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Subchondral and intra-articular injections of bone marrow concentrate are a safe and effective treatment for knee osteoarthritis: a prospective, multi-center pilot study

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Abstract

Purpose Subchondral bone is becoming a treatment target for knee OA patients, with promising early findings on the use of bone marrow aspirate concentrate (BMAC). The aim of this prospective, multi-centric pilot study was to evaluate safety as well as clinical and MRI outcomes of a combined approach of intra-articular and subchondral BMAC injections.

Methods Thirty patients (19 men, 11 women, 56.4 ± 8.1 years) with symptomatic knee OA were treated with a combination of an intra-articular and two subchondral BMAC injections (femoral condyle and tibial plateau). Patients were evaluated at baseline and at 1–3–6–12 months of follow-up with the IKDC subjective, VAS, KOOS, and EQ-VAS scores. The MRI evaluation was performed with the WORMS score.

Results No major complications were reported and only two patients were considered treatment failures, requiring a new injective or surgical treatment. The IKDC subjective score improved significantly from 40.5 ± 12.5 to 59.9 ± 16.1 at 3 months, 59.1 ± 12.2 at 6 months, and 62.6 ± 19.4 at 12 months ($p < 0.0005$). A similar improvement was reported for VAS pain and all KOOS subscales at all follow-ups, while EQ-VAS did not show any significant improvement. The MRI analysis showed a significant bone marrow edema reduction ($p = 0.003$), while the remaining WORMS parameters did not show any significant changes.

Conclusion The pilot evaluation of this combined BMAC injective treatment showed safety and positive outcome up to 12 months of follow-up in patients with symptomatic knee OA associated with subchondral bone alterations. These findings suggest that targeting both subchondral bone and joint environment can provide promising results, and that BMAC can be a valid option for this combined approach to treat knee OA.

Keywords Osteoarthritis · Knee · Subchondral · Intra-articular · Injective · Bone marrow · BMAC

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Introduction

Knee osteoarthritis (OA) is a common disease characterized by progressive deterioration and loss of articular cartilage with concomitant structural and functional changes in the entire joint, involving synovium, meniscus, periarticular ligaments, and subchondral bone [30]. Non-surgical strategies may substantially diminish symptoms and disability, but their action is generally temporary and they are not able to arrest the underlying disease process [14]. The definitive treatment is represented by total knee arthroplasty (TKA), which is invasive and not free from complications, especially in young and active patients [4]. Thus, to delay or avoid TKA, research efforts have been made to find new minimally invasive and potentially disease-modifying procedures to address knee OA.

In this light, the use of orthobiologics is gaining increasing interest due to the availability of several promising products, ranging from blood derivatives (platelet-rich plasma—PRP) to minimally manipulated mesenchymal stromal cells (MSCs) from bone marrow or adipose tissue [7, 12, 16]. Although the intra-articular use of these products for the treatment of knee OA provided positive results, the improvement in terms of pain relief and function remains partial and not always satisfactory [1, 7]. Thus, a new application has been recently proposed to further exploit the potential of biologic products by also targeting the subchondral bone [21]. This strategy is supported by the substantial evidence revealing that subchondral bone alterations may play a critical role in both the pathophysiology and progression of knee OA [40]. Moreover, the presence of subchondral alterations seems to correlate with the severity of clinical symptoms [13, 26]. Accordingly, subchondral bone is increasingly becoming a target to address knee OA patients, not only with bone substitutes, but also with biologic products such as bone marrow aspirate concentrate (BMAC). However, beside promising early findings, evidence is limited, and it would be clinically relevant to provide prospective data with both clinical and imaging evaluations of the potential of this biological approach combined with the more documented intra-articular injections [22, 36, 37, 41].

The aim of this prospective, multi-centric pilot study was to evaluate the safety as well as the clinical and imaging outcomes at 1 year follow-up of a novel approach combining intra-articular and subchondral injections of autologous BMAC for the treatment of knee OA. The hypothesis was that this combined approach, addressing both the whole joint environment and the subchondral bone, could be effective in patients with symptomatic knee OA.

Materials and methods

Study design and patient selection

The current prospective multi-center study was approved by the Hospital Ethics Committee and institutional Internal Review Board. The following centers have been involved in the clinical trial: Rizzoli Orthopaedic Institute (Bologna, Italy) and Humanitas Clinical and Research Center (Rozzano, MI, Italy). The trial was registered at clinicaltrials.gov (NCT03110666). Patients were enrolled from November 2016 to July 2019. Informed consent for study participation was obtained from each patient. Treatment indications included the presence of subchondral bone alterations on a recent MRI (performed within 3 months from the enrollment visit) in symptomatic knee OA. Moreover, the following inclusion criteria were used for selection: women and men aged between 40 and 75 years; predominant femoro-tibial OA either of the medial or lateral compartment with pain in the involved compartment; radiographic severity grades ≥ 2 according to the Kellgren–Lawrence classification; failure after at least 6 months of conservative treatment (patients not responsive to pharmacologic therapy with nonsteroidal anti-inflammatory drugs and analgesics, lack of improvement with rehabilitation or after an intra-articular injective treatment); patients physically and mentally able to comply with the study requirements and with scheduled clinical and radiographic follow-up. Conversely, the following exclusion criteria were considered: patients lacking understanding capacity; patients with bilateral knee OA or with evidence of predominant patello-femoral involvement; any previous trauma to the index knee within 6 months; intra-articular knee injection in the past 6 months; knee surgery in the last 12 months; patients with untreated knee instability; lower limb malalignment $> 10°$; history of malignant neoplasia or rheumatic diseases; presence of metabolic disorders including diabetes mellitus, thyroid metabolic diseases, history of alcohol or drug abuse; body mass index (BMI) < 18 or > 35.

Thirty patients were prospectively and consecutively enrolled according to the inclusion/exclusion criteria. Among them, 19 patients were men and 11 women, with mean age 56.4 ± 8.1 years. In this cohort, 16 patients had already undergone previous surgery in the affected knee, including meniscectomy (10 patients), cartilage surgery (4 patients), or other surgery (2 patients). Further baseline patient characteristics and demographic data are summarized in Table 1.

Table 1 Baseline characteristics of the included patients

Gender (male/female)	19/11
Age, years (mean ± SD)	56.4 ± 8.1
BMI, kg/cm^2(mean ± SD)	25.5 ± 3.5
Side (left/right)	21/9
Compartment (medial/lateral)	25/5
Symptoms duration, months (range)	58.7 (6–240)
Kellgren–Lawrence	Grade 2: 11 Grade 3: 19
Previous surgery	16
Previous cartilage surgery	4

BMI body mass index, *SD* standard deviation

Procedure

All patients were treated by orthopedic surgeons with established experience in cartilage regenerative procedures. The treatment consisted of a combination of an intra-articular BMAC injection and two subchondral BMAC injections (at the femoral condyle and tibial plateau, respectively, either in the medial or lateral compartment according to the patient OA pain location evaluated through patient clinical history and examination). The procedure was performed with the patient in supine position under spinal loco-regional anesthesia. The ipsilateral hip and tibia were sterilely prepared and draped for both anterior iliac crest and tibial bone marrow aspiration. Anatomical landmarks for a small surgical incision were at the anterior superior iliac spine and on the medial side of the tibial tuberosity, per standard technique [24, 38]. Trocars were inserted and advanced with clockwise/counter clockwise motion, bone marrow was harvested in two syringes (a 60 cc syringe for the aspirate from the iliac crest and, to avoid further harvesting from the iliac crest, a 30 cc syringe for the aspirate from the proximal tibia) coated with glucose citrate anticoagulant, and concentrated through the BioCUE BMA Concentration System Kit (Zimmer Biomet, USA). In detail, iliac and tibial aspirates were centrifuged independently at a rate of 3200 RPM for 15 min, removing the plasma and red blood cells components, thus obtaining 9 cc of BMAC (6 from the iliac crest and 3 from the tibial aspirates) for the injections.

Subchondral BMAC injections were performed with an 8 Gauge trocar, which was manually introduced into the bone under fluoroscopic control (Fig. 1). Once the trocars were placed in the desired position, corresponding to the subchondral bone alterations previously visualized on MRI, 3 cc of iliac crest derived BMAC were injected into the subchondral bone of both femoral condyle and tibial plateau. Finally, 3 cc of tibia-derived BMAC were injected intra-articularly using a lateral suprapatellar approach. It was decided to use the iliac BMAC for the subchondral injections, both at the femur and tibia, and the tibial BMAC for the intra-articular injection to avoid re-injecting the tibia aspirate in the subchondral tibia, while being consistent with subchondral injections from the same source.

Postoperatively, patients were discharged on the same day of the procedure or the day after, based on patient condition. Pain control was prescribed as needed with analgesic only in the immediate period after treatment, and thromboembolic prophylaxis was prescribed for 2 weeks. Weight bearing was partial the first 7 days and progressive for the following 7 days. Cryotherapy was started within the first 24 h. Passive mobilization and quadriceps isometric exercises were started at the beginning of the rehabilitation program. Patients were permitted to return to most of their daily activities, as tolerated, once they reached full weight-bearing. No other conservative treatments were prescribed during the study period. Joint impact sport activities were discouraged.

Patients' evaluation

All patients were clinically evaluated before the injective procedure and at the follow-up visits at 1, 3, 6 and 12 months. To evaluate the treatment safety, all complications and adverse events were assessed and reported by the patient during the assessments at all follow-ups or through patients-physician communication in between the follow-ups. Serious adverse events were defined as any event that

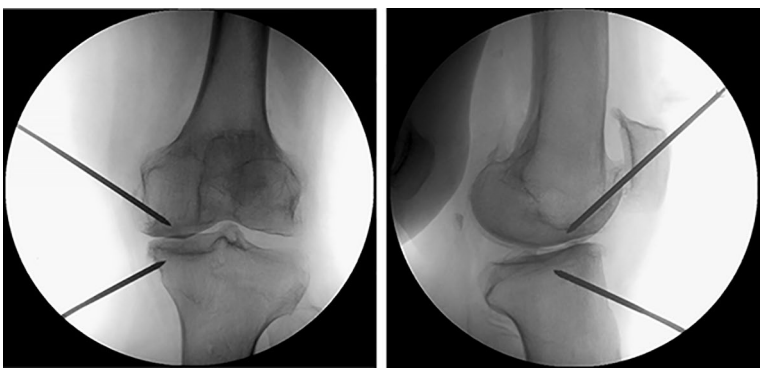

Fig. 1 Trocars are placed under fluoroscopic control in the subchondral area of tibial plateau and femoral condyle of the affected knee compartment

resulted in death or were life-threatening, required hospitalization or intervention to prevent permanent impairment or damage. Minor adverse events were defined as the presence of significant pain or swelling (i.e. a pain or swelling limiting the daily activities) of the treated knee for over five days as reported by patients at the first follow-up. The primary clinical outcome was determined by the change in the International Knee Documentation Committee (IKDC) subjective score at 12 months after the procedure. Moreover, further scores were used for patient evaluation, including the Visual Analogue Scale (VAS) for pain, the Knee injury and Osteoarthritis Outcome Score (KOOS) for subjective functional improvement, and the EuroQol-Visual Analogue Scale (EQ-VAS) for patient generic health status. At 1-month follow-up, only VAS score and adverse events were determined, while a complete clinical score assessment was performed at 3 months, 6 months, and 12 months postoperatively.

The treated knees were evaluated with high-resolution (1.5 T) MRI: one at baseline and one after the procedure between 6 and 12 months of follow-up. The evaluation was performed by two independent, experienced musculoskeletal radiologists in consensus, who blindly assessed and reviewed the images. The Whole-Organ Magnetic Resonance Imaging Score (WORMS) was used to assess seven features of the treated knees: articular cartilage morphology, bone marrow edema, subchondral cysts, articular profile, marginal osteophytes, meniscal integrity, and synovitis [36].

The treatment was deemed to have failed if the patient needed a new surgical procedure on the articular surface (e.g., total or partial knee replacement) or an injection procedure (e.g., new intra-articular treatment with steroids, hyaluronic acid, or PRP) because of persistence or worsening of knee symptoms. For failed patients, the clinical evaluation before the new treatment was considered for further evaluations.

Statistical analysis

All continuous data were expressed in terms of the mean and the standard deviation of the mean, the categorical data were expressed as frequency and percentages. The Shapiro–Wilk test was performed to test normality of continuous variables. The Repeated Measures General Linear Model (GLM) with Sidak test for multiple comparisons was performed to assess the differences at different follow-up times. The Friedman non-parametric test, followed by the Wilcoxon post hoc pairwise comparison corrected by Bonferroni method for multiple comparisons, was used to the differences at different follow-up times of not normally distributed scores. The ANOVA test was performed to assess between groups differences of continuous, normally distributed and homoscedastic data; the Mann–Whitney test was used otherwise. The ANOVA test followed by the Scheffè post hoc pairwise comparison was used also to assess among groups differences of continuous, normally distributed and homoscedastic data; the Kruskal–Wallis test followed by the Mann Whitney test with the Bonferroni correction for multiple comparison was used otherwise. The Spearman rank Correlation was used to assess correlation between continuous data. The Kendall tau correlation was used to assess correlation between ordinal data. With 30 patients and assuming an effect size equal to 0.25 according to Cohen, a post hoc power equal to 0.9 was obtained. For all tests, $p < 0.05$ was considered significant. All statistical analysis was performed using SPSS v.19.0 (IBM Corp., Armonk, NY, USA).

Results

No major complications and adverse events were reported during follow-up evaluation and an overall significant improvement in the clinical scores was observed. In particular, the IKDC subjective score improved significantly at all follow-up times compared to the basal evaluation, changing from 40.5 ± 12.5 to 62.6 ± 19.4 at 12 months ($p < 0.0005$). No significant differences were reported among the different follow-ups (Fig. 2). The VAS pain score improved from 6.3 ± 1.8 at baseline to 3.7 ± 2.3 at 1 month ($p < 0.0005$), remaining stable up to 12 months. A significant improvement was also reported for all KOOS subscales at all follow-ups, while the EQ-VAS did not report any significant improvement at all follow-ups as reported in Table 2. Two patients failed at 6 months of follow-up: a 57-year-old woman with previous surgical treatments to the affected knee (meniscectomy, arthroscopic debridement, and microfractures) showed no clinical improvement after the procedure and was treated with a tibial osteotomy in another institute; a 67-year-old man showed persistent symptoms after the procedure and was treated with a TKA.

The MRI findings of 28 knees, analyzed with the WORMS score, showed a significant improvement ($p = 0.003$) in terms of bone marrow edema reduction from baseline (2.8 ± 1.3) to the follow-up evaluation (1.5 ± 1.1). In particular, a reduction of the bone marrow edema was found in 17 patients (61%) after treatment (Fig. 3). The remaining six WORMS parameters did not show any significant changes after the injection, neither as improvement nor as signs of disease progression (Table 3).

Further analysis was performed to determine the parameters that influenced the clinical and radiological outcomes at follow-up. Previous surgeries negatively influenced the KOOS symptoms subscale at 3 months of follow-up (71.9 ± 16.4 vs 85.4 ± 12.9, $p = 0.014$), with worse findings in patients previously treated with knee surgery, although no significant differences were confirmed at 6–12 months. Conversely, sex, age, BMI, knee side, affected compartment, and

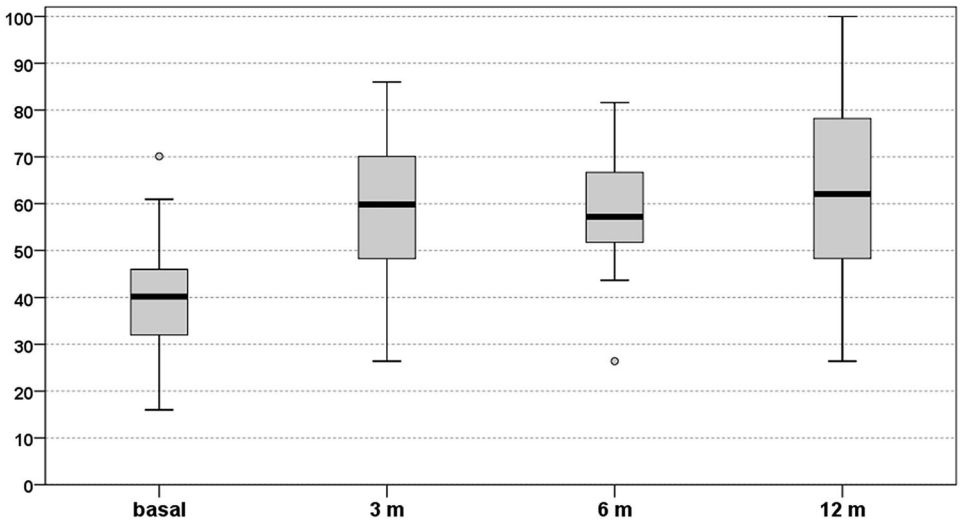

Fig. 2 The International Knee Documentation Committee (IKDC) subjective score at basal level and at 3, 6, and 12 months of follow-up

Table 2 Outcome scores at baseline and at 3–6–12 months after injective procedure

Outcome	Baseline	3 months	6 months	12 months	*p*
KOOS pain	59.5 ± 14.0	78.0 ± 14.0	80.7 ± 12.4	79.3 ± 19.0	< 0.0005
KOOS symptoms	63.1 ± 17.1	78.2 ± 16.1	77.9 ± 15.8	76.8 ± 20.8	< 0.0005
KOOS ADL	70.7 ± 13.6	86.9 ± 11.4	89.0 ± 10.0	86.3 ± 19.0	< 0.0005
KOOS Sport/Rec	25.4 ± 19.0	56.5 ± 25.6	56.6 ± 25.1	55.7 ± 30.2	< 0.0005
KOOS QoL	35.7 ± 15.8	51.3 ± 19.5	54.2 ± 20.2	58.2 ± 24.3	< 0.0005
EQ-VAS	74.1 ± 11.8	77.2 ± 12.0	74.6 ± 13.5	73.7 ± 13.2	n.s
VAS pain	6.3 ± 1.8	3.4 ± 1.9	3.9 ± 1.7	3.0 ± 1.9	< 0.0005

ADL function in daily living, *EQ-VAS* EuroQol-visual analogue scales, *KOOS* knee injury and osteoarthritis outcome score, *IKDC* international knee documentation committee subjective score, *n.s.* not significant, *QoL* quality of life, *Sport/Rec* function in sport and recreation, *VAS* visual analogue scale

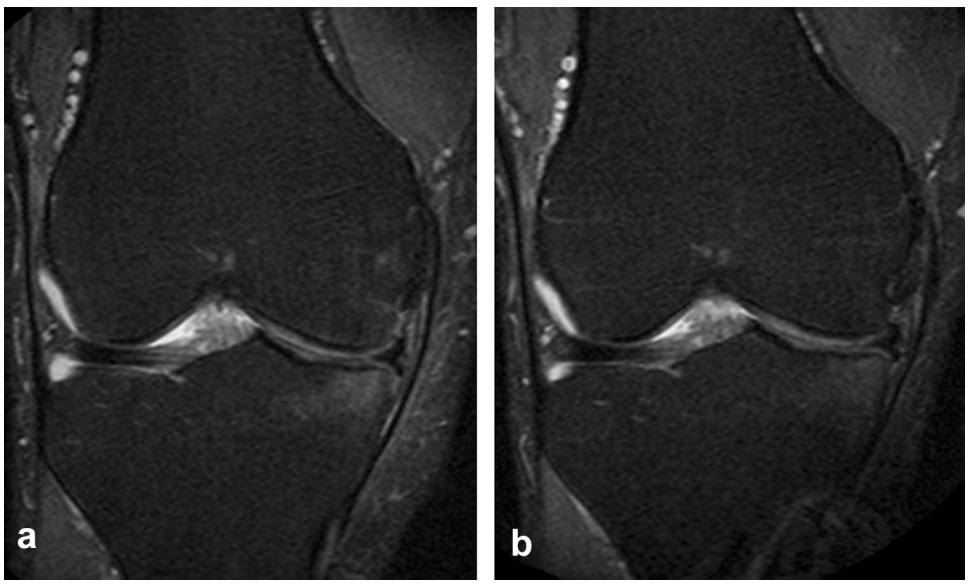

Fig. 3 MRI evaluation at baseline **a** and at 12 months of follow-up **b** after the BMAC injective treatment in a 56-year-old man with predominant medial knee OA. A reduction of the bone marrow edema was found after treatment

Table 3 The Whole-Organ Magnetic Resonance Imaging Score (WORMS) evaluation

	WORMS score	Baseline	Post-injection	*p*
1	Articular cartilage morphology	5.3 ± 1.3	4.7 ± 1.6	ns
2	Subchondral cysts	0.6 ± 1.1	0.6 ± 1.1	ns
3	Bone marrow edema	2.8 ± 1.3	1.5 ± 1.1	0.003
4	Articular profile	0.6 ± 0.7	0.7 ± 0.8	ns
5	Marginal osteophytes	2.0 ± 1.8	2.1 ± 1.9	ns
6	Meniscal integrity	2.4 ± 1.6	2.3 ± 1.5	ns
7	Synovitis	0.4 ± 0.5	0.3 ± 0.5	ns

n.s. not significant

Kellgren–Lawrence grade did not significantly influence the post-injective clinical outcome. No correlations were found between WORMS score and basal parameters. Regarding correlations between WORMS subscales and clinical scores, a higher basal WORMS 3 (i.e. worse bone marrow edema) was associated with a higher IKDC improvement at 12 months after the treatment ($r = 0.397$; $p = 0.008$). Lower WORMS 1 (i.e. better articular cartilage morphology) at final follow-up was significantly correlated to better IKDC at 12 months ($r = -0.342$; $p = 0.022$), IKDC improvement at 12 months ($r = -0.308$; $p = 0.039$), and KOOS pain at 12 months ($r = -0.375$; $p = 0.014$). Finally, lower WORMS 4 (i.e. better articular profile) at final follow-up was significantly correlated to better KOOS pain at 12 months ($r = -0.358$; $p = 0.021$).

Discussion

The most important finding of the present study was that the combination of subchondral and intra-articular BMAC injections proved to be safe and provided a significant clinical improvement up to 12 months in patients affected by symptomatic knee OA with subchondral bone alterations. Moreover, the MRI evaluation of the treated knees showed an improvement of the subchondral bone status by reducing the bone marrow edema.

BMAC is considered a promising product among the orthobiologics currently available, being a combination of biologically active cells and proteins obtained through a technically easy and mini-invasive procedure [19]. Recently, BMAC has increasingly been used as injective treatment for knee OA, with the rationale relying on the transplantation of the entire bone marrow niche, containing MSCs, hematopoietic precursors, monocytes, endothelial cells, as well as a great array of soluble factors [15, 18]. In particular, bone marrow MSCs possess immunomodulatory, anti-inflammatory, anti-apoptotic, proliferative, and chemoattractive functions, and can coordinate the differentiation process of functional tissue regeneration in host cells [20]. Bone marrow MSCs have the capacity to differentiate toward several lineages (i.e. chondrocytes, osteoblast, adipocytes) and to produce soluble factors, which may positively affect the joint homeostasis and eventually contribute to relieve pain and to improve joint function [28, 34]. Moreover, BMAC contains a high number of growth factors, cytokines, and chemokines, including transforming growth factor-beta (TGF-β), interleukin-1 receptor antagonist (IL-1ra), platelet-derived growth factor (PDGF), bone morphogenetic protein (BMP)-2 and -7, and vascular endothelial growth factor (VEGF). These growth factors are involved in several pathways crucial for cell maintenance and function, differentiation, extracellular matrix production, and regulation of cell catabolic/anabolic activity [6, 17]. Thanks to these characteristics, BMAC could have the potential to positively influence joints affected by OA [19]. The principal use of BMAC for the treatment of knee OA has generally been the intra-articular administration. In a recent systematic review, 13 clinical studies evaluated the safety and effectiveness of this intra-articular approach in knee OA patients, showing promising clinical results in terms of pain relief and knee function improvement [7].

A new application has been recently proposed to further exploit the potential of BMAC by targeting directly the subchondral bone. In fact, the possible role of subchondral bone in OA etiopathogenesis has become more evident with the understanding of the intercorrelation between subchondral bone and articular cartilage, a connection referred to as the osteochondral functional unit [10, 39]. In particular, subchondral bone is a source of vessels whose perfusion rate enables an important nutritional route for articular cartilage, and any damage to this microvasculature affects venous bony circulation, thereby altering cartilage and chondrocyte function [25]. Subchondral bone undergoes changes in patients with OA including microcracks and structural defects, vascularization of channels, nerve growth, and a progressive replacement of the subchondral marrow with fibro-neurovascular mesenchymal tissue changes [31]. Several studies have suggested that subchondral bone alterations may progress to the destruction of the overlying articular surface: longitudinal studies revealed that the deterioration of the subchondral bone structure and the loss of cartilage volume and thickness were interdependent in knee OA patients, underlining the importance of subchondral bone lesions in OA physiopathology [5, 33]. To date, the structural changes of the subchondral bone can be readily observed using MRI, which is able to reveal the presence of bone marrow lesions such as edema-like lesions or cysts [27]. The presence of these lesions in patients with knee OA showed to be strongly associated with the severity of symptoms [10, 13, 43]. Thus, the idea to target directly the subchondral bone area has recently

gained a growing interest for the treatment of knee OA patients with bone marrow lesions, with the aim to address more successfully the symptomatic impairment [8, 9].

In this scenario, researchers initially proposed the use of injectable bone substitute materials with osteoconductive properties, such as calcium phosphate, with the aim to add biomechanical strength and structural integrity, theoretically allowing subchondral bone remodeling and addressing OA progression [10, 41]. However, even though several studies suggested possible benefits from this approach [11], these materials could have the disadvantage of altering the physiological properties of the subchondral bone [2, 3]. Thus, the use of subchondral injections with biologic products has been proposed to allow strengthening and healing of subchondral bone without jeopardizing its properties [41]. The first evidence on biologic subchondral injections to address patients with knee OA described the use of PRP, a blood derivate with a high concentration of growth factors and bioactive molecules [35]. Sànchez et al. evaluated subchondral PRP injections combined with intra-articular PRP injections for the treatment of knee OA with associated bone marrow lesions, reporting the safety of this procedure, with rare and minor complications, and its effectiveness in reducing pain and improving functional status, with a relatively low rates of conversion to TKA [35, 37]. More recently, they reported a superior clinical outcome at 6 and 12 months for the combination of subchondral and intra-articular PRP injections when compared to intra-articular injections alone in 60 patients with a prevalent grade 3 knee OA according to Ahlbäck scale, confirming the importance of directly targeting also the subchondral bone area [36]. Lychagin et al. evaluated the levels of the serum cartilage oligomeric matrix protein (COMP), an early and promising biomarker for the remodeling of articular cartilage, in OA patients treated with subchondral PRP injections [29]. They found a consistent increase of serum COMP levels after the procedure, which could reflect the effects on cartilage turnover resulting from the subchondral treatment.

The effectiveness of subchondral bone injections in knee OA patients was also confirmed for the use of bone marrow aspirate (BMA). Vad et al. used tibial BMA for the combined subchondral and intra-articular treatment of ten patients with unicompartmental knee OA, showing improvements in pain and function up to 12 months [42]. The first evidence on the subchondral BMAC injections to address knee OA has been documented in a randomized clinical trial (RCT) by Hernigou et al., which treated 30 young patients (mean age 28 years) with bilateral knee OA secondary to osteonecrosis with BMAC injections on one side, and with TKA on the other side [21]. In this study, subchondral BMAC injections provided similar clinical results compared with TKA, with a lower complication rate and a quicker recovery. In a similar study by the same authors, 140 adult patients (mean age 75 years), planned to undergo staged-bilateral TKA for medial knee OA, were treated with subchondral BMAC injections on one side and with TKA on the other side [23]. The authors reported that subchondral BMAC injections provided a sufficient effect on pain to postpone or avoid the TKA up to 15 years of follow-up, with only 25 patients requesting the TKA in joint treated with BMAC. Severe bone marrow lesions were predictive factors for future TKA in the knee with subchondral BMAC treatment. Finally, in a recent RCT, Hernigou et al. demonstrated the superiority of subchondral BMAC injections over the intra-articular BMAC injections in 60 patients with bilateral knee OA, showing in the subchondral group higher clinical and MRI improvements at 2 years of follow-up, and a lower yearly arthroplasty incidence (1.3% versus 4.6%) [22].

However, previous studies did not combine BMAC intra-articular and subchondral injections. The current study documented the combined effect of subchondral and intra-articular BMAC injections for the treatment of symptomatic knee OA with bone marrow alterations. This approach proved to be safe and showed promising clinical results up to 12 months of follow-up, with reduction of pain and knee functional improvement in the majority of patients, even though no significant changes were reported in patients' perception of their general health status. Positive results were also obtained in patients with Kellgren–Lawrence grade 3, proving the effectiveness of this treatment also in case of moderate knee OA. Out of 30 patients, only 2 failed and required a surgical treatment, and no severe complications were reported after the procedure and during the follow-up period. MRI analysis with the WORMS score confirmed the effectiveness of the subchondral and intra-articular BMAC approach, detecting a reduction in subchondral bone edema at the short-term evaluation, while the other independent parameters such as cartilage morphology, articular profile, bone marrow cyst, osteophytes, meniscal condition, and synovitis did not present further degeneration.

This study presents several limitations. First of all, the lack of a control group, inherent to the pilot nature of this study, hindered the possibility to prove the real efficacy of this procedure compared to other strategies, to the natural course of the disease, and to the isolated intra-articular vs. combined subchondral approach. Placebo is a major component of the effect of every intra-articular injection and, as recently pointed out in a meta-analysis of Previtali et al., this effect can be substantial [32], thus underlining the need for placebo controlled studies to evaluate the real potential of this approach. Further studies should explore the most suitable dose for this BMAC application, also based on the MRI evaluation of the subchondral bone alterations. Test–retest reliability was not performed in this study, as this study used commonly applied measurements methods, but this could represent a limitation as well. A further limitation is that the reduction of bone marrow edema,

obtained after the treatment, might also be caused by the limited weight-bearing period, although the weight-bearing was reduced for only a few days. Moreover, the small number of the included patients precluded a significant sub-analysis of the factors correlating with the final outcome. Nevertheless, this multi-centric pilot study allowed to obtain for the first time preliminary results on safety and effectiveness of this combined BMAC approach for the treatment of patients with knee OA. Another limitation was the absence of a radiographic evaluation to detect a possible OA progression, still unlikely considering the short-term follow-up. Moreover, long leg radiograph views were not performed for all patients, but only for those with a clinical doubt of malalignment at enrollment visit, hindering the possibility to correlate clinical outcomes with the lower limb alignment. Nevertheless, the MRI evaluation, though not performed in the same follow-up for all patients (from 6 to 12 months), represents a major strength of the current study, documenting the effectiveness of this combined approach in reducing the OA-associated bone marrow lesions. Overall, the clinical relevance of the study is the documentation of the safety and potential of this new biological strategy to address OA. Starting from these promising preliminary findings, larger high-level, placebo-controlled studies should be carried out to confirm the efficacy of this minimally invasive combined intra-articular and subchondral injective approach for the treatment of knee OA.

Conclusions

The pilot evaluation of this combined subchondral and intra-articular BMAC injective treatment showed to be safe and provided an overall positive outcome in patients with symptomatic knee OA associated with subchondral bone alterations. In particular, a significant clinical improvement was documented up to 12 months of follow-up, and the MRI evaluation showed a reduction of bone marrow edema. These findings suggest that targeting both subchondral bone and the joint environment can provide promising results, and that BMAC can be a valid option for this combined approach to treat knee OA.

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Compliance with ethical standards

Conflict of interest EK reports consulting for Carihealldt, Green Bone, Geistlich, and Bioveex, and speaking for Zimmer Biomet and Fidia Farmaceutici SPA. MM reports consulting for Green Bone and royalty for Rejoint. SZ reports non-financial support from personal fees from I+SRL, grants from Grants from FidiaFarmaceutici SPA, Cartihealltd, IGEA clinical biophysics, BIOMET, and Kensey Nash, outside the submitted work. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results. The other authors declare no conflict of interest.

Ethical approval Approval was obtained by the Hospital Ethics Committee and Istitutional Internal Review Board of IRCCS Istituto Ortopedico Rizzoli (prot. gen. n. 0013097).

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