Safety and Efficacy of Bone Marrow–Derived Mesenchymal Stem Cells for Chronic Patellar Tendinopathy (With Gap >3 mm) in Patients

12-Month Follow-up Results of a Phase 1/2 Clinical Trial

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Background: In a previous study, the authors found that at 6 months after treatment with a 20×10^6 dose of bone marrow–derived mesenchymal stem cells (BM-MSCs), patients showed improved tendon structure and regeneration of the gap area when compared with treatment using leukocyte-poor platelet-rich plasma (Lp-PRP). The Lp-PRP group (n = 10), which had not seen tendon regeneration at the 6-month follow-up, was subsequently offered treatment with BM-MSCs to see if structural changes would occur. In addition, the 12-month follow-up outcomes of the original BM-MSC group (n = 10) were evaluated.

Purpose: To evaluate the outcomes of all patients (n = 20) at 12 months after BM-MSC treatment and observe if the Lp-PRP pretreated group experienced any type of advantage.

Study Design: Cohort study; Level of evidence, 2.

Methods: Both the BM-MSC and original Lp-PRP groups were assessed at 12 months after BM-MSC treatment with clinical examination, the visual analog scale (VAS) for pain during daily activities and sports activities, the Victorian Institute of Sport Assessment–Patella score for patellar tendinopathy, dynamometry, and magnetic resonance imaging (MRI). Differences between the 2 groups were compared with the Student *t* test.

Results: The 10 patients originally treated with BM-MSCs continued to show improvement in tendon structure in their MRI scans ($P < .0001$), as well as in the clinical assessment of their pain by means of scales ($P < .05$). Ten patients who were originally treated with Lp-PRP and then with BM-MSCs exhibited an improvement in tendon structure in their MRI scans, as well as a clinical pain improvement, but this was not significant on the VAS for sports ($P = .139$). Thus, applying Lp-PRP before BM-MScs did not yield any type of advantage.

Conclusion: The 12-month follow-up outcomes after both groups of patients (n = 20) received BM-MSC treatment indicated that biological treatment was safe, there were no adverse effects, and the participants showed a highly statistically significant clinical improvement ($P < .0002$), as well as an improvement in tendon structure on MRI ($P < .0001$). Preinjection of Lp-PRP yielded no advantages.

Keywords: regenerative medicine; mesenchymal stem cells; patellar tendinopathy; jumper's knee; sports injury

In patellar tendinopathy, elite and nonelite running and jumping athletes[9,14] experience anterior knee pain and reduced function, often for prolonged periods.[16] Although modification of training and implementation of load management programs may be effective,[5] it is still unclear what the optimal strategies are.[17] In addition, the results of nonoperative management are unpredictable, and returning to sport is not certain despite rigorous adherence to rehabilitation programs.[5,14,19,22]

Shockwave therapy,[12] prolotherapy,[20] high-volume injection,[21] and corticosteroid injection[1,21] have been tried as treatments for patellar tendinopathy, and recently, platelet-rich plasma (PRP) injections have become popular, although the ideal composition of PRP is still

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unclear.[10,25,26] Increased apoptotic cell death is a feature of patellar tendinopathy, and it contributes to the pathophysiology of patellar tendinopathy.[13] The management of tendinopathy remains a major challenge.[2] These nonoperative treatments usually obtain unsatisfactory results at the level required by professional sports persons. Moreover, 10% of patients do not respond to any treatment and require a surgical option. Currently, these surgical options include debridement without bone resection and removing the neovascularization and the innervation linked to the degenerative area, with good functional results in 80% of cases.[7,18] Nonetheless, the surgical option is not free of complications, and it is not attractive to athletes, as it involves recovery times of at least 5 months. In addition, failure after surgery means that this option can bring about a poor prognosis.[3,16]

Regenerative medicine with autologous expanded bone marrow–derived mesenchymal stem cell (BM-MSC) therapy has recently been introduced in the management of tendon pathology.[6] In 2021, we published the 6-month follow-up results of a prospective, double-blind, randomized, 2-arm, parallel, active-controlled, phase 1/2, single-center clinical study investigating the effects of ultrasound-guided intratendinous and peritendinous injections of BM-MSCs or leukocyte-poor PRP (Lp-PRP) on clinical outcomes in younger patients (age, 18-48 years) with patellar tendinopathy.[24] At 6 months after treatment, patients who received BM-MSC treatment demonstrated significantly greater improvement in tendon structure as measured by 2-dimensional ultrasound and magnetic resonance imaging (MRI) when compared with patients who received Lp-PRP.[24] There were no significant differences in patient-reported outcomes (visual analog scale [VAS] for pain or Victorian Institute of Sport Assessment–Patella [VISA-P] score) between the study groups,[24] indicating that injections of BM-MSCs or Lp-PRP, together with rehabilitation, were both effective in chronic refractory patellar tendinopathy to reduce pain and improve activity levels in these patients.

After completion of the first phase of the study,[24] a new hypothesis was formulated that included a planned 12-month analysis,[23] and the current study reports the 12-month follow-up results. The original hypothesis of the investigation was that Lp-PRP and BM-MSC injections into the patellar tendon gap, in combination with exercise-based rehabilitation, would decrease pain in those experiencing patellar tendinopathy and improve functional outcomes.[24] We also hypothesized that improvement in tendon structure consistent with regeneration would occur only in patients in whom BM-MSCs were injected.[24]

METHODS

Study Design and Participants

The study was a prospective, active-controlled clinical trial with a 12-month follow-up period. The study protocol received ethics committee approval, and all participants provided written informed consent. All participants who met the inclusion criteria were recruited and monitored for 12 months after BM-MSC treatment according to the study protocol.[23]

The original study[24] included 20 adult men aged 18 to 48 years who had sustained unilateral chronic patellar tendinopathy with an intratendinous lesion >3 mm in longitudinal diameter in the patellar tendon, just distal to the lower pole of the patella, such as a grade 2 partial tear (<25%) in accordance with the Popkin-Golman classification.[11] All lesions were confirmed on MRI according to standard diagnosis criteria. Patients were evaluated with focal load-related pain near the attachment of the patellar tendon at the lower pole of the patella and palpable tenderness in that area for >4 months, having been unresponsive to nonoperative management and rehabilitation.[15]

Procedure and Study Design

Eligible participants were recruited from December 2017 to November 2018 and randomly assigned to receive BM-MSCs (n = 10) or Lp-PRP (n = 10).[24] At 6 months after treatment, the study was unblinded. Between 6 and 14 months after treatment (median, 11.6 months), the 10 patients in the Lp-PRP group were transferred to receive BM-MSC treatment. This cross resulted in a protocol addendum that was accepted by the ethics committee of our institution. The patients in the original BM-MSC group

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continued to be evaluated for an additional 6 months, while the patients in the original Lp-PRP group received their initial treatment with BM-MSCs. Thus, for the current study we evaluated the 12-month follow-up results after BM-MSC treatment in 20 patients with recalcitrant patellar tendinopathy, with the final follow-up evaluations occurring 6 months later for patients in the original Lp-PRP group. The study flow diagram is shown in Figure 1.

Study Dosing

BM-MSCs are mesenchymal stem cells from autologous bone marrow, isolated and expanded according to good manufacturing practice guidelines for clinical application based on the criteria of the International Society for Cellular Therapy (ISCT). We followed the MIBO (Items for Minimum Information for Studies Evaluating Biologics in Orthopedics) guidelines and checklist for clinical studies evaluating BM-MSCs.

BM-MSC Characteristics and Processing

For all participants, bone marrow was obtained from the posterior iliac crest in an outpatient surgery session. Briefly, the patient was sedated with midazolam, placed prone, and kept under the effects of local anesthesia (20 mL of 1% lidocaine diluted vol/vol with saline) applied in both posterior iliac crests. Bone marrow aspiration was performed on both sides of the iliac crest with an 11-gauge trocar. Through use of sequential aspirations of 1 to 2 mL each, 100 mL of bone marrow was collected in a heparinized container, conditioned, refrigerated at 4°C, and processed within the first 24 hours. All processed bone marrow samples were confirmed negative for microbiological contamination, human immunodeficiency virus (HIV), hepatitis A and B, and rapid plasma reagin syphilis.

The mononuclear fraction was isolated by means of gradient centrifugation (Ficoll-Plaque, GE Healthcare Bio-Sciences AB), resuspended, and cultured in MSC expansion culture medium in a 175-cm^2 tissue culture flask, in a humidified incubator at 37°C with 5% CO_2, with periodic washing to remove nonadherent cells. When the cells reached 80% confluence, they were trypsinized and replanted, and the process was repeated for 2 more passes. Isolations were carried out with the following parameters (mean ± SD): bone marrow volume, $10^3 \pm 8$ mL; number of mononuclear cells obtained, $1.1 \pm 0.5 \times 10^9$; expansion time, 22 ± 2 days; and viability >98%. At the end of this period (23 days), cells were harvested and resuspended in an isotonic medium composed of Ringer lactate solution containing 0.2% human albumin (CSL Behring GmbH) and 5 mM glucose. The product was kept stable for 8 hours at 4°C to 2°C and was transported to the hospital facilities on the scheduled treatment date. The product was supplied in two 5-mL syringes, one containing 10×10^6 MSCs suspended in a 2-mL solution and the other containing 10×10^6 MSCs suspended in a 4-mL solution. The total dose of cells supplied was $(20 \times 10^6) \pm (2 \times 10^6)$ MSCs. In addition to quality control tests (mycoplasma negative), endotoxin levels were below 0.5 IU/mL, and flow cytometric immunophenotypic profiles were determined at this stage (cluster of differentiation [CD] 14–, CD34–, CD45–, human leukocyte antigen–DR isotype–, CD105+, CD166+, CD73+, CD90+). The antigenic profile conformed to the ISCT criteria for MSCs.[8]

BM-MSC Injection Procedure

With the patient positioned supine and under sedation with midazolam and propofol, a specialist in ultrasound-guided injection (R.S.) administered the solutions of BM-MSCs: a 2-mL solution containing 10×10^6 MSCs was injected into the tendon gap, a 2-mL solution containing 5×10^6 MSCs was injected into the medial peritendinous area, and a 2-mL solution containing 5×10^6 MSCs was injected into the lateral peritendinous area accounting for the Hoffa fat pad, as described in the study protocol.[23]

Rehabilitation Protocol

All patients performed the same posttreatment progressive rehabilitation protocol as described in Appendix Table A1, based on isometrics exercises, concentric isotonic exercises, and eccentric exercises[21] progressively at 3 days, 2 weeks, and 4 weeks, respectively, after injection of BM-MSCs.

Data Collection

The following data were collected: physical examination, pain during daily life, and pain during sports activities as recorded on the VAS and VISA-P scale, which is the most condition-specific patient-reported outcome measure used to assess symptom severity in athletes with patellar tendinopathy, especially for jumper's knee.

Also collected were blood sample parameters and serologies, which were reviewed as part of the inclusion criteria for the original study[24] (complete blood count, platelet count, C-reactive protein, erythrocyte sedimentation rate; coagulation factors: prothrombin time, partial thromboplastin time; comprehensive metabolic panel: glucose, calcium, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, albumin, total protein; serology: hepatitis C, hepatitis B surface antigen [HBsAg], antibody to hepatitis B core antigen, hepatitis B type e antigen, antibody to HBsAg, HIV antibody test, and rapid plasma reagin [syphilis]). In addition, we evaluated quadriceps force measured on manual dynamometry and MRI parameters. Patients were closely monitored after recruitment at 1, 2, 3, 6, 8, and 10 weeks and 3, 4, 5, 6, and 12 months through questionnaires and physical examination. Blood tests were performed at baseline and 3 months. Pain scales, dynamometry, and MRI scans were collected at baseline and 3, 6, and 12 months after BM-MSC inoculation.

Adverse events (AEs) were recorded using the Medical Dictionary of Regulatory Activities (MedDRA) Version 16.1 throughout the trial. Monitoring for AEs occurred at each review. Serious AEs were recorded and assessed by both

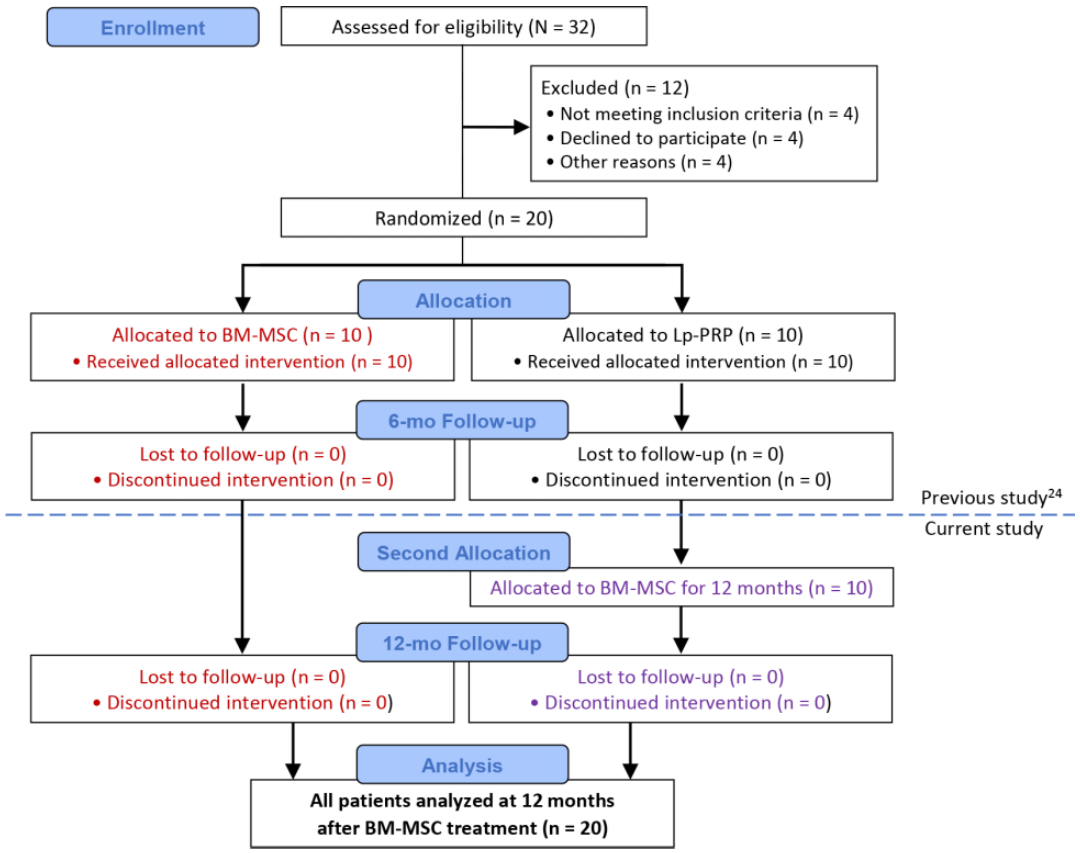

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flowchart diagram. BM-MSC, bone marrow–derived mesenchymal stem cell; Lp-PRP, leukocyte-poor platelet-rich plasma.

the site investigators and a blinded medical monitor. For serious AEs, a notification was sent to the Spanish Agency for Medicines and Healthcare Products through the local Clinical Research Organization (Adknoma Health Research), in accordance with standard operating procedures.

Outcome Evaluation

The primary study endpoints were symptoms, exploration of the tendon, patient-reported outcomes (VAS pain and VISA-P), manual dynamometry to evaluate the strength assessment of the extensor muscle group, and MRI for measuring changes in the structure of the patellar tendon. Dynamometry was used for measuring the quadriceps force with maximal voluntary isometric contraction measured with a handheld dynamometer (Mark-10 Serie 3; Mark-10 Corp) fixed to an examination table. Participants were in a standard sitting position with 90° of knee flexion and were instructed to progressively apply maximum strength toward knee extension.[12] Participants completed a maximal voluntary isometric contraction for 5 seconds for 3 repetitions, with 30 seconds of rest between each contraction. Quadriceps strength was recorded, and pain levels during the test were recorded with a numeric rating scale in both legs.[19,27]

The MRI scans (Vantage Galan 3-T; Canon Medical) were performed using various sequences (coronal T2-weighted gradient echo, 0.5-mm slice thickness; sagittal proton-density [PD] fat-saturated, 2-mm slice thickness; coronal PD fat-saturated, 1.5-mm slice thickness; axial PD fat-saturated, 2-mm slice thickness; and sagittal T2-weighted mapping, fast spin-echo T2 with 4 echo times 20, 60, 100, and 140 milliseconds for parametric reconstruction image) on all study patients during the first evaluation before treatment as well as at the 3-, 6-, and 12-month follow-up visits. In addition to measuring lesion size (longitudinal, transverse, anteroposterior) and volume, we graded the following MRI parameters as per our previous study[24] on a 0- to 100-point scale, with 100 indicating complete restoration of the tendon structure:

1. Lesion size on coronal T2-weighted fat-saturated image (no lesion = 40 points; ≤3-mm gap = 30 points; >3- to 6-mm gap = 20 points; >6- to 10-mm gap = 10 points; and >10-mm gap = 0 points)
2. Appearance of homogeneous tissue and passage of fibers/fascicles on sagittal and coronal T2-weighted fat-saturated images (32 points)
3. Hypersignal on coronal T2-weighted spin-echo image (10 points)

4. Edema of the Hoffa body on sagittal and axial T2-weighted fat-saturated images (9 points)
5. Bone edema of the lower patella on sagittal and axial T2-weighted fat-saturated images (9 points)

This scoring system was devised by us and was validated by 4 independent radiologists.[24]

Statistical Analyses

The safety population comprised all patients who received BM-MSCs. As the intention-to-treat and per-protocol populations were the same for all visits, efficacy results were represented by groups (BM-MSC group [n = 10], original Lp-PRP group [n = 10]) and by the overall BM-MSC study cohort (N = 20) at the 12-month follow-up after treatment.

Descriptive statistics for the characteristics of the study participants were recorded as absolute and relative frequencies for categorical variables and as mean ± SD with ranges as applicable for continuous variables. For continuous variables, the study groups were compared using parametric (Student *t* test) or nonparametric (Mann-Whitney *U* test) tests, in accordance with the characteristics of the variable (assumption of normality). In addition, for comparisons of continuous variables between different evaluation times, parametric (Student *t* test for paired data) or nonparametric (Wilcoxon signed-rank test) tests were used, as appropriate. A statistical significance level of .05 was applied to all statistical tests, and the statistical analyses were performed with the SAS statistical software package (Version 9.4 or later, SAS Institute). The statistician was not part of the medical team and undertook analysis without any knowledge of the participants.

Regarding sample size calculation, in the first part of our original study, a sample size of 10 patients per group was required to detect an effect size of 0.6 with a power of 80%, assuming balanced allocation to treatment groups, and 5% type 1 error probability.[24] The second part of the original study was a phase 1/2 clinical study in which the feasibility of the proposed procedure and the safety of the product under study were evaluated. It also sought to determine the efficacy of the experimental treatment. Hence, the sample size for the current study was set at 20 patients diagnosed with patellar tendinopathy who met the inclusion criteria and did not have any exclusion criteria.

RESULTS

The patient and anthropometric characteristics (recorded at the inclusion visit of the original study[24]) are shown in Table 1 for all patients as well as by group.

The mean bone marrow volume obtained was 101.00 ± 4.47 mL. The mean time elapsed between bone marrow retrieval and ultrasound-guided MSC implantation was 21.65 ± 2.89 days. All participants received the same BM-MSC injection procedure: intratendinous injection (volume, 10.0×10^6/mL), medial peritendinous injection (volume, 5.0×10^6/mL), and lateral peritendinous injection (volume, 5.0×10^6/mL), for a total cell dose of $20 \times 10^6 \pm 2 \times 10^6$ BM-MSCs in 6.0 mL. No complications occurred during the procedures.

All clinical parameters improved at 12 months. While 25% of patients had swelling at the inclusion/baseline visit, none of the patients had swelling at 12 months. In addition, 5% of patients showed signs of inflammation at the baseline/inclusion visit, but no such signs were present at 12 months. Finally, 95.0% of the patients had pain on tendon palpation at the baseline/inclusion visit, compared with only 5% at 12 months.

Table 2 shows the comparison of VAS pain and VISA-P scores from baseline to the 12-month follow-up, overall and by group. The BM-MSC group showed posttreatment improvement on all scales ($P < .05$ for all), and the patients from the original Lp-PRP group showed improvement in VAS for pain during daily activities ($P = .0039$) and VISA-P ($P = .0019$), with no significant improvement in VAS for pain during sports activities ($P = .139$). The results for all 20 patients at 12 months of BM-MSC treatment indicated improvement on all scales ($P < .01$ for all). When comparing the change from baseline to the 12-month follow-up ($\Delta_{\text{baseline–12 mo}}$), there were no statistically significant differences between the study groups for VAS pain during daily activities ($P = .3668$), VAS pain during sports activities ($P > .999$), or VISA-P score ($P = .0863$).

Table 3 reports the MRI findings at the baseline and 12-month visits. Regarding the grading of MRI parameters, the BM-MSC group showed significant posttreatment improvement in the grading of lesion size ($P = .0039$), passage of fibers ($P < .0001$), and hypersignal ($P = .0028$). The group originally treated with Lp-PRP showed no significant improvement on any MRI parameter except the passage of fibers ($P = .0426$). Regarding the overall grading, the BM-

TABLE 1
Patient and Anthropometric Characteristics by Study Group and Overall[a]

	BM-MSC Group (n = 10)	Original Lp-PRP Group (n = 10)	All Patients (N = 20)
Age, y	35.80 ± 10.03 (18-45)	32.00 ± 9.45 (20-46)	33.90 ± 9.68 (18-46)
White ethnicity and race	10 (100)	10 (100)	20 (100)
Weight, kg	83.65 ± 7.39	79.95 ± 13.47	81.80 ± 10.74
Height, cm	1.80 ± 6.60	1.81 ± 7.90	180.10 ± 7.76
BMI, kg/m^2	25.75 ± 2.25 (21.91-29.68)	24.54 ± 1.91 (22.30-28.34)	25.14 ± 2.13 (21.91-29.68)
Side affected, left/right	6 (60)/4 (40)	8 (80)/2 (20)	14 (70)/6 (30)

[a]Data are presented as mean ± SD (range) or n (%). All data were normally distributed. No patients were lost between the 6- and 12-month follow-ups. BMI, body mass index; BM-MSC, bone marrow–derived mesenchymal stem cell; Lp-PRP, leukocyte-poor platelet-rich plasma.

TABLE 2
Patient-Reported Outcomes From Baseline to 12 Months by Study Group and Overall[a]

Outcome Measure	Baseline	12-mo Follow-up	$\Delta_{\text{baseline–12 mo}}$[b]	*P*
BM-MSC group (n = 10)				
VAS pain during daily activities[c]	4.23 ± 2.13	1.83 ± 2.85	2.40 ± 2.93	**.0294**
VAS pain during sports activities[c]	6.91 ± 1.11	2.58 ± 3.35	4.44 ± 3.00	**.0041**
VISA-P scale[d]	42.30 ± 16.29	72.00 ± 25.76	29.70 ± 22.26	**.0022**
Original Lp-PRP group (n = 10)				
VAS pain during daily activities[c]	2.61 ± 2.11	0.76 ± 0.83	1.86 ± 1.82	**.0039**
VAS pain during sports activities[c]	3.50 ± 2.19	0.98 ± 2.41	2.53 ± 2.19	.139
VISA-P scale[d]	62.22 ± 21.80	81.22 ± 13.95	19.00 ± 12.59	**.0019**
Overall (n = 20)				
VAS pain during daily life[c]	3.34 ± 2.23	1.32 ± 2.16	2.14 ± 2.42	**.0011**
VAS pain during sports activities[c]	5.05 ± 2.49	1.93 ± 2.69	3.48 ± 2.73	**.0001**
VISA-P scale[d]	54.00 ± 22.96	76.37 ± 20.99	24.63 ± 18.66	**.0002**

[a]Data are presented as mean ± SD. Boldface *P* values indicate a statistically significant difference between baseline and the 12-month follow-up ($P < .05$, Student *t* test). BM-MSC, bone marrow–derived mesenchymal stem cell; Lp-PRP, leukocyte-poor platelet-rich plasma; VAS, visual analog scale; VISA-P, Victorian Institute of Sport Assessment–Patella.
[b]Positive values indicate improvement.
[c]The VAS is graded from 0 points (no pain) to 10 points (unbearable pain).
[d]The VISA-P score is graded from 0 points (least satisfactory) to 100 points (most satisfactory).

MSC group was evaluated with significant improvement between baseline and the 12-month follow-up ($P < .0001$), while the group previously treated with Lp-PRP showed no changes ($P = .0907$).

The MRI findings for the entire study group indicated significant improvement from baseline to the 12-month follow-up in the overall grading of parameters ($P < .0001$) and significant improvement in the individual parameter grading with the exception of lower patellar bone edema ($P = .1250$) (Table 4).

Figure 2 shows the structural improvement of the tendon on MRI in 3 patients from the BM-MSC group, and Figure 3 shows the MRI evolution in 3 patients who had originally been treated with Lp-PRP and then received BM-MSCs. None of the patients initially treated with Lp-PRP had shown regenerative changes in their patellar tendon by 6 months posttreatment with Lp-PRP (middle images). However, at 12 months after injection of BM-MSCs, positive structural changes in their patellar tendon could be readily appreciated, although in all main MRI aspects these changes were not significant ($P = .0907$).

Safety Profile

A total of 12 patients reported 24 AEs during the 12 months of the study. The most frequent were musculoskeletal complaints (50%): patellofemoral pain (20.8%; 5 cases), arthralgia (12.5%; 3 cases), myalgia (8.3%; 2 cases), back pain (4.2%; 1 case), and swelling (4.2%; 1 case). Other events were otitis, gastritis, hand fracture, allergy, migraine, and headache.

Only 5 of the 24 (20.8%) AEs were considered to be related to the injections, and all were mild and transient, related to pain and swelling in the area of injection. Patients were prescribed analgesic medication such as acetaminophen or ibuprofen, and all events cleared up after a few days of treatment. Only 3 of the AEs were still present by the end of the study, and all were patellofemoral pain.

DISCUSSION

When we analyzed clinical function in the 20 patients treated with BM-MSCs at 12 months after treatment, all parameters had improved in a statistically significant and clinically meaningful way, although 1 patient from the Lp-PRP group still experienced some pain at palpation of the tendinopathic area. In our preliminary outcomes after 6 months, the patients in the Lp-PRP group experienced positive changes regarding pain, with these changes being even greater than those for the BM-MSC group, but there were no statically significant differences between the groups on VAS pain or VISA-P scores.[24] Now, considering 12 months of treatment with BM-MSCs in both groups, regardless of whether the patients had previously received Lp-PRP–based therapies, both groups saw improvements in pain.

The improvement in mean VAS pain scores during daily activities was significant in both study groups at 12 months after treatment with BM-MSCs, but the improvement in mean VAS pain during sports activities was not significant in the group originally treated with Lp-PRP ($P = .139$), although the reduction in score was notable. These results are probably because of the small sample size (n = 10). On the other hand, significant differences were observed in the VISA-P score ($P = .0002$) between the 12-month visit and the inclusion/baseline visit, with the mean score at the 12-month visit being higher than at the inclusion/baseline visit. There were no statistically significant differences in the relative percentage change in the VISA-P total score at 12 months between patients treated with BM-MSCs and patients initially treated with Lp-PRP. Therefore, both

TABLE 3
MRI Findings From Baseline to 12 Months in the Study Groups[a]

	Baseline	12-mo Follow-up	$\Delta_{\text{baseline–12 mo}}$[b]	*P*
Lesion size				
Longitudinal, mm				
BM-MSC group	6.75 ± 4.20	3.15 ± 2.94	3.60 ± 5.10	**.0098**
Original Lp-PRP group	6.00 ± 2.49	4.10 ± 5.26	1.90 ± 6.01	.0801
Transverse, mm				
BM-MSC group	4.30 ± 2.16	4.50 ± 2.92	–0.20 ± 1.87	.7435
Original Lp-PRP group	6.40 ± 2.32	3.15 ± 1.97	3.25 ± 2.55	**.0030**
Anteroposterior, mm				
BM-MSC group	5.10 ± 2.33	2.90 ± 2.14	2.20 ± 1.67	**.0024**
Original Lp-PRP group	4.25 ± 1.44	2.15 ± 1.94	2.10 ± 2.25	**.0160**
Lesion volume, mm^3				
BM-MSC group	140.10 ± 136.44	89.10 ± 135.76	51.00 ± 100.61	.0840
Original Lp-PRP group	192.00 ± 196.86	40.55 ± 67.12	151.45 ± 209.95	**.0092**
Grading of MRI parameters[c]				
Lesion size				
BM-MSC group	15.00 ± 7.07	28.00 ± 7.89	13.00 ± 10.59	**.0039**
Original Lp-PRP group	17.50 ± 5.00	22.50 ± 9.57	5.00 ± 5.77	.5000
Passage of fibers/fascicles				
BM-MSC group	14.30 ± 5.17	29.10 ± 1.97	14.80 ± 4.80	**<.0001**
Original Lp-PRP group	20.30 ± 8.31	27.10 ± 3.81	6.80 ± 9.11	**.0426**
Hypersignal				
BM-MSC group	4.00 ± 1.25	7.50 ± 2.01	3.50 ± 2.72	**.0028**
Original Lp-PRP group	6.00 ± 1.63	7.50 ± 1.51	1.50 ± 2.46	.0860
Hoffa fat edema				
BM-MSC group	3.80 ± 2.30	6.50 ± 2.37	2.70 ± 4.08	.0661
Original Lp-PRP group	6.40 ± 1.96	7.60 ± 1.58	1.20 ± 2.30	.1333
Bone edema of lower patella				
BM-MSC group	5.40 ± 2.17	7.50 ± 2.01	2.10 ± 3.28	.1250
Original Lp-PRP group	7.00 ± 2.21	7.70 ± 1.34	0.70 ± 2.11	.3217
Overall				
BM-MSC group	42.50 ± 8.05	78.60 ± 9.72	36.10 ± 14.71	**<.0001**
Original Lp-PRP group	64.00 ± 7.35	72.25 ± 12.18	8.25 ± 6.70	.0907

[a]Data are presented as mean ± SD. Boldface *P* values indicate a statistically significant difference between baseline and the 12-month follow-up ($P < .05$, Student *t* test). BM-MSC, bone marrow–derived mesenchymal stem cell; Lp-PRP, leukocyte-poor platelet-rich plasma; MRI, magnetic resonance imaging.
[b]Positive values indicate improvement.
[c]Graded on a scale from 0 to 100, with 100 indicating complete restoration of the tendon structure.

TABLE 4
MRI Findings From Baseline to 12 Months in All Patients (N = 20)[a]

	Baseline	12-mo Follow-up	$\Delta_{\text{baseline–12 mo}}$[b]	*P*
Lesion size				
Longitudinal, mm	6.38 ± 3.38	3.63 ± 4.18	2.75 ± 5.49	**.0010**
Transverse, mm	5.35 ± 2.43	3.83 ± 2.52	1.53 ± 2.81	**.0252**
Anteroposterior, mm	4.68 ± 1.93	2.53 ± 2.03	2.15 ± 1.93	**<.0001**
Lesion volume, mm^3	159.74 ± 15.85	35.17 ± 21.38	101.23 ± 168.32	**.0014**
Grading of MRI parameters[c]				
Lesion size	16.50 ± 5.87	26.43 ± 8.42	10.71 ± 9.97	**.0010**
Passage of fibers/fascicles	17.30 ± 7.41	28.10 ± 3.13	10.80 ± 8.19	**<.0001**
Hypersignal	5.00 ± 1.75	7.50 ± 1.73	2.50 ± 2.72	**.0006**
Hoffa fat edema	5.10 ± 2.47	7.05 ± 2.04	1.95 ± 3.32	**.0206**
Bone edema of lower patella	5.40 ± 2.17	7.60 ± 1.67	2.10 ± 3.28	.1250
Overall	49.30 ± 3.93	76.68 ± 3.40	36.10 ± 14.71	**<.0001**

[a]Data are presented as mean ± SD. Boldface *P* values indicate a statistically significant difference between baseline and 12-month follow-up ($P < .05$, Student *t* test). MRI, magnetic resonance imaging.
[b]Positive values indicate improvement.
[c]Graded on a scale from 0 to 100, with 100 indicating complete restoration of the tendon structure.

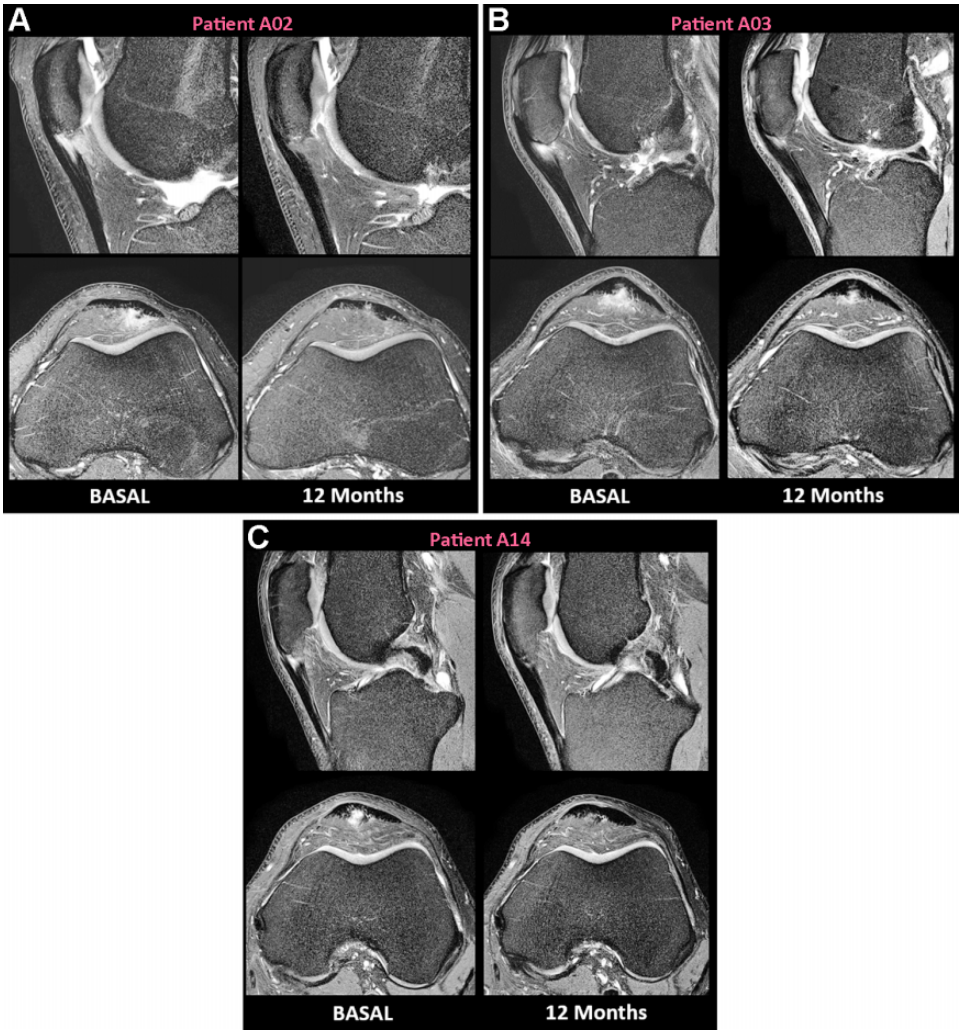

Figure 2. Sagittal (top row) and axial (bottom row) T2-weighted fat-saturated magnetic resonance imaging sequences of 3 patients who were initially treated with bone marrow–derived mesenchymal stem cells (BM-MSCs). Shown are images from baseline and 12-month follow-up after BM-MSCs.

treatments were effective in terms of pain management, but the injection of BM-MSCs produced significant favorable changes in intratendinous structure. We still do not know, however, whether this may be associated with a lower rate of recurrence after BM-MSC injection.

Analyzing the MRI data in detail, at 12 months in the overall group, we observed only statistically significant differences in lesion cross-sectional sizes, but a different phenomenon was observed between groups. In the group of patients who were initially treated with BM-MSCs, the mean cross-sectional lesion size increased, and the longitudinal and anteroposterior sizes decreased, as well as there being a decrease in volume, while in the group of patients who initially were treated with Lp-PRP, the mean cross-sectional size decreased. These differences can be explained by the sample size being too small. Thus, the important thing is to observe that in the analysis of lesion size and volume in all patients, all aspects improved significantly ($P < .05$ for all) (Table 4). This coincides with the closure of lesions observed through the MRI scans and the reduction of VAS pain scores. By 12 months, there was better tendon restructuring, with progressive evidence of significant tendon tissue regeneration in all patients who participated in the study (Table 4).

Treatment with BM-MSCs was effective in the overall group in terms of pain, size of the lesion, and changes on the MRI scans, while prior treatment with Lp-PRP did not show any great changes in terms of lesion repair. Overall, the main aspects checked with the MRI were significant. These data are not conclusive because we would probably need a larger sample size; however, they do indicate that pretreatment with Lp-PRP does not provide any benefit to subsequent treatment with MSCs, and the only treatment to cause changes to the lesion when studied with MRI was BM-MSCs.

When comparisons of the different dynamometry parameters were made between the 3-week visit (first visit where the dynamometry test was performed) and the 12-

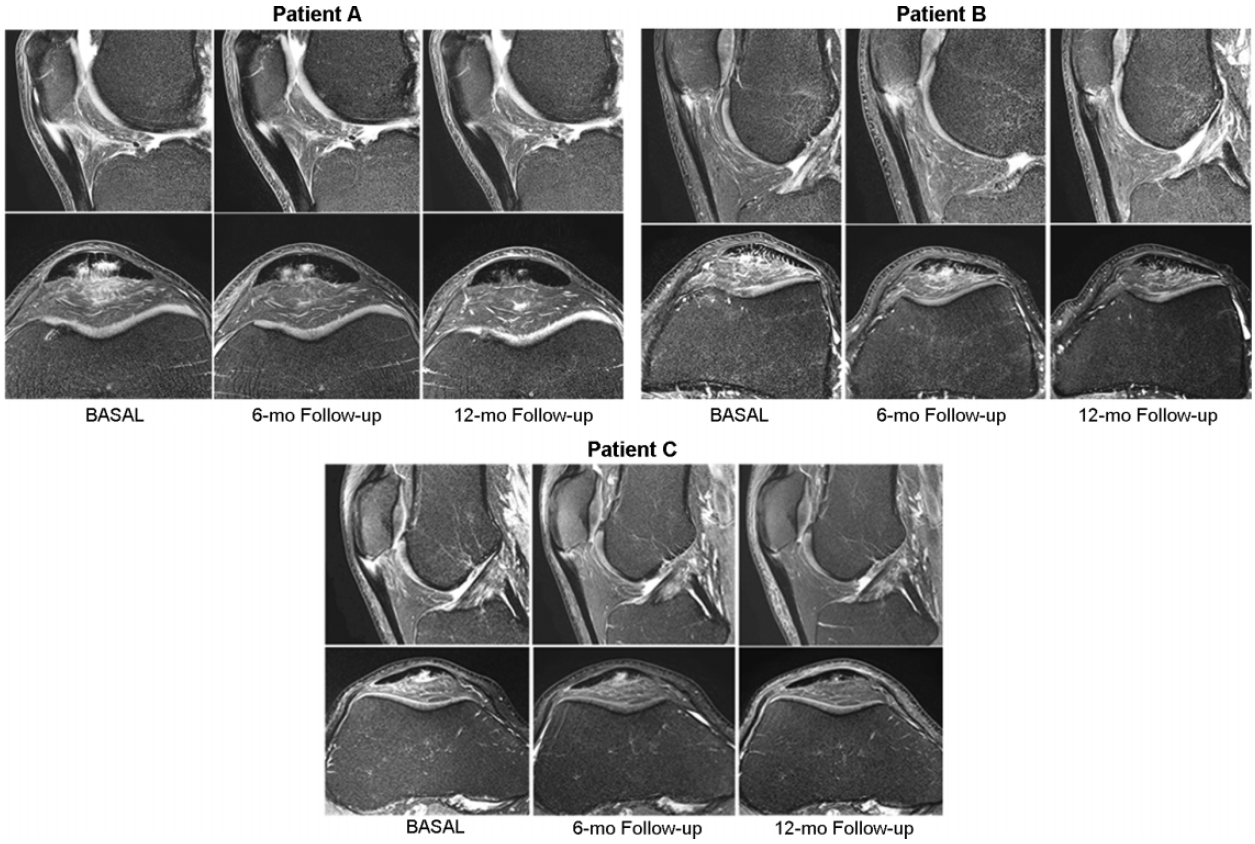

Figure 3. Sagittal (top row) and axial (bottom row) T2-weighted fat-saturated magnetic resonance imaging sequences of 3 patients who were initially treated with leukocyte-poor platelet-rich plasma (Lp-PRP) and then with bone marrow–derived mesenchymal stem cells (BM-MSCs). Shown are images from baseline, the 6-month follow-up (after Lp-PRP), and the 12-month follow-up (after BM-MSCs).

month follow-up visit, no statistically significant differences were observed in any of the dynamometry test results. This can probably be explained by the absence of pain from the third month of treatment, which positively influenced the good dynamometry results. Furthermore, there were no statistically significant differences in the percentage change in the dynamometry test measurements at 12 months between patients initially treated with BM-MSCs and patients treated initially with Lp-PRP.

Statistically significant differences were observed in some parameters used to determine the primary and secondary objectives of the study, with clinical improvement in the patients included, satisfactory patient assessment, and a good safety profile. In terms of safety, treatment with BM-MSCs was well tolerated by all 20 patients, regardless of whether they came from the Lp-PRP group or BM-MSC group. No serious treatment-related AEs were reported.

Limitations

One limitation of this research was the fact that no control group existed in which placebo injections could have been performed, and other limitations include the fact that we cannot rule out the possibility that further structural changes would not have occurred without the treatment change. BM-MSCs, unlike Lp-PRP, have a true regenerative effect, improving the structure of the tendon. Obviously, the cellular and molecular events behind this fact are beyond the scope of this study. It is possible that the appropriate biochemical and mechanical stimuli[8] induce tenogenic differentiation of resident precursor cells.[12,19] These factors may mediate key aspects of tendon tissue repair and may be more evident than when platelets are injected.[27]

The dose used in this trial was 20×10^6 MSCs. This stems from our previous experience of safety and efficacy in other tissues because it has not been established how many cells are required to have an effect. In the case of the patellar tendon, it can be said that the dose applied (10×10^6 MSCs suspended in 2 mL) is half of the maximum possible since the volume of the cell suspension cannot exceed that admissible in the tendon defect. On the other hand, cell hyperconcentration $>10 \times 10^6$ MSCs/mL compromises survival. It is important to advance in the establishment of the best effective dose, since dose reduction is partly equivalent to a reduction in the cost of the biological drug. Preliminary studies in animal models with doses of 1×10^6, 4×10^6, and 8×10^6 MSCs did not yield additional benefits at higher doses.[4]

CONCLUSION

At 12 months after treatment, BM-MSC injection for patellar tendinopathy was found to be a safe procedure and produced a significant decrease in pain, allowing for a safe return to sporting activities. Unlike Lp-PRP, however, these injections were associated with significant favorable changes in tendon structure during imaging. The reason for the favorable structural change may be because of a better anti-inflammatory and analgesic effect compared with Lp-PRP. However, it is not known whether such effects are long-lasting or whether an improved structure reduces future clinical episodes. Further longer-term studies are needed on the efficacy and cost-effectiveness of BM-MSCs compared with other treatments. Nonetheless, given these results, cultivated BM-MSC treatment shows promise in patients' refractory and conventional treatment and opens new holistic approaches to tendinopathy that provide positive results before resorting to surgery.

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APPENDIX

APPENDIX TABLE A1
Rehabilitation Protocol[a]

Exercise	Start Day	Type	Repetitions/Series	Frequency
Isometric	3 d after treatment	• Gym/therapist supervised: leg extension/leg press, 0°-30° knee extension. • Home exercises: 45° squat against wall, progress to 90°.	30 s/5 reps for 2 wk. Progress home exercises to 45 s/rep. Rest every 2 min.	Daily
Concentric isotonic	2 wk after treatment or VAS pain during sports <4	• Gym/therapist supervised: leg extension/leg press, ROM 20°-30°, consider adding 20-30 kg. Controlled speed 4 s up/4 s down. Continue with isometrics exercises. Introduce "Russian belt" exercises at 45°-90° of knee flexion. • Home exercises: progressive 10°-60° of knee extension against elastic resistance, elliptical or bicycle as pain allows.	8 reps/3 sets. Every 3 d add 1 set. Rest every 2 min.	Every other day
Eccentric	4 wk after treatment or VAS pain during sports <2	• Gym/therapist supervised: squat exercises on 25°-30° inclined plane progress from bilateral to unilateral. Controlled speed 4 s up/4 s down. Initiate downhill walk. • Home exercises: eccentric unilateral sidestep. Initiate running, progressively increase speed, change of direction, and jump.	8 reps/3 sets, rest after 2 min. Increase 1 set every 3 d.	Daily

[a]Gym, gymnasium; reps, repetitions; ROM, range of motion; VAS, visual analog scale.