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Intra-articular injection of bone marrow concentrate protocol for Osteoarthritis – A preliminary report with 12 months follow up

VR Ravi[1,*], Avinash Gandhi[2], S. Sankaranarayanan[3], K. Manimaran[4]

[1]Consultant Orthopedic Surgeon, Maruti Hospital, Trichy, [2]Sc. Co-ordinator, [3]Director, Mothercell Regenerative Center, Trichy, Tamil Nadu, [4]HOD, Dept. of Oral & Maxillofacial Surgery, KSR Institute of Dental Science & Research, Tamil Nadu

***Corresponding Author:**
Email: ravidhyas20@yahoo.com

Abstract

Osteoarthritis – a degenerative joint disorder by loss of cartilage leading to pain and physical function of the patients. Most existing Intra articular injections show promising results in pain relief but none have regenerative potentials. With the existing published evidences and clinical trial an attempt was made by Intra articular injection of autologous bone marrow concentrate in 10 Osteoarthritis patients with 12 months follow up. Bone marrow was concentrated using protocols of Biosafe Sepax and on an average 7ml of concentrate was injected in each defective knee. Patients showed improvements by pain relief and physical function. Apart from WOMAC pain score and VAS the improvement was also found in increase of joint space and cartilage volume evident from post-operative X ray and MRI, which might be due to the regeneration of cartilage. No adverse events were noted in this study.

Keywords: Bone marrow Concentrate, Bone marrow mononuclear cells, Knee osteoarthritis, Biological regeneration

Introduction

Osteoarthritis (OA) is a degenerative condition of the connective tissues of the joints which is characterized by loss of articular cartilage, hypertrophy of bone at the margins, sub-chondral sclerosis and range of biochemical and morphological alterations of the synovial membrane and joint capsule. This degeneration is due to the imbalance in the ongoing breakdown and repair of the joint tissue.(1) Most common symptoms are joint pain, stiffness and limited mobility. The disease progression is slow but finally ends up in joint pain and functional disability. As a degenerative joint disorder the cartilage material is found to decrease with chondrocyte apoptosis, matrix loss and bone spur (osteophytes) formations are also noted. Therapies for OA can be of non-regenerative approach (physical therapy, NSAID, Steroids and visco supplements) or Regenerative approach which includes treatment with Platelet Rich Plasma (PRP), Biologicals, growth factors and stem cells based approaches. This approach has attempted to elicit the role of regenerative techniques restoring the articular cartilage (chondrocyte and the matrix) and other soft tissues like injured menisci, capsule and ligaments.(2) Intra articular steroids have been used for more than 40 years and Intra articular hyaluronic acid injections approved by FDA have been used for 20 years, yet the problem is unresolved. Most existing Intra articular injections show promising results in pain relief but none have regenerative potentials. Intra articular steroids have been reported to worsen degeneration / deterioration of articular cartilage and hence repeated injections are avoided.(3)

It was so far believed that cartilage defects cannot heal spontaneously and the poor self-healing capacity is probably due to the poor blood supply and low metabolic activity in cartilage. Recent research has identified that biophysical alterations are bought by the cytokines (small proteins from cells) and matrikines (peptides from Matrix) in the joint. It is possible to reverse joint pathology by favourably altering cytokine/ matrikine level through regenerative techniques.(4) If injured cartilage is not treated properly, it affects the biomechanics of the joint, and ultimately degenerates into OA. Along with joint dysfunction and cartilage loss the degeneration includes changes in bone and synovial membrane. The regeneration of cartilage does occur at childhood but gets reduced as it is aged.(5) As reported by few studies it is suggestive that the biological mechanism & pathophysiology for the cause of OA knee can be reversed by cell based therapy.(6) In this article we have reported the case series for 10 individuals with OA (grade 2 & 3) with autologous bone marrow aspirate concentrate as an approach based on regenerative therapeutics. Several Indian and international(7) studies are reported using various sources of stem cells both cultured and fresh isolates from bone marrow(8) and fat(9) as autologous & umbilical cord MSCs as allogenic source.

In our present study we want to evaluate the safety and efficacy of autologous bone marrow derived stem cells in treatment of Osteoarthritis for therapeutic chondrogenesis through delivery of stem cells into the knee joint space in 10 Indian patients and the objective is to record the pain relief and functional improvement by scoring scales.

Materials and Method

This case series was conducted in Dept. of Orthopaedics, Maruti Hospital, Trichy, Tamilnadu. This

study protocol was approved by the Maruti Hospital institutional ethical committee. Informed consent was obtained from all 10 individual participants included in the study. The following inclusion and exclusion criteria was fulfilled to take up,

Inclusion Criteria:

1. Both Male and Female subjects 40 years of age or older
2. Subjects must have symptomatic OA > 3 months.
3. Osteoarthritis may be primary or secondary. Knees must have Kellgren-Lawrence Grades 2 & 3.
4. Subjects must have previously tried 6 weeks of one of the following conservative treatments Activity modification, weight loss; physical therapy, anti-inflammatory or injection therapy
5. Patients can provide written informed consent after the nature of the study is fully explained

Exclusion Criteria:

1. Patients with abnormal hematology, serum chemistry, or urinalysis screening laboratory results.
2. Patients taking anti-inflammatory medications (prescription or over-the-counter), including herbal therapies, within 14 days of baseline visit.
3. Patients taking anti-rheumatic disease medication (including methotrexate or other antimetabolites) within the 3 months prior to study entry.
4. Patients receiving intra articular injections to the treated knee within 2 months prior to study entry.
5. Patients who are pregnant or currently breast-feeding children.
6. Patients with systemic, rheumatic or inflammatory disease of the knee or chondrocalcinosis, hemochromatosis, inflammatory arthritis, arthropathy of the knee associated with juxta-articular Paget's disease of the femur or tibia, ochronosis, hemophilic arthropathy, infectious arthritis, Charcot's knee joint, villonodular synovitis, and synovial chondromatosis.
7. Patients with ongoing infectious disease, including HIV and hepatitis
8. Patients with clinically significant cardiovascular, renal, hepatic, endocrine disease, cancer, or diabetes
9. Acute traumatic or sports related injuries

In 10 patients, 5 males and 5 females (aged between 55-80 years) were treated with autologous bone marrow aspirate concentrate. The radiological evaluation of all 10 patients with knee pain was done and OA severity was scored using Kellgren-Lawrence scale,[10] The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Score and visual analogue score (VAS). The patients were followed up for more than 12 months. WOMAC and VAS data for first 6 months is being provided in this study.

Primary Outcome

1. Safety and tolerance, assessed by adverse events
2. Patient's Assessment of Arthritis Pain

- Osteoarthritis-Pain Visual analog scale (VAS)

Secondary Outcome

1. Change from baseline in the WOMAC OA (Western Ontario and McMaster Universities Osteoarthritis) Index -pain subscale score
2. Reduction in intake of analgesic tablets
3. WOMAC Osteoarthritis stiffness Index
4. Increased joint space width after 6 and 12 months in standing x ray
5. Increased cartilage volume in the different regions of the knee after 6 and 12 months in MRI

Aspiration of Bone Marrow & Processing: Under aseptic condition and spinal anaesthesia for 8 patients & local with IV sedation for 2 patients in supine position a stab incision was made at anterior third superior iliac crest. Bone marrow aspiration needle (11G, 10cm, Osteo-Site, Cook) was inserted and placed in the marrow cavity, marrow was collected from multiple sites (serial withdrawal & angulation) via heparin rinsed 50ml Syringe (BD Precise, leure lock). The collected volume (average 125ml) of bone marrow was mixed with ACD solution in a blood transfer bag in a ration 8:1 respectively. This bone marrow was concentrated using Bone Marrow Processing Kits of BioSafe Sepax (S-100) with the protocol UCB-HSC-V218and the instructions were followed as per instrument manual.[11] The final bone marrow concentrate (BMAC) volume was fixed to 12ml. 10ml of bone marrow concentrate was injected by intra-articular injection in both knees (Fig. 1). 2 mL was taken for *in vitro* culture studies.

Fig. 1: Bone marrow Concentration Procedure A) Aspiration of bone marrow with Cooks Osteo-Site 11G/10cm Needle B) Pooling of collected bone marrow C) Separation of BMMNC via Sepax Protocol D) Separated BMMNC E) Collected Bone marrow concentrate F) intra articular injection of BMMNC

Counting of injected cells: The obtained BMAC has a mixed population of cells including RBC and other nucleated cells including a small population of stem

cells and its precursors.[12] 20µl of BMAC was taken in slide for staining with trypan blue dye (1:1) to assess the viability of mononuclear fraction and number of cells injected.[13]

***In-vitro* Studies – Colony Forming Units and presence of MSC:** In order to find the presence of mesenchymal stem cells present in the bone marrow concentrate obtained by sepax procedure, 2 ml of it was taken for *in vitro* culture studies[14] at Mothercell Regenerative Centre Pvt Ltd Fig. 2. After confluence the cultured cells were evaluated via flow cytometry(FACS) for mesenchymal marker Fig. 3.

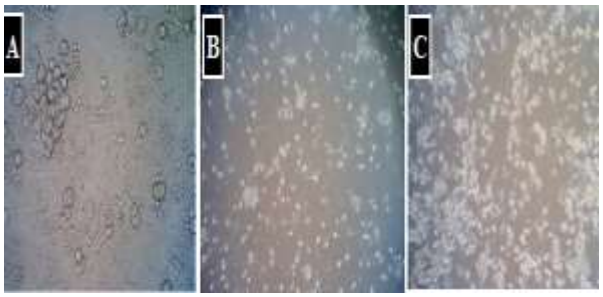

Fig. 2: In-vitro culture of BMMNC A) CFU Formation 40x B) Day 7 culture shows fibroblast morphology like cells C) Cells at day 21

Fig. 3: Flow cytometric analysis cultures (Passage 3) BMMNC showing significant concentration of mesenchymal fraction

Data analysis

Statistical methods: Descriptive statistics were generated for all variables. Continuous data following a normal distribution were summarized with means and standard deviations (SDs).

Result

All 10 patients were monitored for 24hrs after BMAC injection for acute adverse events and none were reported. Two patient had slight pain at aspiration site and none at injected site. No fever or systemic symptoms due to injection were reported.

The preliminary study group with 10 patients aged between 55 to 79 years (Mean 62.6 ± 5.73) with an BMI of 31.35 kg/m^2 (SD 3.8 kg/m^2) at the time of injection. An average of 125 ml (SD: 17.16ml) of bone marrow was aspirated and concentrate to final volume 10ml (SD: 2.75ml). Out of 10 patients 7 had bilateral osteoarthritis and 3 had in one knee, hence 7 patients received intra articular injection for both knees and 3 received for 1 knee. The BMAC was counted for the Total nucleated cell (TNC) before injection and an average of 134 x 10^9 TNC/ml SD (17.26 x 10^9 TNC/ml) was injected in each knee. There was no much difference in the no. of nucleated cells with age or sex in our preliminary study as the sample size is small. It was also noted that the amount of yellow marrow increased with aged patients (Table 1).

Table 1: Patient Demographic Characteristics at Base line and Total nucleated cells injected

Case	Sex	Age	BMI	KL Grade	WOMAC Total	VAS	Bone Marrow		TNC injected (Cells x 10^9/ml)
							Volume Collected (ml)	Final volume (ml)	
1.	F	55	31.6	2	70.45	60	110	10	135
2.	F	66	28.9	2	90.90	78	135	7	112
3.	F	70	33.6	3	94.31	69	125	10	105
4.	F	60	32.4	3	94.31	80	90	7	128
5.	F	62	39	3	93.18	72	130	10	146
6.	M	79	30.1	3	82.14	66	140	15	132
7.	M	66	34.8	3	88.57	75	135	10	125
8.	M	55	32	2	93.18	83	120	7	151
9.	M	62	33.3	2	90.90	74	115	10	147
10.	M	58	28.1	2	78.55	85	150	14	159
Mean ± SD		62.6 ± 5.7	31.85 ± 3.8		87.65 ± 8.04	74.2 ± 7.77	125 ± 17.16	10 ± 2.74	134 ± 17.26

BMI – Body Mass Index
WOMAC – Western Ontario and McMaster University

VAS – Visual Analog Scale
TNC – Total Nucleated Cells

From the WOMAC scores of base line and the post-operative follow up of 6 months it is evident that the BMAC treatment is helping in the patients' improvement. As per the VAS scale the pain relief was from 76 to 24 (Fig. 4). The total WOMAC score was reduced from 86.24±8.4 to 31.01±3.5 (Fig. 5) and the physical function WOMAC score gradually decreased from 77.18 ± 7.12 to 28.14 ± 1.2 (Fig 8) similarly the WOMAC Score for Pain and stiffness was also found to be reduced from 87.5±8 to 24±5.2 and 81.25±14.7 to 27.5±5.3 respectively (Fig. 6 & 7). There was also case in which the post-operative X ray showed increase in joint space, which might be due to the regeneration of cartilage (Fig. 8). The MRI report of another patient showed improvement in cartilage regeneration (Fig. 9). 8/10 patients did not have visual evidence of cartilage regeneration but had relief of pain and improvement in functional activities which was evident from VAS and WOMAC Score.

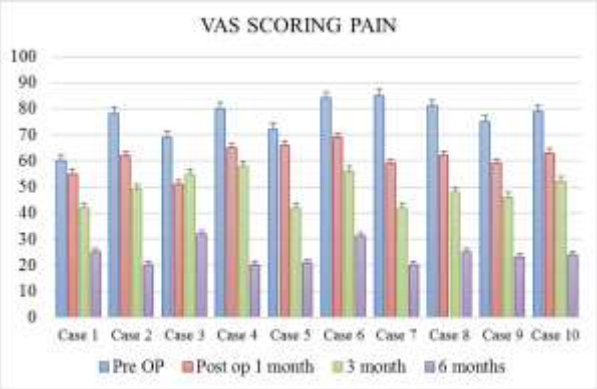

Fig. 4: Visual Acute Score for Function in Sports and Recreation at time 0, 30, 90 and 180 days. The standard deviation at each data point is represented by the vertical black lines

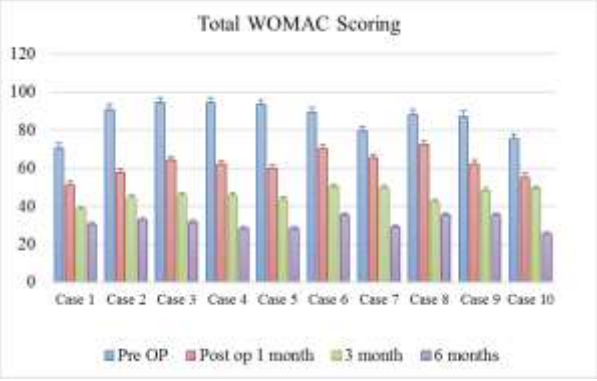

Fig. 5: Total WOMAC Score for Function in Sports and Recreation at time 0, 30, 90 and 180 days. The standard deviation at each data point is represented by the vertical black lines

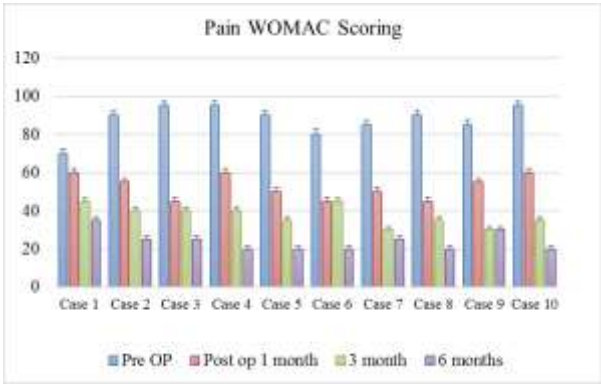

Fig. 6: Pain WOMAC Score for Function in Sports and Recreation at time 0, 30, 90 and 180 days. The standard deviation at each data point is represented by the vertical black lines

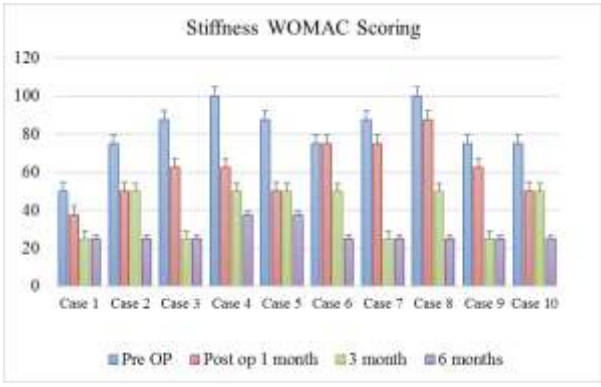

Fig. 7: Stiffness WOMAC Score for Function in Sports and Recreation at time 0, 30, 90 and 180 days. The standard deviation at each data point is represented by the vertical black lines

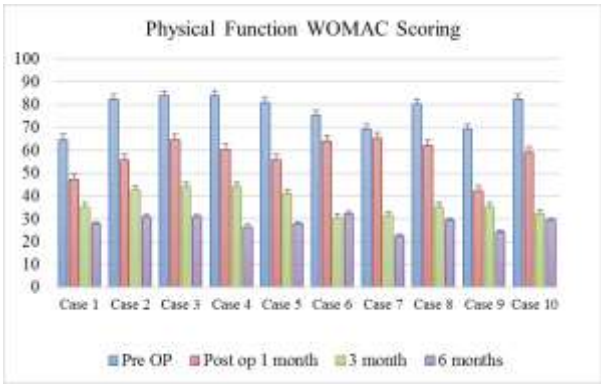

Fig. 8: Physical Function WOMAC Score for Function in Sports and Recreation at time 0, 30, 90 and 180 days. The standard deviation at each data point is represented by the vertical black lines

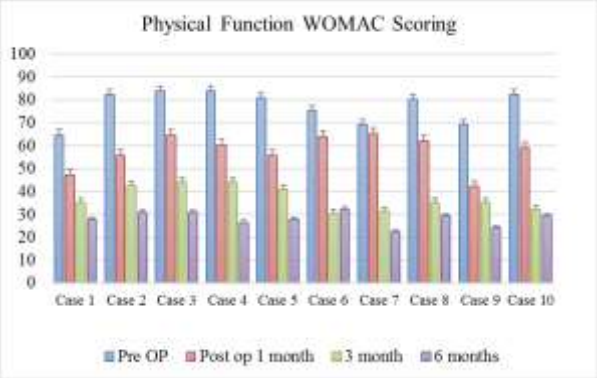

Fig. 8: Physical Function WOMAC Score for Function in Sports and Recreation at time 0, 30, 90 and 180 days. The standard deviation at each data point is represented by the vertical black lines

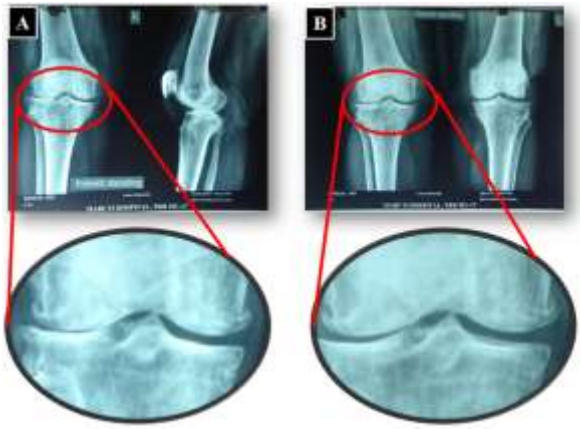

Fig. 9: X-Ray evidence of increase in joint space after intra articular injection of BMMNC (Case 1) (A) Pre operative X ray (B) X-ray - Post Operative 6 months with increased joint space

Fig. 10: MRI evidence of increase in joint space after intra articular injection of BMMNC A) Pre operative X ray B) X-ray - Post Operative 6 months with increased joint space

Discussion

In this study we have investigated the efficacy of intra articular injection of BMMNC for OA pain patients and found that the BMMNC are effective in pain relief and improvement in functional status.

In OA the biochemical and chemical changes of the cartilage makes the knee unable to withstand normal stress. This is due to the inadequate synthesis of extracellular matrix. Self-regeneration of cartilage is a slow process, which includes the formation of chondrocytes, cartifibers etc. The production of inflammatory cytokines (TNF-α, IL6 and nerve growth factor) by the synovial membrane & subchondral bone has been reported to be the cause for OA pain.[15,16] Injection of stem cells, bone marrow mono nuclear cells into the space is to support self-healing. The notable outcome will be the relief from OA symptoms (pain) and finally improvement in the functional status of the patient. This relief of pain might be due to the anti-inflammatory characteristics of BMMNC that could have altered the inflammatory cytokine atmosphere around the defective site. Jo, Lee[7] have stated that there are more number of platelets present in bone marrow concentrate that helps in pain reduction by peripheral endocannabinoid related pathway & NFκB pathway there by producing more endogenous hyaluronic acid.[17] The work of Wu, Leijten[18] also add evidence that stem cells has cytokines including Transforming Growth Factor beta (TGFβ), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF) and an array of bioactive molecules that stimulate tissue repair. These factors also helps in the formation of chondrocytes and cartilage matrix. In our study we have noted that 10/10 where relieved from pain after 1 week of BMMNC injection. The stiffness was also to be reduced with gradual increase in functional activities. The single injection of BMMNC had a relief of pain for more than 9 months.

The use of biologicals like PRP, BMMNC[19] and cultured cells[20] for the treatment of OA knee has made a revolution for the past few decades as minimally invasive procedures.[19] The bone marrow concentrates or BMMNC procedures has been used in many traumatic and degenerative joint disorders such as meniscus tears, osteochondral lesions, Avascular necrosis[21] and Osteonecrosis.[22]

OA was considered to be found reportedly in elderly individuals but meta-analysis of Ajuied, Wong[23] provided evidence for young individuals being affected with symptomatic OA knee due to sports/ accident related anterior cruciate ligament tear or meniscal tear. In our study the least age was 55 years.

Most of the published clinical data for OA knee with Bone marrow concentrate has used VAS, KOOS, WOMAC scores as a parameter of evaluation[24] and in our preliminary report we have also used VAS & WOMAC scores along with the visible evidence of increase of joint space in one patient in X-ray and another showed cartilage volume growth in MRI results and 8/10 did not have a visible improvement via x-ray but were free of pain and where able to do their regular day to day functions without any discomfort.

Basic research on animal model studies have reported promising results for using bone marrow concentrate/ BMMNC in treating cartilage pathology.[25-27] The autologous bone marrow concentrate contains heterogeneous population of

nucleated cells including mononuclear cells, hematopoietic stemcells, mesemchymal stem cells & platelets along with bioactive molecules like cytokines and cells with anti-inflammatory properties. Iliac crest derived BMC has been reported to contain the highest numbers of MSCs.[28] In our study we have injected nearly 134±17.26 x 10^9 cells /ml via intra articular route. The number of MSC present in the sample was taken for separate in vitro study. The cultured MSCs showed plastic adherent potential and fibroblast morphology. Similar reports of plastic adherence and fibroblast morphology of bone marrow derived MSCs were published by Alvarez-Viejo, Menendez-Menendez.[29] They have also stated that the number of MSC in BMMNC varies between individuals and it ranges from 0.0017 to 0.0201% of the total nucleated cells. The number of progenitors and no of colony forming units influence the regeneration of cartilage. The results of Hernigou, Poignard[22] published a clinical data of their experience that the prevalence of connective tissue progenitors in bone marrow in the iliac crests of patients was approximately one per 30000 nucleated cells. In our study we have injected 7 ml of bone marrow concentrate for one defective knee, which had 134 ± 17.26 x 10^9 mononuclear cells /ml.

In the field of orthopaedics the use of stem cells was proposed as early as 1985 by Dr. Hernigou. He uses patient's bone marrow to treat a debilitating disease known as avascular necrosis over the past 30 years and has not reported events like malignancies and mortality etc. His clinical experience with aspiration of bone marrow in more than 1000 patients had no complications. Recent publications have confirmed the efficiency of bone marrow aspiration procedure also.[21]

The works of Centeno and team has given more clinical results with bone marrow concentrate, stromal vascular fraction, MSC or combinations cell along with growth factors by means of platelet rich plasma and platelet lysates injected in subsequent follow-up visits for better results.[20] In their first published clinical results they have proved both chondral volume[30] and meniscus volume.[31] In their extensive clinical data on OA Knee a larger sample of 339 patients have seen pain relief from 50 to 70% and where followed up for 11 months.[32]

Conclusion

Intra-articular injections of BMMNC have resulted in pain and functional improvement in a number of preclinical and clinical trials. Our preliminary results of 10 cases with follow up of 1 year had good results in OA with relief of pain and restoration of functional activities in patients with no adverse events recorded. With improving MRI and other imaging technologies like cartigram & using nanomaterial for stem cell tracking we can show evidence of cartilage regeneration more convincingly in further studies, possibly differentiating between fibrocartilage and hyaline cartilage.

References

1. Hutton, C., Osteoarthritis: the cause not result of joint failure? Annals of the rheumatic diseases, 1989. 48(11): p. 958-961.
2. Centeno, C., et al., Efficacy of autologous bone marrow concentrate for knee osteoarthritis with and without adipose graft. BioMed research international, 2014. 2014.
3. Hauser, R.A., The deterioration of articular cartilage in osteoarthritis by corticosteroid injections. Journal of Prolotherapy, 2009. 2: p. 107-23.
4. Docheva, D., et al., Biologics for tendon repair. Advanced drug delivery reviews, 2015. 84: p. 222-239.
5. Goldring, M.B. and S.R. Goldring, Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. Annals of the New York Academy of Sciences, 2010. 1192(1): p. 230-237.
6. Koelling, S. and N. Miosge, Stem cell therapy for cartilage regeneration in osteoarthritis. Expert opinion on biological therapy, 2009. 9(11): p. 1399-1405.
7. Jo, C.H., et al., Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. Stem cells, 2014. 32(5): p. 1254-1266.
8. Sampson, S., et al., Intra-articular bone marrow concentrate injection protocol: short-term efficacy in osteoarthritis. Regenerative Medicine, 2016. 11(6): p. 511-520.
9. Tantuway, V., et al., Use of autologous adipose tissue derived stromal vascular fraction in treatment of knee osteoarthritis and chondral lesions.
10. Kellgren, J. and J. Lawrence, Radiological assessment of osteo-arthrosis. Annals of the rheumatic diseases, 1957. 16(4): p. 494.
11. Gastens, M.H., et al., Good manufacturing practice-compliant expansion of marrow-derived stem and progenitor cells for cell therapy. Cell transplantation, 2007. 16(7): p. 685-696.
12. Martin, D.R., et al., Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. Experimental hematology, 2002. 30(8): p. 879-886.
13. Darlington, G.J., Viability Staining of Mammalian Cell Cultures. Cold Spring Harbor Protocols, 2007(6): p. pdb.prot4769.
14. Pösel, C., et al., Density gradient centrifugation compromises bone marrow mononuclear cell yield. PLoS ONE, 2012. 7(12): p. e50293.
15. Orita, S., et al., Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee. BMC musculoskeletal disorders, 2011. 12(1): p. 144.
16. Charlier, E., et al., Insights on Molecular Mechanisms of Chondrocytes Death in Osteoarthritis. International Journal of Molecular Sciences, 2016. 17(12): p. 2146.
17. Sundman, E.A., et al., The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. The American journal of sports medicine, 2014. 42(1): p. 35-41.
18. Wu, L., et al., Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation. Tissue Engineering Part A, 2011. 17(9-10): p. 1425-1436.
19. Kim, J.-D., et al., Clinical outcome of autologous bone marrow aspirates concentrate (BMAC) injection in

degenerative arthritis of the knee. European Journal of Orthopaedic Surgery & Traumatology, 2014. 24(8): p. 1505-1511.
20. Centeno, C.J., et al., Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells. Medical hypotheses, 2008. 71(6): p. 900-908.
21. Hernigou, P., et al., Stem cell therapy for the treatment of hip osteonecrosis: a 30-year review of progress. Clinics in orthopedic surgery, 2016. 8(1): p. 1-8.
22. Hernigou, P., et al., Cell therapy of hip osteonecrosis with autologous bone marrow grafting. Indian journal of orthopaedics, 2009. 43(1): p. 40.
23. Ajuied, A., et al., Anterior cruciate ligament injury and radiologic progression of knee osteoarthritis: a systematic review and meta-analysis. The American journal of sports medicine, 2014. 42(9): p. 2242-2252.
24. Chahla, J., et al., Concentrated bone marrow aspirate for the treatment of chondral injuries and osteoarthritis of the knee: a systematic review of outcomes. Orthopaedic journal of sports medicine, 2016. 4(1): p. 2325967115625481.
25. Saw, K.-Y., et al., Articular cartilage regeneration with autologous marrow aspirate and hyaluronic acid: an experimental study in a goat model. Arthroscopy: The Journal of Arthroscopic & Related Surgery, 2009. 25(12): p. 1391-1400.
26. Fortier, L.A., et al., Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. The Journal of Bone & Joint Surgery, 2010. 92(10): p. 1927-1937.
27. Khan, W.S., D.S. Johnson, and T.E. Hardingham, The potential of stem cells in the treatment of knee cartilage defects. The Knee, 2010. 17(6): p. 369-374.
28. Hermann, P.C., et al., Concentration of bone marrow total nucleated cells by a point-of-care device provides a high yield and preserves their functional activity. Cell transplantation, 2007. 16(10): p. 1059-1069.
29. Alvarez-Viejo, M., et al. Quantifying mesenchymal stem cells in the mononuclear cell fraction of bone marrow samples obtained for cell therapy. in Transplantation proceedings. 2013. Elsevier.
30. Westminster, C., et al., Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. Pain Physician, 2008. 11(3): p. 343-353.
31. Centeno, C.J., et al., Partial regeneration of the human hip via autologous bone marrow nucleated cell transfer: a case study. Pain Physician, 2006. 9(3): p. 253-6.
32. Centeno, C.J., et al., Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. Curr Stem Cell Res Ther, 2011. 6(4): p. 368-378.