Journal of Orthopaedic Surgery

Journal of Orthopaedic Surgery
32(1) 1–9
© The Author(s) 2024
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/10225536231219970
journals.sagepub.com/home/osj
Sage

Biomaterials augmented with filtered bone marrow aspirate for the treatment of talar osteochondral lesions. A comparison of clinical and cellular parameters

Matic Kolar[1,2], Matija Veber[3], Lenart Girandon[3] and Matej Drobnič[1,2]

Abstract

Background: Biomaterials augmented with Bone Marrow Aspirate Concentrate (BMAC) are becoming increasingly utilized in the cartilage treatment. However, the potential role of cellular parameters in the intraoperatively applied BMAC have yet to be elucidated.

Purpose: (A) To evaluate clinical outcomes and safety of a combined single-step approach with scaffolds (fibrin glues, collagen gels, collagen-hydroxyapatite membrane) and filtered Bone Marrow Aspirate (fBMA) for the treatment of osteochondral lesions of the talus (OLTs). (B) To identify significant factors for postoperative improvements, considering cellular parameters as potential predictors.

Methods: All the patients operated on due to OLTs by the combination above were selected from the hospital registry database (35 pts, years 16–55, and minimally 1 year follow-up). Treatment outcomes were followed clinically with Patient-reported outcome measures (PROMs), and by pursuing serious adverse events (SAE) and graft failures (GF). Cellular parameters of the injected fBMA were determined. Pre- and postoperative PROMs values were compared to evaluate postoperative improvements. Multivariable regression models were applied to identify potential factors (demographics, medical history, joint and lesion characteristics, scaffold type, surgical and cellular parameters) that predict the treatment outcomes.

Results: At the mean follow-up of 32.2 (12.5) months, all Foot and Ankle Outcome Score (FAOS) and European Quality of Life in Five Dimensions Three-Level (EQ-5D-3 L) values improved significantly. 4 (11%) SAE (3 arthrofibrosis, one hardware removal), and 3 (9%) GF occurred. Female gender and concomitant procedures were the main negative predictors for postoperative outcomes. The number of fibroblast colony forming units (CFU-F) or their proportion among total nucleated cells (CFU-F/TNC) were positively correlated with the improvements of some PROMs.

Conclusions: Scaffolds augmented with fBMA proved as an adequate and safe approach for OLTs treatment. Cellular parameters seem to influence the treatment outcomes, thus further attention should be given to the intraoperatively applied products.

[1]Department of Orthopaedic Surgery, University Medical Centre Ljubljana, Ljubljana, Slovenia
[2]Chair of Orthopaedics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[3]Educell Ltd, Trzin, Slovenia

Corresponding author:
Matic Kolar, Department of Orthopaedic Surgery, University Medical Centre Ljubljana, Zaloška cesta 9, Ljubljana SI-1000, Slovenia.
Email: matic.kolar@kclj.si

Data Availability Statement included at the end of the article

Level of evidence: Level IV.

Keywords
talus, osteochondral, scaffold, bone marrow aspirate, mesenchymal stem cells

Date received: 15 October 2023; Received revised 27 November 2023; accepted: 25 November 2023

Introduction

Mesenchymal stem/stromal cells (MSCs) are adult, non-hematopoietic, multipotent stem cells which gained one of the central roles in the rapidly growing field of tissue engineering.[1–3] Bone marrow has been the most common and longest utilized source of MSCs with Friedenstein's pioneering research from 1966.[4] MSCs represent only 0.001%–0.01% of the total mononuclear cells in the bone marrow aspirates (BMA).[5,6] For increasing the density of MSCs, various concentration methods, like recovery of cells from filters and clotting and/or bone marrow density gradient centrifugation, have been developed to obtain products, known under the general term Bone Marrow Aspirate Concentrate (BMAC). To emphasize the involvement of filtration in the concentration process, the expression filtered Bone Marrow Aspirate (fBMA) has been introduced.[7]

Bone Marrow Aspirate Concentrate in the combination with biomaterials is becoming an increasingly studied and practiced approach in the orthopaedic surgery for the treatment of chondral and osteochondral lesions, which can greatly affect the patients' functioning and quality of life.[8–10] The rationale for BMAC augmentation is to leverage the established positive mechanisms of action of MSCs in cartilage regeneration by introducing additional MSCs at the lesion site. Simultaneously, scaffolds are used as a temporary biocompatible and biodegradable 3-dimensional microenvironment, supporting MSCs retention, progenitor cell migration, and facilitating adhesion, proliferation, and differentiation toward the desired phenotypes.[3,11,12] A wide array of materials have been utilized for the production of such scaffolds, including synthetic or natural polymers, and the combination of both. Various structural, compositional and architectural designs have been employed, mainly ranging from membranes to gels, and from monophasic to bi-/multiphasic scaffolds.[13] Some of them have already demonstrated promising clinical results, both in stand-alone application and with biological augmentation.[14–16]

In the present study, the absorbable gels: homologous (Beriplast, CSL Behring,Germany) and autologous (Vivostat, Alleroed, Denmark) fibrin glues or collagen gels (CartiFill, Seoul, Korea, and ChondroFiller, Meidrix Biomedicals, Esslingen, Germany), and multilayered biomimetic membrane: MaioRegen (Finceramica, Faenza, Italy) consisting of different ratios of equine collagen type I and hydroxyapatite (HA), with fBMA have been used to treat osteochondral lesions of talus (OLTs).

Although, the literature is limited to some case series, the available results of treating OLTs with BMAC augmented scaffolds are promising.[17–24] To the best of our knowledge, this is the first study reporting outcomes of such combined approach that evaluates cellular parameters as one of the possible prognostic factors. The hypotheses of the present study were: (1) combined single-step procedure with scaffolds and fBMA for OLTs treatment significantly improves clinical status of patients; (2) such therapeutic approach is safe; (3) cellular parameters of the fBMA have the potential to impact the treatment outcomes.

Methods

Study design

The present study was a retrospective case series. Clinical investigational plan was approved by the National Medical Ethics Committee (permit No. 0120-99/2019/4). It was part of a larger ankle research project registered at ClinicalTrials.gov: NCT04132076. All investigations were conducted in conformity with the ethical principles of Declaration of Helsinki, and written informed consent was signed by all enrolled patients. Adult subjects operated on OLTs with combined single-step approach (scaffold and fBMA), between July 2014 and December 2018, that met the inclusion criteria were enrolled. At the time of the index surgery, all patients were registered in a hospital database. The following inclusion criteria were defined: symptomatic OLTs, unresponsive to conservative treatment, Hepple grades III-V,[25] size $\geq$1.5 cm^2, minimum follow up of 12 months, only mild osteoarthritis (Kellgren-Lawrence grades 1-2),[26] and necessary concomitant procedures around the ankle were allowed. The main exclusion criteria were associated medical conditions (inflammatory, metabolic, neoplastic, etc.) that could directly handicap the musculoskeletal system or indirectly impact the quality of life.

Subjects

20 women and 15 men with the mean age of 34.5 (13.7) and BMI 26.8 (4.8) kg/m^2 were enrolled. 26 lesions were located on the medial (74%), and nine on the lateral talar dome

(26%). The mean lesion size was 2.4 (1.0) cm^2. In the majority of cases[26] full arthroscopic implantation was utilized, while medial malleolar osteotomy exposure was performed in six and arthrotomy in three cases. One or more concomitant ankle procedures were performed in 26 patients (74%), and 21 patients (60%) underwent previous attempt of cartilage treatment in the index ankle. Details on patients' demographics, medical history, and operative procedures are presented in Table 1.

Surgical intervention

All procedures were performed by the senior coauthor (M.D.) at a single university orthopedic center. Patients were under general or spinal anesthesia, placed supine on the operation table. Initially, an arthroscopic evaluation of the ankle was conducted to evaluate the joint, size and location of the OLTs, and perform some of the concomitant procedures, if necessary (decompression, debridement, etc.). Subsequently, the harvesting of 18–50 mL of bone marrow aspirate (BMA) from the ipsilateral iliac crest and its processing followed, according to the protocol already described in detail by Veber et al.[7] For the processing of BMA, the filter-based cell separation device CellEffic BM (Kaneka Corp., Osaka, Japan) was used, strictly following the manufacturer's instructions. After the isolation of nuclear cells, around 50 mL of cell suspension in saline was obtained. Small aliquots (2–4 mL) were sampled for cellular evaluation. The remaining cell suspension was centrifuged for 5–10 min at 400–800 g and concentrated to the mean final volume of 3.3 (1.6) mL fBMA. In the meantime, the remaining steps of lesion preparation, scaffold implantation and potential concomitant procedures were completed. During the OLTs debridement, the exposure and removal of the diseased subchondral bone cannot be avoided. However, in none of the cases were additional drilling or hole-picking performed. For the implantation of multilayered biomimetic scaffold MaioRegen, either medial malleolar osteotomy exposure or arthrotomy is required, while absorbable collagen (ChondroFiller, CartiFill) and fibrin (Beriplast, Vivostat) gels allowed a full arthroscopic approach. MaioRegen was implanted into the lesions in a press-fit manner, and additionally fixed with a fibrin glue (Beriplast, CSL Behring, Marburg, Germany). In the MaioRegen cases, the intra-operative tourniquet was released before the injection of the processed fBMA, so that fresh blood soaked the membrane, which is especially important for its early stability, while gels were injected with fBMA before the tourniquet release.[27] Typically, 2/3 of the prepared fBMA was injected into the scaffolds, while the remaining 1/3 was applied freely into the joint at the end of the surgery.

Table 1. Demographics, medical history, and operative details for the 35 patients enrolled in the study. Categorical variables are presented as frequencies (percentages), while continuous variables are presented as mean (standard deviation).

Age	34.5 (13.7) years
Gender	
Female	20 (57%)
Male	15(43%)
BMI	26.8 (4.8) kg/m^2
Medical history (lesion etiology)	
Spontaneously	3 (9%)
Injury	11 (31%)
Previous surgery	21 (60%)
Symptoms duration	
<1 year	0 (0%)
1–3 years	3 (9%)
3–5 years	5 (14%)
>5 years	6 (17%)
Revision surgery	21 (60%)
Previous surgery	
None	14 (40%)
Microfractures	13 (37%)
Scaffold	3 (9%)
Debridement	2 (6%)
Autologous chondrocyte implantation	2 (6%)
Fragment fixation	1 (3%)
Lesion size	2.4 (1.0) cm^2
Lesion location	
Medial talar dome	26 (74%)
Lateral talar dome	9 (26%)
Surgical approach	
Arthroscopy	26 (74%)
Arthrotomy	3 (9%)
Medial malleolar osteotomy	6 (17%)
Scaffold	
MaioRegen	9 (26%)
ChondroFiller	12 (34%)
CartiFill	9 (26%)
Fibrin glue	5 (14%)
Concomitant procedures	
None	9 (26%)
Decompression	20 (46%)
Tibial cartilage treatment	5 (12%)
Achilles tendon lengthening	3 (7%)
Alignment correction	2 (5%)
Ligament reconstruction	1 (2%)
Others	1 (2%)

Rehabilitation

Post-operatively, the subjects were restrained from weight-bearing for 6–8 weeks, with special attention to the healing of malleolar osteotomy in MaioRegen cases. During the first 2–3 weeks, the immobilization in a removable posterior short-leg plastic splint was applied, and only passive range of motion exercises were allowed. From that time onwards, the subjects were allowed active and passive dorsal/plantar exercises without side motions. Stationary cycling was allowed after 1 month. At 2 months, the crutches were discontinued, and patients progressed to proprioceptive exercise, aquatic rehabilitation, and gait training. After 3 months, they began strength training, including elliptical trainers, and Nordic walking on stable grounds. If the ankle's functional status permitted, they were allowed activities on uneven ground, including easy jogging, at 8 months after the surgery. Return to full activities, even the participation in strenuous sport activities (such as ball games, martial arts, athletics training, etc.) was permitted only 1 year after the procedure.

Cellular evaluation

Samples for cell counting (100 µL), CFU-F assay (1–2 mL) and flow cytometry (1–2 mL) were taken before and after BMA processing with filter-based separation device. The total nucleated cell (TNC) count was determined by counting white blood cells with hematology analyzer COULTER® Ac·T diff2™ (Beckman Coulter, Fullerton, CA, USA). MSCs content was estimated with flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA) by determining CD271 positive ($CD271^+$) and CD45 negative ($CD45^-$) cells, and concomitant assessment of their viability using 7-amino-actinomycin D (7-AAD) dye. Also, the fibroblast colony forming units (CFU-F) assay, which is a functional assay for measuring the frequency of MSCs with the colony forming abilities, was performed. Briefly, cells were seeded at least in duplicates into six well culture plates at 3.125×10^4, 62.5×10^4, 1.25×10^5, 2.5×10^5 or 5×10^5 nucleated cells/well and cultured in standard conditions: 37°C, 5% CO_2. After 14 days, colonies containing ≥50 fibroblastic cells were manually counted under the stereomicroscope. Detailed protocols for cellular evaluation were described in Rebolj et al. study.[28]

Clinical and radiological evaluation

Subjects were clinically followed using Patient Reported Outcome Measures (PROMs): Foot and Ankle Outcome Score (FAOS),[29] European Quality of Life in Five Dimensions Three-Level Time-Trade-Off (EQ-5D 3L TTO), EuroQol-Visual Analogue Scale (EQ-VAS)[30] and Tegner activity scale (TAS).[31] The weight-bearing ankle radiographs were repeated at all evaluation time-points. The preoperative and latest available radiographs were used for the assessment of concomitant ankle OA, according to Kellgren-Lawrence classification system (K-L OA score).[26] Serious adverse events (SAE), defined as any hospitalization or revision surgery (arthrofibrosis, synovitis, infection, hardware problem, etc.), and graft failures (GF), defined as revision surgery to the lesion or confirmed indication for it, were recorded.

Statistical analysis

Descriptive statistical analysis was used to describe patients' demographics, ankle joint status, lesions' characteristics, surgical and cellular parameters for the whole cohort of 35 patients. Continuous variables were presented as means with standard deviations (SD), and categorical variables as frequencies with corresponding percentages. Paired Student t test was applied to compare preoperative and postoperative values of all PROMs. Clinical outcome measures (FAOS subscales, EQ-5D 3L TTO, EQ-VAS) and potential predicting factors (patients' age, gender, BMI, lesion size and location, medical history, symptoms duration, K-L OA grade, concomitant procedures, previous surgeries, surgical approach, scaffold type, length of follow up, and cellular parameters: CFU-F, TNC, CFU-F/TNC, $CD271^+$ $CD45^-$ 7-AAD$^-$, cell viability) were further analyzed. Multivariable regression models were used to assess the influence of proposed predictors on postoperative improvements in the outcome measures. Statistical analysis was performed with SPSS (version 25.0; IBM, Chicago, IL, USA). The level of statistical significance was set at $p < 0.05$.

Results

At the mean follow-up time of 32.2 (12.5) months, 27 (77%) patients returned their postoperative PROMs questionnaires. All FAOS scores significantly increased at postoperative evaluation compared to preoperative values: Symptoms from 60 to 71 ($p = 0.04$), Pain from 55 to 70 ($p < 0.01$), ADL from 69 to 80 ($p < 0.01$), Sport from 30 to 58 ($p < 0.01$), QoL from 25 to 47 ($p < 0.01$), and Total Score from 56 to 71 ($p < 0.01$). Improved quality of life was also observed with significant increase in EQ-5D 3L TTO score from 0.47 (0.15) preoperatively to 0.66 (0.28) at the time of final follow-up ($p < 0.01$). Only the scores of TAS and EQ-VAS were not significantly increased. In the whole cohort of 35 enrolled patients, 4 (11%) SAE (3 arthrofibrosis, one hardware removal), and 3 (9%) GF occurred. The postoperative progression of K-L OA in the operated ankles was statistically insignificant ($p = 0.08$). Detailed results are presented in Table 2.

In two of the 27 responding patients, cellular sampling was incomplete. Therefore, cellular analysis and corresponding regression models were performed for the 25 patients with the complete data set. Intraoperatively prepared fBMA contained on average 140.9 (31.7) × 10^6 TNC with 90.9 (5.0) % viability, among which 39.7 (26.7) × 10^3 were $CD271^+$ $CD45^-$ 7-AAD$^-$ cells and 7878.0 (5579.1) CFU-F. Proportion of CFU-F among all nucleated cells (CFU-F/TNC) was 0.0056 (0.0037) %. Cellular parameters are summarized in Table 3.

Table 2. Joint specific patient outcomes, quality of life, activity level and radiographic ankle OA status of the 27 responding patients treated operatively with scaffolds and fBMA for OLTs. Data is presented as means with standard deviations. Significant postoperative improvements (differences between post- and pre-operative scores) are marked with *. The *p* values are reported.

	Respondents (*N* = 27)			
	PRE-OP	POST-OP	Difference (POST-OP – PRE-OP)	*P* value
FAOS symptoms	60 (16)	71 (22)	11 (25)*	0.04
FAOS pain	55 (17)	70 (25)	15 (21)*	<0.01
FAOS ADL	69 (15)	80 (23)	11 (17)*	<0.01
FAOS sport	30 (21)	58 (32)	28 (32)*	<0.01
FAOS QoL	25 (18)	47 (26)	22 (23)*	<0.01
FAOS total	56 (13)	71 (23)	15 (18)*	<0.01
EQ-VAS	66 (15)	72 (2)	6 (25)	0.18
EQ-5D 3L TTO	0.47 (0.15)	0.66 (0.28)	0.19 (0.21)*	<0.01
Tegner activity scale	2.9 (1.6)	3.3 (1.6)	0.4 (1.8)	0.25
K-L OA	1.6 (0.8)	1.7 (0.7)	0.1 (0.3)	0.08

OLT: osteochondral lesion of talus; FAOS: Foot and Ankle Outcome Score; ADL: Activities of Daily Living; QoL: Quality of Life; EQ-VAS: EuroQol-Visual Analogue Scale; EQ-5D 3L TTO: EuroQol-5D 3-Level Time-Trade-Off; K-L OA–: Kellgren-Lawrence ankle Osteoarthritis score.

Table 3. Cellular parameters of the intraoperatively prepared and injected fBMA for the 25 patients with complete laboratory evaluation.

Parameters of FBMA	Mean (SD)
TNC count	140.9 (31.7) × 10^6
TNC/mL	125.8 (228.0) × 10^6
$CD271^+$ $CD45^-$ $7\text{-}AAD^-$ cells	39.7 (26.7) × 10^3
$CD271^+$ $CD45^-$ $7\text{-}AAD^-$ cells/mL	67.1 (158.5) × 10^3
CFU-F Number	7878.0 (5579.1)
CFU-F/mL	8811.5 (21291.0)
Viability of the cells	90.9 (5.0) %
CFU-F/TNC	0.0056 (0.0037) %

fBMA: filtered Bone Marrow Aspirate; SD: Standard Deviation; TNC: Total Nucleated Cell; CD: Cluster of differentiation; 7-AAD: 7-amino-actinomycin-D; CFU-F: Fibroblast Colony Forming Units; TNC/mL: TNC concentration per milliliter; $CD271^+CD45^-7\text{-}AAD^-$ cells/mL: $CD271^+CD45^-7\text{-}AAD^-$ cells concentration per milliliter; CFU-F/mL: CFU-F concentration per milliliter; CFU-F/TNC: portion of CFU-F among all TNC.

Multivariable regression models revealed that male gender was correlated with greater increase of FAOS subscales Symptoms ($p = 0.03$), Activities of Daily Living (ADL) ($p < 0.01$), and FAOS Total Score ($p < 0.01$). Concomitant procedures negatively affected the improvements of FAOS subscales Symptoms ($p = 0.02$), ADL ($p < 0.01$), Quality of Life (QoL) ($p < 0.05$), FAOS Total Score ($p < 0.01$), and EQ-5D 3L TTO questionnaire ($p = 0.04$). OLTs located on the medial talar dome had higher postoperative FAOS subscale QoL scores, compared to the lateral ones ($p = 0.02$). Patients with more advanced preoperative K-L OA improved significantly in FAOS subscale ADL ($p = 0.01$). The impact of cellular parameters on postoperative course also turned out as significant in some PROMs. The number of CFU-F or their proportion among all nucleated cells (CFU-F/TNC) in the injected fBMA preparations were positively correlated with greater increase in FAOS subscale ADL ($p < 0.01$), and FAOS Total Score ($p < 0.01$), while TNC count turned out as a negative predictor for postoperative improvements of FAOS subscales Sport ($p = 0.02$) and QoL ($p = 0.02$). Other factors evaluated as potential predictors: patients' age, BMI, medical history, length of follow up, scaffold type, lesion size, surgical approach, symptoms duration, and previous surgeries have not turned out as significant for any of the outcome measures. Results of regression models are presented in Table 4.

Discussion

The main findings of the present study were: (1) combined single-step approach with scaffolds and fBMA for OLTs treatment proved to significantly improve patients' perceived ankle joint status, functioning and quality of life; (2) the procedure is safe with relatively small number of SAE (only three related to the graft) and GF; (3) female gender and concomitant procedures were the main negative predicting factors for postoperative improvements; (4) cellular parameters, the number of CFU-F and TNC, in intraoperatively applied fBMA turned out as potential predictors of the treatment outcomes.

The literature of treating cartilage lesions in the ankle with BMAC augmented scaffolds is limited to some reports. The favorable results of the present cohort, regarding postoperative improvements and good safety profile, are in line with the findings of other studies.[17–24] In their works, Giannini et al. used porcine collagen powder or hyaluronic acid membrane in combination with BMAC for the

Table 4. Results of multivariable regression models for the significant predictors of postoperative improvements in significantly increased PROMs. Only statistically significant independent variables are reported ($p < 0.05$). Gender was coded: Female = 1, Male = 2; and Location: Lateral = 1, Medial = 2.

	Dependent variable	Significant predictors	B	*P*-value
Difference: POST-OP to PRE-OP values	FAOS symptoms	Concomitant procedures	−.438	0.02
		Gender	.394	0.03
	FAOS pain	/	/	/
	FAOS ADL	Gender	.671	<0.01
		CFU-F/TNC	.638	<0.01
		Concomitant procedures	−.591	<0.01
		K-L OA	.364	0.01
	FAOS sport	TNC count	−.450	0.02
	FAOS QoL	Concomitant procedures	−.334	<0.05
		TNC count	−.393	0.02
		Location	.384	0.02
	FAOS total	Concomitant procedures	−.496	<0.01
		CFU-F	.527	<0.01
		Gender	.667	<0.01
	EQ-5D 3L TTO	Concomitant procedures	−.421	0.04

PROMs: Patient Reported Outcome Measures; OLT: osteochondral lesion of talus; FAOS: Foot and Ankle Outcome Score; K-L OA: Kellgren-Lawrence ankle Osteoarthritis score; BMI: Body Mass Index; SAE: Serious Adverse Event; GF: Graft failure; POST-OP: Postoperative; PRE-OP: Preoperative; ADL: Activities of Daily Living; QoL: Quality of Life; EQ-5D 3L TTO: EuroQol-5D 3-Level Time-Trade-Off; TNC: Total Nucleated Cell; CFU-F: Fibroblast Colony Forming Units; CFU-F/TNC: portion of CFU-F among all TNC.

treatment of OLTs. Both, in vitro and in vivo results were encouraging, as in vitro experiment confirmed that bone marrow-derived cells are able to differentiate into chondral and osseous lineages, and clinical outcomes were favorable and durable at short-to mid-term follow-up. The significant improvement of AOFAS score was documented at 48 months postoperatively, however some decrease was observed from 24 months' follow-up onwards. Histological and Magnetic Resonance Imaging (MRI) evaluations revealed the presence of new cartilaginous tissue with various degrees of tissue remodeling toward hyaline cartilage, which represented around 80% of the repaired tissue at 24 months' follow-up. The percentage of hyaline like cartilage showed a tendency to correlate with clinical results.[17–19] The combination of collagen I/III bilayer matrix with BMAC proved to be safe and effective in chondral treatment of the ankle in Murphy et al. and Richter et al. studies.[20–23] Moreover, Buda et al. reported similar clinical results in groups treated with Autologous Chondrocyte Implantation (ACI) and hyaluronic acid membrane augmented with BMAC at 48 months' follow-up. In addition to already known advantages of single-step procedures, there was even higher presence of hyaline-like cartilage and lower incidence of fibrocartilage in the BMAC group.[24]

More evidence of such combined approach with scaffolds and BMAC have been gathered in the knee joint, where some systematic reviews, documenting at most 13 clinical studies, have been published.[32] Likewise to the ankle, all clinical studies in the knee, independent of the study group or level of evidence, reported improved clinical outcomes and higher macroscopic, magnetic resonance imaging, and histology scores at different short-to mid-term follow-ups.[8,9,32,33] Interestingly, the comparative trials favored BMAC over microfracture, especially at mid-term follow-ups and reported equivalent outcomes between BMAC and Matrix-induced Autologous Chondrocyte Implantation.[34,35]

To the best of our knowledge, only five studies, all in the knee joint, have reported some cellular parameters of intraoperatively applied BMAC.[7,36–39] In 2 case series, Gobbi et al. documented the concentration of CFU-F per milliliter (CFU-F/mL) that ranged between 2000 and 5700/mL, which is close to the mean concentration of 8811.5/mL observed in the present cohort.[36,37] Skowronski and Rutka reported only the TNC per milliliter (TNC/mL), ranging from 4.5×10^5 to 9×10^5 cells/mL, which is approximately 100-fold less than the mean TNC concentration of 125.8×10^6/mL in the current group.[38] In Martinčič et al. study, the final products contained on average 1156 CFU-F, while Veber et al. performed the most detailed evaluation of the final cell suspensions and their processing, reporting the mean dosage of 1571 CFU-F administered to the patients.[7,39]

However, there have not been analysed any cellular parameters of the intraoperatively applied BMAC as possible prognostic factors for the treatment outcomes. In the present study, the regression models identified some cellular parameters that influence the treatment outcomes. Based on the results, the total number of CFU-F, which represent the

MSCs with colony forming abilities in the injected BMAC, seemed as the most important cellular parameter that positively impacted the treatment outcomes. The established positive mechanisms of action of MSCs, mainly their multipotency, immunomodulation and trophic effects, are consistent with the observed results.[2,3] Moreover, the analysis indicated that the proportion of CFU-F among all TNC, albeit being very small, may have the potential to positively influence the results. This is to some extent in line with the observed negative impact of TNC number on the postoperative improvements in some PROMs. According to the literature, the majority of TNC represent white blood cells (WBC) with ambiguous role in orthopedic surgery due to the possibility of increased expression of proinflammatory cytokines and inferior outcomes compared to the leukocyte-poor preparations, or potential advantages with eliciting the healing and other antiinflammatory responses in the cascade on the other hand.[40–42]

Female gender and concomitant procedures turned out as the main risk factors for postoperative improvements. This is in line with the findings of previous studies, in which females were more affected by osteochondral lesions preoperatively and had less favorable results of operative interventions.[10,43] It is interesting to note that higher preoperative OA K-L scores were positively correlated with improvements in patients' daily activities. This could be attributed to the greater preoperative functional impairments in such patients with concurrent OA, which was limited to K-L grade 2, and therefore not too advanced, leaving the potential for restoring joint function and homeostasis. Interestingly, the other variables analyzed as potential predictors: patients' age, BMI, medical history, length of follow up, scaffold type, lesion size, surgical approach, symptoms duration, and previous surgeries, did not prove as significant for any of the outcome measures.

The need to interpret the results of the present study in light of several limitations has to be emphasized. It was a limited patient series with short-term follow-up and non-randomized, non-comparative, and un-blinded study design. The majority of patients underwent concomitant procedures, which proved to be statistically significant, and as such present a direct source of bias. Moreover, three different types of scaffolds were used, however the scaffold type did not significantly influence the results. The entire patient cohort was treated at a single university orthopedic center by one foot and ankle consultant orthopedic surgeon, therefore indications, harvesting and processing of bone marrow aspirates, and surgeries were standardized. Furthermore, the findings are based on clinical follow-up with PROMs and complications, while radiological and histological assessment of the repaired tissue was not performed. Nevertheless, the present study demonstrated that the combined single-step procedure with scaffolds and fBMA is an adequate and safe approach for OLTs treatment, and primarily recognized the potential impact of cellular parameters on the treatment outcomes, thus introducing an additional aspect in the osteochondral treatment.

Conclusions

The findings of the present study allow to confirm the first hypothesis, as the combined single-step approach with scaffolds and fBMA significantly improved almost all measures of patients' perceived ankle status and quality of life. Moreover, the relatively small number of SAE and GF, and observed impact of the CFU-F and TNC number in the injected fBMA on the treatment outcomes are in favour of the second and third hypotheses. The study primarily identified additional aspects in the field of cartilage treatment, with an emphasis on the intraoperatively applied products.

Author contributions

All authors made substantial contributions to:
- the conception and design of the study (MK, MV, LG, MD), acquisition of data (MK, MV), analysis and interpretation of data (MK, MD)
- drafting the article (MK) or revising it critically for important intellectual content (MV, LG, MD)
- final approval of the version to be published (MK, MV, LG, MD).

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: MD is clinical consultant for Finceramica, Faenza, Italy. Educell Ltd., Trzin, Slovenia (co-authors MV, LG) is a commercial cell and tissue institution that is providing separated MSCs and other advanced cell therapies.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Matic Kolar https://orcid.org/0000-0003-0197-2744

Data availability statement

The data used and/or analyzed in the present study are available from the corresponding author on a reasonable request.

References

1. Caplan AI. Mesenchymal stem cells: the past, the present, the future. *Cartilage* 2010; 1(1): 6–9. DOI: 10.1177/1947603509354992.
2. Pittenger MF, Discher DE, Péault BM, et al. Mesenchymal stem cell perspective: cell biology to clinical progress.

NPJ Regen Med 2019; 4: 22. DOI: 10.1038/s41536-019-0083-6.

3. Filardo G, Madry H, Jelic M, et al. Mesenchymal stem cells for the treatment of cartilage lesions: from preclinical findings to clinical application in orthopaedics. *Knee Surg Sports Traumatol Arthrosc* 2013; 21(8): 1717–1729. DOI: 10.1007/s00167-012-2329-3.
4. Friedenstein AJ, Piatetzky-Shapiro II and Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* 1966; 6(3): 381–390. DOI: 10.1242/dev.16.3.381.
5. Hass R, Kasper C, Böhm S, et al. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal* 2011; 9: 12. DOI: 10.1186/1478-811X-9-12.
6. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284: 143–144. DOI: 10.1126/science.284.5411.143.
7. Veber M, Vogler J, Knežević M, et al. Combination of filtered bone marrow aspirate and biomimetic scaffold for the treatment of knee osteochondral lesions: cellular and early clinical results of a single centre case series. *Tissue Eng Regen Med* 2020; 17(3): 375–386. DOI: 10.1007/s13770-020-00253-9.
8. Gianakos AL, Sun L, Patel JN, et al. Clinical application of concentrated bone marrow aspirate in orthopaedics: a systematic review. *World J Orthoped* 2017; 8(6): 491–506. DOI: 10.5312/wjo.v8.i6.491.
9. Cotter EJ, Wang KC, Yanke AB, et al. Bone marrow aspirate concentrate for cartilage defects of the knee: from bench to bedside evidence. *Cartilage* 2018; 9(2): 161–170. DOI: 10.1177/1947603517741169.
10. Kolar M, Brulc U, Stražar K, et al. Patient-reported joint status and quality of life in sports-related ankle disorders and osteoarthritis. *Int Orthop* 2021; 45(4): 1049–1055. DOI: 10.1007/s00264-020-04747-y.
11. Iwasa J, Engebretsen L, Shima Y, et al. Clinical application of scaffolds for cartilage tissue engineering. *Knee Surg Sports Traumatol Arthrosc* 2009; 17(6): 561–577. DOI: 10.1007/s00167-008-0663-2.
12. Vannini F, Filardo G, Kon E, et al. Scaffolds for cartilage repair of the ankle joint: the impact on surgical practice. *Foot Ankle Surg* 2013; 19(1): 2–8. DOI: 10.1016/j.fas.2012.07.001.
13. Wasyłeczko M, Sikorska W and Chwojnowski A. Review of synthetic and hybrid scaffolds in cartilage tissue engineering. *Membranes* 2020; 10(11): 348. DOI: 10.3390/membranes10110348.
14. Kon E, Roffi A, Filardo G, et al. Scaffold-based cartilage treatments: with or without cells? A systematic review of preclinical and clinical evidence. *Arthroscopy* 2015; 31(4): 767–775. DOI: 10.1016/j.arthro.2014.11.017.
15. Kolar M and Drobnič M. Multilayered biomimetic scaffolds for cartilage repair of the talus. a systematic review of the literature. *Foot Ankle Surg*. 2023; 29(1): 2–8. 2022 Nov 2: S1268-7731(22)00205-3. Epub ahead of print. DOI: 10.1016/j.fas.2022.10.007
16. Cavinatto L, Hinckel BB, Tomlinson RE, et al. The role of bone marrow aspirate concentrate for the treatment of focal chondral lesions of the knee: a systematic review and critical analysis of animal and clinical studies. *Arthroscopy* 2019; 35(6): 1860–1877. DOI: 10.1016/j.arthro.2018.11.073.
17. Giannini S, Buda R, Vannini F, et al. One-step bone marrow-derived cell transplantation in talar osteochondral lesions. *Clin Orthop Relat Res* 2009; 467(12): 3307–3320. DOI: 10.1007/s11999-009-0885-8.
18. Giannini S, Buda R, Cavallo M, et al. Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: from open field autologous chondrocyte to bone-marrow-derived cells transplantation. *Injury* 2010; 41(11): 1196–1203. DOI: 10.1016/j.injury.2010.09.028.
19. Giannini S, Buda R, Battaglia M, et al. One-step repair in talar osteochondral lesions: 4-year clinical results and t2-mapping capability in outcome prediction. *Am J Sports Med* 2013; 41(3): 511–518. DOI: 10.1177/0363546512467622.
20. Richter M and Zech S. Matrix-associated stem cell transplantation (MAST) in chondral defects of foot and ankle is effective. *Foot Ankle Surg* 2013; 19(2): 84–90. DOI: 10.1016/j.fas.2012.11.005.
21. Richter M, Zech S and Andreas Meissner S. Matrix-associated stem cell transplantation (MAST) in chondral defects of the ankle is safe and effective - 2-year-followup in 130 patients. *Foot Ankle Surg* 2017; 23(4): 236–242. DOI: 10.1016/j.fas.2016.05.320.
22. Richter M and Zech S. Matrix-associated stem cell transplantation (MAST) in chondral lesions at the ankle as part of a complex surgical approach- 5-year-follow-up in 100 patients. *Foot Ankle Surg* 2019; 25(3): 264–271. DOI: 10.1016/j.fas.2017.11.004.
23. Murphy EP, Fenelon C, Egan C, et al. Matrix-associated stem cell transplantation is successful in treating talar osteochondral lesions. *Knee Surg Sports Traumatol Arthrosc* 2019; 27(9): 2737–2743. DOI: 10.1007/s00167-019-05452-z.
24. Buda R, Vannini F, Castagnini F, et al. Regenerative treatment in osteochondral lesions of the talus: autologous chondrocyte implantation versus one-step bone marrow derived cells transplantation. *Int Orthop* 2015; 39(5): 893–900. DOI: 10.1007/s00264-015-2685-y.
25. Hepple S, Winson IG and Glew D. Osteochondral lesions of the talus: a revised classification. *Foot Ankle Int* 1999; 20(12): 789–793. DOI: 10.1177/107110079902001206.
26. Kellgren JH and Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis* 1957; 16(4): 494–502. DOI: 10.1136/ard.16.4.494.
27. Filardo G, Drobnic M, Perdisa F, et al. Fibrin glue improves osteochondral scaffold fixation: study on the human cadaveric knee exposed to continuous passive motion. *Osteoarthritis Cartilage* 2014; 22: 557–565. DOI: 10.1016/j.joca.2014.01.004.

28. Rebolj K, Veber M, Drobnič M, et al. Hematopoietic stem cell and mesenchymal stem cell population size in bone marrow samples depends on patient's age and harvesting technique. *Cytotechnology* 2018; 70(6): 1575–1583. DOI: 10.1007/s10616-018-0250-4.
29. Roos EM, Brandsson S and Karlsson J. Validation of the foot and ankle outcome score for ankle ligament reconstruction. *Foot Ankle Int* 2001; 22(10): 788–794. DOI: 10.1177/107110070102201004.
30. EuroQol Group. EuroQol--a new facility for the measurement of health-related quality of life. *Health Pol* 1990; 16(3): 199–208. DOI: 10.1016/0168-8510(90)90421-9.
31. Tegner Y and Lysholm J. Rating systems in the evaluation of knee ligament injuries. *Clin Orthop Relat Res* 1985; 198: 43–49. DOI: 10.1097/00003086-198509000-00007.
32. Cavinatto L, Hinckel BB, Tomlinson RE, et al. The role of bone marrow aspirate concentrate for the treatment of focal chondral lesions of the knee: a systematic review and critical analysis of animal and clinical studies. *Arthroscopy* 2019; 35(6): 1860–1877. DOI: 10.1016/j.arthro.2018.11.073.
33. Chahla J, Dean CS, Moatshe G, et al. Concentrated bone marrow aspirate for the treatment of chondral injuries and osteoarthritis of the knee: a systematic review of outcomes. *Orthop J Sports Med* 2016; 4(1): 2325967115625481. DOI: 10.1177/2325967115625481.
34. Gobbi A and Whyte GP. One-stage cartilage repair using a hyaluronic acidebased scaffold with activated bone marrowederived mesenchymal stem cells compared with microfracture: five-year follow-up. *Am J Sports Med* 2016; 44: 2846–2854. DOI: 10.1177/0363546516656179.
35. Gobbi A, Chaurasia S, Karnatzikos G, et al. Matrix-induced autologous chondrocyte implantation versus multipotent stem cells for the treatment of large patellofemoral chondral lesions: a nonrandomized prospective trial. *Cartilage* 2015; 6: 82–97. DOI: 10.1177/1947603514563597.
36. Gobbi A, Karnatzikos G, Scotti C, et al. One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in fullthickness knee cartilage lesions. *Cartilage* 2011; 2: 286–299. DOI: 10.1177/1947603510392023.
37. Gobbi A, Karnatzikos G and Sankineani SR. One-step surgery with multipotent stem cells for the treatment of large fullthickness chondral defects of the knee. *Am J Sports Med* 2014; 42: 648–657. DOI: 10.1177/0363546513518007.
38. Skowronski J and Rutka M. Osteochondral lesions of the knee reconstructed with mesenchymal stem cellsdResults. *Ortop Traumatol Rehabil* 2013; 15: 195–204. DOI: 10.5604/15093492.1058409.
39. Martinčič D, Leban J, Filardo G, et al. Autologous chondrocytes versus filtered bone marrow mesenchymal stem/stromal cells for knee cartilage repair-a prospective study. *Int Orthop* 2021; 45(4): 931–939. DOI: 10.1007/s00264-020-04727-2.
40. Holton J, Imam M, Ward J, et al. The basic science of bone marrow aspirate concentrate in chondral injuries. *Orthop Rev* 2016; 8(3): 6659. DOI: 10.4081/or.2016.6659.
41. Cassano JM, Kennedy JG, Ross KA, et al. Bone marrow concentrate and platelet-rich plasma differ in cell distribution and interleukin 1 receptor antagonist protein concentration. *Knee Surg Sports Traumatol Arthrosc* 2018; 26(1): 333–342. DOI: 10.1007/s00167-016-3981-9.
42. Riboh JC, Saltzman BM, Yanke AB, et al. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. *Am J Sports Med* 2016; 44(3): 792–800. DOI: 10.1177/0363546515580787.
43. Brulc U, Drobnič M, Kolar M, et al. A prospective, single-center study following operative treatment for osteochondral lesions of the talus. *Foot Ankle Surg* 2022; 28(6): 714–719. DOI: 10.1016/j.fas.2021.08.008.