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Original article

Bone Marrow Aspirate Concentrate vs Autologous Conditioned Serum in the Treatment of Knee Osteoarthritis

Matteo Vitali[1], *Marco Ometti*[1], *Pierluigi Pironti*[2], *Damiano Salvato*[3], *Alice Sandrucci*[4], *Orlando Leone*[3], *Vincenzo Saliniv*[1]

[1]San Raffaele Scientific Institute, Department of Orthopedics and Traumatology (Milan, Italy); [2]University of Milan, Residency Program in Orthopedics and Traumatology (Milan, Italy); [3]San Raffaele University, Residency Program in Orthopedics and Traumatology (Milan, Italy); [4]San Raffaele University (Milan, Italy)

Abstract. *Background and aim:* The aim of this study was to compare the efficacy of a single Bone Marrow Aspirate Concentrate (BMAC) with a cycle of 4 Autologous Conditioned Serum (ACS) injections in the treatment of early-stage knee osteoarthritis (OA). *Methods:* Two groups of 12 patients with degenerative knee OA were treated with a single BMAC injection and with a cycle of 4 ACS injections respectively. Follow-up was set at baseline (t0), one-month (t1) and six-months (t2) evaluating VAS for pain, WOMAC index and range of motion (ROM). *Results:* We reported a significant improvement in WOMAC after BMAC injection both at t1 (p=0,001) as well as t2 (p< 0,001), plus a reduction of VAS values in BMAC group at six months follow-up (p=0,024). In contrast, no significant differences in ROM between the two groups were observed. *Conclusions:* Both the approaches are safe and effective in the treatment of knee OA, with a major efficacy of BMAC. (www.actabiomedica.it)

Key words: knee osteoarthritis, biological therapies, Bone Marrow Aspirate Concentrate, Autologous Conditioned Serum

Introduction

Osteoarthritis (OA) is a chronic degenerative and debilitating joint disease which affects primarily people over the age of 65 years (1,2). Concerning the World Health Organization (WHO), more than 150 million people are affected by OA worldwide (1). The most involved sites are knees, hips, finger interphalangeal joints, first metacarpal joints, first metatarsophalangeal joints, and apophyseal joints of the lower cervical and lower lumbar spine (1). Knee OA represents over 80% of the total disease burden (3). OA consists of degeneration and loss of the articular cartilage with subsequent synovitis, subchondral bone degeneration and osteophyte formation (4,5), resulting in chronic pain, stiffness and functional limitation of the affected joint (5). The whole process is likely to be triggered by an imbalance between intra-articular anabolic and catabolic cytokines which leads to a low-grade inflammation able to elicit symptoms and to accelerate disease progression (4,6). Indeed, some of the cartilage matrix catabolic products can likely activate macrophages as well as other innate immune cells to release inflammatory cytokines which influence chondrocyte activity, enhancing the cartilage damage progression (6). Current treatments in the early phase of OA aim to relieve inflammation and pain with no impact on the natural progression of the disease (4). In particular, the first step of management of knee OA consists of weight loss, physical therapy and medications such as

non-steroidal inflammatory drugs (NSAIDs), analgesics, corticosteroid and/or hyaluronic acid injections as well as oral supplementation of glucosamine chondroitin sulphate (3,4). Nowadays, the most successful treatment for end-stage knee osteoarthritis (OA) is total knee arthroplasty (TKA) which results in substantial symptomatic relief and functional improvement (4,7). Since the interest in regenerative medicine has been growing in recent years, different biological treatments have been proposed to decelerate the OA progression. Among these options, intra-articular injections of autologous conditioned serum (ACS) represent a viable choice for the treatment of symptomatic OA, and has been employed by physicians for more than 10 years (8). It contains a high concentration of interleukin-1 receptor antagonist (IL-1Ra) as well as various other anti-inflammatory cytokines and growth factors aiming to contrast the inflammatory cascade which leads to cartilage degeneration (8,9). Most recently, the administration of injectable therapies based on mesenchymal stem cells (MSCs) obtained from autologous bone marrow aspirate concentrate (BMAC) are becoming a suitable option for the treatment of knee OA (4,9). To the authors knowledge, there are still no studies that directly compare these two biological therapies. Therefore, the purpose of this study was to compare the effects of single injection BMAC therapy with a cycle of 4 injections of ACS therapy in patients affected by knee OA.

Methods

Between December 2017 and January 2019, 24 patients with clinical and radiological signs of degenerative knee OA were enrolled for this study at IRCCS San Raffaele Hospital, department of Orthopedics and Traumatology, Milan. All the patients underwent plain radiographs to assess and classify the OA. Specifically, OA was classified using the Kellgren Lawrence radiological scale (10,11). Furthermore, MRI was prescribed in case of clinical suspicion of concomitant meniscal injury. The patients were therefore subdivided for each pathology into two equal sized groups of 12 patients: Group 1 was treated with a single BMAC injection and the Group 2 was treated with 4 injections (once per week for 4 weeks) of ACS at the site of OA. Our inclusion criteria were chronic knee pain unresponsive to conservative treatments in patient < 65 years old. Patients presenting knee instability, meniscal injuries, severe malalignment, flexion contracture >10, Kellgren Lawrence grade IV, inflammatory arthritis (such as rheumatoid arthritis and ankylosing spondylitis), muscle pain, hematologic disorders, septicemia, coagulopathy, active infection, and immune deficiency disorders were excluded from this study. Autologous bone marrow aspirate (BMA) was harvested from the anterior iliac crest then concentrated with a standardized technique using a single-spin manual method (**Figure 1**), and finally injected in the patient's knee with a single injection (**Figure 2**).

Specifically, patients were placed in the supine position and underwent a local anesthesia involving the periosteum as well as the overlaying tissues. Then 60 ml of bone marrow were harvested from iliac crest, using a SmartPreP2 Bone Marrow Procedure Pack (Harvest Technology, USA). The BMA was filtered at first and then drawn from the filter bag. Subsequently, BMA was transferred into a disposable sterile container and concentrated via single spin centrifugation technique for 15 minutes at 2800 RPMs obtaining

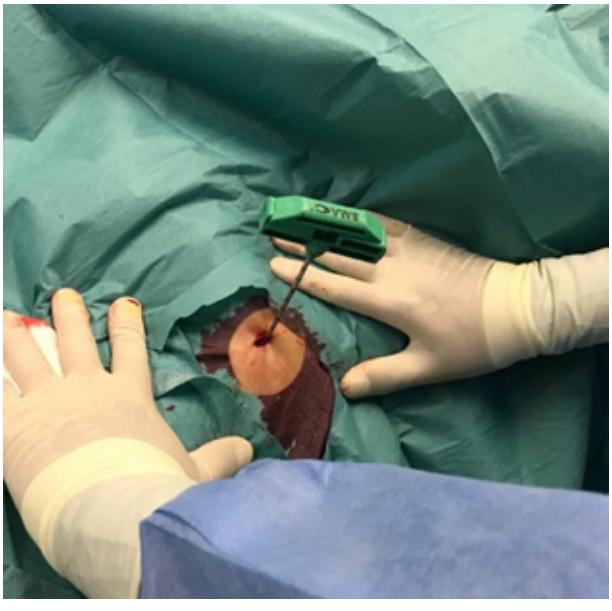

Figure 1. BMA single-spin manual harvesting method from anterior iliac crest.

BMAC. Eventually, 7-10 ml of BMAC was aspired through a syringe and injected in the patient's affected knee (**Figure 2**). Since the bone marrow aspiration requires sterile conditions, the whole process must be performed in the operating room (12).

Besides, ACS was isolated from 50mL of whole blood obtained using syringe containing CrSO4-treated glass beads and subsequently incubated for 7 hours (**Figure 3**). Then, after a centrifugation the serum supernatant was filtered and aliquoted into four 3mL portions (**Figure 4**).

Since the injections were not performed immediately after the serum preparation, the aliquots were stored at -20°C (13,14). Both the blood draw and the injections were performed on an outpatient basis.

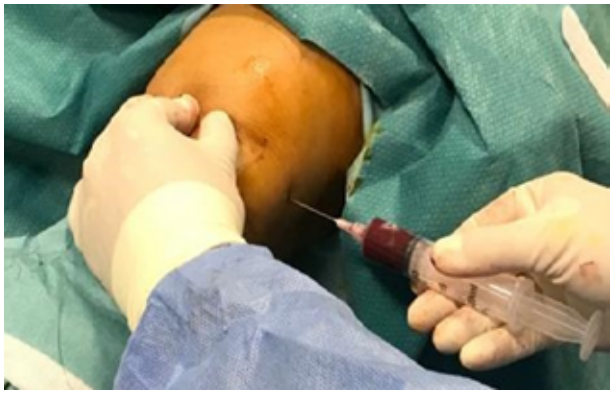

Figure 2. BMAC injection performed to the superolateral margin of the patella of the affected knee.

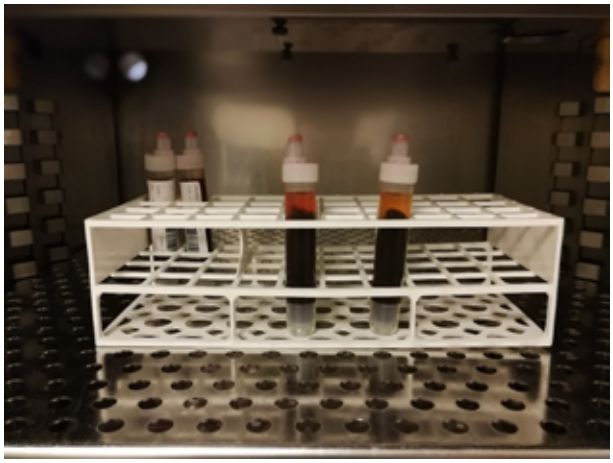

Figure 3. Incubation of whole product at 37°C for 7 hours.

The injections were performed without any ultrasonography support, with the patient in the supine position in both the groups. The site of injection was the superolateral margin of the patella of the affected knee. All the patients were evaluated before treatment, and additional 1 and 6 month follow-up post-treatment using visual analog scale (VAS) for pain, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and a range of motion (ROM) evaluation.

This study was conducted following the principles of the Declarations of Helsinki and with the patients' agreement expressed through a written consent.

Statistical Analysis

Statistical analysis was performed using IBM Statistics (SPSS Inc., Chicago, IL). Numerical variables were tested by Shapiro-Wilk test to assess normal distribution. T test or Mann-Whitney U test were used according to the results of normality test to evaluate between groups differences. Fisher's exact test (or, if not applicable, Chi-square test) was used to test differences between groups in regard to categorical variables. Data are reported as mean ± standard deviation. P values < 0.05 were considered statistically significant.

Figure 4. Final ACS product in syringe.

Results

Both the study groups were similar and uniform in terms of distribution of age, sex, and affected side (Table 1).

Pre-treatment group values both for VAS (p=0,187) and WOMAC (p=0,101) were comparable between the two groups (Table 2). In BMAC group, mean VAS values at one-month follow-up decreased of 2,58 points (SD 2,96) compared to the ACS group with 2,45 (SD 1,48), showing no statistically significant difference between the two groups (p=0,234). However, at final follow-up VAS values showed a significant decrease in BMAC group compared to the ACS group (p=0,024), with a mean reduction of 3,542 (SD 3,0411) and 2,29 (SD 1,41) points respectively (**Figure 5**).

Besides, WOMAC values in BMAC group already showed a significant reduction at one-month follow-up compared to ACS group (p=0,001), with mean reduction of 22,92 (SD 21,43) and 12,02 (SD 8,37) points respectively. Moreover, at final follow-up we observed a higher reduction of WOMAC values in BMAC group compared to ACS group (p<0,001). Mean WOMAC reduction was 39,87 (SD 23,53) and 11,13 (SD 8,48) points respectively (**Figure 6**).

Mean VAS and WOMAC values, pre-treatment and at each follow-up, are summarized in Table 2, as well as p-values between the two groups (Table 2). Concerning ROM, pre-treatment values were also comparable between the analyzed groups (Table 3). At one-month follow-up, we observed mean improvement of 7,0 flexion degrees (SD 12,89) as well as 3,5 degrees (SD 5,2) extension deficit reduction in BMAC group. Besides, ACS group showed only 2,0 degrees (SD 5,38) of mean flexion improvement, and 1,5 degrees (SD 3,51) extension deficit reduction. Moreover, at final follow-up the mean flexion improvement was 13,33 degrees (SD 12,12), and extension deficit reduction 4,33 degrees (SD 7,13) in BMAC group. Concerning the ACS group, the improvement of flexion was 4,33 degrees (SD 10,07) and the extension deficit reduction 1,5 degrees (SD 3,50).

However, despite the higher improvement in ROM values showed by BMAC group, our analysis did not show significant differences between BMAC group and ACS group at one-month follow-up (flexion: p=0,193; extension: p=0,193) as well as at final follow-up (flexion: p=0,077; extension: p=0,409) (Table 3). Additionally, we observed a marked reduction of NSAIDs consumption in BMAC group compared to ACS group at 6 months follow-up (**Figure 7**).

Table 1. Mean age in years, male and female subdivision, mean average of affected side both for BMAC as well as ACS group.

	BMAC	**ACS**
Number of patients **Age (SD)**	12 57,92 (15,74)	12 59,67 (14,2)
Male	5 (41,7%)	6 (50%)
Female	7 (58,3%)	6 (50%)
Right side	8 (66,7 %)	6 (50%)
Left side	4 (33,3%)	6 (50,%)

Table 2. Pretreatment, 1 month and 6 months after treatment outcomes. Means of WOMAC and VAS values are showed along with standard deviations (SD). P-values between groups are given both for WOMAC as well as VAS pretreatment and at each follow-up.

	t0 (Pretreatment)	**t1 (1 Month)**	**t2 (6 Months)**
BMAC	12	12	12
WOMAC (SD)	60,42 (16,30)	37,50 (12,22)	20,55 (15,41)
VAS (SD)	5,96 (1,86)	3,38 (1,97)	2,42 (2,35)
ACS	12	12	12
WOMAC (SD)	69,92 (10,26)	57,90 (14,84)	58,79 (14,84)
VAS (SD)	6,83 (1,21)	4,38 (2,03)	4,54 (1,90)
P-value WOMAC	0,101	0.001	< 0,001
P-value VAS	0,187	0,234	0,024

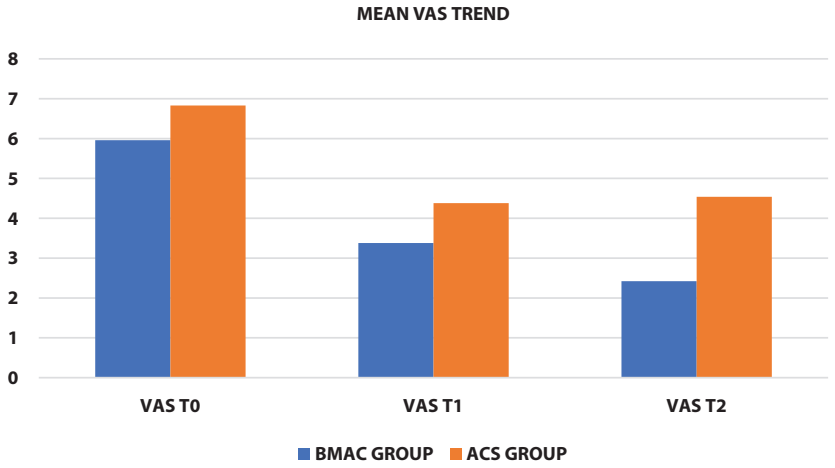

Figure 5. Mean VAS values trend at each evaluation, showing a significant decrease in BMAC group at t2 as compared to ACS group.

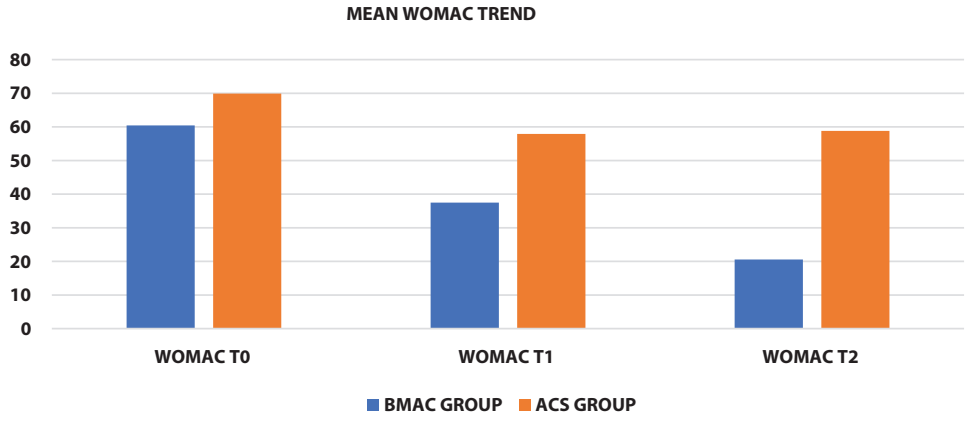

Figure 6. Mean WOMAC values at each evaluation, showing a significant reduction in BMAC group at t1 as well as t2 as compared to ACS group.

Table 3. ROM characteristics, pretreatment at 1 month and 6 months follow-up. Flexion degrees and Extension deficit are shown in means along with standard deviations (SD). P-values are given for Flexion and Extension deficit between groups pretreatment as well as at each follow-up.

	t0 (Pretreatment)	t1 (1 Month)	t2 (6 Months)
BMAC	12	12	12
FLEX	121,67 (16,28)	128,67 (12,3)	135 (7,98)
EXT	5,58 (7,60)	2,08 (3,97)	1,25 (3,11)
ACS	12	12	12
FLEX	114 (28,95)	116 (30,29)	118,33 (30,1)
EXT	1,92 (3,61)	0,42 (1,44)	0,42 (1,44)
P-value FLEX	0,432	0,193	0,077
P-value EXT	0,151	0,193	0,409

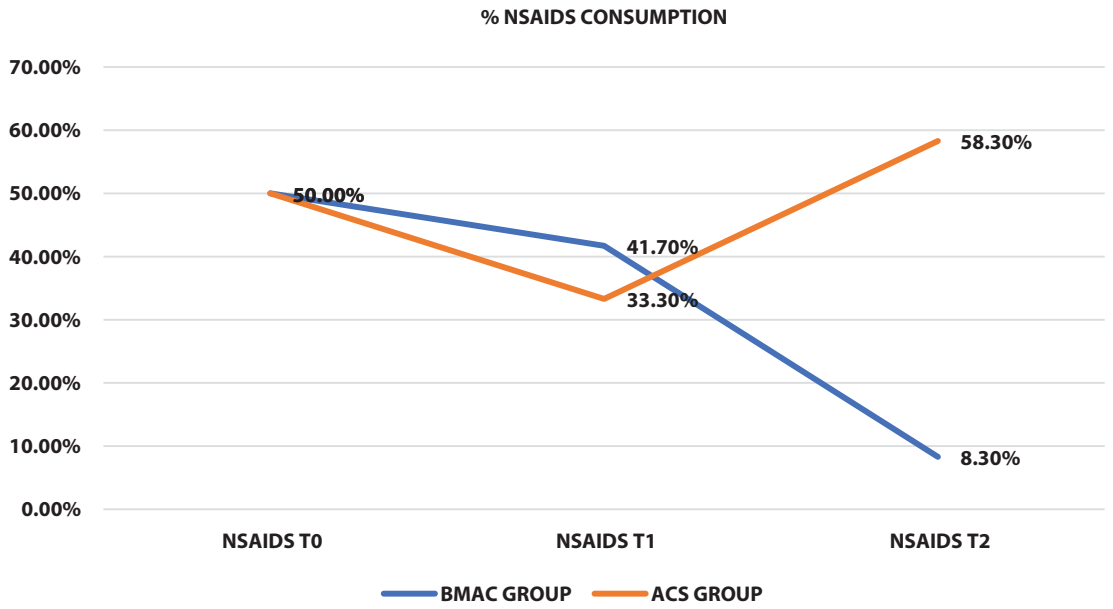

Figure 7. Mean NSAIDs consumption at each evaluation for both the studied groups.

Discussion

OA is a chronic inflammatory and degenerative joint disease in adults, representing a huge socioeconomic health care burden (15,16). Particularly, the knee is one of the most affected sites showing an upward trend (17). Standard treatment regimens for early OA consist of rest, cryotherapy, oral supplements, hyaluronic acid as well as medications such as steroids and NSAIDs (4). All these approaches represent a valid option for pain control, nevertheless none of these can modify the natural course of the disease (18). In recent years, there has been a growing interest about the use of biological therapies for the treatment of OA. Particularly, in the past decade the administration of autologous growth factors such as intra-articular injections of platelet rich plasma (PRP) obtained a large consensus in the literature (19). PRP is a fraction of whole blood harvested by centrifugation of autologous blood, obtaining thus a higher platelets concentration than normal values (19,20). Even though the mechanisms of PRP are not completely understood, this therapy could interfere with catabolic and inflammatory activation with subsequent improvement of anabolic response. Indeed, besides the cytokines, thrombin and growth factors contained in the plasma, platelet degranulation releases additional growth factors responsible for a variety of crucial tissue healing mechanisms (20). Further treatment options include ACS. ACS is an autologous blood product augmented in the interleukin-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of interleukin-1 (IL-1), developed by Meijer et al. in 2003(9,21). It contains a high concentration of interleukin 1 receptor antagonist (IL-1Ra) as well as other anti-inflammatory cytokines and growth factors such as interleukins 4 (IL-4), 6 (IL-6), and 10 (IL-10) epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF1), platelet-derived growth factor (PDGF), and transforming growth factor beta 1 (TGFβ1) (9,13). Since IL-1 plays a critical role in osteoarthritis pathogenesis through the stimulation of chondrocytes as well as synovial fibroblasts to upregulate matrix metalloproteases able to damage the cartilage, its blockade leads to a slowdown of OA progression (9). Yang et al (22) performed a prospective, randomized, double-blinded study that evaluated 167 patients with knee OA to determine the effects of ACS versus saline solution intra-articular injections. Their results state significant improvements

in functionality and pain control in the ACS group compared to the control group. Moreover, Barreto et al. (23) evaluated the efficacy of ACS in 100 patients affected from knee OA showing a significant improvement of joint function as well as an overall pain reduction. However, a most recent study conducted by Zarringam et al. (24) shows how ACS was not able to significantly delay the need for knee arthroplasty in patients with severe knee OA, when compared to a placebo control group at 10 years follow-up. Considering the recent increase of interest about regenerative medicine, several studies investigating the regeneration of articular cartilage through MSCs are actively underway (18). MSCs are multipotent stem cells with a strong capacity for self-renewal as well as a differentiation capacity to form chondrocytes, adipocytes and osteocytes (4). Bone marrow and adipose tissue have been the most source for harvesting MSCs (1,4). Particularly, BMAC represents one of the safest and most feasible sources of MSCs and its intra-articular administration has shown pain reduction, functional improvement and most likely a tissue regeneration. BMAC is obtained through density gradient centrifugation of BMA. It has been shown to deliver high levels of hematopoietic stem cells (HSCs), MSCs, platelets, chemokines and cytokines including PDGF and transforming growth factor beta (TGF-β) (4). It must be noticed that growth factors can be both contained in alpha granule of platelets as well as actively secreted by MSCs. For that reason, BMAC holds anti-inflammatory, angiogenic, trophic and immunomodulatory properties which most likely induce cartilage repair (4). In addition, MSCs have been shown to be able to suppress all immune cells playing a crucial role both in OA pathogenesis and progression, due to their immunoregulatory abilities (1). Kim et al. (18) performed a study on 41 patients affected from knee OA treated by a single BMAC articular injection, demonstrating the efficacy of this therapy. Moreover, Chahal et al. (16), confirmed the effectiveness of a single BMAC injection in the treatment of knee OA, with an overall improvement in symptoms and pain as well as a reduction of the synovial inflammation. Another study performed by Mautner et al. (3) shows a significant improvement both in functionality as well as pain in patients affected from knee OA, with no substantial difference between micro-fragmented adipose tissue (MFAT) and BMAC. Thus, according to the literature and considering the biological characteristics of BMAC as well as ACS, we believe that both these therapies could represent a valid choice for the treatment of early-stage knee OA. Our preliminary results show how both the therapies can likely be effective in term of pain control as well as functional improvement in the treatment of early-stage knee OA. However, the two groups comparison showed a greater and significant reduction of VAS values in BMAC group at six months follow-up (**Figure 5**). Moreover, we observed a greater reduction of WOMAC values both at one-month follow-up as well as at final follow up (Table2) (**Figure 6**). Additionally, we noticed that BMAC group showed a greater reduction in NSAIDs consumption at final follow-up compared to the ACS group (**Figure 7**). In contrast, we did not observe significant differences in ROM improvement between the two groups (Table 3).

Limitations

Although our encouraging results, several limitations must be addressed. Specifically, the small number of patients for each group as well as the lack of randomization in group allocation can increase selection biases. However, the reason for the lack of randomization was in order to take into consideration regimen compliance and allergies of patients. Moreover, we do not have functional scores for the patients after 6 months follow-up, as well as an instrumental monitoring with X-Rays or MRI after the therapy.

Conclusions

Our results show how, after an accurate patient selection, both single BMAC injection as well as a cycle of 4 injections of ACS therapy can likely represent a valid biologic treatment option for early-stage knee OA, showing better outcomes with a single BMAC injection. Considering our positive experience, we believe it could be appropriate to better analyze the comparison of these two biological therapies in order to clarify their roles. Moreover, considering the poor

literature about the comparison of these biological therapies, another purpose of this study is also to give a starting point for further research in order to enlarge the number of studies about these specific approaches.

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Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Correspondence:
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Pierluigi Pironti, MD
University of Milan, Residency Program in Orthopedics and Traumatology
Via Festa del Perdono 7
Milan, 20122 Italy
Phone: 3385362097
E-mail: pierluigi.pironti@libero.it