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Volume and location of bone regeneration after autologous expanded mesenchymal stromal cells in hip osteonecrosis

A PILOT STUDY

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Aims

Successful cell therapy in hip osteonecrosis (ON) may help to avoid ON progression or total hip arthroplasty (THA), but the achieved bone regeneration is unclear. The aim of this study was to evaluate amount and location of bone regeneration obtained after surgical injection of expanded autologous mesenchymal stromal cells from the bone marrow (BM-hMSCs).

Methods

A total of 20 patients with small and medium-size symptomatic stage II femoral head ON treated with 140 million BM-hMSCs through percutaneous forage in the EudraCT 2012-002010-39 clinical trial were retrospectively evaluated through preoperative and postoperative (three and 12 months) MRI. Then, 3D reconstruction of the original lesion and the observed postoperative residual damage after bone regeneration were analyzed and compared per group based on treatment efficacy.

Results

The mean preoperative lesion volume was 18.7% (SD 10.2%) of the femoral head. This reduced to 11.6% (SD 7.5%) after three months ($p = 0.015$) and 3.7% (SD 3%) after one year ($p < 0.001$). Bone regeneration in healed cases represented a mean 81.2% (SD 13.8%) of the initial lesion volume at one year. Non-healed cases (n = 1 stage progression; n = 3 THAs) still showed bone regeneration but this did not effectively decrease the ON volume. A lesion size under mean 10% (SD 6%) of the femoral head at three months predicted no ON stage progression at one year. Regeneration in the lateral femoral head (C2 under Japanese Investigation Committee (JCI) classification) and in the central and posterior regions of the head was predominant in cases without ON progression.

Conclusion

Bone regeneration was observed in osteonecrotic femoral heads three months after expanded autologous BM-hMSC injection, and the volume and location of regeneration indicated the success of the therapy.

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Keywords: Femoral head osteonecrosis, Mesenchymal stromal cell, Bone regeneration, Osteonecrosis volume, Cell therapy efficacy

Article focus

- Volume and location of bone regeneration in osteonecrosis of the femoral head (ONFH) treated with expanded, autologous bone marrow-derived mesenchymal stem cells (BM-MSCs).
- Comparison between healed and non-healed ONFH in patients.

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Key messages

- Bone regeneration was observed in all treated cases, independently of the ONFH volume and location.
- A mean threshold of 10% (SD 6%) residual lesion volume was established. If the remaining lesion at three months exceeded this threshold, the head might collapse despite the cell injection.

Strengths and limitations

- Non-controlled clinical trial data on expanded, autologous BM-MSCs.
- Robust imaging assessment permits conclusions about bone regeneration.
- As in any pilot clinical trial, the number of cases is limited.

Introduction

The efficacy of cell therapy treatments on early non-traumatic osteonecrosis of the femoral head (ONFH) is frequently assessed as the absence of osteonecrosis (ON) stage progression and/or the avoidance of total hip arthroplasty (THA). Histopathological findings suggest that femoral head injury associated with ON may be due to one single event, with rare progression of the necrosis,[1] and that symptoms and progression are related to insufficient bone repair. Creeping substitution has been evoked as the repair mechanism in non-traumatic ONFH.[2] The reparative process, including marginal sclerosis and intralesional changes, has been associated with cessation of collapse,[3] while the boundary between the necrosis and the sclerosis may be at the origin of fracture and collapse.[4] Osteoblast activity is centred at the sclerotic rim, while osteoclast activity is predominant in the region of femoral head collapse, with subchondral bone resorption.[5] These findings have also been reported in early ON cases.[6] However, osteoblasts from the femoral head in patients with ONFH have shown both reduced viability and reduced mineralized nodule formation, compared with osteoblasts from the intertrochanteric region.[7] The cellular microenvironment in the bone marrow (BM) of the osteonecrotic femoral head, with adipocyte enlargement potentially affecting bone remodelling,[8] is another open research area justifying cell therapy intervention.

Collapse of the femoral head is the end stage of symptomatic ON and prompts articular degeneration.[9] Up to 73% of small, stage I lesions may progress to collapse six months after becoming symptomatic.[10] Effective mechanical support is required to avoid head collapse, particularly if the interface between viable and necrotic bone develops in a transverse plane.[11] Maximum area of bone resorption in the coronal plane (anterolateral or lateral column) may predict a rapid progression to head collapse,[12] and this area should be monitored and treated. Following this reasoning, bone needs to be preserved or recovered in the lateral pillar to avoid collapse.[13] Large necrotic lesions extending laterally to the acetabulum edge (the so-called C2 lesions in the Japanese Investigation Committee (JIC) classification, according to Sugano et al[14]) are associated with femoral head collapse, even in asymptomatic hips without treatment.[15]

Core decompression (CD) alone is not effective in preventing collapse in early-stage ON.[16] The CD failure rate is higher in hips with moderate-to-severe lesions (with an estimated volume higher than 15% of the femoral head), and in hips with more lateral lesions in the coronal plane.[17] Higher extension of fibrosis after CD may be a predictor of treatment failure and lower femoral head survivorship.[18] Treatments that obtain enough bone regeneration under the subchondral area may avoid fracture and femoral head collapse.

Advanced cell therapy claims to regenerate bone based on a study by Hernigou and Beaujean,[19] confirming the benefits of BM cells injected through the forage. Different cell therapy approaches substantially improved the classic CD[20] through the bone marrow concentration (BMC) or cell expansion techniques to deliver a high number of mesenchymal stromal cells (MSCs).[21] Ex vivo expanded bone marrow-derived mesenchymal stem cells (BM-MSCs; two million MSCs per hip) were claimed to outperform CD in delaying or avoiding femoral head collapse in ONFH under stage IIC.[22] The MSCs remained within the injected femoral head in preclinical studies,[23] confirming the tropism of injected BM-derived MSCs for the bone surface. These treatments presume bone regeneration, but proof of this regeneration, subsequently avoiding head collapse or stage progression, is limited. Clinical and experimental results thus sustain the hypothesis of bone regeneration within the osteonecrotic lesion in the cell-injected femoral head.

The aim of this study was to evaluate the amount and location of bone regeneration after injecting expanded autologous BM-derived MSCs in the femoral head with ON stage II, from clinical trial data. Bone regeneration measurements, based on the osteonecrosis volume (ONV) changes, were compared between the group of successfully treated femoral heads and the group of failures (where the treatment did not avoid progression into the next ON stage or into THA).

Methods

A total of 22 patients were treated (between March 2014 and June 2015) for ONFH under the Ortho 2 clinical trial (EudraCT 2012-002010-39) in five clinical centres from four European countries (France, Germany, Italy, and Spain) under the REBORNE EU-funded project (Regenerating Bone defects using New biomedical Engineering approaches; FP7 HEALTH-2009 to 1.4-2; Grant Agreement 241879).[24]

The clinical trial and its results have been presented elsewhere.[24] In short, patients were treated per protocol with percutaneous forage plus implantation of 140 million expanded, autologous MSCs (clinical grade) from BM in a single injection of up to 7 ml of albumin (dose of 20×10^6 cells/cc[3]). All included patients agreed to their participation in the trial and signed an appropriate informed consent form (Ethics Committee authorization

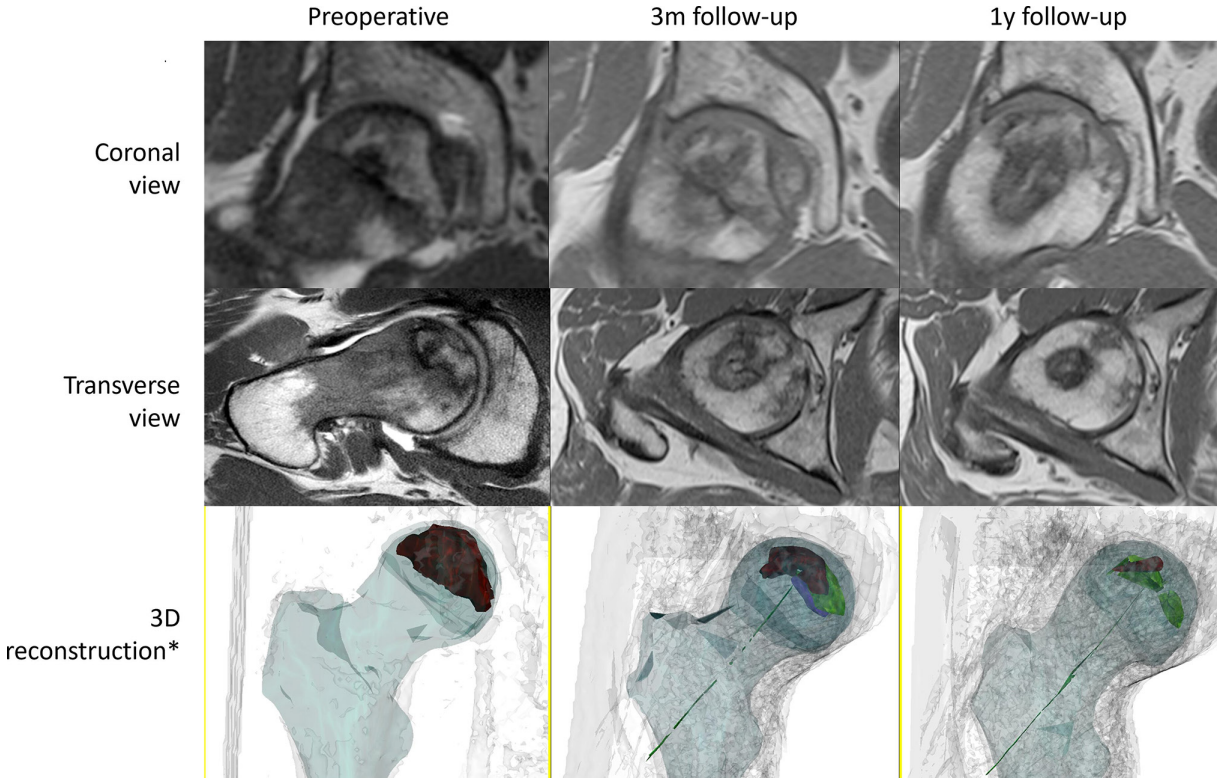

Fig. 1

Example of the osteonecrosis evolution in healed cases. A healed case (no. 101) changed the osteonecrosis volume (ONV) from 19.9% to 12.6% at three-month follow-up, and to 7.5% at 12-month follow-up. *See Supplementary Video 1.

code, coordinating centre: HULP 3875). Two patients dropped out of the study early (at three and five months after surgery). After 12 months of follow-up (FU), 16/20 treated patients healed (pain was under 30/100 in a visual analogue scale (VAS) and head sphericity was maintained in radiological imaging), while 4/20 were considered non-healed (three of them undergoing THA at 12 months and one showing progression in the ON stage at subsequent radiograph exams without THA). Preoperatively, included cases were all classified as stage 2 or II by Arlet and Ficat[25,26] or ARCO,[27] all symptomatic, acute, or subacute. No differences were found between the healed and non-healed groups (h/nh) in terms of mean age ($\bar{x}_h$ = 43.0 (standard deviation (SD) 10.6)/$\bar{x}_{nh}$ = 46.1 (SD 9.9); p = 0.617, unpaired *t*-test), obesity (19%/25%; p = 0.539, Fisher's exact test), male sex (81%/100%; p = 0.905, Fisher's exact test), smoking habit history (50%; p = 0.999, Fisher's exact test), mean months of evolution of ON ($\bar{x}_h$ = 2.4 (SD 2.4)/$\bar{x}_{nh}$ = 1.9 (SD 1.5); p = 0.481, Mann-Whitney U test), ON aetiology (55%/75% idiopathic, 18%/25% corticosteroid, and 31%/0% other non-traumatic; p = 0.623, Fisher's exact test), mean preoperative VAS for spontaneous pain ($\bar{x}_h$ = 30.4 (SD 20.2)/$\bar{x}_{nh}$ = 30.1 (SD 25.4); p = 0.928, Mann-Whitney U test), mean preoperative VAS for weightbearing pain ($\bar{x}_h$ = 58.7 (SD 20.9)/$\bar{x}_{nh}$ = 54.9 (SD 19.7); p = 0.787, Mann-Whitney U test), and mean preoperative Harris Hip Score ($\bar{x}_h$ = 65.4 (SD 14.1)/$\bar{x}_{nh}$ = 68.7 (SD 8.6); p = 0.654, Mann-Whitney U test).

The specific surgical technique and its variability has also been described elsewhere.[28] In brief, patients were positioned supine on a fracture table, and anteroposterior (AP) and axial views of the femoral neck and head were checked under fluoroscopy with a radiological C-arm. A guiding wire was drilled into the femoral head osteonecrotic lesion, and a 4 mm cannulated drill along the drilling guide was placed into the femoral head under fluoroscopy. One single administration of 140 million MSCs suspended in 5% human albumin (7 ml) was administered into the femoral head. Radiographs and MRI were obtained during the clinical trial FU (with 1.5 to 3.0 T MRI equipment, as available in the collaborating clinical centres).

Anonymized imaging preoperatively and three and 12 months postoperatively (MRI T1 and/or T2 images in the coronal, sagittal, and/or transverse planes) was included from the 20 patients who completed the FU. Volumetry was performed on sets of coronal MRI sections on Digital Imaging and Communications in Medicine (DICOM) format using the OsiriX MD licensed software (Pixmeo, Switzerland) for macOS (Apple, USA), and 3D reconstructions of the osteonecrotic lesion and subsequent bone regeneration were obtained. To interpret

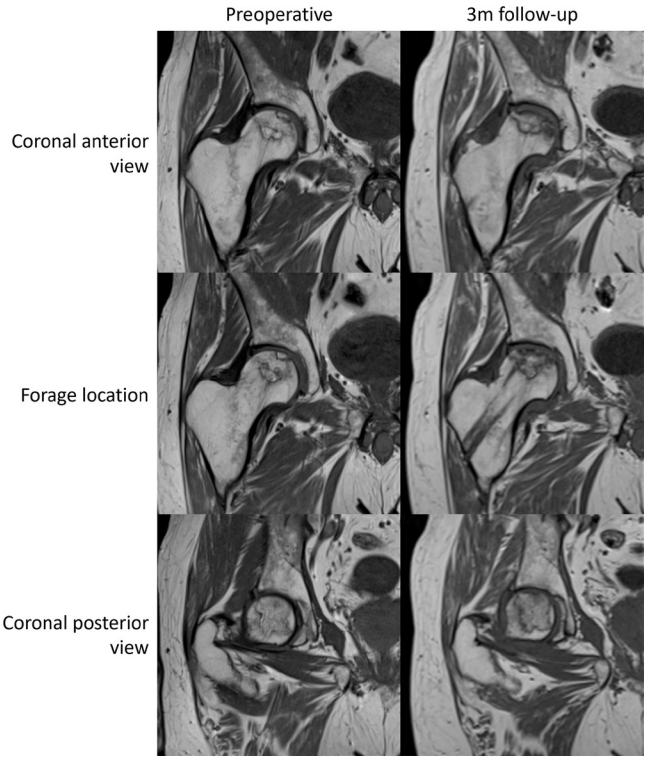

Fig. 2

Osteonecrosis (ON) evolution in non-healed cases. Non-healed case (no. 203) with ON stage progression three months after cell implantation surgery, finally receiving total hip arthroplasty (THA) at 11 months. The ON volume (ONV), in this case, changed from 43.4% preoperative to 28.7% at three-month follow-up (3mFU). The developing subchondral fracture site at 3mFU on anterior view was observed, while no lesion change was observed in the posterior section from preoperative to 3mFU.

the ON lesion, we identified the low-intensity, sclerotic rim, which is usually detected as cortical-like bone. Compared with the rest of the bone, the affected tissue showed an irregular topography. Regions of interest (ROIs) were outlined on each slice manually, both for the ON lesion and for the femoral head, following the anatomical contour. After selecting all the ROIs within one series, OsiriX automatically calculated the volume by multiplying the surface for each slice thickness and then summing up individual slice volumes. The final ONV was estimated as a percentage of the femoral head volume (FHV) following the formula: 100*(ONV/FHV). Bone regeneration was represented by the percentage of the recovered volume, comparing the initial and the remaining lesion volume at three and 12 months, following the formula: $100*(ONV_1/ONV_0)$.

To determine the coronal location of the necrotic lesion, we used the zones indicated in the 2001 classification system proposed by the JIC (JIC 2001)[14] on coronal MRI preoperative and three and 12 months postoperative sections. Zones were analyzed and recorded zone by zone (yes/no) to identify the extension of regeneration in the coronal plane during the FU. A lesion type C2 covering from A to C2 was recorded as A = 1 (yes), B = 1 (yes), C1 = 1 (yes), and C2 = 1 (yes).

The lesion location in the transverse plane was similarly recorded on MRI transverse sections through the anterior-central-posterior (ACP) method.[28] Anterior, central, and posterior zones of the femoral head were identified at the central transverse section, with the acetabulum as the reference (from the anterior to the posterior edge). If the lesion surpassed the anterior acetabular edge, the notation was A2 (Anterior 2). The ACP location of the lesion was also recorded per zone, to identify the lesion extension in the transverse plane during the FU. A lesion A1CP covered from A1 to P and was recorded as A2 = 0 (no), A1 = 1 (yes), C = 1 (yes), and p = 1 (yes).

Statistical analysis. All images were processed, measured, and classified with OsiriX software (Pixmeo, Switzerland).[29] For statistical analysis, we used Stata Statistical Software: Release 12 (StataCorp, USA). The mean and SD or standard error (SE), as well as proportions were reported as appropriate. For demographic details and basal variables, *t*-test, Mann-Whitney U test, or Fisher's exact test were used to compared healed and non-healed groups, where deemed appropriate. For the ON volume variable, paired *t*-test was used for comparison between FU visits. The location variables (as per JIC 2001 and ACP) were described, and Fisher's exact test differences were estimated, by FU visits and healing.

A difference-in-differences (DID) estimation model for panel data was conducted to test differences in the ONV (dependent variable) over healing groups (control group = non-healed) before and after treatment (preoperatively over three months and one year postoperatively, and three months over one year postoperatively) with no covariate adjustment. DID estimator (function intercept) was interpreted as the mean treatment effect (reduction of ONV) on healed cases compared with non-healed ones.

To explain the behaviour of healing (1 = yes, 0 = no) in the function of ONV and time (preoperative, three-month FU (3mFU), 12-month FU (12mFU)), we modelled a Probit regression, assuming that the larger the ONV observed, the lower the probability of healing, adjusted for months of ON evolution (< three months, ≥ three months) and age (< 50 years, ≥ 50 years), and clustered by participants. A 'probability of healing' variable, after Probit analysis, was generated to identify the mean ONV that predicts healing. Probit models fix the threshold of healing in $P_i < 0.5$; therefore, if the estimated probability is below 0.5, the case is interpreted as non-healed; and above 0.5, the case would be healed.[30] Statistical significance was set at $p < 0.05$ using Wald Chi-Square test.

Results

Osteonecrosis volume. The ONV is illustrated over time in one healed case (Figure 1 and Supplementary Video 1) and one non-healed case (Figure 2). More detailed reconstructions are shown in the Supplementary Material. The mean overall ONV was 7.2 mm^3 (SD 4.5) preoperatively, 4.5 mm^3 (SD 3.2) at three-month FU, and 1.5 mm^3 (SD 1.7) at one-year FU. As a percentage of the femoral

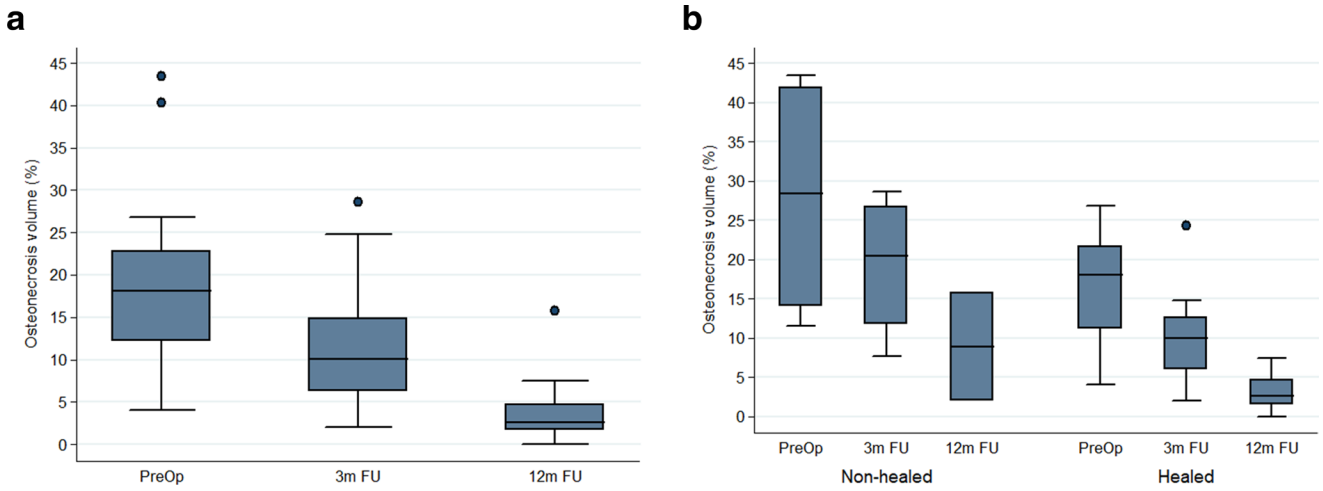

Fig. 3

Distribution of the osteonecrosis volume (ONV) over follow-up time (FU). a) Boxplot ONV, as a percentage of the femoral head volume (FHV). From preoperative imaging, the mean ONV decreased by 7.3% at three-month FU (3mFU) ($p < 0.001$, paired *t*-test; power determination (β) = 0.73) and by 15% at 12-month FU (12mFU) ($p < 0.001$, paired *t*-test; β = 0.99). b) Boxplot showing the median ONV, as a percentage of the FHV, by healing group. Based on the difference in differences model, the mean ONV was higher in the non-healed group than in the healed group, preoperatively (mean ONV difference = 11.26, standard error (SE) = 4.5; $p = 0.017$, paired *t*-test) and at 3mFU (mean ONV difference = 9.7, SE = 4.5; $p = 0.040$, paired *t*-test). Equivalent ONVs were observed at 12mFU (mean ONV difference = 5.9, SE = 5.5; $p = 0.293$, paired *t*-test).

head, the mean overall preoperative ONV was 18.9% (SD 10.2%) (Figure 3a). This volume was reduced to mean 11.6% (SD 7.5%) at the three-month FU ($p < 0.001$, paired *t*-test) and 3.6% (SD 3.7%) after one year post-surgery ($p < 0.001$, paired *t*-test).

Healed cases displayed a similar pattern (Figure 3b) with a mean preoperative ONV of 16.7% (SD 7.2%), as a percentage of the femoral head, dropping to 9.5% (SD 5.6%) at the 3mFU ($p = 0.004$) and 2.9% (SD 2.1%) after 12 months ($p < 0.001$, both paired *t*-test). The mean percentage of ONV in the non-healed group dropped from preoperative (27.9% (SD 16.2%)) to 19.2% (SD 9.4%) at 3mFU, and 8.8% (SD 9.7%) at 12mFU, but the number of cases (n = 4 at three months, n = 2 at 12 months) does not allow any conclusive quantitative analysis. DID estimations were not conclusive in favour of the healed group, suggesting that the mean reduction of ONV over time was similar in both the healed and non-healed groups ($DID_{Preop/3mFU} = 1.5$, $p = 0.809$; $DID_{Preop/12mFU} = 5.33$, $p = 0.446$; and $DID_{3mFU/12mFU} = 3.7$, $p = 0.453$, all paired *t*-test).

The Probit regression model fitted well ($p = 0.899$) to explain the healing behaviour, depending on the percentage of ONV ($\beta = -0.15$, $p < 0.001$), FU time ($\beta_{(Preop)}$ = Ref; $\beta_{(3mFU)} = -1.01$, $p = 0.009$; $\beta_{(12mFU)} = -1.72$, $p < 0.001$), months since diagnosis ($\beta_{(< 3m)} = 1.52$, $p = 0.104$), and age category ($\beta_{(> 50\text{-year-old})} = -1.62$, $p = 0.093$, all Wald Chi-Square test). The model correctly classified 85.4% of cases (sensitivity = 95.5%; specificity = 40%). The mean percentage of ONV that predicted healing after completing the 12mFU was 15.7% (SD 6.5%) at preoperative, and 10.1% (SD 6.2%) at 3mFU. Therefore, these data provide thresholds of ONV that can be healed with the single MSC injection technique, as performed in the current clinical trial.

The overall bone regeneration, understood as the percentage of the lesion volume that healed from one FU visit to the other, can be best seen in 3D reconstructions (Supplementary Figure a). Regeneration was estimated in mean 38.3% (SD 19.8%) of the initial lesion volume at 3mFU, and in 76.5% (SD 23.5%) at 12mFU ($p < 0.001$, paired *t*-test). In healed cases, the mean bone regeneration was estimated in 41.2% (SD 20.2%) of the initial lesion volume at 3mFU, and in 81.2% (SD 13.8%) at 12mFU ($p < 0.001$, paired *t*-test). In non-healed cases, the mean bone regeneration was 27.4% (SD 16.0%) at 3mFU and 43.8% (SD 28.8%) at 12mFU ($p = 0.483$, paired *t*-test).

Spatial distribution of bone regeneration. Using the JIC 2001 classification method for preoperative ON coronal extension, 19/20 cases displayed lesions in the medial third of the weightbearing area (WBA) (A), 20/20 in the central third (B), 20/20 occupied the lateral third without surpassing the acetabular edge (C1), and 14/20 laterally surpassed the acetabular edge (type C2). Figure 4 shows that regeneration in the medial third (A) one year after surgery was seen in 8/19 cases ($p = 0.033$), regeneration in the central third (B) was seen in 8/20 cases ($p = 0.014$), in the lateral third (C1) in 9/20 cases ($p = 0.005$), and laterally to the acetabular edge (C2) regeneration was obtained in 13/14 of the lesions ($p < 0.001$, all Fisher's exact test).

Using the ACP method for transverse ON extension, 15/20 of the preoperative lesions extended in the anterior region and surpassed the anterior acetabular edge (A2), 20/20 covered the anterior region but did not surpass the anterior acetabular edge (A1), 20/20 covered the central region (C), and 14/20 the posterior region (P). Figure 5 shows that one year after surgery, regeneration at the A2 region was seen in 10/15 cases ($p = 0.482$), in 8/20

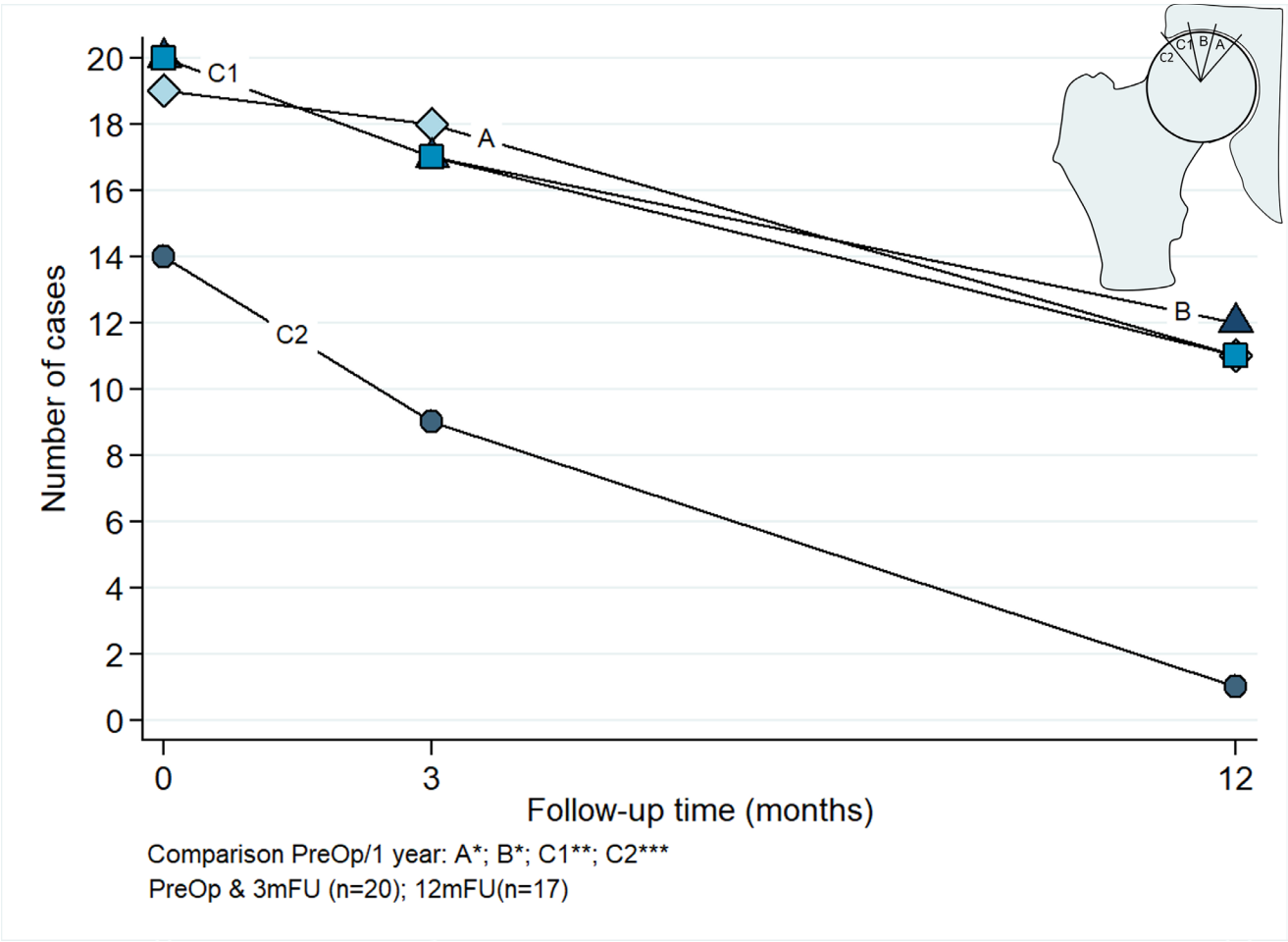

Fig. 4

Location of bone regeneration during follow-up (FU) using the coronal Japanese Investigation Committee JIC 2001 classification system.[14] JIC 2001 classification: type A when osteonecrosis (ON) occupies the first third of the weightbearing area (WBA); type B when ON occupies the second third of the WBA; type C1 when ON occupies the final third of the WBA but not surpassing it; and type C2, which is like type C1 but laterally surpasses the WBA and the acetabular rim. Sample size: preoperative and three months' FU (3mFU) (n = 20), 12 months' FU (12mFU) (n = 17). Fisher's exact test comparison from preoperative to 12mFU per location: A*; B*; C1**; C2***. *p < 0.05; **p < 0.01; ***p < 0.001.

cases at the A1 region (p = 0.014), in 12/20 cases at the C region (p < 0.001), and in 11/14 cases at the P region (p = 0.003, all Fisher's exact test).

Discussion

Bone regeneration was observed in all treated patients during the FU period, with a statistically significant volume reduction of the osteonecrotic lesion at three and 12 months after surgically injecting expanded, autologous BM-MSCs. Preserved femoral heads one year after treatment confirmed this significant ONV decrease. However, the detected regeneration in the non-healed femoral heads was insufficient to avoid collapse or progression.

The first issue under discussion relates to the calculation of the reference FHV, needed to express the ONV as a percentage. Manual calculation in all MRI sections, a cumbersome task, has also been criticised due to the potential inter- and intraobserver variability in determining the reference FHV.[31-33] We performed both manual (based on the anatomical contour) and automatic (based on a spherical model) calculations of the femoral head, as mentioned in Supplementary Figure b. Data shown in the results are obtained as a percentage of the manual measured FHV. Still, the model of the manual femoral head (FHV) was equivalent to the sphere adjusted volume (SAV) calculation estimating the amount of ON (reducing 22% of the sphere volume in the case of males, or reducing 29% of the sphere volume if females), as observed in Supplementary Table ii. The comparison of FHV and SAV (Supplementary Figures c and d) showed perfect agreement (rho = 0.963, p < 0.001) and proved concordance between both measures (δ = mean 0 (SD 3.6); p = 0.738, Bradley-Blackwood test; see methodology in the Supplementary Material). SAV can be useful for systematic calculations in future studies.

An ON lesion larger than 25% of the femoral head has been identified as a predictor of femoral head collapse after one year following diagnosis in case of patients

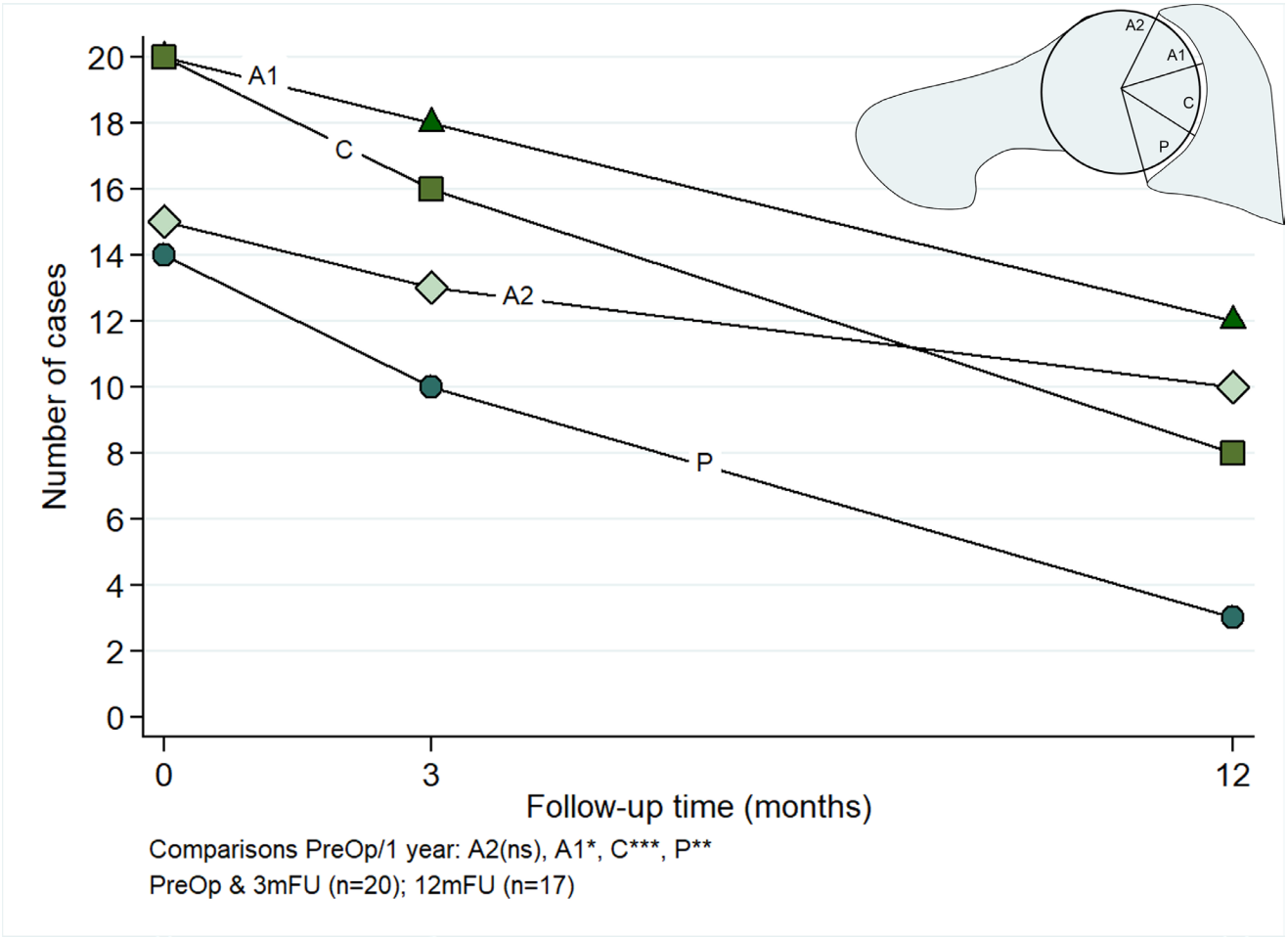

Fig. 5

Location of bone regeneration during the follow-up (FU) using the transverse anterior-central-posterior (ACP) method. ACP classification: type A1 when osteonecrosis (ON) occupies the anterior section but does not surpass the acetabular edge; type A2, which is like A1 but surpasses the acetabular edge; type C when ON occupies the central area of the head; and type P when ON occupies the posterior head. Sample size: preoperative and three months' FU (3mFU) (n = 20), 12 months' FU (12mFU) (n = 17). Fisher's exact test comparison from preoperative to 12mFU per location: A2 (ns); A1*; C***; P**. *p < 0.05; **p < 0.01; ***p < 0.001. ns, not significant.

under conservative treatment or untreated.[31,34] In a series of cases secondary to systemic lupus erythematosus under pharmacological treatment, the mean ON volume of small lesions improved (from 7.20% (SD 5.54%) to 2.51% (SD 3.70%); p < 0.001, paired *t*-test), while those with larger volume did not change (from 38.6% (SD 7.5%)to 35.1% (SD 11.9%); n.s., paired *t*-test) after six-year FU.[34] Our data found that preoperative volumes lower than 22% of the femoral head tend to heal after one year of BM-MSC implantation, while failure was observed in higher volumes. Nevertheless, an important debate is placed on the timing of regeneration. Our series evaluated the regeneration after three and 12 months, to understand if early regeneration may be needed to avoid collapse. Higher amount of regeneration occurred three months after surgery in healed cases, with a mean rate of 2.4% lesion volume decrease per month, compared with 1.5% per month in the next nine months of FU. When the remaining lesion was under mean 10% (SD 6%) of the femoral head at three months due to early regeneration, no further ON stage progression or THA conversion was seen at 12 months. Hence, this therapy would aim to decrease the ONV to a lesion under mean 10% (SD 6%) of the femoral head in the first three months. We can hypothesize that if the remaining lesion at three months exceeded the 15% volume, the femoral head might collapse despite the cell injection. Therefore, a second intervention might be recommended because the final regeneration could be insufficient to avoid ON progression or THA.

The number of available non-healed cases was insufficient to confirm the amount of regeneration (n = 4 at three months, and n = 1 at 12 months due to THA conversion of three other cases). However, non-healed cases at three months still showed a mean regeneration similar to the healed group (although the comparison was underpowered). In these non-healed cases, the amount of regeneration could not bring the volume of

the lesion under mean 10% (SD 6%) of the femoral head. This could be the reason why ON stage progression or even collapse occurred in all four cases, motivating THA in three of them by one year. A recent meta-analysis suggests the superiority of cell therapy after CD versus CD alone to eliminate necrotic areas in the osteonecrotic femoral head, reducing the risk of THA conversion.[35] When comparing CD with CD plus cell therapy, studies suggest a significant improvement in both clinical and radiological outcomes, using the combined treatment.[36] Yet, no quantitative data on the amount of regeneration needed to outperform CD with added cell therapy have been previously communicated.

The 3D reconstructions and the location distribution of the ON lesions allowed us to evaluate the topography of the bone regeneration. It was initiated around the forage location but spread in different femoral head regions. However, not all regions within the treated femoral heads showed similar regeneration. After one year, traces of the osteonecrotic lesions in most cases were still present and remnants were frequently seen as encapsulated cysts.

It was clearly shown that the lateral region of the femoral head in the coronal plane, particularly lateral to the acetabulum edge, was strongly regenerated in those femoral heads that did not progress to collapse, as previously proposed.[37,38] On the contrary, in the coronal section more medial areas showed slower and less consistent bone regeneration.[37,38]

Anterior, central, and posterior areas of the lesion were also evaluated in the transverse plane, and regeneration was detected in all of them. However, the transverse plane may under-represent the lesion, frequently located in the proximal subsurface of the femoral head. Anterior regeneration remained low, both at three and 12 months postoperatively, while central and posterior areas of the femoral head at 12 months showed regeneration in the healed cases. This would suggest that residual cysts may remain more frequently in the anterior region, which is consistent with Baba et al[39] who found a high bone-resorptive volume in the anterior femoral head of resected hips. A recommendation to aim the injection at the anterior femoral head, as previously suggested,[28] could be supported by this finding.

The main limitation of this study is the reduced sample size, because the clinical trial was designed as a pilot. Consequently, some comparisons were underpowered. Although the regression model fits, larger samples will be needed to confirm our findings. The intrinsic variability in these lesions is another limitation. The complex 3D spherical spread of the osteonecrotic lesions further compounds the result evaluation, but this was solved by a systematic approach to coronal and transverse topographical studies supported by 3D reconstructions. A third limitation is surgery and the injection placement of the cell therapy product, as analyzed in a recent paper.[28] However, the current study confirms that regeneration may extend in different areas of the femoral head lesion. Other aspects (tissue density and permeability, cell distribution after injection, cell progression, or survival within the lesion) may apply to explain variable bone regeneration. More information from the regenerated bone in histological studies might be helpful to interpret the amount and quality of bone regeneration.

As a conclusion, bone regeneration was observed in all cases of ONFH after injection of a cell therapy product based on 140 million autologous BM-derived expanded MSCs. However, a residual lesion of 15% at three months postoperatively would suggest that regeneration may be insufficient to prevent progression or collapse. This being the case, a second injection may be required but further studies are needed to evaluate this repeated injection as the best strategy to improve the current results.

Supplementary material

Methodology to perform the volume calculation, figures from different cases, and a 3D reconstruction video showing the evolution of the treated osteonecrotic lesion from preoperative to three and 12 months postoperative.

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Ethical review statement:
- The clinical trial was performed in accordance with the Declaration of Helsinki, after approval at the Ethics Committees of all the participating hospitals (with the authorization code at the coordinating hospital: HULP 3875). All included patients agreed to their participation in the trial and signed an appropriate informed consent form.

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