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Long-Term Results of Treatment with Mesenchymal Stem Cells, Growth Factors and Neural Regulation in Post-Traumatic Osteoarthritis of the Ankle

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Keywords: Post-traumatic Osteoarthritis; Mesenchymal Stem Cells; Platelets Rich Plasma.

Abstract

Background: Post-Traumatic Osteoarthritis (PTOA) continues to impose a big challenge on orthopedic surgery worldwide. Biological interventions, such as injections with growth factors and stem cells, combined with improving Extracellular Matrix (ECM), intend to regenerate damaged tissues or minimize further degeneration. The ECM provides structural support and is a fundamental component of stem cell niches. Mesenchymal Stem Cells (MSCs) have shown to possess potent anti-inflammatory and immune modulatory properties, in addition to their ability to form cartilage and bone. The success of tissue regeneration can potentially be improved with the addition of adjuncts like Platelets Rich Plasma (PRP).

Aim and methods: We present 7 patients (mean age 30.9 years, SD 11.5) with PTOA of the ankle ranging from Kellgren-Lawrence grades 2 to 4 treated with MSCs and PRP. Mean follow-up was 4.6 years (SD, 3.1). At baseline, the Visual Analogue Scale (VAS) score for pain was 5.9 and the Foot & Ankle Disability Index (FADI) Score was 51.2. Bone Marrow Mesenchymal Stem Cells (bmMSC), Adipose Tissue

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Mesenchymal Stem Cells (adMSC) and PRP were injected simultaneously, via percutaneous intra-articular injection, followed by a second PRP injection 2 months later.

Results: Six months after the procedure the mean VAS score improved to 3 and the FADI score improved to 76.2. After 1 year, the mean VAS score (2.85) and FADI score (78.1 SD, 25.1) remained almost unchanged. No systemic or local adverse events were observed.

Conclusion: The results of our study are encouraging and show that intra-articular injection of a combination of bmMSCs, adMSCs, and PRP is safe and effective for reducing pain and improving function and quality of life in patients with PTOA of the ankle.

Introduction

Ankle fractures are among the most common surgically treated fractures and are the leading cause of post-traumatic osteoarthritis (PTOA) of the ankle. Tissue degeneration of the ankle, decreased function and disabling pain are typically seen[1]. In young patients with end-stage ankle PTOA, Ankle Joint Arthrodesis (AJA) is considered the gold standard procedure even though overall complication rates of up to 60% and non-union rates between 5% and 37% have been reported for this treatment modality [2].

Another procedure available for PTOA is Ankle Joint Replacement (AJR), of which the effectiveness compared to AJA continues to be a debated topic among foot and ankle surgeons. Although AJA can offer rapid pain relief, this procedure is associated with premature deterioration of other joints in the foot, which eventually will lead to arthritis, joint dysfunction and pain [3].

A systematic review showed that the intermediate results of AJR appear to be similar to that of AJA [4]. Although the two treatment options are characterized by their own merits and demerits, both continue to be associated with severe complications [5,6,7].Therefore, less invasive procedures that can delay or even reverse the degenerative changes related to PTOA of the ankle are currently being investigated with great interest.

Most patients with PTOA suffer not only from musculoskeletal pain, but also from neuropathic pain. Burning, hyperalgesia, and allodynia are the result of neuroinflammation. Neuroinflammation is a localized inflammation in the Peripheral Nervous System (PNS) and Central Nervous System (CNS). A characteristic feature of neuroinflammation is the activation of glial cells in dorsal root ganglia, spinal cord, and brain which leads to the production of pro inflammatory cytokines and chemokines in the PNS and CNS that drives peripheral sensitization and central sensitization [8].

A complex interplay between various injured tissues, vascular, autonomic nervous system, and central and peripheral nervous systems compromise the ECM. This interstitial compartment is a highly dynamic structure that is present in all tissues and provides not only structural support, but also remodeling, regeneration and tissue homeostasis [9]. ECM is a fundamental component of the stem cell niche and its remodeling affects stem cell fate [10].

Repair of tissue after injury depends on the synthesis of a fibrous ECM to replace lost or damaged tissue [11]. Acute inflammation, re-epithelialization, and contraction all depend on cell–extracellular matrix interactions and contribute to minimize infection and promote rapid wound closure [11].

ECM proteins provide biochemical cues interpreted by cell surface receptors, such as the integrins [12] and initiate signaling cascades controlling cell survival, cell proliferation, differentiation and stem cell state [13, 14] Neural inputs transmit distant physiological cues to the stem cell microenvironment [15]. In our experience these impulses can be achieved with the correct procaine injection in the injured tissues. Among the neuroendocrine mechanisms involved in restoring homeostasis, the sympathetic nervous system plays a central role in mediating acute counter-regulatory stress responses to injury [16]. The application of a local anesthetic has a dampening effect in the case of pathologically increased sympathetic activity and thus promotes the mechanisms of auto regulation.

The strength of the sympathetic stimulation is dependent on the strength of the stimulus. The very short acting ester-structured Procaine is the first choice among the local anesthetics. Membrane stabilization of all tissue structures reached by procaine leads to the matrix and neural regulation [17]

ECM improvement through neural regulation and MSC might be able to support tissue regeneration in PTOA after ankle trauma. We present 7 cases of ankle PTOA treated with adult autologous mesenchymal stem cells from bone marrow and adipose tissue.

PRP is a procedure that has been used clinically in humans since the 1970s, mainly for its enhanced wound healing properties that are attributed to high levels of growth factors, secretory proteins and paracrine activity [8,19]. These growth factors in PRP can promote the recruitment, proliferation and differentiation of cells that contribute to tissue regeneration [20]. In order to improve and ensure that regenerative medicine procedures using autologous cells are successful, incorporating these kind of factors is essential.

The concept of a fibrin network, serving as a scaffold holding cells and platelets together, has been suggested [21]. Xu et al. [22] have recently shown in vitro that activated PRP has the ability to promote the proliferation and differentiation of human adult MSCs. Furthermore, several preclinical studies have already demonstrated the efficacy of co-transplantation of adult MSCs and PRPs in a wide range of model systems [23-26].

Our study aims to expand the current knowledge about PTOA of the ankle with MSCs and PRP injections. In this paper, we would like to present our long-term results of treating 7 patients with PTOA of the ankle.

Methods

Design: Descriptive case series. A written informed consent was obtained in all patients.

Patients: n=7, patients with PTOA of the ankle ranging from Kellgren-Lawrence27 grades 2 to 4 were included. Mean age was 39.9 (SD, 11.5) years with a mean follow-up of 4.6 (SD, 3.1) years.

Baseline characteristics of patients are present in **Table 1**.

Table 1: Demographic and Baseline characteristics of 7 patients included in this study.

PATIENT	SEX	AGE	PTOA GRADE	VAS	FADI SCORE	FOLLOW UP
1	F	23	4	7	29.8/100	9y 7m
2	M	47	4	7	43.3	7y 5m
3	M	29	2	4	88	5y 2m
4	M	46	3	5	61.5	4y 7m
5	M	33	2	6	65.4	2y 7m
6	M	47	2	4	74	2y 1m
7	M	54	4	8	20.2	1y 0m

Figure 1 illustrates the severity of PTOA of the ankle in our first patient.

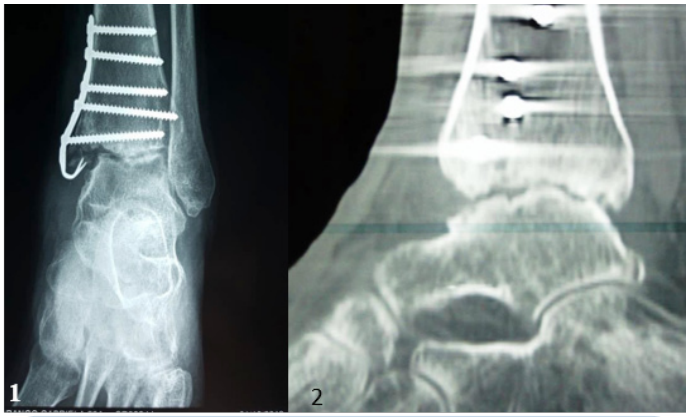


Figure 1&2: Patient 1. 23-year-old female patient with severe PTOA of the ankle, secondary to a Tibial Pilon Fracture type 43 C 1.3 according to the AO classification 28 treated with internal fixation. X ray and ankle CT scan 10 months after initial surgery showing severe joint line erosion.

Procedures

Matrix and neural regulation.

All patients were prepared before the application of stem cells with injections of 2% Procaine. One application per week for 3 weeks in the surgical scars - intradermal - and intra articular of the ankle, fluoroscopy guided. The repolarization of the cell membrane that is produced by the injection of procaine reduces pain at the level of the scar and increase joint mobility at the level of the intra-articular space.

Figure 2: Intradermic and intraarticular procaine injections in affected area in order to improve ECM and neural regulation.

Bone Marrow - MSC procedure (bmMSC).

Bone marrow aspiration was performed using a fluoroscopically guided percutaneous puncture of the posterior iliac crest. A total of 60 ml of blood was aspirated and decanted into 6 green (heparin) tubes and then centrifuged at 3000 rpm for 10 minutes. Subsequently, approximately 1 cc of the buffy layer of each of the six tubes was aspirated. A total of 5 cc of bmMSCs was obtained.

Adipose Tissue - MSC procedure (adMSC)

A simple method of liposuction and washing adipose tissue was used to isolate the cells. Local anesthesia with Klein tumescent solution was applied in the gluteal and trochanteric region. 40 ml of pure fat was extracted with two 60 ml syringes and a 2.5 to 3 mm diameter cannula with equal luer lock spike. Physiological solution was added and subsequently decanted in order to discard infranatant and remove traces of tumescence and blood. The technique involved mechanical agitation which breaks down the adipose tissue and releases the stromal cells. The decanting procedure was repeated 4 times or until blood was no longer present in the syringe. Five cc of washed adipose tissue was obtained. Next, the adMSCs were joined with the bmMSCs in the same syringe and percutaneously injection into the ankle joint with a total volume of around 5 cc.

Platelet Rich Plasma (PRP)

All patients received a percutaneous intra-articular injection of PRP during the 2nd or 3rd month after the initial MSC injection. PRP was obtained through a centrifugation process of 30 cc of ulnar venous blood. The acquired blood was centrifuged for 5 minutes at 2800 rpm in a plasma separation kit (Neogenesis PRP). The buffy coat obtained from the kit was around 1 or 2 cc. Using fluoroscopic or CT scan guidance, PRP was injected into the affected ankle.

We performed clinical evaluations every month and radiological evaluations every 6 and 12 months **(Figure 3).**

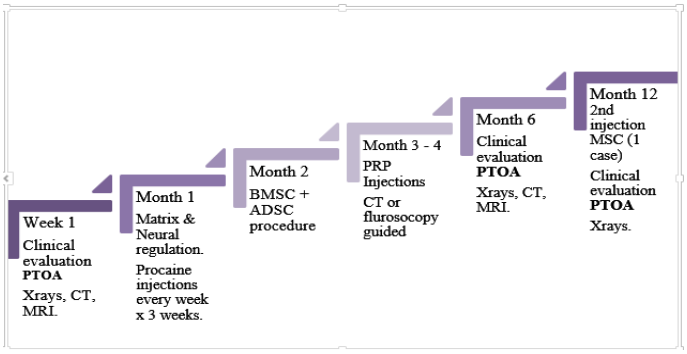


Figure 3: Timeline of the Protocol.

Outcome measures

For the evaluation of the grade of PTOA we used the Ankle and Hindfoot Radiographic Osteoarthritis Scoring and the modified Kellgren-Lawrence (KL) Grade.27 To rate stability, pain, and activity limitations we used the Foot & Ankle Disability Index (FADI) Score29 and Visual Analogue Scale(VAS) - both completed by the patients themselves.

Results

All patients received a single intra-articular injection of bmMSC & adMSC. Patient No. 1 received a 2nd injection of MSC (both bmMSC & adMSC) due to the severity of the case at 12 months. All patients received PRP during the 2nd month after the procedure.

Six months after the procedure the mean VAS score improved to 3 and the FADI score improved to 76.2. After 1 year, the mean VAS score (2.85) and FADI scores (78.1 SD, 25.) remained almost unchanged. The pain, edema and functional limitations of the affected ankle improved considerably in almost all cases, allowing patients to return to their level of physical activity and work prior to trauma, except for the last patient. Patient 7, in whom we did not get establish any improvement, was the only patient in whom we did not perform a neural regulation with procaine injections, prior to MSC procedures.

X rays and MRI showed evidence of recovery of the joint congruency in most of the patients. (Table 2, Figure 4 and Supplement 1). Two patients went from a KL score 4 before intervention to a KL score 2 after interventions; four patients from grades 3 and 2 went to grade 1. And 1 patient with grade 4 showed no improvement.

Obtained results are summarized in **Table 2 and Figure 4**.

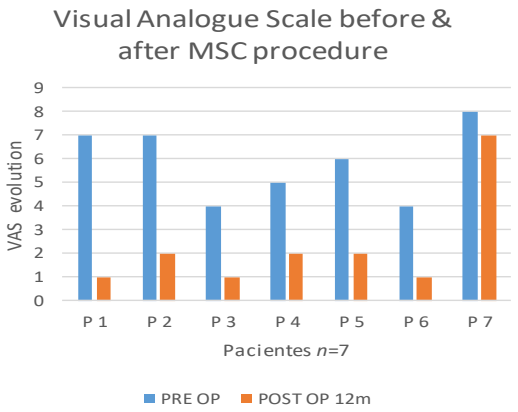


Figure 4: VAS evolution before and after interventions.

Imaging results

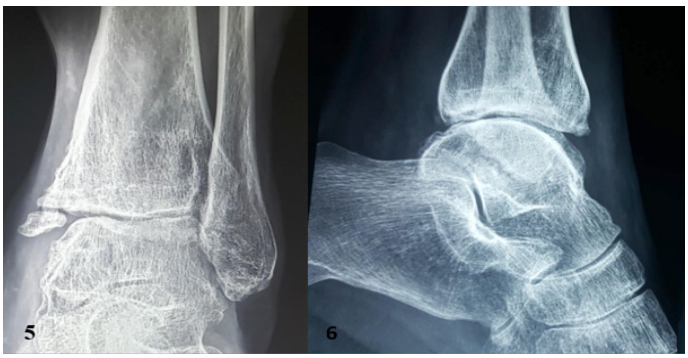


Figure 5&6: Patient 1. 6 years and 9 months after 2 MSC procedures and 2 PRP injections.

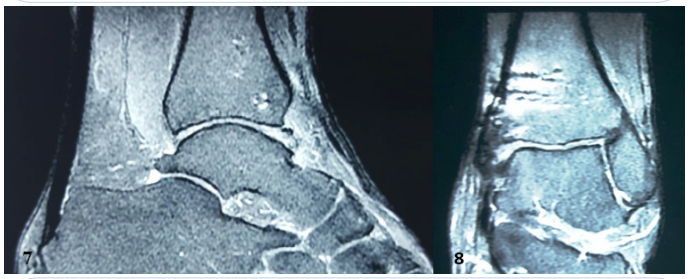


Figure 7&8: MRI. Joint line congruency is conserved. No pain or activity restriction at present time.

Table 2: FADI Results & follow up of the 7 patients after MSC & PRP injections.

PATIENT	AGE	PTOA Kellgren Lawrence grade	FADI (score) before MSC	FADI 6 months post MSC	FADI 12 months post MSC	FADI At the end of follow up	Total follow up
1	23	4	29.8/100	69.2	71.2	94.2	9y,7m
2	47	4	43.3	86.5	85.6	89.4	7y, 5m
3	29	2	64.4	88.5	91.3	93.3	5y,2m
4	46	3	61.5	93.3	94.2	93.3	4y,7m
5	33	2	65.4	96.2	94.2	95.2	2y 7m
6	47	2	74	76.9	86.5	90.4	2y, 8m
7	54	4	20.2	23.1	24.0	24.0	12m

All patients expressed satisfaction with the results of their treatment. Except one patient. There were no systemic or local adverse events observed after MSCs or PRP injections or throughout the entire follow-up period.

Table 3: Radiological & range of motion evolution after 12 months of treatment.

Patient	Kellgren Laurence PTOA Before MSC	Kellgren Laurence PTOA After MSC	Ankle & hindfoot range of motion Before MSC	Ankle & hindfoot range of motion After MSC
1	4	2	Marked restriction (less than 25%)	Normal or mild restriction (75%-100% normal)
2	4	2	Marked restriction (less than 25%)	Moderate restriction (25-74%)
3	3	1	Moderate restriction (25-74%)	Normal or mild restriction (75%-100% normal)
4	2	1	Moderate restriction (25-74%)	Normal or mild restriction (75%-100% normal)
5	2	1	Moderate restriction (25-74%)	Normal or mild restriction (75%-100% normal)
6	2	1	Moderate restriction (25-74%)	Normal or mild restriction (75%-100% normal)
7	4	4	Marked restriction (less than 25%)	Marked restriction (less than 25%)

Imaging results of the 7 patients are in the Supplement 1.

Supplement 1

Patient 1

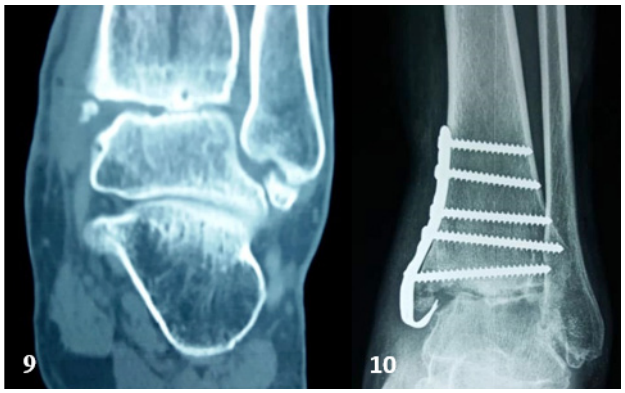
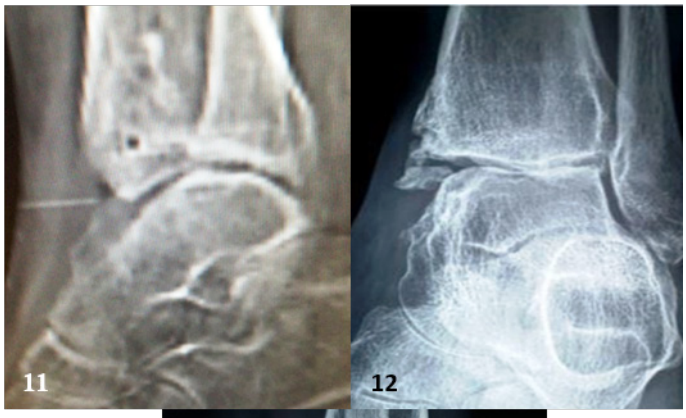


Figure 9&10: Patient 1. 23-year-old female patient with severe PTOA of the ankle, secondary to a Tibial Pilon Fracture. PTOA grade 4. CT and X ray before MSCs procedures.



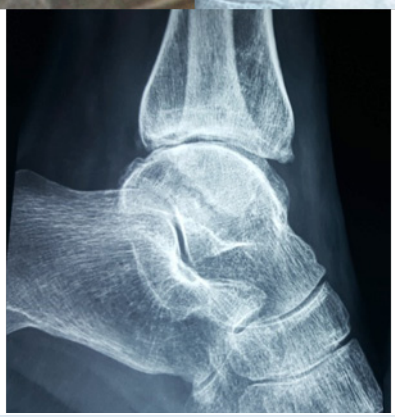


Figure 11,12&12.1: PRP guided MSCs intra articular injection, after hardware removed. **12 & 12.1**: Joint line remodeling 6 years after 2 MSCs treatments and 2 PRP procedures.

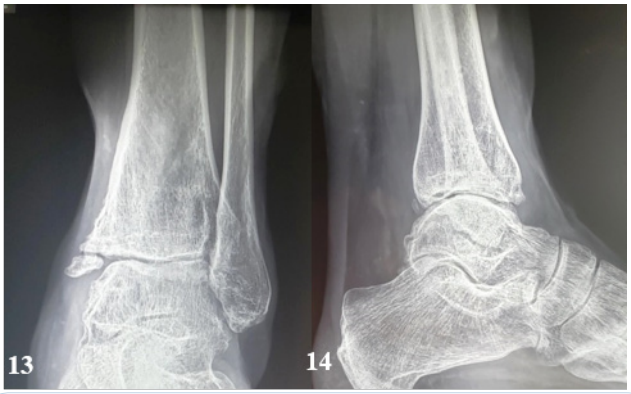
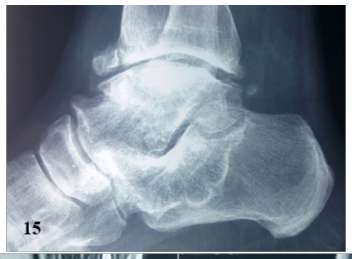


Figure 13&14: X rays views 8 years 3 months after bmMSCs and adMSC treatments.

Patient 2



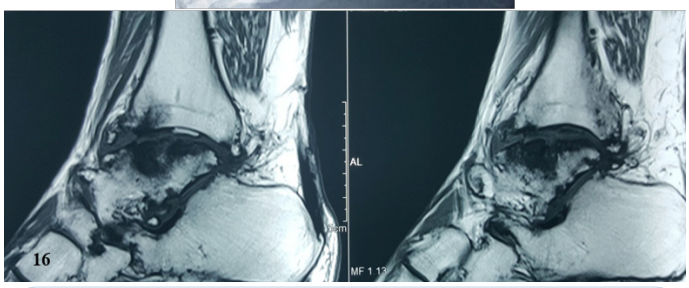


Figure 15&16: Patient 2. 47 years old male patient with grade 4 PTOA after Talus fracture fixation. X ray and ankle MRI, 9 years after fracture. FADI score before MSC and PRP treatment was 43.8.

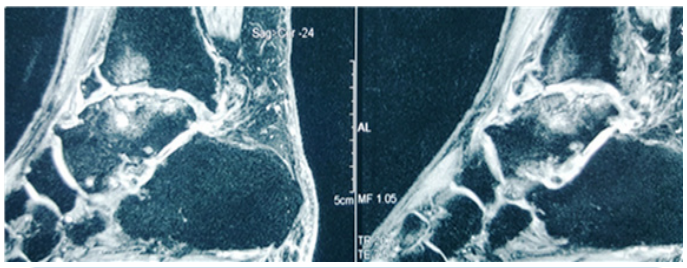


Figure 17: Patient 2: 47 years old male patient with grade 4 PTOA after Talus fracture fixation. Ankle MRI, 9 years after fracture and before MSC treatments.

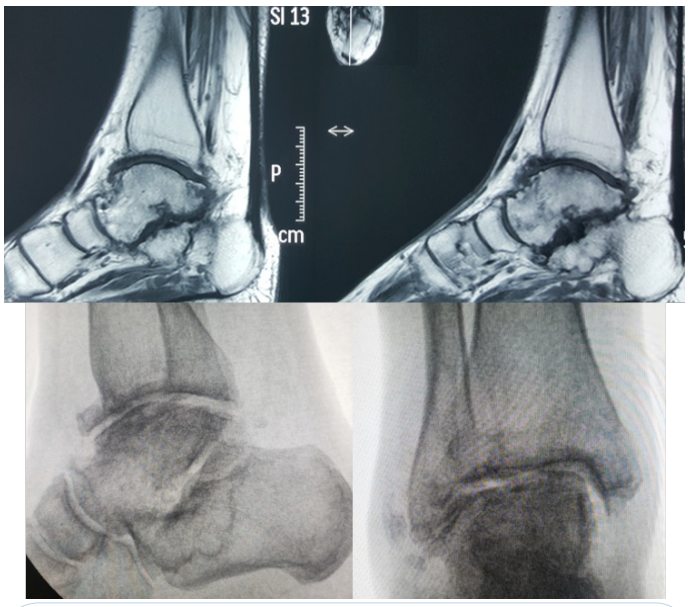


Figure 18&19: Patient 2. MRI 18 months after MSCs procedure.

Figure 19 Patient 2: X rays after MSCs procedure. VAS score at the end of follow up is 1. FADI score at the end of follow up 81.7.

Patient 3

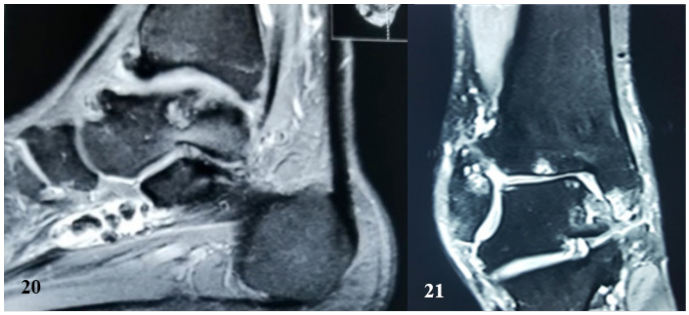


Figure 20&21: Patient 3: 46 years old male patient with PTOA Kellgren Lawrence grade 3, secondary to ankle sprain grade III. T1 and T2 ankle MRI.

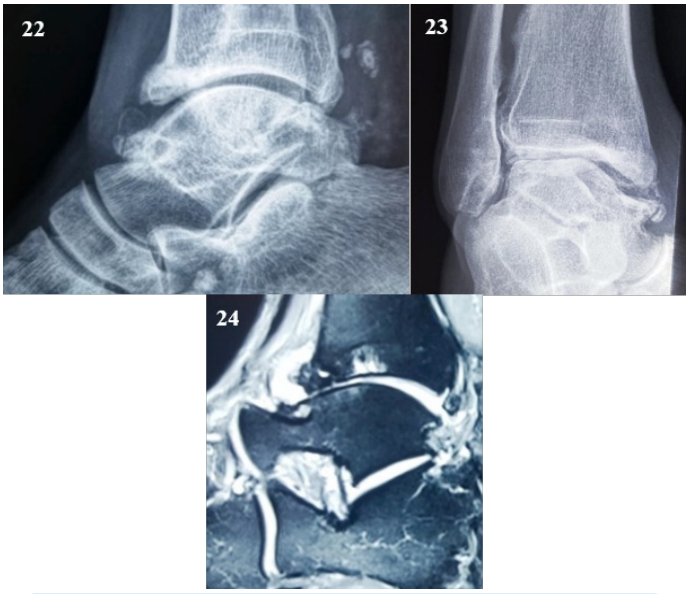


Figure 22,23&24: Follow up with X ray & MRI after 5 years. VAS: 1/10. Patient runs 4 miles daily.

Patient 4

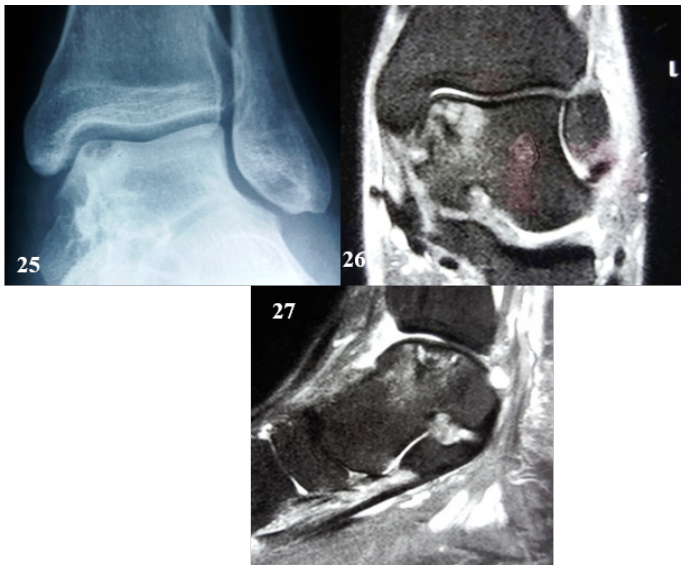


Figure 25,26&27: Patient 4: A 33 years old male patient with PTOA Kellgren Lawrence grade 2, secondary to talus osteochondritis post ankle sprain.

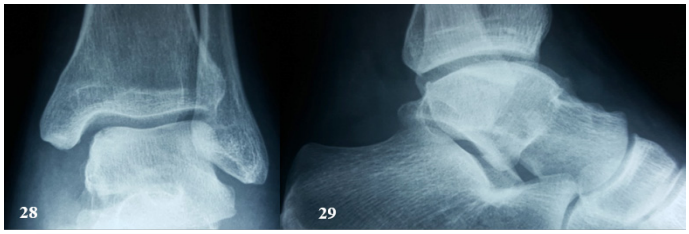


Figure 28&29: Patient 4: AX ray 8 months after MSC procedure. FADI score 94.2 at the end of follow up.

Patient 5

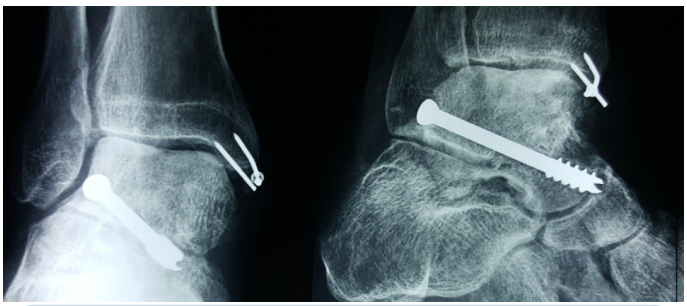


Figure 30: Patient 5: Ankle X ray of a 29 y/o male patient. PTOA post Astragalus & Tibial malleolus fracture.

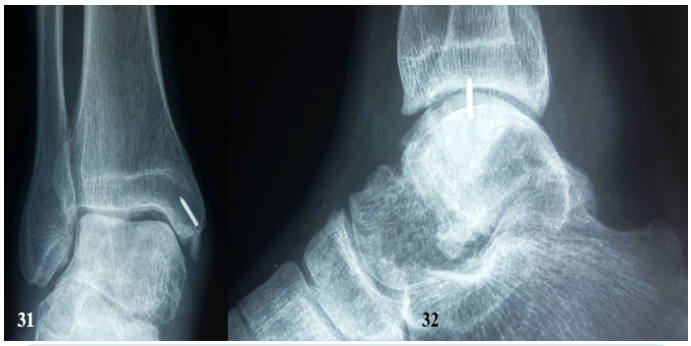


Figure 31&32: Patient 5: X rays 6 months after hardware removed and MSC & PRP injections. No pain very and very little restriction of physical activity. FADI at the end of follow up 5 years 10 months was 80.8.

Patient 6

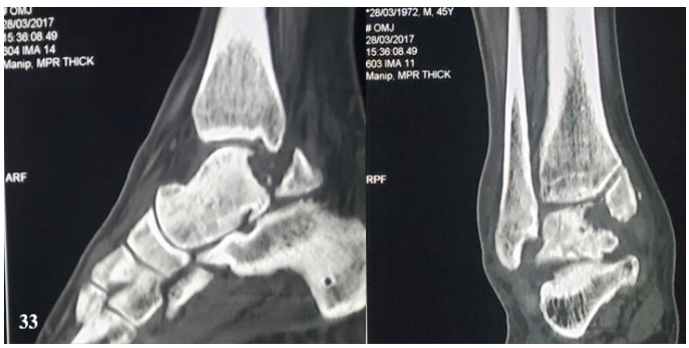


Figure 33: Patient 6: CT scan of 45 years old male patient with a Talus fracture treated 1 year before with external fixation without reduction of the astragalus. Patient denied to have ankle arthrodesis. VAS 6/10. FADI score 48.1.

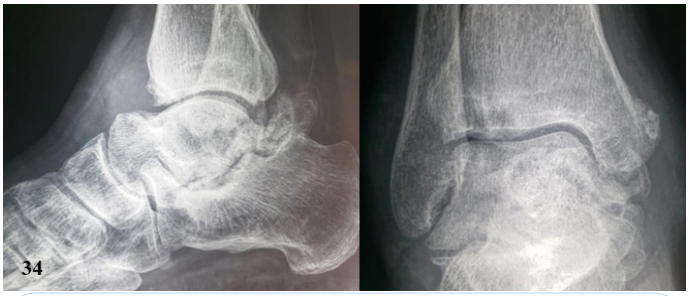


Figure 34: Patient 6: X rays 2 years 8 months after MSC and PRP procedures. Pain score is 1/10. FADI score is 74. Ankle & hindfoot range of motion after MSC changed from moderate to mild restriction.

Patient 7

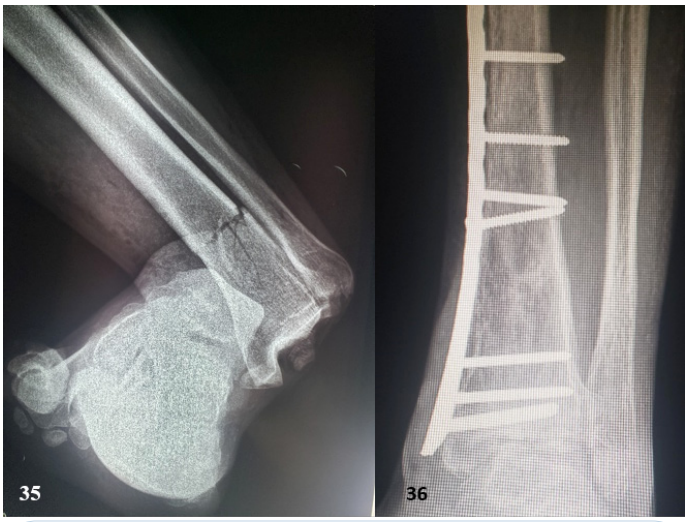


Figure 35&36: 39 y/o. Severe compression trauma with mine cart. Open distal tibial fracture grade 2. Two debridments surgeries and finally osteosinthesis.

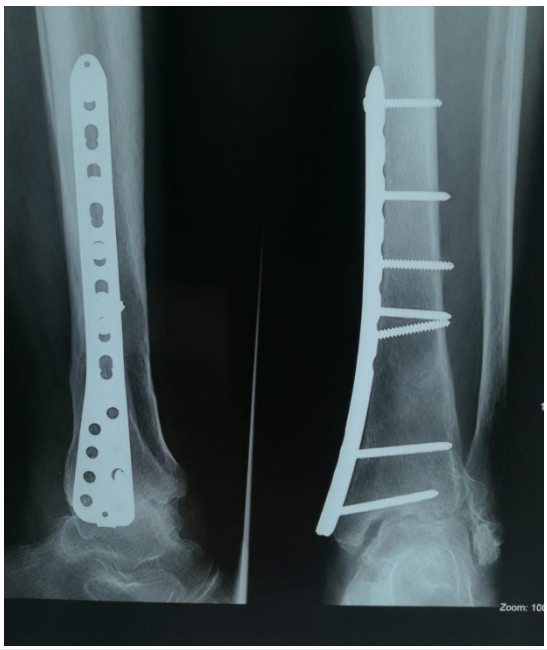


Figure 37: 12 months post op. MSC procedure and 3 distal screws removed. No radiological and clinical improvements. No reural regulation performed because patient live out of city.

Procedures

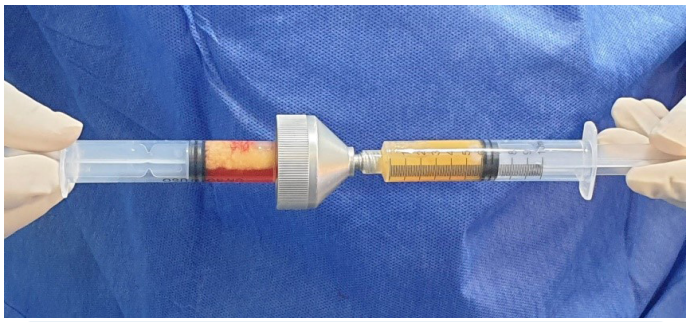


Figure 38: bmMSC and adMSC mixing before injection.

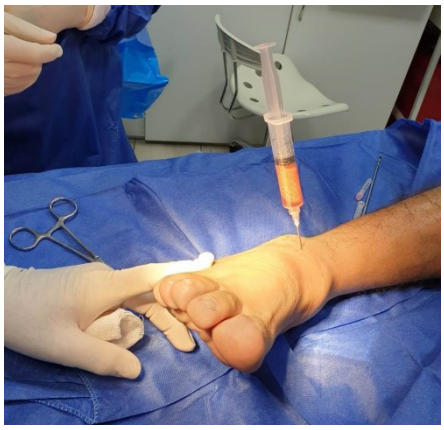


Figure 39: bmMSC and adMSC ankle injection.

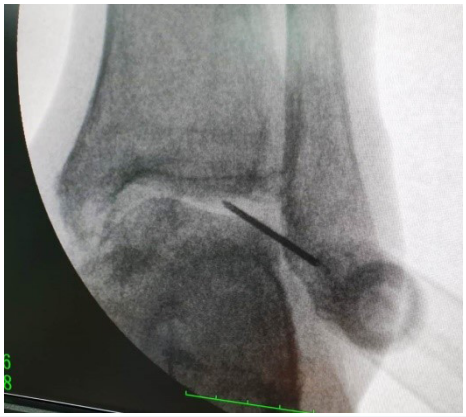


Figure 40: bmMSC and adMSC fluoroscopic guided ankle injection.

Discussion

The current study investigated the clinical and radiological evolution after intra-articular injections with MSCs and PRP in patients suffering from PTOA of the ankle for a mean period of 4.6 years. Patients' clinical symptoms and pain improved significantly at 6 and 12 months after the procedure, except in 1 patient, where neural and ECM regulation was not performed. Range of motion increased in all patients and no adverse events were encountered.

The perception of pain is typically associated with inflammation, a complex biological response of the somatosensory, immune, neuronal, autonomic and vascular/circulatory system to tissue damage, pathogens, or irritants [30]. In chronically inflamed tissues, aberrant ECM expression and fragments of the ECM that are derived from tissue-remodeling processes can influence immune cell activation and survival, thereby actively contributing to immune responses at these sites [31].

Peripheral sensitization, which is marked by a state of hypersensitivity and hyperexcitability of nociceptors as a result of tissue injury and inflammation, is caused by the activation of a varied collection of ion channels [32,33], sodium channels [34,35]. Local anesthetics block voltage-gated sodium channels in the axon, and exert beneficial effects on pain and can affect the inflammatory response and the hemostatic system [36]

The objective of procaine injections is the generation of a directed stimulus (through the needle) and the selective extinction of other stimuli (through the LA), thus affecting the nervous system and tissue perfusion [37] and allowing the interruption of positive feedback loops (vicious circles) of pain and other pathological processes [38,39].

Regarding MSC safety and complications, Centeno et al [40]. reported that complications were generally infrequent, transient and usually resolved with simple therapeutic measures. We did not find complications related to the procedure, except in patient 6, who presented pain and post-operative edema in the ankle injection area.

The concept that MSCs may prevent PTOA after intra-articular fracture is consistent with the role of endogenous stem cells after bone and cartilage injury. After a long bone fracture, MSCs reach the fracture site to instigate endochondral ossification as part of the physiological repair process [21]. The results obtained by Wittig et al.[41] support the use of autologous MSCs and PRP for the treatment of non-union fractures.

Regenerative Medicine according to Caplan [42], is based on the ability of MSCs to provide a home for injured tissues, as well as being part of the response to injury, providing a wide range of paracrine factors through their trophic activity, cytokines/growth factors, such as PDGF, in the wound area [42].

While some authors [4-45] have used PRP with bone marrow concentrate in cell therapies, the vast majority have used it with adMSC [46,21,47]. This cell–PRP interaction may increase stemness and prolong the survival time and rate of cells in the PRP.[46] This is why we intend to keep regulation of the extracellular matrix at the injury tissues through a 2nd injection of PRP during the 2nd or 3rd month post MSC injection. The effect of an autologous MSC/PRP admixture on bone regeneration has illustrated nicely in a rat calvarial defect model and was attributed to the osteogenic potential of MSCs, which in turn received stimulus from cytokines released by the platelets [25].

AdMSCs, like bmMSCs, have shown promise in regenerative medicine[48]. Clinical and pre-clinical studies show that autogenous adMSC demonstrably remain alive after transplantation, show pluripotent differentiation [49-52], and disclose anti-inflammatory properties, and the formation of new blood vessel growth [53-57]. We performed all procedures mixing bmMSC and adMSC in order to get a higher number of cells and different functions like chondrogenic and angiogenic potentials. Adipose tissue contains approximately 500-2500 times more mesenchymal stem cells compared to the same volume of bone marrow [58]. While AdMSCs are capable of proliferative capacity, and immunomodulatory effects, bmMSCs show osteogenic and chondrogenic differentiation potential [59].

Other studies uncovered the potential of intra-articular injections of MSC's in preventing PTOA., Using such a technique, Diekman et al.[60] were able to prevent the development of PTOA in mouse knees 8 weeks after intra-articular fracture.

Emadedin et al. [61] demonstrated in patients with knee, hip and ankle OA that transplantation of BM-derived autologous MSCs was not associated with adverse effects and was considered completely safe. Their study furthermore showed that this procedure had therapeutic benefits regarding pain and function.

Strengths and Limitations

Our study is a case series with small sample size and a clinical trial is needed to confirm our results.

Future studies should be performed to determine the standard number of MSCs required for each injection. Cytokines and growth factors control cell proliferation. We need to determine the quantity and timing for PRP injections to establish standard procedures.

Conclusions

Our study demonstrated that autologous injection of bmMSC, adMSC and PRP in patients with PTOA of the ankle is a safe and effective treatment, capable of reducing pain, increasing joint movement and improving quality of life in patients. Neural regulation and ECM improvement through procaine injections before MSC procedures showed differences. These results justify further exploration of this promising technique.

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