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Use of bone marrow derived mesenchymal stem cells for the treatment of osteoarthritis: A retrospective long-term follow-up study

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ABSTRACT

Background: Available studies suggest that bone marrow concentrate, highly enriched in mesenchymal stem cells, is a potentially encouraging treatment for knee osteoarthritis. The aim of this retrospective study was to evaluate the clinical outcome in patients affected by this condition after treatment with autologous bone marrow aspirate concentrate (BMAC).
Methods: 55 patients who had undergone a single intra-articular injection of BMAC were administered two questionnaires to clinically evaluate their condition based on patient-reported outcome measures before treatment and at follow-up.
Results: Analysis of the data collected indicates that patients experienced improvements in Tegner, VAS and WOMAC scores and that all outcomes at the follow-up improved in a statistically significant manner compared to outcomes at baseline.
Conclusions: The changes observed in the different scores examined suggest that a single BMAC injection seems to be a beneficial and safe treatment for knee osteoarthritis.

1. Introduction

Osteoarthritis is a widespread degenerative joint disorder in adults, and it is a disabling condition with a significant and growing health impact.[1,2] The knee is the most common site of osteoarthritis accounting for roughly 85% of the total global burden.[3] Osteoarthritis has a prevalence of more than 13% in the male population and 10% in the female population over the age of 60,[4] and is predicted to become the fourth leading cause of disability worldwide by 2020.[5,6] In the near future, the incidence will steadily increase due to increasing life expectancy and obesity in the modern Western world.[7–9] Osteochondral lesions and osteoarthritis not only affect the aging population, but according to a recent study,[10] 89% of elite athletes have a damaged joint surface that may eventually develop into early osteoarthritis.

A variety of treatments for injured articular cartilage are available including non-pharmacological, pharmacological, surgical and regenerative medicine approaches.[1,11,12] Conservative treatment of this condition, which includes the use of drugs such as NSAIDs, hyaluronic acid (HA), platelet-rich plasma (PRP), and steroids,[13–15] has proven to be rather ineffective as it tends to relieve symptoms rather than improve the overall condition. Furthermore, since resident chondrocytes lack healing potential due to poor vascularization and innervation, pharmacological treatments are likely to provide only temporary relief. Other treatment options include surgical procedures such as debridement with microfracturing and autologous chondrocyte implantation,[16] but this method have proven to be less effective and more costly and complex.[17,18]

Conservative and surgical treatments have been reported to show an effect on slowing osteoarthritis, but are unable to definitively block its progression which can be effectively halted only by total knee replacement. Hower, since a 10–34% proportion of patients still report long-term pain after this procedure and the incidence of osteoarthritis is increasing in the younger population, it is in the best interest of the patient to delay joint replacement as long as possible.[19]

Recently, regenerative medicine has been used as an alternative to traditional surgery, with the goal of creating new tissue with properties similar to natural cartilage.[20–22] Injection of

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mesenchymal stem cells (MSCs), adult multipotent cells with the ability to self-renew and differentiate, recently emerged as a key tool for orthopedic surgeons.[23–25] Although MSCs are found in a broad range of tissues, for clinical purposes they are most commonly obtained either from bone marrow concentrate or adipose tissue. Adipose tissue has recently been reported to contain a higher percentage of MSC compared to bone marrow (approximately 1% vs. 0.01%) and proponents of autologous microfragmented adipose tissue injection maintain that it provides a concentration of stem cells and growth factors that triggers a paracrine effect at the injection site; this paracrine effect initiates a healing process of the host cells through the release of cytokines and chemokines.[26] Conversely, bone marrow concentrate is known to contain numerous growth factors, such as Platelet Growth Factor (PGF), Transforming Growth Factor-beta (TGF-beta), and Bone Morphogenic Protein 2 and 7 (BMP2 - 7), which provide a direct anti-inflammatory and anabolic effect at the injected site.[23] The inflammatory environment of the injured joint affects the repair potential[27] and thus it is crucial to suppress the local inflammation to restore a physiological environment in the affected joint.[9,23,25]

The main aim of this retrospective study was to evaluate the clinical outcome in patients affected by osteoarthritis of the knee treated with a single intra-articular injection of autologous bone marrow aspirate concentrate (BMAC) harvested from the anterior-superior iliac spine.

2. Materials and methods

Our orthopedic team, at Conegliano city hospital (Treviso, Italy), retrospectively assessed 92 consecutive patients affected by knee disorders and treated with BMAC injections from May 2011 to March 2019 such that all patients had received treatment at least a year prior to the start of the study. The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Patients were selected based on the following criteria: i) diagnosis of gonarthrosis or osteochondral lesions; ii) presence of symptoms affecting activities of daily living lasting longer than 6 months; iii) unsuccessful conservative treatments such as corticosteroid injections, oral administration of NSAIDs, and physical therapy. Exclusion criteria included: acute knee trauma in the past 6 months, previous surgery on the affected knee, and immature bone structure as in the pediatric population. The research study focused on degenerative cartilage defects and knee osteoarthritis and patients with other conditions affecting the knee were excluded from the study. Of the 92 patients initially considered, 55 were eventually included in this study.

Every patient was assessed and treated by two expert knee surgeons at our facility. The diagnosis of knee osteoarthritis was based on clinical examination and radiological or MRI findings, and if suitable, the patient was scheduled for knee BMAC injection. Because the study was conducted during the COVID-19 outbreak and it was not possible to perform the follow-up visits and administer the questionnaires at our facility, we relied on patients' records to determine clinical status at their last outpatient visit. Patients were contacted and were administered two questionnaires at once by telephone. The first questionnaire was used to quantify symptom severity before knee injection and the second one to evaluate changes in their clinical condition after knee injection.

Regardless of the possible outcomes, it was not deemed necessary to perform an arthroscopic second look as a follow-up because the risks of subjecting our patients to an unnecessary procedure far outweighed the benefits. Therefore, in the majority of the cases studied, only a clinical examination were included in the standard follow-up. Only in cases where another procedure was already scheduled, such as removal of an osteotomy plate, was a second look performed. However, this was only a small proportion of our patients, so we have not included these results in this manuscript.

The primary objective of this study was to evaluate the efficacy of BMAC injections in alleviating knee osteoarthritis symptoms. The degree of degenerative arthritis was evaluated according to the Kellgren–Lawrence (K-L) grading scale. The clinical evaluation of BMAC injection was based on patient-reported outcome measures before treatment and at the final follow-up, collected retrospectively by telephone. The study evaluated the degree of pain of patients through the visual analogue scale (VAS) scores, the level of activity of patients using the Tegner activity scale and the levels of pain, stiffness, and physical functioning of the knee through the WOMAC index. The secondary objective was to assess the safety of BMAC injections by monitoring the occurrence and severity of adverse events.

Data were described as mean ± standard deviation (SD). Nonparametric analysis was performed with Wilcoxon rank sum test to analyze differences in the clinical outcomes between preoperative and post-procedure scores. P values less than 0.05 were considered statistically significant.

2.1. BMAC isolation

The bone marrow was harvested from the superior iliac crest as follows: the surgeon and a qualified nurse filled a 30-ml syringe with 5 ml of anticoagulant. After performing a small stab incision over the iliac crest, the bone marrow was aspirated three times, each time adding fresh anticoagulant, to obtain a total volume of approximately 60 ml. The bone marrow aspirate was then passed through a sterile filter into a separate 60-mL syringe to remove particulate matter and transferred to a suitable vessel that was placed in a SMART PREP2 centrifuge (Terumo BCT, INC, Lakewood, Colorado) to concentrate it. Typically 10–11 ml of bone marrow concentrate was obtained in this procedure. The technology of separation that leads to the preparation of bone marrow concentrate is shown in Fig. 1.

2.2. Surgical technique

Patients were placed supine on a standard operating table with a padded tourniquet applied proximally to the previously marked surgical leg. A lateral leg holder, at the same height and side of the tourniquet, was used to provide light weight bearing to the knee during the arthroscopic portion of the procedure. General anesthesia was administered throughout the procedure and two surgical fields were prepared: the knee and the area corresponding to the superior-anterior iliac spine (SAIS) were classically draped and prepared in a sterile fashion.

The knee joint was evaluated by diagnostic arthroscopy with a 30°, 4.0-mm arthroscope. A classic antero-lateral portal was created and a sheath with scope inserted into the joint. An anteromedial portal was made via needle localization to insert the required tool during surgery. After the required additional procedures (selective meniscectomy, high tibial osteotomy, and ACL reconstruction), the BMAC knee injection was performed intra-articularly into the knee and the surgical portals were sutured without placement of a drain.

3. Results

In total, 55 patients were treated. 17 (31%) were females and 38 (69%) were males and the mean age at the time of treatment was 45.3 ± 9.6. All patients underwent a single, intra-articular injection of BMAC monolaterally, 23 in the left knee and 32 in the right knee.

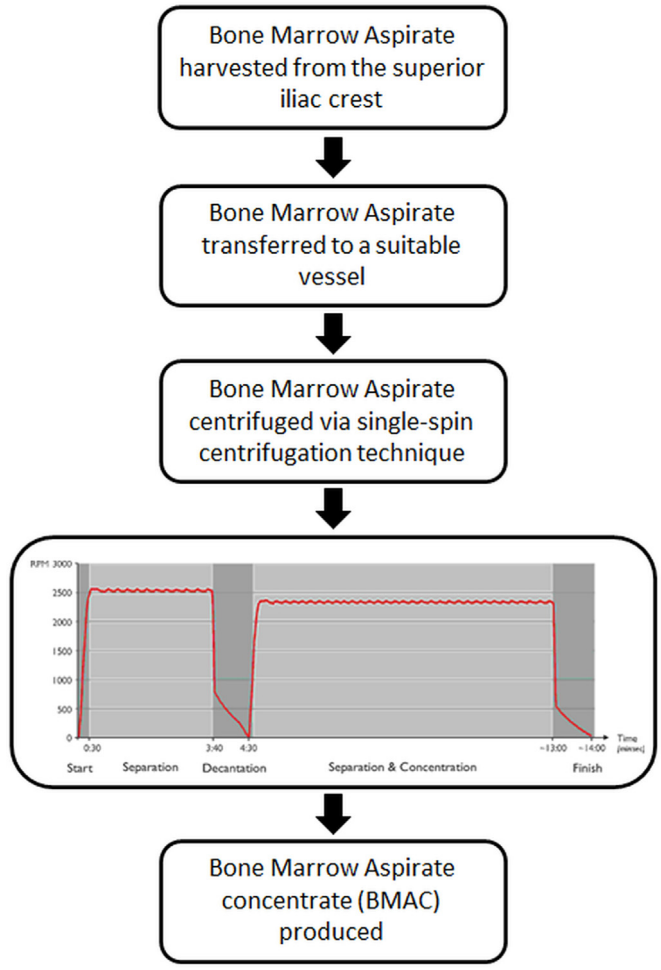

Fig. 1. Schematic representation of the procedure. The bone marrow was harvested from the superior iliac crest and transferred to a suitable vessel that was placed in a SMART PREP2 centrifuge (Terumo BCT, INC, Lakewood, Colorado) to concentrate it. The disposable was loaded and the process started. During an initial 3 min spin, the disposable moves to a horizontal position and blood components separate based on density (start and separation). The first spin cycle slows to a stop, and the contents above the floating shelf automatically transfer to a second "plasma" chamber (decantation). After decantation, a second spin cycle begins (~10 min cycle), designed to isolate the platelet and cell mixture in a discrete "buffy coat" in order to maximize the final concentration of the final product (separation and concentration). Processing stops and the disposable returns to a vertical position (finish). Excess platelet poor plasma is removed, and the concentrated biologic is aspirated, ready for application. The entire process is automated and takes only about 14 min.

Follow-up ranged between 18 and 111 months and mean follow-up was 57.6 months after the procedure. Furthermore, 29 (52.7%) of the 55 subjects included in the study underwent previous additional procedures: 18 had high tibial osteotomy to treat varus alignment, 7 had microfracture surgery, 2 had lateral meniscectomy and one each underwent lateral release and medial meniscectomy.

Patients in the study were almost equally split based on the severity of osteoarthritis: 29 (52.7%) had grade 2 and 26 (47.3%) had grade 3 osteoarthritis according to the Kellgren and Lawrence system. Analysis of the data clearly shows that BMAC injection resulted in a substantial improvement of the clinical status across all parameters examined. Compared with baseline, patients reported a decrease in the level of pain, activity and overall clinical condition experienced when they responded to the follow-up questionnaire, with a statistically significant shift (Table 1). . Likewise, the results were statistically significant also within K-L grade 2 patients (Table 2) as well as within K-L grade 3 patients (Table 3). Importantly, during the course of this study no patient-reported adverse effects occurred indicating the safety of BMAC injections.

Table 1
Clinical status across all parameters examined before and after treatment reported as Mean ± SD.

	Baseline	BMAC injection	*p* value
VAS	7.8 ± 1.9	3.3 ± 2.5	<0.001
Tegner scale	53.8 ± 14.9	77.3 ± 15.1	<0.001
WOMAC scale	60.5 ± 10.6	76.2 ± 7.8	<0.01

Since over 50% of the patients underwent prior surgical treatments, we investigated whether stratification using this parameter could influence the outcome of BMAC injection. Analysis of the data, however, showed that there was no statistically significant difference when comparing the pre-BMAC and post-BMAC injection scores in the group of patients that had undergone previous surgery and those of the group of patients who had not, suggesting that BMAC was similarly effective in both populations.

Taken together, the changes in the different scores examined suggest that a single BMAC injection is a beneficial and safe treatment for knee osteoarthritis. Two representative cases are depicted in Fig. 2.

4. Discussion

The primary intervention for knee osteoarthritis is a non-surgical treatment. Although current non-surgical approaches can provide symptomatic relief, they frequently fail, leaving knee arthroplasty as the only option. Thus, there is a need for more effective knee osteoarthritis treatments that arrest or even reverse disease progression without involving knee replacement. The results from our study demonstrate that a significant improvement in knee osteoarthritis symptomatology can be achieved with intra-articular injections of autologous bone marrow concentrate. Interestingly, we did not find a correlation between lower severity and greater improvement of the patients self-rated functional and pain scores. In fact, comparable results were obtained in both K-L 2 and K-L 3 group patients and although none of the patients in our study was affected by severe K-L 4 osteoarthritis, our findings could imply that BMAC injection is effective within an ample spectrum of disease severity, independently of the starting condition.

Our study is interesting for at least three reasons. Firstly, the mean age of the patients involved in the study was definitely lower than in most of the previously published reports on the use of BMAC injections,[9,15,28,29] with some of the patients being in their twenties and thirties. Although the reduced number of younger patients did not allow us to statistically correlate better outcomes with younger age, our observations suggest that this procedure could be used advantageously also to treat a younger population more prone to sport-related knee injuries.[30] As a consequence of the relatively young age of the participants, however, the beneficial effects observed in our study might be partially skewed. Similar to the age-related decline in clinical efficacy of cartilage repair and restoration procedures, the efficacy of BMAC may show a decline

Table 2
Clinical status across all parameters examined before and after treatment in K-L grade 2 patients reported as Mean ± SD.

K-L grade 2 patients	Baseline	BMAC injection	*p* value
VAS	8 ± 1.6	3.3 ± 2.7	<0.001
Tegner scale	55.3 ± 11.4	80.4 ± 15.1	<0.001
WOMAC scale	60.2 ± 11.4	76.2 ± 15.1	<0.001

Table 3
Clinical status across all parameters examined before and after treatment in K-L grade 3 patients reported as Mean ± SD.

K-L grade 3 patients	Baseline	BMAC injection	*p* value
VAS	7.7 ± 2.3	3.4 ± 2.2	<0.001
Tegner scale	52.3 ± 18.2	73.8 ± 14.7	<0.001
WOMAC scale	60.8 ± 18.2	76.2 ± 14.7	<0.001

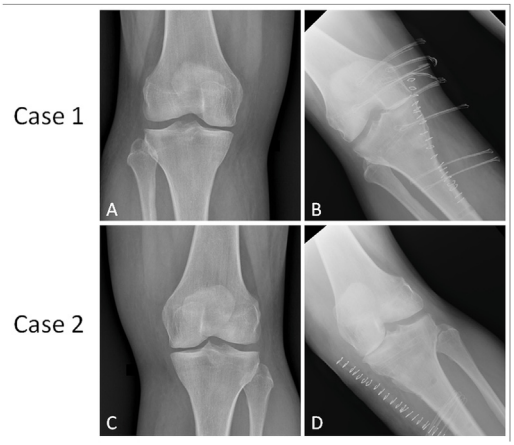

Fig. 2. Two representative cases affected by knee disorders with K-L 2 grade osteoarthritis, that underwent high tibial osteotomy and were treated with BMAC injections. A) and C): pre-operation standing anteroposterior knee radiography. B) and D): knee radiography taken 3 and 2 years after BMAC treatment respectively.

with age.[31,32] Controlled and more specifically population-targeted studies will be required to determine the effects of patient age at time of BMAC harvest and confidently extend our findings to the older population.

Secondly, in contrast to most published studies we have not used adjuvant therapies, such as adipose tissue grafts, platelet-rich plasma, hyaluronic acid, or collagen matrices, in conjunction with BMAC. While it is not clear whether the effects seen in these previous studies are a direct result of BMAC injection, adjuvant therapies, or due to a synergic effect, the positive outcomes we have observed can be ascribed solely to BMAC injections. Furthermore, the efficacy of BMAC is also independent of prior knee surgeries. Indeed, even though a subset of our patients had other surgical treatments before receiving BMAC, this was not associated with a better outcome. Lack of significant differences between the surgically-treated and untreated populations clearly indicates that the reported improvements were due to BMAC injections.

Finally, compared to other studies, our results are based on an unusually long follow-up period of about 57 months on average, which was 18 months for the shortest occurrence. Despite the growing interest in biological alternatives for the treatment of knee pathologies, only a small number of studies has investigated BMAC, and these are generally weakened by a rather short follow-up period, among other drawbacks.[23] Our work, instead, provides preliminary evidence that a single BMAC injection has a lasting effect on knee osteoarthritis and helps to address some concerns about the durability of these treatments.[23]

This study, of course, has several limitations, which is why we consider it only preliminary evidence. First, it is a retrospective analysis that also lacks a control arm treated with placebo, which could lead to bias due to self-assessment of subjective response variables. Second, a number of patients underwent concurrent surgical treatment, which could influence the results, and the cohort of patients studied was heterogeneous. Third, in order to assess the results more objectively, it would be important to perform MRI of the knee joint and secondary arthroscopy, as well as biopsy of the regenerated joint tissue at the follow-up. Fourth, we should have performed flow cytometry to characterize MSC's in the final BMAC concentrate before injecting it to the patient in order to better understand their role in the improvement in knee osteoarthritis symptoms. Future evaluations will be conducted to better understand the efficacy of BMAC in the treatment of knee OA and to investigate its usefulness in different types of patients with OA as well as restricting the study group to only one K-L grade of osteoarthritis patients in order to remove the impact of this confounding variable. Nevertheless, based on our preliminary results, we can conclude that patients experienced significant improvement in knee osteoarthritis symptoms after a single intra-articular injection of BMAC, suggesting high efficacy and safety of BMAC for these patients. Furthermore, our study shows that it is possible to monitor patients remotely and continuously assess their condition even in difficult situations.

Fundings

None.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contribution

All authors have contributed substantially to the conception, design, analysis and interpretation of the data.

Declaration of competing interest

None.

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