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THE EFFECT OF COMBINED BONE MARROW ASPIRATE, LIPOASPIRATE, AND PLATELET-RICH PLASMA INJECTIONS ON PAIN, FUNCTION, AND PERCEIVED CHANGE AMONGST INDIVIDUALS WITH SEVERE KNEE OSTEOARTHRITIS

Morey J. Kolber
Department of Physical Therapy, Nova Southeastern University, Fort, Lauderdale, FL, USA
Joseph Purita
Institute of Regenerative Medicine, Boca Raton, FL, USA
José Fabio Santos Duarte Lana
The Bone and Cartilage Institute, Cidade Nova, Indaiatuba/SP, Brazil
Paul A. Salamh
University of Indianapolis, Indianapolis, IN, USA
William J. Hanney
School of Kinesiology & Physical Therapy, University of Central Florida, Orlando, FL, USA

Author for correspondence: Morey J. Kolber: kolber@nova.edu

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Abstract

Background: Owing to a paucity of research on minimally processed orthobiologics, we sought to investigate the efficacy of minimally processed bone marrow aspirate (BMA) and fat graft with a leukocyte-rich, platelet-rich plasma (PRP) intra-articular injection series on pain, function, and global rating of change (GROC) among patients with severe knee osteoarthritis (OA).

Methods: Thirty-one adults (23 females and 8 males, mean age 67 years) with clinical and radiographic evidence of knee OA (Kellgren–Lawrence ≥ 3) were included. During the initial visit, patients were examined and administered the patient-specific functional scale (PSFS) and a numerical pain rating scale ranging from 0 to 10. Patients then underwent procedures to obtain 4–6 mL of PRP, a minimally processed 6 mL fat graft, and 10 mL of BMA. Patients returned twice over 6-week intervals for booster PRP injections. At each follow-up (F1 and F2), the GROC questionnaire and prior outcome measures were completed.

Results: Patients returned at an average of 41 days for the second PRP (F1) and 90 days from initial visit for the third PRP injection (F2). Friedman Chi Square analysis indicated statistically significant improvements in pain (best and worst) and PSFS from initial to F1 and F2 ($P \leq 0.001$). Post hoc Wilcoxon signed-ranks analysis with Bonferroni correction identified improvement from initial to F1 and F2, as well as F1–F2 for pain, PSFS, and GROC ($P \leq 0.013$). Effect sizes ranged from $r = 0.32$ to 0.51. Change, based on established minimum clinically important differences, indicated pain, GROC, and PSFS met thresholds at F2.

Conclusion: A minimally processed fat graft with BMA and a series of three PRP injections improved pain and function among individuals with severe knee OA who were previously recalcitrant to conservative care. Although results indicated significant improvement, clinically important change did not occur until F2. A one-arm design is a limitation of this study.

Keywords: *joint diseases; knee; platelet-rich plasma; stem cells*

INTRODUCTION

Knee osteoarthritis (OA) affects up to 33% of the population and is associated with pain, functional decline, and a considerable socioeconomic burden.[1,2] While efficacious interventions have been identified for the treatment of knee OA, a subgroup of individuals are recalcitrant to conservative care and may be steered toward pharmacological therapies with undesirable effects (e.g., opioids) or surgical care such as joint replacement.[3] Although patients generally experience improved physical activity following joint replacement, surgery is costly and some patients may experience chronic postoperative pain and complications.[4,5] When considering surgical costs[6] and potential postoperative complications, nonsurgical interventions that have the potential to decelerate the disease process and improve function are of interest.

Novel regenerative medicine products (e.g., orthobiologics) have gained considerable attention in the musculoskeletal specialties, owing to the promise of decelerating the disease process and potentially offering a superior long-term solution to existing conservative treatments. Autologous orthobiological interventions such as bone marrow aspirate (BMA), platelet-rich plasma (PRP), and adipose tissue derivatives may be a viable option for individuals with OA who are recalcitrant to conservative care. These interventions have an excellent safety profile and require minimal post-procedural downtime, allowing individuals to pursue a timely resumption of physical activity.[7–9] Furthermore, failure to respond to orthobiologics does not preclude future treatments.[10]

Of the injectable orthobiologics, PRP is most performed, due to ease of procurement and reduced cost. Evidence suggests that PRP products contain a supraphysiological concentration of cells, namely, platelets, as well as a reservoir of growth factors (e.g., insulin-derived growth factor (IGF-1), proangiogenic factors (e.g., platelet-derived growth factor), and anti-inflammatory cytokines (e.g., interleukin 1 receptor agonist (IL-1RA) and interleukin-10). The interest in BMA and adipose-derived products resides in the immunomodulatory capacity of the cells found in the final product. The benefits of these procedures resides in the capability of the progenitor cells to manipulate the microenvironment through immunomodulation and anti-inflammatory influences.[9,10,13–20] Furthermore, in addition to cellular content, adipose and BMA possess bioactive molecules such as cytokines, pro-angiogenic and anti-apoptotic substances, as well as trophic factors.[3,8,10,18,21–23] Although BMA is rich in hematopoietic stem cells, a decline in mesenchymal stem cell (MSC) numbers occur with aging.[24] Thus, procuring a fat graft via lipoaspirate provides the needed MSCs that are deficient in BMA.[24]

Evidence from published clinical trials support the use of autologous injectable procedures such as PRP, BMA concentrate (BMAC), and culture expanded or processed adipose procedures as a treatment for knee OA. In published systematic reviews with meta-analysis,[25,26] PRP has been superior to placebo or control groups as well as corticosteroid and viscosupplementation injections for improving pain and self-reported function at time points ranging from 3 to 12 months. PRP is not a stem cell product and current options for obtaining autologous MSCs include both BMA and adipose derivatives. Evidence underpinning the efficacy of BMA is mainly limited to procedures that have used BMAC, whereas evidence for minimally processed adipose tissue is limited due to most studies using cell expansion or stromal vascular fraction procedures. Systematic reviews on BMAC, processed fat, and bone marrow expanded cells generally suggest that these orthobiologics are efficacious for the treatment of individuals with knee OA as related to improved function and pain.[3,21] Furthermore, the incidence of adverse events has been comparable to control groups.[3] With regard to cartilage regeneration and bone marrow edema, evidence suggests improvement from BMAC and adipose-based procedures that used either culture expansion or a stromal vascular fraction.[9,21,27] Evidence for the use of combined injections has been favorable; however, a superior effect when adding a PRP injection to culture expanded BMA has not been identified.[28] Centeno et al.[29] compared an approach that utilized BMAC and leukocyte-poor PRP with and without a minimally processed fat graft and reported no superiority in the group receiving the fat graft.

Although a body of evidence supports autologous orthobiologics, regulatory requirements and cost

may present a barrier for use. For example, geographic restrictions limit physicians in their ability to perform procedures requiring more than minimal manipulation.[30–32] The United States Food and Drug Administration, under Title 21 of the Code of Federal Regulations Part 1271.10 (a & b) and Part 1271.3 (c and f), provides a criterion for minimal manipulation of orthobiologics.[30] In their documents, minimal manipulation is defined as processing that does not alter original relevant characteristics of the cells or tissues. As such, processing adipose tissue to isolate cellular components and produce a stromal vascular fraction or using methods to enzymatically digest the tissue would be considered more than minimal manipulation.[30] Thus, techniques using a lipoaspirate fat transfer without enzymatic degradation must be performed. Furthermore, using BMA or fat to produce terminally differentiated cells by culturing would exceed minimal manipulation thresholds. The clinical application of orthobiologics that are more than minimally manipulated results in the need to satisfy regulatory requirements such as an approved research trial or an Investigational New Drug application prior to patient care.[10,30–32] Treatments based on expanded cell cultures and expensive processing kits or enzymes also involve a higher cost of care which may limit general population access.[3]

While evidence for PRP is promising for knee OA, a majority of BMA and adipose-based procedures used in published studies do not fall under the regulatory criteria of minimal manipulation. Such regulations have incentivized physicians to develop treatment strategies for delivering orthobiological agents with minimal manipulation.

An interventional approach that concurrently utilizes a minimally processed lipoaspirate (fat graft) and BMA, with leukocyte-rich PRP amongst individuals with knee OA has not been previously investigated. Thus, the purpose of this study was to investigate the efficacy of a combined minimally processed BMA and fat graft with a leukocyte-rich PRP intra-articular injection series on pain, function, and global rating of change (GROC) among individuals with knee OA who were recalcitrant to conservative care. We hypothesized that, although the patients were recalcitrant to conservative care, significant improvements in pain, function, and perceived change would be identified at follow-up.

METHODS

The study was approved by the Institutional Review Board at Nova Southeastern University. We retrospectively reviewed records of patients who completed orthobiological treatments for unilateral knee OA between February 2018 and January 2019. Specifically, 31 patients seeking care at an outpatient facility for unilateral knee OA who received minimally processed BMA, a lipoaspirate fat graft, and a series of three leukocyte-rich PRP injections were included in the study. Eligibility criteria included fulfillment of radiological (Kellgren–Lawrence grade 3 minimum) and clinical criteria according to the American College of Rheumatology (ACR).[33,34] Clinical criteria included knee pain plus three or more of the following: age > 50 years, morning stiffness less than 30-min, bony tenderness, crepitus on motion, bone enlargement, and no palpable warmth of synovium. In addition, patients were required to have been recalcitrant to conservative care including physiotherapy, viscosupplementation, and corticosteroid injections. Patients were excluded if they did not complete the three-series PRP protocol, refused to complete outcome measures, or had a corticosteroid injection within the past week of the initial visit or during the post treatment follow-up points.

Procedures

On the initial visit, patients were evaluated by a single board-certified orthopedic surgeon (JP) with a subspecialty in regenerative medicine. Both radiological and clinical examinations were completed by the orthopedic surgeon. Kellgren–Lawrence grading was based on radiograph interpretation by the orthopedic surgeon. Once diagnosis was confirmed, a medical assistant had patients complete the self-reported outcome measures, which included a numerical pain scale, rating pain from best to worst, ranging from 0 = no pain to 10 = worst pain. Numerical pain scales have a reported intraclass correlation coefficient (ICC) of 0.95.[35] For patients with knee OA, the numerical pain scale has been reported to have a minimum clinically important difference (MCID) of two points, indicating a change of two points is needed to be

clinically meaningful.[36,37] Patients also completed the patient-specific functional scale (PSFS), which is a self-reported outcome measure documenting and quantifying key activity impairments with a ranked level of difficulty. The PSFS has been reported to have excellent reliability (0.84) for patients with knee dysfunction[38] and the MCID has been reported at two raw points when multiple items are averaged.[38]

Following the clinical examination and completion of outcome measures, patients underwent an antecubital venipuncture to obtain 40 mL of blood using a 21-guage needle. The blood was collected into tubes containing sodium citrate to prevent clotting. The blood was then manually processed using a double spin centrifugation technique. Specifically, the blood tubes were placed in the centrifuge for 10 min of slow spinning at a rate of 1600 spins per minute, which converts to a relative centrifugal force of 200 g. The tubes were then processed to remove the top layer of clear plasma. The tubes were then placed in the centrifuge once again for the second centrifugation at 3800 spins per minute (2500 g) for 10 min and processed to retain buffy coat; however, given manual processing, some of the bottom layer of erythrocytes and platelets were captured. The retained samples were then resuspended yielding 4–6 mL of leukocyte-rich PRP for injection. Following the PRP blood draw, a manual liposuction was performed at the flank region. The flank region was used based on availability and accessibility of adipose tissue. For this procedure, patients assumed the lateral decubitus position, and the donor site was first anesthetized with 1% lidocaine. After the initial anesthetic injection, a tumescent solution containing epinephrine, Ringer's lactate, and 2% lidocaine was injected into the region. A liposuction cannula was then used to manually aspirate approximately 20 mL of adipose which contained injected tumescent fluid. Following the procedure, the donor site was cleansed, and steri-strips were applied. The lipoaspirate procured fat graft was then exposed to gravity to allow migration of the infranatant in the collection tube, which was then discarded. The remaining adipose (6 mL) was passed between two syringes with normal saline twice in a manner that would irrigate the fat and allow intra-articular injection using an 18-gauge needle. The final volume of adipose tissue was separated into two 3 mL syringes for injection. In keeping with guidelines for minimal processing, the fat graft was not subjected to enzymatic degradation or centrifugation.

The BMA was performed at the posterior ilium with patients positioned prone under fluoroscopic guidance. Once positioned, the harvest site periosteum was first anesthetized with both 1% lidocaine and a Marcaine solution. The aspiration needle was then advanced to collect the BMA. A mallet was used to advance the aspiration needle to progressive depths yielding a total of 10 mL of noncentrifuged BMA for injection. Specifically, two 10 mL collection syringes, each yielding 5 mL of BMA, were used. Each syringe contained heparin to prevent clotting. Following the procedure, the harvest site was cleansed, and steri-strips were applied. Patients were then transferred to a treatment room to prepare for the knee injections.

The PRP vials underwent photoactivation for 10 min using low-level integrated LED light (AdiLight-2, AdiStem Ltd. Carnegie, VIC, Australia), whereas the fat graft and BMA underwent 20 min of photoactivation. No additional activation methods were used. Injections were performed during the same visit with an anterior approach to the joint space. Once injections were completed, steri-strips were applied to the injection site with ice application for 10 min. Patients were sent home with instructions for icing the knee, physical activity was encouraged as tolerated to begin the next day, and patients were advised to weight-bear as tolerated. Patients were advised to return twice over 6-week intervals for additional PRP injections using the same processing methods.

The outcome measures completed at baseline were reissued at each follow-up visit along with the GROC questionnaire. The GROC is a self-report outcome measure that documents the patients' perceived change in condition compared to baseline and is rated on a 15-point ordinal scale, from −7 to +7 (much better), with 0 = no change. Evidence suggests that the GROC has an MCID of ±3 points.[39]

Statistical analysis

Data were entered into SPSS version 27 for Windows software program (IBM SPSS, Armonk, New York, USA) for analysis. Descriptive data and outcome measure scores were calculated as appropriate using

frequency counts and means ± standard deviation (SD). For the outcome measures, averages were reported as mean values, as opposed to median values, based on standard clinical application for scoring and interpreting change scores using the MCID. Outcome measure comparison points were analyzed as nonparametric data utilizing a Friedman Chi Square analysis, with $\alpha = 0.05$. Post hoc analysis with a Wilcoxon signed-rank test was conducted with a Bonferroni correction applied, resulting in a significance level set at $P < 0.017$. Effect sizes were calculated using Z scores from the Wilcoxon signed-rank test using the formula, $r = Z/\sqrt{N}$, where N = number of observations for which Z is based.[40,41] Interpretation of effect sizes were based on recommendations for nonparametric tests such that a large effect is 0.5 or greater, a medium effect is 0.3, and a small effect is 0.1.[41,42]

RESULTS

Thirty-one patients, including 23 females and 8 males, met inclusion criteria and were included in the analysis. No adverse events were reported other than increased pain and swelling for the first few days following the first procedure in approximately 20% of patients, based on telephone follow-up contact and upon reporting at the first follow-up visit. Patients returned an average of 41 days (SD ± 14) after the initial injection for the second PRP injection [follow-up 1 (F1)] and 90 days (SD ± 20) from the initial visit for the third PRP injection [follow-up 2 (F2)]. Results from the outcome measures including mean ± SD and p-values are illustrated in Table 1. Friedman Chi Square analysis indicated statistically significant differences in pain at best and worst, and patient-perceived function (based on PSFS) from baseline to both outcome points as well as between the first and second follow-up points ($P < 0.001$). Post hoc analysis with Wilcoxon signed-rank test with a Bonferroni correction applied compared change between individual time points. A statistically significant difference indicating improvement from baseline to F1, baseline to F2, as well as from F1 to F2 for patient-perceived function using the PSFS, pain at best, and pain at worst ($P \leq 0.013$) was identified.

Although statistical significance was identified at both follow-up points, effect size estimates were calculated to determine change magnitude. Effect size calculations (Table 2) indicated a moderate effect from baseline to both F1 and terminal follow-up point F2 ($r \geq 0.32$) for all outcome measures except for patient-perceived function on the PSFS, which had a large effect of $r = 0.51$.

Pain improvements at best and worst did not satisfy MCID of two points between baseline and F1, although they exceeded thresholds at F2 (Figure 1).

Table 1. Change Scores and Probability Analysis of Outcome Measures

	Baseline (mean ± SD)	F1	F2	*P*
Pain-best	2.7 (2.0)	1.7 (1.8)	0.81 (1.3)	0.001*
Pain-worst	7.5 (2.1)	6.3 (2.3)	3.8 (2.3)	≤ 0.001*
PSFS	3.2 (1.7)	5.0 (2.3)	6.7 (2.2)	≤ 0.001*
GROC	N/A	1.7 (2.0)	4 (2.5)	< 0.001**

*PSFS, patient-specific functional scale; GROC, global rating of change; F1, First follow-up; F2, Second follow-up; *Friedman Chi Square; **Wilcoxon signed-rank; SD, standard deviation.*

Table 2. Effect Size Estimates (r) for Outcome Measures

	Baseline to F1	Baseline to F2	F1–F2
Pain-best	0.33	0.42	N/A
Pain-worst	0.32	0.48	N/A
PSFS	0.44	0.51	N/A
GROC	N/A	N/A	0.46

PSFS, patient-specific functional scale; GROC, global rating of change; F1, First follow-up; F2, Second follow-up. F1–F2, effect size magnitude of change from first follow-up to second follow-up.

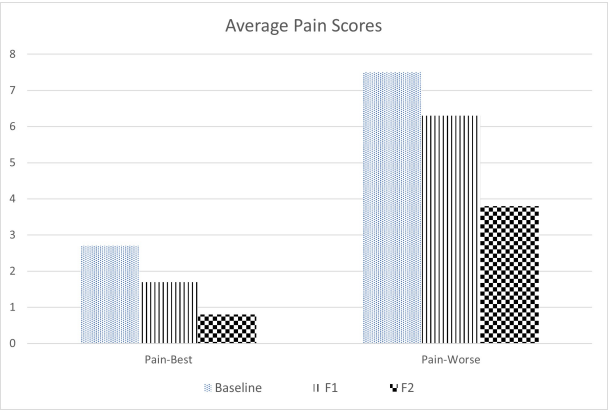

Figure 1. Pain score comparison. Pain scores based on a numeric pain scale, with rating of pain from 0 (no pain) to 10 (worst possible pain). F1, Follow-up 1; F2, Follow-up 2.

Improvements in patient-perceived function (using PSFS) exceeded MCID threshold at F2 only. With regard to the GROC, F1 indicated an average report of +2.0, which indicates patient-perceived improvement; however, scores did not meet MCID of +3.0. On F2, the average GROC score was +4, which indicates patient-perceived overall improvement based on the MCID of +3.0. Wilcoxon signed ranks testing indicated statistically significant improvements in the GROC from F1 to F2 ($P \leq 0.001$).

DISCUSSION

Evidence for the treatment of knee OA with PRP, adipose, and bone marrow derivatives is promising, although variability in procedures and processing restrictions challenges generalization.[10,18,29,30,43] A majority of studies have indicated efficacy; however, procedures often involve expansion of MSCs and the use of processing procedures that are unavailable to many physicians as a result of regulatory requirements. Furthermore, expensive processing kits and procedures imply a cost to the patient, which ultimately limits population access.

The novel aspect of the study is based on procedures, whereby both BMA and adipose were minimally processed and processing kits were not used. Furthermore, all patients received an injection series of three leukocyte-rich PRP injections and all vials underwent photoactivation.

The study results indicate that despite being recalcitrant to prior conservative measures, patients derived a statistically significant benefit with regard to reduced pain, improved function, and perceived change at both follow-up points. Statistical significance does not offer an interpretation of the clinical importance or magnitude of change. Magnitude may be determined through effect sizes, whereas a comparison of change scores and their ability to meet published MCID thresholds helps to determine clinical importance. As a result, effect sizes were calculated, and they indicated a moderate effect approaching large effects at the second follow-up suggesting an appreciable magnitude of change. Furthermore, clinical application was a priority; thus, change scores were compared to previously established MCID values. While the differences were statistically different, implying improvement from baseline to F1, actual scores did not meet the threshold for clinically important change until F2 which was on average 90 days from baseline. Thus, clinically important outcomes may be achieved on a more long-term basis, which is in line with what would be expected in a cohort previously recalcitrant to conservative care.

The study findings are consistent with previous investigations of orthobiologics for knee OA.[26,29,44] While there are variations in preparation, one area of debate is the use of leukocytes in PRP.[45,46] A concern over potential proinflammatory effects of leukocytes[46,47] may steer practitioners toward leukocyte-poor products, despite evidence to the contrary.[48,49] In one study, subjects with knee OA received leukocyte-rich PRP using a protocol of 3-weekly injections.[48] In the aforementioned study, peripheral blood and synovial fluid was tested for proinflammatory cytokines and growth factors before and after the intervention. Results indicated similar proinflammatory levels prior to and after treatment. Furthermore, results from a systematic review indicated that there was no clear relationship between clinically relevant inflammatory reactions and the concentrations of leukocytes in PRP.[50]

Reports of post-procedural pain and swelling in this study were the only adverse events identified. In a previously published multicenter study, the overall reported adverse events were 12.1%, with 29% of the events the result of post-procedural pain.[7] Reports of

post-procedural pain in our study may be higher as a result of the patients having severe knee OA based on a minimum Kellgren–Lawrence grade of 3. Another relevant area to consider is the number of PRP injections, as evidence from comparison studies has suggested a superior benefit for three injections as compared to a single injection for the treatment of knee OA.[44,51] Moreover, results from a systematic review with meta-analysis indicate that while no differences in pain were present when comparing single versus multiple injections, three injections were superior to single and double injections for outcomes.[52] This particular evidence prompted the use of a series of three leukocyte-rich RPR injections in our patients.

The novel aspect of our study is the concurrent use of minimally processed BMA, lipoaspirate, and leukocyte-rich PRP. One study, which may be used for comparison, was that of Centeno et al.,[29] who reported that a combination of BMAC, adipose graft, and leukocyte-poor PRP was efficacious; however, no difference was reported in outcomes when comparing with those who received adipose graft in addition to BMA and PRP. Despite results from the aforementioned study, a review of the results suggested that patients in the group who received the lipoaspirate achieved superior clinical change in function when using a clinimetric assessment. Two key differences in our procedures should be noted as compared to that of Centeno et al.[29] First, we used a leukocyte-rich product which may have contributed to our outcomes particularly as leukocytes are a key component of healing.[45,49] Another difference in the procedures used in this investigation is nonconcentrated BMA as compared to BMAC.

The major difference in our study when compared with many of the published studies is the absence of cell expansion and the use of minimal processing. While it is not clear if these procedures may produce a comparable cellular and molecular product, it is cost-efficient and within permitted geographical regulatory guidelines. Despite minimal processing, satisfactory outcomes were achieved, and the utilization of best practice may have contributed to the results. We utilized the posterior ilium for BMA which has been shown to possess 1.6 times greater cell yield than other regions.[18,43,53,54] Moreover, the use of a 10-mL syringe has been shown to produce the best cell yields.[43,55] Furthermore, aspirating bone marrow from the iliac crest using small volumes with a 10-mL syringe has been proposed for harvesting bone marrow aspirate as a standard technique to avoid blood dilution.[55] Lastly, photoactivation may have had an effect on outcomes. Although high-quality studies with large sample sizes do not exist to support the efficacy of LED light exposure, evidence does exist to suggest an increase in interleukin-10 along with a reduction in proinflammatory cytokines (TNF-alpha and IL-6).[56] Our understanding of the potential benefits of using photoactivation comes from the data published by Zhevago et al.[56] who exposed human peripheral blood to transcutaneous and in vitro irradiation with polychromatic visible and infrared polarized light. In the study, a decrease in the level of pro-inflammatory cytokines TNF-alpha, IL-6, and increases in IL-10 were reported.

Study limitations

A limitation resides in the utilization of a one-arm design and short terminal follow-up point of mean = 90 days. One aspect to consider when evaluating this limitation is the inclusion criteria of being recalcitrant to prior care. Essentially, patients served as their own controls having had prior physiotherapy, viscosupplementation, and corticosteroid injections. Another limitation was the lack of magnetic resonance imaging (MRI) at baseline and lack of follow-up radiographs. Our reasoning for using standard radiographs at baseline for diagnosis was patient cost. Inclusion criteria were strengthened via utilization of ACR guidelines for the clinical diagnosis of knee OA, which has a sensitivity of 95%.[34] While follow-up imaging may have been of value to determine structural changes, our priority was clinical change, particularly as imaging may be discordant to clinical findings.[57] Nevertheless, we acknowledge this study limitation. Furthermore, cell counts were not obtained, thus limiting the precise understanding of the products used in the study. Lastly, the use of a combined procedure limits the ability to identify causation, as it is not clear if benefits were derived from the combined approach or a single component of care.

CONCLUSION

Owing to regulatory requirements, research of minimally processed orthobiologics is of considerable value. This study suggests that a combined, minimally processed procedure using BMA, fat graft, and PRP series may improve pain and function for those individuals with severe knee OA who did not recover with conservative care. Although the results of a single group study limit generalization and prohibit superiority claims, the outcomes are of clinical value. Future research comparing minimally processed orthobiologics to culture expanded procedures is indicated to further guide clinical practice. Patients should be educated that improvements are most likely to occur over a longer duration when compared to pharmacological therapies.

Level of Evidence: IV

AUTHORS' CONTRIBUTION

All authors contributed to the concept/design, data/patient or study materials acquisition/provision, analysis, and drafting/final approval of the manuscript.

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