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Effectiveness of a single intra-articular bone marrow aspirate concentrate (BMAC) injection in patients with grade 3 and 4 knee osteoarthritis

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Abstract

Aim: To evaluate the clinical efficacy and safety of an intra-articular injection of bone marrow aspirate concentrate (BMAC) as a treatment option for osteoarthritis (OA) of the knee.

Materials and methods: Between June 2014 and February 2017, data from 233 patients with knee osteoarthritis treated with BMAC injection at a single center, were retrospectively evaluated. Only patients with idiopathic osteoarthritis were included. Exclusion criteria were post-traumatic osteoarthritis, previous knee surgery, age less than 50 years old or more than 85 years old, active infection, uncontrolled diabetes mellitus, rheumatological or other systemic disease, malignancy, or treatment with immunosuppressive drugs. Bone marrow from the iliac crest was aspirated/concentrated with a standardized technique using a single-spin manual method. Patients were evaluated before and after the

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procedure, using the numeric pain scale (NPS) and Oxford knee score (OKS). Mean follow-up period was 11 months, range (6−30 months).

Results: A total of 121 of 233 patients had completed data as previously defined and were included in the statistical analysis. There were 85 females and 36 males, with mean age 70 years (range 50−85). Compared to baseline, the mean NPS decreased from 8.33 to 4.49 ($p < 0.001$) and the mean OKS increased from 20.20 to 32.29 ($P < 0.001$) at final follow-up. There were no complications.

Conclusion: A single intra-articular injection of BMAC is a safe and reliable procedure that results in clinical improvement of knee OA.

Keywords: Evidence-based medicine, Surgery

1. Introduction

Osteoarthritis (OA) is a chronic progressive degenerative disorder consisting of degeneration and loss of articular cartilage with accompanying synovitis, subchondral bone remodelling, and osteophyte formation [1, 2]. It constitutes a major cause of disability with pain, stiffness, resulting in severe functional limitations [3]. OA is the most common joint disorder in the United States, affecting 1 in 5 Americans over 60 years of age [4] and with projected 67 million patients in the United States alone by 2030 [5]. This has huge socio-economic implications [6, 7] and a substantial financial burden on the economy [8], and it is estimated that it accounts between 1% and 2.5% of the gross national product of Western world [9].

Although OA is a process of articular cartilage "wear and tear", its changes are biochemically mediated [10], through an imbalance between intra-articular anabolic and catabolic cytokines [11]. This results in cartilage loss, synovial inflammation and eventually leads to mechanical and biological dysfunction of the joint [12].

The articular cartilage due to its avascular nature and the limited self-renewal [13] capacity of chondrocytes has remarkably poorer regenerative ability than other tissues [14, 15].

Current treatments for early phase of degenerative arthritis focus on relieving inflammation and pain [16], but have no effect on the natural progression of the disease [17] because it does not improve the biochemical environment (homeostasis) of the joint.

Conservative treatments including medications such as non-steroidal anti-inflammatory drugs (NSAIDs) and steroids as well as supplements including glucosamine, chondroitin sulphate, omega-3 fatty acids and intra-articular use of viscosupplementation [16, 18], cannot alter the natural history of the disease. Specifically, viscosupplementation is efficient in the early stage of osteoarthritis but pain relief is limited to a few months, offering only a temporary benefit [19, 20], whereas injection of

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corticosteroids provides short-term improvement of symptoms while posing the risk of aggravating cartilage damage and producing tissue atrophy [21].

Costly total knee arthroplasty (TKA) then follows when other treatment options have been exhausted [22], however patients experience a higher risk of death from mental and inflammatory musculoskeletal diseases, with a serious adverse event rate of 5.6% and a 0.2% mortality rate [23].

With recent increase of interest in field of regenerative medicine, research has been directed towards the development of treatment strategies to provide a symptomatic improvement by influencing joint homeostasis [24]. Recently, extraction of the mesenchymal stem cells (MSCs) obtained from autologous bone marrow (BMAC) followed by concentration was introduced represents the next generation of injectable intra-articular orthobiologic therapy for patients with cartilage disease [25, 26].

MSCs are multipotent cells that exhibit strong self-renewal abilities, combined with a differentiation capacity to form chondrocytes, adipocytes, and osteocytes [27]. These cells also have very important local paracrine affects to alter their local micro-environment to conditions favourable for regeneration and repair [22, 28].

BMAC represents the safest and most feasible source of MSCs. Intra-articular application has resulted in pain reduction, functional improvement and/or tissue regeneration [29]. BMAC is obtained through density gradient centrifugation of bone marrow aspirate (BMA) typically aspirated from the iliac crest [30]. BMAC has been shown to provide elevated levels of hematopoietic stem cells (HSCs), MSCs, platelets, chemokines and cytokines including PDGF and TGF-β [31]. These growth factors (GFs) are not only contained within the alpha granules of platelets, but they are also secreted by MSCs [32, 33] and can induce chondrogenesis of MSCs [32, 33, 34]. GFs also initiate stem cell migration to the injury site and provide adhesion sites for the migrating stem cells [35]. Moreover BMAC possesses in general, anti-inflammatory, angiogenic trophic and immunomodulatory properties that can potentially have anabolic and anti-inflammatory effects enhancing cartilage repair [32, 33, 36].

To date, there have been several studies that have looked at BMAC for the treatment of osteoarthritis, with conflicting results secondary to the differences and/or inconsistencies in methodologies used throughout the studies [37]. Therefore, the role of BMAC in osteoarthritis is not yet been established.

Encouraged by the positive preliminary results of BMAC-induced bone regeneration [22, 38, 39, 40, 41, 42], the authors initiated a retrospective clinical trial to evaluate the results of a single, intra-articular injection of BMAC with knee OA. This study is one of the largest cohorts in the literature.

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2. Materials & methods

Between June 2014 and February 2017, data from 233 patients with knee osteoarthritis, treated with BMAC injection, were retrospectively evaluated. All procedures were performed at one Institution by the Authors. The Mediterraneo Hospital Scientific Committee approved the study protocol and informed consents were obtained from each participant.

Inclusion criteria were a longstanding knee pain from idiopathic osteoarthritis unresponsive to activity modification, weight loss, physical therapy, bracing, analgesics, nonsteroidal anti-inflammatory drugs, injection therapy or arthroscopy for at least 6 weeks with a Kellgren–Lawrence [43] grade III or higher radiographic OA.

Exclusion criteria included post-traumatic osteoarthritis, previous knee surgery, age less than 50 years old or more than 85 years old, active infection, uncontrolled diabetes mellitus, rheumatological or other systemic disease, malignancy, treatment with immunosuppressive drugs. Although in cases of bilateral osteoarthritis both knees were treated, the worst knee was taken into account for comparison analysis. Patients who elected to participate in the study and had a follow-up time of less than 60 days were also excluded. Additionally, patients who elected to proceed with total knee arthroplasty before their post-procedure evaluation, were also excluded.

As BMAC treatment works by stimulating the normal inflammatory healing mechanisms, medications such as NSAIDs [44] or corticosteroids [45] that can impair soft tissue healing and also reduce MSC proliferation, were discontinued at least 10 days and 4–6 weeks prior to the procedure respectively. In addition, no patient received pre-procedural antibiotics. If the patient was under anticoagulants, routine bridging was performed with subcutaneous enoxaparin until the day before the procedure.

2.1. Procedure

With the patient in a supine position on the operating table, the iliac crest was surgically prepped and draped in the usual fashion. A combination of conscious sedation and local anesthesia (1% lidocaine) was used. Prior to aspiration, an 11-Gauge Bone Access Needle (Medtronic, Inc) and eight 10ml-syringes are flushed with heparin (5000 U/20ml) and then filled with 1 ml heparin solution.

Using a stab incision, the bone access needle is inserted and advanced through the periosteum of the Anterior Superior Iliac Spine. After the periosteum is pierced, the driver and stylet are removed and a 10ml syringe containing 1ml of heparin dilution is used to aspirate 8 ml of bone marrow [Fig. 1]. There was extra care to hold the needle still throughout the procedure. The needle was not advanced nor rotated after each successive 10 mL aspiration, in contrast to other authors who recommend this practice in order to reduce peripheral blood contamination (hemodilution) [46]. All

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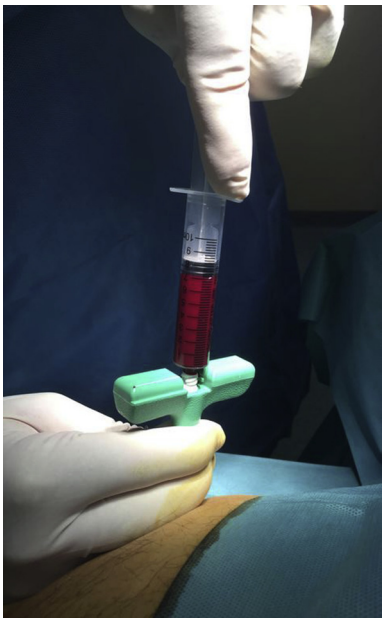

Fig. 1. Bone marrow aspiration.

aspirations were performed by the same surgeon (IMK). The total amount of bone marrow harvested (BMA) was 80 ml for the treatment of both knees and 60 ml for one knee. Following bone marrow aspiration, the bone access needle is withdrawn, pressure is applied to the skin entry site, followed by dressing application.

Following extraction, the aspirate is transferred to sterile tubes and is carefully processed by hand in a separate room under sterile conditions to isolate the buffy coat through centrifugation [Fig. 2]. A single-spin centrifugation technique (Hettich®

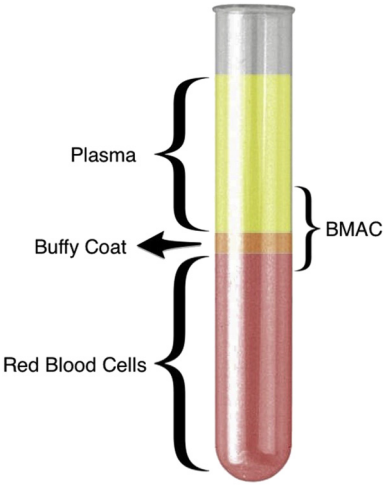

Fig. 2. Cell separation layers starting from the top: Plasma mainly containing platelets, buffy coat with mononuclear HSCs and MSCs and bottom fraction with red blood cells (RBCs). BMAC consists mainly from buffy coat, a supernatant with platelets and small size MSCs and the very top of the RBC layer containing the largest size MSCs.

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Rotofix 32A centrifuge, 15 min at 2800 RPMs) yielded approximately 20ml of BMAC for every 80 ml of BMA which was then transported back to the operating room and under sterile conditions, each knee joint was injected with 10 ml of BMAC. The supernatant part of the 80 ml aspirate was preserved and additionally used as prolotherapy at the level of the joint line. All injections were performed using an anterolateral approach to the knee joint by the same physician (G.S.T.) without anaesthesia in order to prevent any interaction with the BMAC. Immediately following injection, the knee joint was passively moved throughout its range of motion to disseminate the fluid throughout the joint. A summary flow diagram of the procedure is shown in Fig. 3. Mean duration of the procedure was approximately 1 hour, while the time between extraction and injection was approximately 35 minutes.

The patients were allowed full weight bearing and instructed to return to light activity as tolerated avoiding oral NSAID's and corticosteroids for at least four weeks post procedure. Within six weeks' patients were allowed to return to full activities. There was no other therapeutic intervention (bracing, physical therapy, etc). There were no adverse events or complications and all patients recovered completely.

2.2. Outcome measurements

Outcome was assessed using a numeric Pain Scale (NPS) (0−10) for pain intensity, (NPS has eleven levels of pain ranging from 0 for no pain, to 10, indicating worst possible pain), and a validated Oxford knee score (OKS) questionnaire for functional assessment [47]. OKS is a self-administered questionnaire, which is designed specifically for the knee joint. The questionnaire includes 12 items with a maximum total score of 48 indicating maximum function.

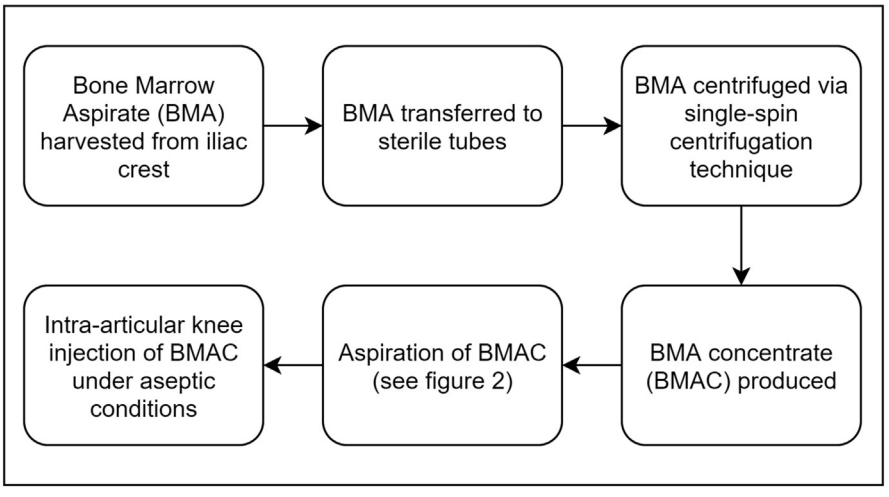

Fig. 3. Flow diagram showing a summary of the procedure.

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Evaluations were performed prior to the administration of treatment and post-procedure in varying time periods (mean follow-up time: 11 months, range, 6–30 months) via phone calls and direct patient evaluations. At final follow-up, the patients were asked if they were satisfied with the procedure and if they would suggest the treatment to someone else.

2.3. Statistical analysis

The pre- and post-treatment scores were compared using the Paired t test. Probability (p) values less than 0.05 were considered statistically significant. All statistical analyses were carried out using SPSS software (SPSS 17.0, SPSS, Chicago, IL, USA).

3. Results

According to the inclusion and exclusion criteria, 121 patients (121 knees) were included in this study. Mean age was 70 years, ranging from 50 to 85 years, and there were 36 (29.7%) males and 85 (70.3%) females, 76 right knees and 45 left knees. The degree of the degenerative arthritis was evaluated by K–L grade (Kellgren–Lawrence grading scale) on standing anteroposterior (AP) view: there were 46 (38%) cases of grade III, and 75 (62%) cases of grade IV. At final follow-up, the mean NPS decreased from 8.33 preoperatively to 4.49 postoperatively ($p < 0.001$). Also the mean OKS increased from 20.20 pre-operatively to 32.92 postoperatively ($p < 0.001$). A total of 6 patients (5%) elected to proceed to total knee arthroplasty, 89 patients (73.5%) indicated that they would repeat the procedure, and 105 patients (86.7%) indicated that they would recommend the procedure to a friend. **The results are summarized in** Table 1. In the group of the current study, knee pain recurrence was not further recorded beyond the follow-up time and this would be a subject for a future follow-up investigation. There was no correlation between age, grade of OA and decreased scores. There were no complications, including pain on the site of the harvest, hematoma or paresthesias.

4. Discussion

Orthobiologics has emerged as a therapeutic option with special emphasis on regenerating damaged or diseased tissue, correcting the systems biology and delaying or

Table 1. Summary of results, 121 patients (121 knees).

Outcome Instrument	Pre-treatment	Post-treatment	p value
NPS	8.33 (range 5–10)	4.49 (range 1–10)	<0.001
OKS	20.20 (range 7–39)	32.29 (range 16–48)	<0.001

NPS: Numeric Pain Scale; OKS: Oxford Knee Score.

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preventing disease progression [36]. The potential of MSCs to differentiate into the cell lineage of interest to form chondrocytes, adipocytes, and osteocytes [27], and the capacity of self-renewal has created huge interest in trauma and orthopedic surgery [28, 48]. Additionally, MSCs may favourably alter the microenvironment conditions for regeneration and repair [49], due to down-regulation of inflammatory cytokines, including interleukin 1 (IL-1), interleukin 6 (IL-6), interferon-γ, and tumor necrosis factor alpha (TNF-α) [50, 51]. Apart from bone marrow, MSCs are present in numerous tissues in the body including adipose, synovium and blood [52], however their chondrogenic potential is lower than that of bone MSCs [36]. BMAC may represent the safest and most feasible source of MSCs for bone tissue regeneration [53], the procedure is technically easy and fast, enabling the harvesting and intraoperative transplantation in one sitting [54]. Additionally, BMAC contains hematopoietic stem cells, platelets, growth factors (GFs), cytokines and chemokines [55]. The GFs are released from platelet alpha granules [32, 33], and mainly include TGF- β, PDGF, VEGF, FGF, BMP, and IGF [56], which additionally help to initiate stem cell migration to the injury site and provide adhesion sites for the migrating stem cells [35].

Currently there are many published studies supporting the feasibility, safety, and efficacy of bone marrow derived MSC therapy in knee OA [22, 36, 57, 58]. Specifically human trials showed improvement in range of motion, pain scores, functional status of the knee, and walking distance even with patients with grade 4 arthritis [22, 59, 60, 61, 62], shortened hospital stay [63], production of cartilage and bone regeneration [58, 60, 64], increase in cartilage thickness, decrease in the size of subchondral edema [15, 58, 61, 62, 65], treatment of patello-femoral cartilage defects [66], complete filling of cartilage defects (MRI-confirmed) [59, 67], combination of BMAC with ACL reconstruction [68], and increase in meniscus volume [62]. In this study we report the clinical results on 134 patients with osteoarthritis who were retrospectively followed after receiving a single, intra-articular injection of BMAC.

The procedure was well tolerated and improved pain and function at both short and long-term follow-up among the vast majority of the patients.

Potential complications of bone marrow aspiration from the iliac crest are rare, (0.05%) with the most common being hemorrhage, while others include, infection, donor site morbidity with persistent pain [69]. No adverse effects were recorded and none of the patients reported worsening of symptoms following the BMAC procedure.

In addition, 86.7% of patients would recommend the procedure to a friend. A number of studies using BMAC for cartilage regeneration and repair have focused on preparations in which MSCs are pre-cultured, assuming that number of delivered MSCs is critical [60, 62, 68]. However, excellent clinical results have been demonstrated

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with low cell numbers compared to culture-expanded techniques [22, 65, 70, 71]. Therefore, it has been suggested that the mechanism of action of MSCs is modulation of the joint homeostasis via paracrine signalling [57], instead of being an actual building block of cartilage [72]. In an effort to obtain the maximum possible number of MSCs, several authors strive to minimize blood "contamination" of the sample; however this minimizes a number of platelets and therefore their growth factors obtained in the final aspirate. Therefore, in this study there has been no effort to increase the number of MSCs and minimize blood contamination as suggested by several authors [73], including multiple sampling sites, multi-hole needles as well as successive needle displacement and/or rotation following each 10 mL aspiration.

Our previous experience (Themistocleous et al., unpublished data) consisted of administration of isolates of large numbers of cultured MSCs that had relatively poor results compared to our current protocol. Therefore, we believe that the administration of MSCs with platelets and therefore growth factors acting similarly to platelet rich plasma would be desirable. Further research is needed in order to establish the optimal fractions of each of the elements. This simple and fast technique applied herein uses a thin (11 gauge) needle to acquire a maximum number of MSCs by a single puncture in order to minimize procedural time and patient discomfort. In addition, the authors did a single spin with low centrifuge settings (580G) in order to maximize stem cell viability and achieve optimal separation between the bone marrow layers. There have been very few studies in which BMAC was injected intra-articularly without any additional processing. A recent study by Centeno et al. [42], in which 424 knees of 373 patients with knee osteoarthritis were injected with BMAC, supported that the final MSC number plays a critical role for optimal outcomes in contrast to our protocol. Sampson et al. [40], performed a single intra-articular injection of BMAC in 73 knee osteoarthritis patients (73 knees) with an average follow-up of 148 days followed by a single platelet rich plasma injection at eight weeks and concluded that this combination is beneficial in the short-term in moderate to severe osteoarthritis. Shapiro et al. [41], conducted a double blinded randomized control trial in 25 patients with bilateral knee osteoarthritis. One side was injected with BMAC whereas the contralateral side was injected with normal saline 0.9%. There was no statistically significant difference in pain relief and function at 6 months. However, given the fact that MSCs administered to any site have the ability to travel to sites of inflammation, it cannot be concluded with certainty that the MSCs injected may have not played a role in improving the contralateral knee symptoms as well [41]. Kim et al. [22], injected with BMAC 75 knees of 41 patients with osteoarthritis degrees ranging from 1 to 4 and showed improved quality of life in all osteoarthritis grades, however even in grade 4 patients the difference was less significant. Our study represents one of the largest cohorts of patients with knee osteoarthritis treated exclusively with a single injection of concentrated BMAC which demonstrates the efficacy of this treatment in the absence of any additional procedure. However, several questions remain yet to be addressed:

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1. What is the ideal/optimal proportion of MSCs and platelet derived growth factors to be injected? More is not always necessarily better.
2. Should treatment with BMAC be combined with and/or followed by other modalities, for example PRP, hyaluronic acid, prolotherapy?
3. Is there any added benefit in repeating treatment with BMAC at regular intervals for maintenance and if so, what is the optimal frequency/timing?

This study has several limitations. First, the results should be interpreted in the light of absence of a control group. Second, the retrospective nature of the study did not allow for data collection at the designated regular intervals and therefore follow up was variable on a case-by-case basis. Third, patients enrolled in this study have advanced (grades 3 and 4) knee osteoarthritis, and therefore the effects of treatment in patients with milder disease were not evaluated. This is greatly attributed to the fact that in our country's culture, a typical patient will seek several orthopedic opinions and undergo several nonoperative treatments and only visit our practice when they desperately try to avoid the total joint arthroplasty surgery that has been proposed to them as final solution. Fourth, another confounding factor may be that many of our "word-of-mouth" patients visiting our practice may be biased in favour of this nonoperative modality, at the expense of other possible operative or nonoperative management options. Lastly, although there was consistency throughout the sampling and injection process, there was no laboratory analysis of the aspirate and BMAC to measure cell numbers, presence of growth factors or other constituents.

5. Conclusion

MSCs are a promising option for the treatment of knee OA. Given the aforementioned limitations, this study showed that a single intra-articular injection of BMAC appears to provide long-term benefits. The procedure is simple, fast, well-tolerated, avoids the need for hospitalization and generated no complications or adverse effects. Further research is needed to better understand the role of BMAC therapy, determine the optimal dose, the best way of separation, delivery and frequency of treatment.

Declarations

Author contribution statement

George S. Themistocleous: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

George D. Chloros: Analyzed and interpreted the data; Wrote the paper.

Ioannis M. Kyrantzoulis: Performed the experiments.

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Ioannis A. Georgokostas: Conceived and designed the experiments; Performed the experiments.

Marios S. Themistocleous, Olga D. Savvidou: Analyzed and interpreted the data; Wrote the paper.

Panayiotis J. Papagelopoulos: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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