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SAFETY AND EFFICACY OF BONE MARROW CONCENTRATE AND PLATELET-RICH PLASMA FOR THE TREATMENT OF MODERATELY ADVANCED POST-TRAUMATIC ARTHRITIS OF THE ANKLE

A RETROSPECTIVE CASE SERIES OF 22 ANKLES WITH A MEAN 2.5-YEAR FOLLOW-UP

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Abstract

Background: Orthobiologics such as mesenchymal stromal cells and platelet-rich plasma are intensely being investigated as treatment options for osteoarthritis. The purpose of this retrospective case series is to evaluate the safety, adverse events, long-term outcomes, and efficacy of bone marrow concentrate (BMC) combined with platelet-rich plasma (PRP) to treat moderate to advanced Kellgren-Lawrence (KL3-4) post-traumatic ankle osteoarthritis.

Methods: Study population at final follow-up included 19 patients with 22 treated ankles with post-traumatic ankle osteoarthritis with a mean duration of symptoms of 56 months before treatment with ultrasound-guided intra-articular injection of BMC and PRP. Patients were followed prospectively from the time of treatment for a mean of 2.6 years at final follow-up. Pre and post Foot and Ankle Disability (FADI) scores were tabulated to assess outcomes, and 7 patients also completed the FADI sports module. Total nucleated cell counts were obtained on all treated patients and Mishra Type 3A PRP was utilized.

Results: A total of 19 of 22 ankles treated showed improvement in their FADI scores. Thirteen of the 19 who had improved FADI scores patients reached MCID ($P = .17$). Three of the six patients who did not reach MCID eventually underwent ankle arthrodesis. The average patient age was 56 years old. There was no correlation between the history of previous surgery, KL grade, sex, or age in terms of outcomes. Seven patients completed the sports module of the FADI, and none reached MCID. Patients with a shorter duration of symptoms had higher post-FADI scores reaching MCID ($P < .001$). The three patients who underwent arthrodesis had the longest duration of symptoms of 84, 84, and 120 months. No adverse effects were reported in the study group. Total nucleated cell count (TNCC) did not correlate with outcomes in this group, and there was no correlation of TNCC based on age and sex.

Conclusion: BMC combined with PRP is a safe treatment option for patients with moderate to advanced ankle arthritis and can provide functional pain relief for a sustained period and delay or possibly obviate the need for future ankle arthrodesis. A shorter duration of symptoms may lead to improved outcomes.

Key Words*: ankle osteoarthritis; bone marrow concentrate; platelet-rich plasma; ultrasound-guided injections*

Degenerative joint disease (DJD) is the most common disabling disorder in orthopedics. Current traditional treatment strategies include pharmacologics, physical therapy, activity modification, bracing, and injection therapies such as corticosteroids, hyaluronic acid, prolotherapy, and surgery.

Ankle osteoarthritis is relatively uncommon, affecting less than 5% of the population, with surgical treatment being less advanced than that for knee, hip, and shoulder arthritis and providing less predictable results.

The role of orthobiologics such as platelet-rich plasma (PRP) and bone marrow concentrate (BMC) is controversial and even more so for ankle osteoarthritis, given the paucity of data and studies to support its use.

Repetto et al. reported a case series of 20 patients with grade 3-4 Kellgren-Lawrence (KL) treated with 4 PRP injections and an 80% satisfaction rate.[1] Sampson reported on 6 ankles treated with one intra-articular bone marrow concentrate followed by a "booster" PRP and found that ankle DJD responded less favorably than the knee, shoulder, and hip DJD in his series of 125 patients.[2] Emadedin used culture-expanded mesenchymal stem cells and found that Foot and Ankle Outcome (FAO) scores improved from 79 at baseline to 49 at 30 months.[3]

This case series presents the efficacy and safety of 19 patients with 22 ankles treated having grade 3-4 KL osteoarthritis treated with BMC and PRP with up to 5-year follow up.

METHODS

A total of 24 consecutive patients were retrospectively evaluated with symptomatic post-traumatic arthritis treated with ultrasound-guided intra-articular BMC/PRP injections. This was performed at the author's clinical practice from December 2014 to December 2019.

Inclusion criteria included age >18 years, >6 months of previously failed treatments including physical therapy, corticosteroid injections, hyaluronic injections, NSAIDS, arthroscopy, previous PRP injections, immobilization and/or bracing, and Kellgren-Lawrence grade 3 or 4 osteoarthritis limited to the tibio-talar joint.

Exclusion criteria included pregnancy or lactation, non-ambulation, systemic disease, CNS disease, and peripheral neuropathy, concomitant autoimmune disease or inflammatory arthritides, active infection or wound ulcers, bleeding diatheses, history of hematologic or lymphatic malignancy, intra-articular hardware, intra-articular corticosteroid injection within 12 weeks, oral corticosteroids within 8 weeks, and inhaled corticosteroids within 3 weeks of treatment, BWC cases, and use of NSAIDS < 1 week before treatment.

All patients received a physical examination, ankle radiographs, and systemic evaluation of pain using the Foot and Ankle Disability Index (FADI) (Table 1) prior to treatment, 12 weeks post treatment, and final follow-up. Higher functioning patients also completed the sports module of the FADI.

Before treatment, each patient completed an IRB-approved informed consent form during which the risks and benefits were reviewed with the patient and obtained informed consent from all patients that pre- and post-treatment questionnaires may be used for clinical and/or research purposes.

Injection Protocol

All patients received an ultrasound-guided intra-articular prolotherapy injection 1 to 5 days before BMC/PRP injection. The 5 mL solution consisted of 25% dextrose and 0.125% ropivacaine and normal saline with the intent to create a brief inflammatory response.[4]

A detailed description of the bone marrow aspiration (BMA) and platelet concentration procedures has been previously described in detail.[5] A total of 60 mL of bone marrow was aspirated from six separate sites and then processed manually under sterile conditions, and the nucleated cells contained within the buffy coat were isolated for reinjection. TNCC was determined by lysing red blood cells from the samples and counting the remaining nucleated cells (Appendix I).[6] A total of 90 minutes elapsed between the start of aspiration, including processing and reinjection.

Ultrasound guidance was then used to inject the BMC intra-articular followed by 1 cc of RBC free Type 3-A PRP according to the Mishra classification.[7,8]

Three to five days after the BMC/PRP injection, the patient returned for a 3-cc injection of RBC-free type 3-A PRP.

TABLE 1 Foot and Ankle Disability Index

Questions†	Sports Module‡
Standing	Running
Walking on even ground	Jumping
Walking on even ground without shoes	Landing
Walking up hills	Squatting and stopping quickly
Walking down hills	Cutting, lateral movements
Going upstairs	Low-impact activities
Going downstairs	Ability to perform with your normal technique
Walking on uneven ground	Ability to perform your sport unlimited
Stepping up and down curves	
Squatting	
Sleeping	
Coming up to your toes	
Walking initially	
Walking 5 minutes or less	
Walking approximately 10 minutes	
Walking 15 minutes or greater	
Home responsibilities	
Activities of daily living	
Personal care	
Light to moderate work (standing, walking)	
Heavy work (push/pull, climb, carry)	
Recreational activities	
General level of pain§	
Pain at rest	
Pain during your normal activity	
Pain first thing in the morning	

†Questions to ask if these items can be done with no, slight, moderate, extreme difficulty or unable to do

‡Questions to ask if these athletic activities can be done with no, slight, moderate, extreme difficulty or unable to do

§Questions to ask regarding pain are asked in terms of no, mild, moderate, severe, or unbearable pain

Ropivacaine was the only anesthetic used for all injections as other local anesthetics have been found to be toxic to mesenchymal stromal cells and hyaline cartilage.[9]

During the peri-injection period, patients were instructed to use either crutches or boot immobilization for 7-10 days to tolerance of pain and then began a six-week standard rehab protocol focusing on range of motion, strengthening, and proprioception. Patients then followed up 8-12 weeks after treatment to monitor progress.

Descriptive statistics (mean, standard deviation) were calculated for all normally distributed continuous variables. Student's t-test was used to evaluate differences between groups. A Bonferroni-corrected significance level of $P = .002$ was used to adjust for multiple comparisons. Frequencies and percentages were calculated for all categorical variables. Relation-ships among categorical variables were calculated using Fisher's Exact Test.

RESULTS

The initial study group consisted of 24 patients with post-traumatic KL grade 3 or 4 osteoarthritis of the ankle. Three patients had new injuries to the ankle and 2 were lost to follow-up and were therefore excluded. A total of 19 patients with 22 treated ankles completed both the pre- and post-treatment FADI and comprised the final study group. The demographic characteristics of the patients are shown in Table 2.

The average patient age was 57±13.1, 14 patients were female, and 13 had previous surgery. Out of those 13 patients, 11 were female ($P = .022$). Eight patients had KL 3, and 14 had KL 4 osteoarthritis. Before treatment, the mean duration of symptoms was 56 months, with an average follow-up period of 32 months.

No complications or adverse effects were noted during the study period or reported by patients other than swelling and pain for 2-5 days following the treatment.

The FADI score was the primary outcome measure. Eighteen of 22 ankles had an improvement in their post-FADI score. Three patients did not complete a post-FADI as they underwent arthrodesis and were considered treatment failures. The minimal clinically significant difference (MCID) was reached by

Table 2 Patient Demographics

	Value
Age (yr)	
Mean ± SD	57±13.1
Range	30-82
Gender	
Male	8
Female	14
Follow up (mo)	
Mean ± SD	32±13.1
Range	13-70
Duration of symptoms prior to treatment (mo)	
Mean ± SD	56±23
Range	19-120
Previous surgery prior to treatment	
Yes	13
No	6
Kellgren-Lawrence Grade	
3	8
4	11

13 out of the remaining 19 patients at final follow-up ($P = .17$). This improvement was maintained for up to 56 months in the patient with the longest follow up.

There was no correlation between KL 3 and KL 4 patients, history of previous surgery, and gender in reaching MCID. Patients with a shorter duration of symptoms had higher post-FADI scores reaching MCID ($P < .001$). The three patients who underwent arthrodesis had the longest duration of symptoms of 84, 84, and 120 months.

Seven patients completed the sports module of the FADI. All had no to minimal improvement in the sports module FADI, and therefore MCID for athletic activities was not achieved. However, 5 out of the 7 of this subgroup reached MCID based on their overall FADI score.

Table 3 illustrates the relationship between patient characteristics and TNCC.

The median TNCC count for the group was 1022×10^6 cells. The median count for men was 1350×10^6 and for females 715×10^6 cells (Table 4). There was no correlation between TNCC in terms of FADI scores, MCID, and patient age.

A secondary outcome measure was the postponement of ankle arthrodesis or arthroplasty. At the time of this writing, only three patients underwent arthrodesis. The remainder of the study group has not undergone surgery, with an average follow-up of 32 months and the longest being 56 months.

DISCUSSION

In this retrospective case series, the most notable finding is that intra-articular BMC/PRP provided significant pain relief and functional improvement for up to five years in most patients. No significant adverse effects were reported from the BMA and intra-articular injection consistent with findings noted by previous authors.[3,10,11]

The role of orthobiologics such as PRP and mesenchymal stem cells is evolving, especially for ankle osteoarthritis, given the paucity of data and studies to support it in ankle joints.

Repetto et al. published a case series of 20 patients with KL grade 3-4 treated with 4 weekly PRP injections and reported an 80% satisfaction rate measured by FAO scores and VAS scores. They had a study group of 18 patients with a mean follow-up of 18 months with 2 patients requiring surgery.[1]

Sampson et al. reported on 6 ankles treated with one intra-articular BMC treatment followed by a "booster" PRP and found that ankle DJD responded less favorably than the knee, shoulder, and hip osteoarthritis in his series of 125 patients. The outcome was assessed using a VAS, and no significant side effects were reported. The average time for follow-up was 148 days. The study did not report if treatment failures of the ankle went on to surgery. No specific rehabilitation protocol was used for the treatment group, and TNCC counts were not reported.[2]

Emadedin used culture-expanded mesenchymal stem cells and found that FAO scores improved from 79 at baseline to 49 at 30 months. The mean walking distance in his group of 6 ankles improved from 1010 m at baseline to 1625 m at 6 months and 2333 m at 30 months. MRI follow-up showed decreased bone marrow edema in 4 of 6 patients and an increase in hyaline cartilage, which is not reported in most studies looking at hyaline cartilage regeneration, nor did the

Table 3. Individual Patient Characteristics and TNCC

AGE	SEX	TNCC†	Pre-FADI	Post-FADI	Pre-Sports FADI	Post-Sports FADI
30	F	567×10^6	52	85	12	22
56	M	1.8×10^9	61	90	10	23
59	M	943×10^6	15	70	14	15
59	M	765×10^6	10	68	14	15
58	M	1.1×10^9	58	87	10	13
72	M	1.7×10^9	39	100	3	16
43	F	1.4×10^9	58	85	1	11
65	F	210×10^6	42	72		
82	F	1.5×10^9	28	67		
42	F	434×10^6	23	77		
63	F	2.3×10^9	40	76		
51	F	841×10^6	72	73		
35	M	1.2×10^9	31	64		
63	M	2.2×10^9	49	87		
69	F	662×10^6	45	75		
69	F	321×10^6	51	87		
39	F	392×10^6	40	85		
48	F	769×10^6	23	20		
71	M	1.5×10^9	37	47		
52	F	1.6×10^9	17	arthrodesis		
60	F	401×10^6	20	arthrodesis		
49	F	1.1×10^6	14	arthrodesis		

TNCC† = total nucleated cell count

Table 4 Total Nucleated Cell Counts for 22 Treated Ankles

Median Count Entire Study Group	1022×10^6 cells
Median Count Males N=8	1350×10^6 cells
Median Count Females N=14	715×10^6 cells

authors report their methodology of measuring the change in hyaline cartilage.[3]

Fukawa followed 20 patients 24 weeks treated with 3 injections of type 4A PRP with moderate to advanced osteoarthritis. The VAS for pain, the Japanese Society for Surgery of the Foot (JSSF) ankle/hindfoot scale, and the Self-Administered Foot Evaluation Questionnaire (SAFE-Q) assessed clinical outcomes.

The investigators found the lowest VAS scores at 12 weeks, with the positive effect being reduced at 24 weeks and those with advanced osteoarthritis responding poorly. No adverse effects were noted.[12]

Other authors have used PRP for various ankle pathologies. Mei-Dan and co workers compared PRP to HA for OCD of the talus and found that PRP led to a better functional outcome at 6 months, but these patients did not have documented advanced DJD of the ankle joint.[13]

Angthong provided a single PRP injection to a heterogeneous group of 12 patients with hindfoot and ankle pathologies, only 5 of which had osteoarthritis with inconclusive results. The PRP utilized was not delineated.[14]

Vannabouathong recently reviewed 27 studies for patients who received any type of intra-articular

injection and found HA and PRP may be more effective than corticosteroids but acknowledged that there is very sparse evidence to support the use of orthobiologics for ankle osteoarthritis.[15]

The current series is the largest known, looking exclusively at moderately advanced post-traumatic ankle osteoarthritis treated with BMC/PRP. PRP with BMC is based on the possible synergism between the two products in that they contain different cytokines and molecules. Very few studies have looked at the combination of BMC and PRP to treat orthopaedic conditions. Kim found that in studying rotator cuff tears, cell proliferation was higher in tendon-derived stem cells while at the same time decreasing the chondrogenic and osteogenic potential of these cells and theorizing this may lead to the therapeutic effects of BMC-PRP.[16] Hakimi, in an animal study involving pigs, found that BMC-PRP provided superior results compared to bone grafting in animals who underwent a lab-induced non-union of the tibia.[17] Bolte found that culture-expanded MSCs combined with platelet growth factors led to better bone union in mice.[18] Bastos treated 18 patients with knee osteoarthritis using culture-expanded stem cells with and without PRP and found that both groups had a positive response, and PRP to the culture-expanded cells did not exceed the outcomes of patients treated with cultured expanded stromal cell alone.[19] The biology and mechanisms between platelet-derived growth factors and MSCs are not clearly understood. Caplan believes that platelet-derived growth factors (PDGF) play a central role in the modulation and activities of MSCs and their release from the vasculature in a synergistic fashion.[20] PRP may also modulate the normal healing cascade and be of benefit, and the combination of BMC and PRP may decrease the concentrations of inflammatory substances in the synovial fluid of joints.[21]

The mechanism of pain relief is not fully understood for mixed PTCPs and PRP. They are believed to decrease the production of inflammatory and catabolic cytokines in osteoarthritis. Production of antagonist molecules such as interleukin 1 receptor antagonist (IRAP) and a negative feedback loop against tumor necrosis factor toward catabolic cytokines within the microenvironment of the joint is beginning to gain acceptance as a possible mechanism.[22] Paracrine effects and exosomes have gained acceptance as mechanisms by which stromal cells exert their therapeutic effect.[23,24]

In addition to the above effects, platelets have also been shown to modulate pain via the production of endogenous endocannabinoids, and exert their therapeutic properties via growth factor release, production of endogenous hyaluronic acid, and other cytokines.[10,25-27]

The majority of patients treated reported long-term "satisfied" or "very satisfied" outcomes, with their FADI scores showing a sustained improvement for many years in this series. A total of 3 patients have undergone subsequent fusion of the ankle joint.

A subset of 7 patients who considered themselves athletically active and completed the subset of the FADI questionnaire and showed improvement at 2 to 5 year follow-up. It is important to note that these patients reported significant improvement in their overall FADI scores, but the improvement in the sports subset of the FADI was significantly less. This means that while patients had significant improvement in their activities of daily living, the treatment did not allow a return to athletic activity that could be considered meaningful.

MRI scans were not obtained routinely in this series as it was felt that plain films and physical exams provided the information to arrive at a clinical diagnosis of ankle osteoarthritis. It would have been of clinical interest to note if the patients had pre-existing bone marrow lesions and if these lesions resolved with treatment over time. There has been a paucity of studies showing regeneration of hyaline cartilage in advanced osteoarthritis of any joint. It is acknowledged that follow-up MRI may have provided information in this regard.

TNCC counts were obtained in all patients, and in this small series, there was no correlation in terms of age, sex, or total TNCC count affecting the outcome. However, Hernigou found in his series of 60 patients treated for non-union of the tibia that TNCC count significantly affected the rate of callus formation. His patients averaged 33 CFU (colony forming units)/million nucleated cells and found that stromal cells decreased with age in women but not men.[28] He also found that CFU in BMA averaged 612/cm^3 versus 2579cm^3 in BMC.

The current group showed that TNCC count was higher in men than in women but found no correlation with age as some of the oldest patients showed the highest TNCC counts.

Centeno et al., studied 373 knees treated with BMC. ROC analysis was used to determine optimal sensitivity and specificity for pain and functional improvement at 4×10^8 cells. A total of 224 patients exceeding this number had a significant reduction in pain scores than 185 with less than 4×10^8 cells.[6]

Pers et al., in a phase 1 escalation trial dividing patients into three groups receiving low, medium, or high dose of cultured expanded adipose-derived stem cells, showed that all improved but only the low dose group showed the improvement to be statistically significant.[29]

The optimal number or "dose" of these stromal cells is yet to be determined for the specific entity being treated and warrants further study.

The current literature seems to favor LP-PRP being best suited to treat osteoarthritis. LR-PRP contains a higher number of growth factors that lead to increased vascularity and synovial inflammation compared to LP-PRP. Le and coworkers, in their review, reported high-quality evidence for the use of LP-PRP for osteoarthritis.[29] In comparison, Riboh et al., in their meta-analysis of 1055 patients including six Level 1 randomized trials, found that LP-PRP produced better WOMAC scores than either placebo, HA, or LR-PRP. IKDC scores showed no difference between LP-PRP and LR-PRP nor was there a difference in adverse events reported.[31] Most recently, Yaradilmis prospectively followed 90 patients treated with either HA, LP, or LR-PRP and found the opposite to be true. At 6 and 12-month follow-up, patients treated with LR-PRP produced better VAS and WOMAC scores.[32] LP-PRP was utilized in this case series due to the current evidence showing it is less likely to produce synovial inflammation. Continued randomized, blinded studies will be needed to answer the question of which PRP preparation produces the most consistent positive outcomes.

The FADI was used as it can quantitate function and ADLs in patients with a disability of the ankle and also includes a module for more active patients.[33] It is acknowledged that there is no consensus as to what FAO questionnaire provides the most validity, but are diagnosis specific. There is also disagreement as to the reliability of classification for post-traumatic ankle osteoarthritis. The KL classification was utilized, as no interobserver agreement was needed for this case series.

The study's strengths include a sustained, positive effect on ankle function over several years, a high compliance and response rate of the study group, no adverse effects, the reporting of cell counts, and cell viability. All injections done in this study were done under ultrasound guidance by a single investigator using a non-toxic local anesthetic. It is also clear that this group of patients had failed all conservative measures given the duration of symptoms before being treated.

Study limitations are the retrospective nature of the case series, its small number of patients, and no control group. MRI was not utilized, and it may have provided information as to how many of the patients had bone marrow edema, and that information may have shed light on responders and nonresponders. The addition of prolotherapy and PRP to the BMC may have influenced the outcomes compared to BMC alone. Future studies need to compare BMC alone vs. BMC with PRP in a randomized controlled fashion. The ideal number of cells contained in BMC has not been determined, nor the need and timing of further orthobiologics treatments.

CONCLUSION

Ultrasound-guided intra-articular injection of BMC combined with PRP is a safe non-operative treatment for patients with moderate to advanced ankle osteoarthritis with little to no adverse effects. The results can be long-term and delay or obviate the need for ankle fusion or arthroplasty. A shorter duration of symptoms before treatment may also portend a positive outcome.

DISCLOSURE

The author has no conflicts of interest to declare.

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APPENDIX I
CALCULATION OF TOTAL NUCLEATED CELL COUNT (TNCC)

TNCC was obtained with the TC20^{TM} Automated Cell counter (Biorad, Hercules, CA).

A 10µl sample of BMC was pipetted into 450µl of sterile water, which was then mixed with 450µl of normal saline for a total dilution of 1:100. 10µl of this mixture was then placed into a microcentrifuge tube and mixed with10µl of trypan blue which is then placed on a slide and placed in the cell counter giving a total cell count per milliliter as well as a live cell count per milliliter. That number is then multiplied by the inverse of the dilution factor to obtain the TNCC.