone marrow grafted group versus the control group approached statistical significance (p=0.052).

Bone marrow implantation induced a decrease of the volume of the necrotic lesion. Overall, the volume of the necrotic lesion decreased significantly in the bone marrow graft group compared to the control group at 24 months (p=0.041) and approached statistical significance at 60 months (p=0.066) (Fig. 4). In the bone marrow graft group, the volume of the lesion decreased compared to baseline by 42% at 24 months and remained stable between 24 and 60 months. In the control group, the volume of the lesion increased by 1% at 24 months and decreased by 22% between 24 and 60 months of follow-up.

Periprocedural safety results were recorded. No serious adverse reactions related to bone marrow aspiration or to the implantation arose during this period. Only 3 patients complained of pain at the

![Fig. 1. Concentrated bone marrow cells were mixed with Technetium-99m labelled leukocytes. Static images of the hips were obtained 24 h after implantation of bone marrow on a Sopha DST gamma camera showing that 60% of the cells remained in the femoral head 24 h after implantation.](image)
level of the bone marrow aspiration. One patient complained of pain and hematoma in the great trochanter region at the site of the core decompression. In another patient, the bacteriological culture of the bone marrow showed coagulase negative staphylococci. The patient was treated with antibiotics but had no clinical symptoms of sepsis.

Discussion

We have shown that autologous bone marrow cell implantation reduces the incidence of fractural stage non traumatic osteonecrosis of the femoral head, delays the progression of stage 1–2 osteonecrosis and decreases hip pain and joint symptoms more efficiently than core decompression during this sixty month follow-up period.

The rate of progression to the fractural stage (ARCO stage 3) was statistically different at 60 months. Indeed, the 23% (three out of thirteen hips) progression of the disease in the bone marrow grafted hips, within this five year period are markedly better than the 80% progression to stage 3 in the natural history of osteonecrosis. The effect of core decompression in the control group is comparable to other studies. Mont et al. assessed forty-two studies in which 71% of the hips treated with core decompression resolved satisfactorily and 35% had a satisfactory result following non-operative interventions. In randomised controlled trials, the efficacy of core decompression measured in terms of decreased proportion of patients having additional surgeries or showing radiological progression to collapse has not been demonstrated. However, core decompression of the hip with the use of an 8-mm trephine is still the most common procedure used to treat early stages of the disease. In this study, a 3-mm trephine had to be used to avoid leakage of the implanted bone marrow. Although there are less data on the efficacy of small diameter trephine on the outcome of osteonecrosis, it seems that small diameter drilling can be at least as efficient as larger diameter trephine. The outcome of osteonecrosis of the femoral head is influenced by the size of the lesion, the stage of disease, the time from the diagnosis and etiological factors. The most important factor in predicting the outcome of early stage osteonecrosis is the size of the necrotic lesion. Many methods have been used to measure the size of the lesion by radiography or MRI among them the exact volumetric analyses of lesions give reliable measurements. Hernigou et al. showed using the same volumetric analysis as in this study, that the percentage volume of osteonecrosis on MRI was a significant predictor of the time to collapse. Eighty percent of the hips with a 10 to 20% involvement had collapsed within 24 months of the diagnosis and 50% of the hips with less than 10% involvement had collapsed within this time period. In our study, the volume of the lesion should be considered as medium size with 17% of involvement. Only 18% of the hips have less than 10% involvement of the femoral head. The hip survival in the control group is indeed similar to the survival of the medium sized lesion reported by Koo et al. The evolution of medium size lesions whatever the methods used is usually towards femoral head collapse between 24 to 36 months. Therefore, a minimum follow-up period of 24 months for the first report was chosen since collapse of the femoral head generally occurs over this time span. However, the long term follow-up was needed to assess the entire period of osteonecrosis evolution. As shown by Nam et al. there is a further 12% evolution to the fractural stage for medium size lesions, between 24 and 60 months of follow-up. Indeed, in the bone marrow grafted hips, between 24 and 60 months, one more hip progressed to the stage 3. Treatment of osteonecrosis can also be gauged on its efficacy in delaying total hip replacement. Bone marrow implantation did not delay the need for total hip replacement but this study was not designed to assess total hip replacement as an endpoint. More patients should be included to evaluate the efficacy of bone marrow implantation in delaying or avoiding total hip replacement.

So far the results obtained with bone marrow cell implantation in osteonecrosis are not fully understood. The effectiveness of bone marrow cells may be related to the availability of mesenchymal and endothelial stem cells endowed with osteogenic and angiogenic properties, arising from an increase in the supply of such cells to the femoral head, via bone marrow implantation. It was suggested in a prospective not controlled study that the efficacy could be related to the number of mesenchymal stem cells implanted but the number of cells needed to induce osteonecrosis repair is still unknown. Our study did not allow studying the relationship between the outcome and the number of cells implanted per volume of lesion. So far, we have been unable to define using imaging techniques the exact location of the cells or which cells might be responsible for the therapeutic effects. Moreover, some of the cells might have leaked through the trephine.

![Fig. 3. A comparison of the bone marrow graft group (solid line) and the control group (dashed line) with respect to the evolution of the scores on the visual analogue scale (VAS) and the Lequesne index over time. The results are shown as the mean and the error bars indicate±standard error of the mean. One asterisk (p<0.05) indicates a significant difference compared to baseline.](image1)

![Fig. 4. A comparison of the bone marrow graft group (solid line) and the control group (dashed line) with respect to the evolution of the volume of the necrotic lesion over time. The results are shown as the mean and the error bars indicate±standard error of the mean. P<0.05 indicate a significant difference compared to baseline.](image2)
or into the circulation of the proximal femur but the greatest part of the bone marrow remained in the area of osteonecrosis as shown by the radionuclide labelling. Another possible explanation for the therapeutic effect is that injected marrow cells supplied in skeletal and angiogenic factors resulting in increased osteogenesis [27] and angiogenesis which would create sufficient repair capacity to make the lesion reversible [28]. Larger trials and other techniques for stem cells homing are needed to fully understand the results of this study.

In conclusion, this study showed that bone marrow cell implantation in the necrotic lesion could be an efficacious treatment of early stages osteonecrosis of the femoral head to delay disease progression, reduce the incidence of fractural stage and relieve symptoms even in long term follow-up.

**Disclosure statement**

None of the authors has a conflict of interest that could inappropriately influence this work.

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**References**


