



Intra-articular injections of expanded mesenchymal stem cells with and without addition of platelet-rich plasma are safe and effective for knee osteoarthritis

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Abstract

Purpose To compare the effectiveness and safety of intra-articular injections of autologous expanded mesenchymal stromal stem cells alone (MSCs), or in combination with platelet-rich plasma (MSCs + PRP), in patients with knee osteoarthritis.

Methods Eighteen patients (57.6 ± 9.6 years) with radiographic symptomatic knee osteoarthritis (Dejour grades II–IV) were randomized to receive intra-articular injections of MSCs ($n=9$) or MSCs + PRP ($n=9$). Injections were performed 2–3 weeks after bone marrow aspiration (± 80 – 100 ml) which was obtained from both posterior iliac crests.

Results The Knee Injury and Osteoarthritis Outcome Score (KOOS) improved significantly throughout the 12 months for both groups ($p < 0.05$). No statistically significant differences between groups were found in KOOS subscales and global score improvements at 12-month end-point (n.s.). The MSCs group showed significant improvements in the pain, function and daily living activities, and sports and recreational activities subscales ($p < 0.05$). Similarly, the MSCs + PRP group showed significant improvements in the pain, function and daily living activities and quality of life subscales ($p < 0.05$). The average number of fibroblast colony forming units (CFU-F) was 56.8 ± 21.9 for MSCs group and 50.7 ± 21.7 for MSCs + PRP group. Minimal adverse effects were seen in both groups (10 adverse events, in 5 patients).

Conclusions Intra-articular injections of expanded MSCs alone or in combination with PRP are safe and have a beneficial effect on symptoms in patients with symptomatic knee osteoarthritis. Adding PRP to the MSCs injections did not provide additional benefit. These results are encouraging and support the recommendation of this minimally invasive procedure in patients with knee osteoarthritis, without requiring hospitalization. The CFU-F results may be used as reference for future research.

Level of evidence Prospective cohort study, Level II.

Keywords Knee · Osteoarthritis · Mesenchymal stem cells · Platelet-rich plasma

Introduction

Knee osteoarthritis (OA) is a chronic progressive disease affecting more than 20% of people older than 45 years [24], involving progressive degeneration of the articular cartilage and subchondral bone, often accompanied by synovitis [18].

A myriad of different treatments have been proposed in the scientific literature [15, 44], and current treatment options include conservative and surgical procedures in which the main objective is to relieve pain and improve function; however, none of these treatment options affect the natural progression of the disease [13]. Nonsurgical options display short-term efficacy and, in severe joint degenerative disease, total knee replacement has become the standard treatment [3, 25]. Due to the avascular nature of articular cartilage and the limited self-renewal capacity of chondrocytes [17, 26], treatment options are generally just palliative. Reconstructive surgical options are invasive, with the potential for complications and high costs [2, 16]. The lack of a gold-standard treatment with long-term relief of symptoms, improvement

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of function and, at the same time, a less invasive procedure with minimum risks and relatively low costs, points out the need for novel treatment options.

More recently, the use of human bone marrow-derived mesenchymal stem cells (MSC) [5, 6], also known as stromal stem cells [31] have emerged as a potential treatment for knee OA [30, 40]. These cells are characterized by their distinct ability to adhere and grow on a substrate *in vitro* and to differentiate into osteoblasts, chondrocytes, adipocytes, and hematopoiesis-supporting reticular stroma when cultured under appropriate differentiation conditions [8]. Considering the degenerative and inflammatory pathophysiology of knee OA [12, 34], the stimulation of local tissue regeneration and the inhibition of immunological responses and inflammatory mediator expression by MSC therapy may be effective in treating knee OA [32]. Moreover, their ease of harvest [7] and reported safety [12, 33] make them an attractive alternative to more invasive approaches. Although early promising results have been reported after MSC treatment for knee OA [19, 22, 32, 42], there is a lack of high-level evidence regarding its effectiveness in humans [32].

Platelet-rich plasma (PRP) action on articular cartilage regeneration has been widely studied, representing a potential biological treatment for knee OA [4, 27]. PRP derives from autologous blood in which platelets are concentrated in plasma and, upon activation, it releases high concentrations of a wide range of growth factors which play key roles in tissue repair [1, 10, 11]. Particularly, transforming growth factor-beta (TGF- β), fibroblast growth factor (FGF), and insulin-like growth factor (IGF) are examples of PRP-derived growth factors that have important chondrogenic properties [39]. Prior studies have reported clinical improvements after intra-articular PRP injections for degenerative cartilage lesions and osteoarthritis [4, 20, 27]. *In vitro*, the use of PRP has also shown to enhance or at least preserve MSC chondrogenic capacity [29, 35]. In animal models, promising results have been reported using PRP in combination with MSCs [28, 43]. However, no clinical studies have compared treatment with MSC alone versus MSC in combination with PRP. Hence, this study aims to compare the effectiveness and safety of intra-articular injections of expanded MSC alone, or in combination with PRP, in

patients with knee OA at 12-month follow-up. The effects of the combination of expanded MSCs and PRP for the treatment of symptomatic knee OA are still not known and may represent a potential useful biologic treatment for improving symptoms and function of OA patients, while potentially delaying the surgical treatment.

Materials and methods

Study design and patients

Patients over age 35 with knee OA (based on American College of Rheumatology criteria) and confirmatory radiographs (Dejour grade 1–4) [9] were included in this study. Exclusion criteria included history of diabetes mellitus, glaucoma, immunodeficiency, chronic use of oral corticosteroid or immunosuppressive therapies, history or presence of malignant disorders and/or use of chemotherapy, infection or active wound in the knee area, history of severe trauma to the knee (post-traumatic OA), presence of systemic inflammation, body mass index (BMI) greater than 40, pregnancy and any other comorbidity that prevented the surgical procedure.

Participants were included after an initial screening visit (visit #1). This included history taking, physical examination, laboratory testing, electrocardiogram, chest radiography, knee radiography [standing anterior–posterior (AP) and lateral views], knee magnetic resonance imaging (MRI) and a survey of recently used medications and supplements. All patients were also invited to complete the Portuguese version of Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire [14]. Additionally, patients were evaluated by a cardiologist for surgical risk stratification. Following this evaluation, bone marrow aspiration procedures were scheduled.

Using the Excel software (Microsoft), the included patients were randomly divided into two groups in a 1:1 ratio using central and permuted-blocks randomization (Fig. 1).

1. Autologous transplantation of expanded bone marrow stromal mesenchymal cells (G1 group);

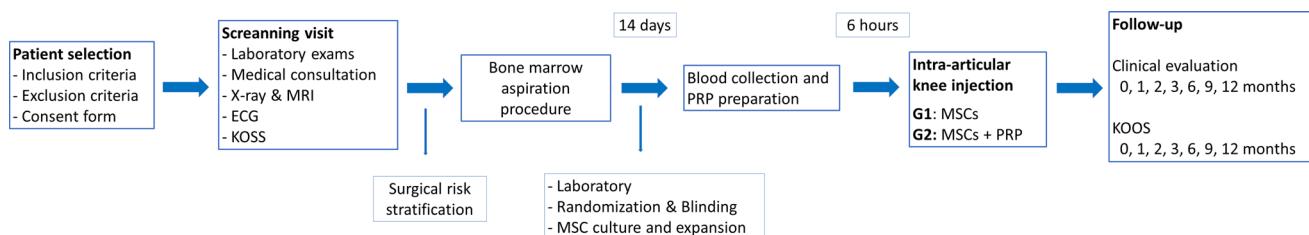


Fig. 1 Study flow chart, from patient selection to treatment follow-up

- Autologous transplantation of expanded stromal bone marrow mesenchymal cells combined with the injection of platelet-rich plasma (G2 group).

Information about the allocated group was obtained by randomization after the bone marrow aspirate and before the scheduled intra-articular injection procedure. The biologist who prepared the material to be injected had password-restricted access to the randomization schedule, which specified the allocated treatment group. The treating orthopedic surgeon, patients, and staff directly involved in the study were blinded to group assignment (G1 and G2). Only the biologist involved with the preparation of the material to be injected knew the experimental group to which the patient was allocated. During treatment preparation, the therapeutic solutions were packed in a standard syringe and covered with a protector to prevent identification of the different treatments.

Bone marrow aspirate surgical harvest procedure

Bone marrow aspirate was obtained after spinal nerve block and sedation were performed. The patient was placed in prone position and the iliac crests were prepared in aseptic conditions. Bone marrow aspirate was obtained percutaneously from both posterior iliac crests with a Jamshid needle (11G × 10 cm). The needle was introduced about 6 cm deep towards the spongy bone of the iliac crest between the inner and outer cortical tables. Approximately 80–100 ml of bone marrow were aspirated through 10-ml EDTA syringes (10 syringes used on the left posterior iliac crest side and 10 syringes used on the right side) that had been rinsed with anticoagulant (heparin). Each syringe was filled to a maximum volume of 5 ml to reduce dilution by the peripheral blood. All bone perforations were performed through the same percutaneous skin incision and were spaced about 2 cm apart to avoid dilution by aspiration of the previous region. The syringes containing bone marrow aspiration were moved to a transfer bag, where they were maintained in sterile conditions and placed in a temperature-controlled box with maintenance of temperature from 4 to 10 °C.

MSCs and PRP preparation procedures

The laboratory procedures for MSCs expansion and PRP preparation are fully described in Supplement 1.

Intra-articular injection

Intra-articular injection of MSCs (G1) or MSCs enriched with PRP (G2) was performed between 2 and 3 weeks after the bone marrow aspiration procedure. After asepsis and adequate antisepsis procedures, intra-articular injection with

a 20G needle was performed in the supero-lateral region of the patella, with the patient in the supine position and the knee in extension.

Post-operative and post-injection care

After the bone marrow aspiration procedure, no functional restrictions nor special care was recommended or required. After intra-articular injection, all patients were instructed to walk with two Canadian-type crutches on the involved limb for a period of 2 weeks. Immediately, patients were instructed to initiate daily physical therapy treatment, following the same protocol for both study groups. For the first 2 weeks, the patients were non-weight bearing, but knee range of motion was not limited. Additionally, isometric quadriceps strengthening and stretching of the thigh, hip and leg muscles for deep venous thrombosis prophylaxis were performed. After 2 weeks, all activities including sports were allowed. Patients were generally recommended to continue their mild-to-moderate level of activities and to increase their activity level gradually as tolerated.

Outcome measures

Medical visits occurred before the bone marrow procedure (screening visit) and at 1, 2, 3, 6, 9 and 12 months after the experimental treatment by the orthopedic team. Pain and knee joint function were evaluated according to KOOS. Using the KOOS subscales and global score, the improvement expected ($IE = 100 - \text{initial score}$), the improvement observed ($IO = \text{final score} - \text{initial score}$) and the percentage achieved of the improvement expected ($CIE = IO/IE$) from baseline to 12-month follow-up was calculated for each patient. The means of these measures were calculated.

During the 12 months of follow-up, other therapies such as anti-inflammatory medications (steroid or non-steroid), hydrotherapy, heat and ultrasound or acupuncture were prohibited, to avoid influencing the results. The use of dipyron 1 g every 6 h (analgesic non-anti-inflammatory) was allowed in case of severe pain.

Ethical approval

The research was approved by the *Comissão Nacional de Ética em Pesquisa* (Brazilian National Ethics in Research Committee)—CONEP, under number 14878813.4.0000.5533 and was conducted in accordance with the principles of the Declaration of Helsinki. All the participants signed the written consent form before inclusion in the study.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science version 22[®] (IBM SPSS, Inc., Chicago). The level of significance for all hypothesis tests (p) was set at 0.05. Absolute (n) and relative (%) frequencies were computed for the categorical variables. Quantitative variables with normal distribution were described using mean and standard deviation, and 95% confidence intervals (CI) for the means were estimated. The association between baseline categorical variables was determined using Fisher's exact tests. The normality of the distribution of quantitative variables was evaluated by Kolmogorov–Smirnov's test and Shapiro–Wilk's test, but due to the small sample size the non-parametric tests were performed in all evaluations. Differences between two independent groups for continuous variables were tested using Mann–Whitney's test. To assess the KOOS improvement from baseline to 12-month follow-up, the Wilcoxon's signed-rank test compared two related measures.

Results

Patient baseline characteristics

Eighteen patients (9 males and 9 females; 57.6 ± 9.6 years old) with radiographic symptomatic knee OA met the inclusion criteria and were enrolled to receive intra-articular injections of either MSCs (G1; $n=9$) or MSCs + PRP (G2; $n=9$). Baseline sociodemographic and clinical characteristics of both groups are summarized on Table 1. At baseline, the sample was homogeneous regarding gender, age, ROM and KOOS subscales and global scores (n.s.).

Clinical outcomes

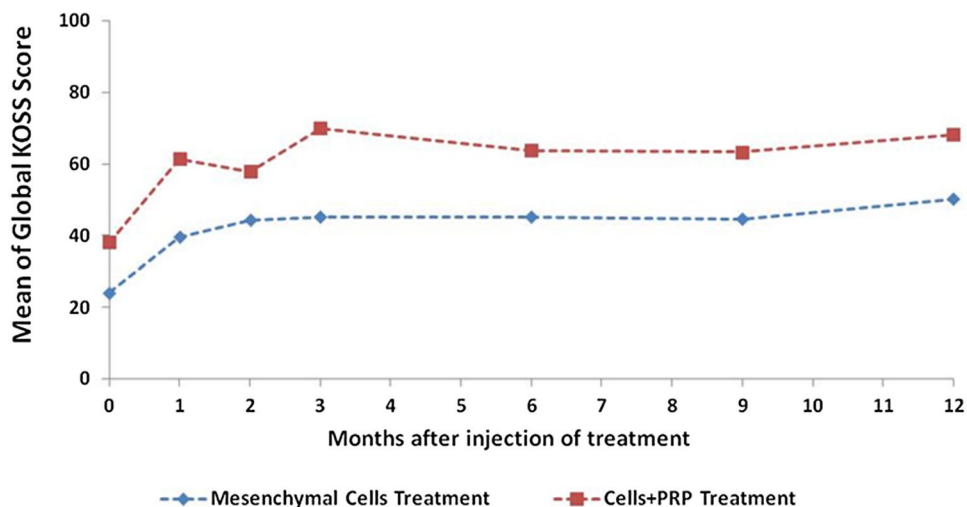
The KOOS score improved significantly throughout the 12 months for both MSCs and MSCs + PRP groups ($p < 0.05$). Additionally, the KOOS score improved significantly from baseline to the first month for MSCs and MSCs + PRP groups ($p < 0.05$), and from the second to third month in the MSCs + PRP group ($p = 0.012$). The progression of mean values of the KOOS score is displayed in Fig. 2. Moreover, there was no statistically significant difference

Table 1 Baseline sociodemographic and clinical characteristics of MSCs and MSCs + PRP groups

Variable	MSCs ($n=9$)	MSCs + PRP ($n=9$)
Gender		
Female	4 (44.4%)	5 (55.3%)
Male	5 (55.3%)	4 (44.4%)
Age, mean \pm SD	54.7 ± 7.2	60.4 ± 11.3
Alignment, no. (%)		
Normal alignment	3 (33.3%)	4 (44.4%)
Varus	0 (0.0%)	2 (22.2%)
Valgus	6 (66.7%)	3 (33.3%)
OA grade, no. (%)		
Grade II	4 (44.4%)	1 (14.3%)
Grade III	3 (33.3%)	5 (71.4%)
Grade IV	2 (22.2%)	1 (14.3%)
ROM, mean \pm SD (95% CI)		
Hyperextension	2.5 ± 3.8 (– 0.7, 5.7)	1.1 ± 3.3 (– 1.4, 3.7)
Flexion	3.8 ± 7.4 (– 2.5, 10.0)	3.9 ± 4.9 (0.2, 7.6)
Total flexion	116.6 ± 25.1 (95.6, 137.6)	112.7 ± 33.0 (87.3, 138.8)
KOOS, mean \pm SD (95% CI)		
Symptoms	35.3 ± 13.6 (23.9, 46.6)	41.7 ± 16.6 (28.9, 54.4)
Pain	31.9 ± 8.0 (24.0, 40.0)	43.5 ± 22.8 (26.0, 61.1)
Function, daily living	26.7 ± 8.2 (19.8, 33.5)	43.6 ± 22.7 (26.1, 61.1)
Sports/recreation	6.3 ± 8.8 (– 1.1, 13.6)	21.1 ± 28.0 (– 0.4, 42.6)
Quality of life	14.0 ± 10.9 (4.8, 23.1)	17.4 ± 13.0 (6.7, 28.0)
Global KOOS score	25.5 ± 8.0 (18.8, 32.2)	38.1 ± 20.4 (22.4, 53.8)

SD standard deviation, CI confidence interval, OA osteoarthritis, ROM range of movement, KOOS Knee Injury and Osteoarthritis Outcome Score

Fig. 2 Progression of mean values of KOOS score for MSCs and MSCs + PRP groups



between the groups in the improvements on the KOOS subscales and global score at 12-month end-point (n.s.).

The mean percentage improvement was greater than 21% for all KOOS subscales and the global score (Table 2). The MSCs group showed significant improvements in the pain ($p=0.035$), function, and daily living activities ($p=0.035$) and sports and recreational activities subscales ($p=0.027$). Similarly, the MSCs + PRP group showed significant improvements in the pain ($p=0.012$), function and daily living activities ($p=0.017$) and quality of life subscales ($p=0.027$). Moreover, the global KOOS score improved 22.65 points (29%) for MSCs group ($p=0.025$) and 26.4 points (30.7%) for MSCs + PRP group ($p=0.012$). At final follow-up, there were no significant

differences between the groups regarding the KOOS total score and respective subscales (n.s.).

Flow cytometry and fibroblast colony forming units (CFU-F)

The average number of CFU-F was 56.8 ± 21.9 (range 18.3–85.0) for MSCs group and 50.7 ± 21.7 (range 19.6–85.7). Mesenchymal progenitors presented a typical myofibroblast pattern of growth. At the edge of the colonies, cells presented a stellate morphology, and at the core of the colonies, cells presented a fusiform monolayer distribution. Cells were evaluated by FACS for the expression of antigenic markers commonly associated with MSCs. After in vitro expansion, cells from all patients expressed

Table 2 KOOS improvement from baseline to 12-month follow-up for MSCs and MSCs + PRP groups

KOOS domain	Groups	Final score (12 months)	Significance between groups at final follow-up	Mean improvement expected	Mean improvement observed	Mean percentage improvement (%)	Significance of improvement ^a
Symptoms	MSCs	54.5 ± 22.2	NS	66.3	19.8	28.0	NS
	MSCs + PRP	63.8 ± 15.3		58.3	21.4	32.2	NS
Pain	MSCs	53.5 ± 28.6	NS	69.4	18.9	26.5	0.035
	MSCs + PRP	76.4 ± 25.0		56.5	31.8	57.1	0.012
Function, daily living	MSCs	55.3 ± 29.8	NS	75.3	24.5	31.1	0.035
	MSCs + PRP	75.0 ± 27.7		56.4	23.0	30.5	0.017
Sports/recreation	MSCs	30.6 ± 33.1	NS	94.4	21.7	22.2	0.027
	MSCs + PRP	55.0 ± 36.6		78.9	27.8	21.6	NS
Quality of life	MSCs	38.3 ± 29.0	NS	86.2	20.2	22.4	NS
	MSCs + PRP	45.3 ± 26.7		82.6	26.4	30.7	0.027
Global KOOS score	MSCs	50.3 ± 26.6	NS	75.9	22.6	29.0	0.025
	MSCs + PRP	68.2 ± 24.7		82.6	26.4	30.7	0.012

MSCs mesenchymal stem cells, PRP platelet-rich plasma, KOOS Knee Injury and Osteoarthritis Outcome Score, NS non-significant

^aPerformed by Wilcoxon's test

similar levels of CD73 (>95%), CD90 (>95%), and CD146 (>55%), and were negative for CD14, CD31, CD34, and CD45.

Safety and adverse events

A total of 10 adverse events (AEs) occurred in 3 patients in the MSCs group (33%) and in 2 of the MSCs + PRP group (22%). All the adverse events reported were treatment-related and none required a specific procedure or hospitalization, and were resolved without any sequelae. The MSCs group reported 5 mild AEs (joint effusion, mild knee pain, low back pain and moderate knee pain), 1 moderate AE (moderate knee pain, treated with concomitant dipyrrone therapy) and 1 severe AE (intense knee pain). In the MSCs + PRP group 2 mild AEs were reported (mild knee pain) and 1 severe AE (intense knee pain, treated with concomitant dipyrrone therapy). The frequency distribution of AEs reported is displayed in Table 3.

Discussion

The main finding of this double-blind randomized clinical trial is that intra-articular injections of expanded MSCs alone or in combination with PRP were safe and result in significant clinical improvement in patients with knee OA, up to 12 months. However, there were no statistically significant differences in the clinical outcomes between the groups. These findings support the use of intra-articular injections of expanded MSCs for the treatment of knee OA.

Intra-articular injections of expanded MSCs alone, or in combination with PRP led to improvements of 22.6 and 26.4 points in the KOOS global score at 12 months, respectively. This improvement corresponded to 29% and 31% of the total improvement that was possible. Additionally, the improvements were most significant in the KOOS pain subscore (26.5% and 57.1% for MSCs and MSCs + PRP groups, respectively). Although the MSCs + PRP group showed higher mean percentage improvement, it must be taken into account that at baseline there was a 11.6 points difference favoring the MSCs group, thus suggesting more room for improvement in the MSCs + PRP group. On the other hand, the mean percentage improvement of quality of life subscore showed a tendency to be higher in the

MSCs + PRP group (accounting for only 3.4 points difference at baseline), suggesting that the addition of PRP to the MSCs may provide greater overall positive effect which translates into higher quality of life scores.

Despite the lack of high-level studies in the scientific literature, the use of MSCs has been emerging as a potential nonsurgical approach to knee OA [21, 23, 32]. Koh et al. [23] reported 2-year follow-up data on a group of patients with knee OA (levels 3 and 4 by the International Cartilage Repair Society Score) who underwent tibial osteotomy and were also treated with concomitant PRP alone ($N=23$) or MSCs + PRP ($N=21$). They reported significant improvements in the symptoms and pain KOOS subscores at 2 years of follow-up (81.2 and 82.8, respectively) in the MSCs + PRP group when compared to patients that underwent isolated PRP intra-articular injections (74.0 and 75.4, respectively). This study results showed smaller values for pain (MSCs group with 50.8 points and MSCs + PRP group with 75.3 points) and symptoms subscores (MSCs group with 55.1 points and MSCs + PRP group with 63.1 points). A significant limitation of this study is the confounding effect of the concomitant high tibial osteotomy.

The studies reported to date have used intra-articular injections of MSCs alone [21, 23, 36]. In the present study it was used expanded MSCs (40×10^6 cells), which is comparable to the studies reported in the literature (15×10^6 to 40×10^6) [37, 38, 41]. However, comparison to prior studies is difficult due to important differences in cell types used and the lack of rigorous reporting of the exact composition and biologic activity of cell preparations. For example, Vega et al. [38] used allogenic bone marrow-derived MSCs, and the biologic activity of such cells may be affected by an immunological response. Additionally, other studies (uncultured MSCs) have used autologous adipose-derived MSCs [21, 23].

The majority of prior studies reported in the scientific literature have used MSC therapy as a concomitant treatment with other surgical procedures to treat knee OA, including high tibial osteotomy [23, 41], arthroscopic debridement [21, 37], and microfracture [41]. These associated procedures may confound the final outcomes, making it difficult to determine the true effect of the isolated use of MSCs therapy. Additionally, Saw et al. [36] combined the use of MSCs and hyaluronic acid, which may also have confounded the final outcomes. Conversely, in the present study it was used intra-articular injections of MSCs alone or MSCs + PRP without any concomitant treatment in order to assess the isolated effect of this therapy.

Concerns regarding the safety of intra-articular injections of MSCs have been raised in the scientific literature [30]. In the present study, minimal adverse effects were seen in both groups. This finding is in accordance with previous reports

Table 3 Frequency distribution of reported AEs

	Mild	Moderate	Severe
MSCs	5 (9.3%)	1 (1.9%)	1 (1.9%)
MSCs + PRP	2 (3.7%)	0 (0%)	1 (1.9%)

MSCs mesenchymal stem cells, PRP platelet-rich plasma

in the scientific literature which also demonstrate the safety of MSCs intra-articular injections [12, 33].

There are some limitations to this study. The primary limitation is the small sample size. This study goal was to perform a pilot study to establish the laboratory and clinical research protocols, to allow for further study. The laboratory analyses of CFUs and flow cytometry studies are labor intensive and costly, limiting the number of patients that could be included in this study. Nevertheless, despite the small number of patients, the results obtained from the CFU analysis may be used as reference in future research. Also, this study did not include a control group with a “gold-standard” treatment, such as steroid or hyaluronic acid injection. The lack of a group with intra-articular injections of PRP alone precludes the direct assessment of PRP, i.e., if it acts by potentiating the MSCs effect or if it has a role in directly relieving OA symptoms. Moreover, it included a heterogeneous population in terms of alignment deformity (varus and valgus) and OA severity in order to assess the treatment efficacy in all groups of the disease; however, this may have led to greater variability in the outcomes. Although a 12-month follow-up provides a reasonable insight into the early outcomes, a longer period of follow-up would provide important information concerning the longer-term effects of these therapies and possible deterioration over time. Moreover, the absence of cartilage follow-up imaging evaluation and/or histologic analysis precluded evaluation of the mechanism which led to the reported symptom relief, i.e., it was not possible to state that the symptom relief was due to repair of the damaged cartilage or, more likely, due to an effect on the inflammatory process of the osteoarthritic knee synovium.

This study showed clinical effectiveness and safety of expanded MSCs and the combination of those with PRP for the conservative treatment of symptomatic and radiographic knee OA. Biological strategies are a promising treatment strategy for degenerative disease and its protocols and outcomes must be further studied to achieve better solutions, and to allow avoidance of more invasive surgical approaches. In addition to the symptom relief and functional improvement, regenerative medicine may bring important contributions in the medical decision-making, including the delay for a joint arthroplasty.

Conclusion

The use of intra-articular injections of expanded MSCs alone or in combination with PRP is safe and has a positive effect on symptoms in patients with knee OA. This preliminary data suggest that there are no important clinical outcome differences between both therapies. These results are encouraging and support the recommendation of this minimally

invasive procedure in patients with knee OA, without requiring hospitalization.

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Compliance with ethical standards

Conflict of interest The authors declare that this study received funding from ESHO Empresa de Serviços Hospitalares S.A. Ricardo Bastos received grants from ESHO Empresa de Serviços Hospitalares S.A. The others authors declare no conflict of interest.

Ethical approval The research was approved by the Comissão Nacional de Ética em Pesquisa (Brazilian National Ethics in Research Committee)—CONEP, under number 14878813.4.0000.5533 and was conducted in accordance with the principles of the Declaration of Helsinki.

Informed consent All the participants signed the written consent form before inclusion in the study.

References

1. Amable PR, Carias RBV, Teixeira MVT, da Cruz Pacheco Í, do Amaral RJFC., Granjeiro JM et al (2013) Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. *Stem Cell Res Ther* 4:67
2. Bastos Filho R, Magnussen RA, Duthon V, Demey G, Servien E, Granjeiro JM et al (2013) Total knee arthroplasty after high tibial osteotomy: a comparison of opening and closing wedge osteotomy. *Int Orthop* 37:427–431
3. Buckwalter JA, Saltzman C, Brown T (2004) The impact of osteoarthritis: implications for research. *Clin Orthop Relat Res* 427:S6-S15
4. Campbell KA, Saltzman BM, Mascarenhas R, Khair MM, Verma NN, Bach BR et al (2015) Does intra-articular platelet-rich plasma injection provide clinically superior outcomes compared with other therapies in the treatment of knee osteoarthritis? A systematic review of overlapping meta-analyses. *Arthroscopy* 31:2213–2221
5. Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 213:341–347
6. Caplan AI (1991) Mesenchymal stem cells. *J Orthop Res* 9:641–650
7. Carstairs A, Genever P (2014) Stem cell treatment for musculoskeletal disease. *Curr Opin Pharmacol* 16:1–6
8. da Silva Meirelles L, Sand TT, Harman RJ, Lennon DP, Caplan AI (2008) MSC frequency correlates with blood vessel density in equine adipose tissue. *Tissue Eng Part A* 15:221–229
9. Dejour H, Carret J, Walch G (1991) Les Gonarthroses. 7èmes Journées Lyonnaises de Chirurgie de Genou. 7èmes Journées Lyonnaises de Chirurgie de Genou, Les Gonarthroses
10. do Amaral RJFC., da Silva NP, Haddad NF, Lopes LS, Ferreira FD, Cappelletti PA et al (2016) Platelet-rich plasma obtained with different anticoagulants and their effect on platelet numbers and mesenchymal stromal cells behavior in vitro. *Stem Cells Int*. <https://doi.org/10.1155/2016/7414036>
11. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA (2009) Platelet-rich plasma: from basic science to clinical applications. *Am J Sports Med* 37:2259–2272
12. Freitag J, Bates D, Boyd R, Shah K, Barnard A, Huguenin L et al (2016) Mesenchymal stem cell therapy in the treatment of

- osteoarthritis: reparative pathways, safety and efficacy—a review. *BMC Musculoskelet Disord* 17:230
13. Gomoll A, Filardo G, De Girolamo L, Espregueira-Mendes J, Marcacci M, Rodkey W et al (2012) Surgical treatment for early osteoarthritis. Part I: cartilage repair procedures. *Knee Surg Sports Traumatol Arthrosc* 20:450–466
 14. Goncalves R, Cabri J, Pinheiro J, Ferreira P, Gil J (2010) Reliability, validity and responsiveness of the Portuguese version of the Knee injury and Osteoarthritis Outcome Score—Physical Function Short-form (KOOS-PS). *Osteoarthritis Cartilage* 18:372–376
 15. Goyal D, Keyhani S, Lee EH, Hui JHP (2013) Evidence-based status of microfracture technique: a systematic review of level I and II studies. *Arthroscopy* 29:1579–1588
 16. Haas DA, Kaplan RS (2017) Variation in the cost of care for primary total knee arthroplasties. *Arthroplast Today* 3:33–37
 17. Heijink A, Gomoll AH, Madry H, Drobnič M, Filardo G, Espregueira-Mendes J et al (2012) Biomechanical considerations in the pathogenesis of osteoarthritis of the knee. *Knee Surg Sports Traumatol Arthrosc* 20:423–435
 18. Ishiguro N, Kojima T, Poole AR (2002) Mechanism of cartilage destruction in osteoarthritis. *Nagoya J Med Sci* 65:73–84
 19. Jo CH, Lee YG, Shin WH, Kim H, Chai JW, Jeong EC et al (2014) Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem cells* 32:1254–1266
 20. Khoshbin A, Leroux T, Wasserstein D, Marks P, Theodoropoulos J, Ogilvie-Harris D et al (2013) The efficacy of platelet-rich plasma in the treatment of symptomatic knee osteoarthritis: a systematic review with quantitative synthesis. *Arthroscopy* 29:2037–2048
 21. Koh Y-G, Choi Y-J (2012) Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis. *Knee* 19:902–907
 22. Koh Y-G, Jo S-B, Kwon O-R, Suh D-S, Lee S-W, Park S-H et al (2013) Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. *Arthroscopy* 29:748–755
 23. Koh Y-G, Kwon O-R, Kim Y-S, Choi Y-J (2014) Comparative outcomes of open-wedge high tibial osteotomy with platelet-rich plasma alone or in combination with mesenchymal stem cell treatment: a prospective study. *Arthroscopy* 30:1453–1460
 24. Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA et al (2008) Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part II. *Arthritis Rheum* 58:26–35
 25. Lohmander LS, Roos EM (2007) Clinical update: treating osteoarthritis. *Lancet* 370:2082–2084
 26. Mazor M, Lespessailles E, Coursier R, Daniellou R, Best T, Toumi H (2014) Mesenchymal stem-cell potential in cartilage repair: an update. *J Cell Mol Med* 18:2340–2350
 27. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD (2016) Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. *Arthroscopy* 32:495–505
 28. Mifune Y, Matsumoto T, Takayama K, Ota S, Li H, Meszaros LB et al (2013) The effect of platelet-rich plasma on the regenerative therapy of muscle derived stem cells for articular cartilage repair. *Osteoarthritis Cartilage* 21:175–185
 29. Mishra A, Tummala P, King A, Lee B, Kraus M, Tse V et al (2009) Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. *Tissue Eng Part C Methods* 15:431–435
 30. Osborne H, Anderson L, Burt P, Young M, Gerrard D (2015) Australasian College of Sports Physicians—position statement: the place of mesenchymal stem/stromal cell therapies in sport and exercise medicine. *Br J Sports Med* 50:1237–1244
 31. Owen M, Friedenstein A (1988) Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found Symp* 136:42–60
 32. Pas HI, Winters M, Haisma HJ, Koenis MJ, Tol JL, Moen MH (2017) Stem cell injections in knee osteoarthritis: a systematic review of the literature. *Br J Sports Med* 51:1125–1133
 33. Peeters C, Leijts MJ, Reijman M, van Osch G, Bos P (2013) Safety of intra-articular cell-therapy with culture-expanded stem cells in humans: a systematic literature review. *Osteoarthritis Cartilage* 21:1465–1473
 34. Pers Y-M, Ruiz M, Noël D, Jorgensen C (2015) Mesenchymal stem cells for the management of inflammation in osteoarthritis: state of the art and perspectives. *Osteoarthritis Cartilage* 23:2027–2035
 35. Rubio-Azpeitia E, Andia I (2014) Partnership between platelet-rich plasma and mesenchymal stem cells: in vitro experience. *Musc Ligaments Tendons J* 4:52–62
 36. Saw K-Y, Anz A, Jee CS-Y, Merican S, Ng RC-S, Roohi SA et al (2013) Articular cartilage regeneration with autologous peripheral blood stem cells versus hyaluronic acid: a randomized controlled trial. *Arthroscopy* 29:684–694
 37. Tan Y, Jiang M, Yu H, Li J, Qing Z (2013) Therapeutic effect of arthroscopy combined with autologous bone marrow stem cell grafting on knee osteoarthritis. *J Tradit Chin Orthop Traumatol* 10:35–38
 38. Vega A, Martín-Ferrero MA, Del Canto F, Alberca M, García V, Munar A et al (2015) Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: a randomized controlled trial. *Transplantation* 99:1681–1690
 39. Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noël D (2009) Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. *Trends Biotechnol* 27:307–314
 40. Wolfstadi JI, Cole BJ, Ogilvie-Harris DJ, Viswanathan S, Chahal J (2015) Current concepts: the role of mesenchymal stem cells in the management of knee osteoarthritis. *Sports Health* 7:38–44
 41. Wong KL, Lee KBL, Tai BC, Law P, Lee EH, Hui JH (2013) Injectable cultured bone marrow—derived mesenchymal stem cells in varus knees with cartilage defects undergoing high tibial osteotomy: a prospective, randomized controlled clinical trial with 2 years' follow-up. *Arthroscopy* 29:2020–2028
 42. Xia P, Wang X, Lin Q, Li X (2015) Efficacy of mesenchymal stem cells injection for the management of knee osteoarthritis: a systematic review and meta-analysis. *Int Orthop* 39:2363–2372
 43. Xie X, Wang Y, Zhao C, Guo S, Liu S, Jia W et al (2012) Comparative evaluation of MSCs from bone marrow and adipose tissue seeded in PRP-derived scaffold for cartilage regeneration. *Biomaterials* 33:7008–7018
 44. Zhang W, Moskowitz R, Nuki G, Abramson S, Altman R, Arden N et al (2008) OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. *Osteoarthritis Cartilage* 16:137–162

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