J Orthop Sports Med 2021; 3 (2): 062- 074 *DOI: 10.26502/josm.511500042*

Research Article

A Review of Bone Marrow Aspirate Concentrate and its Use in Primary Osteoarthritis of the Knee

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Received: 05 October 2020; **Accepted:** 12 October 2020; **Published:** 19 May 2021

Citation: Justin J Ernat, Ingrid K Stake, Naomasa Fukase, Robert A Waltz, Sudheer Ravuri, Johnny Huard. A Review of Bone Marrow Aspirate Concentrate and Its Use in Primary Osteoarthritis of the Knee. Journal of Orthopaedics and Sports Medicine 3 (2021): 062-074.

Abstract

Osteoarthritis (OA) of the knee joint is the most common chronic degenerative joint disease characterized by cartilage deterioration and inflammation. Conservative management to prevent cartilage degradation and future conversion to joint replacement has been the focus of attention, with a number of orthobiologics being applied clinically. Among them, bone marrow aspirate concentrate (BMAC) has been growing in popularity as a source of mesenchymal stem cells (MSC) and growth factors with regenerative capacity as well as anti-inflammatory properties. As it pertains to OA, the anti-inflammatory and regenerative effects of BMAC are promising, although, continued research and standardization of treatment are still necessary. This article aims to provide a review of the characteristics of BMAC from a regenerative medicine perspective

and provide a current summary of the preclinical and clinical results of BMAC when utilized to treat OA of the knee joint.

Keywords: Osteoarthritis; Bone marrow aspirate concentrate; Mesenchymal stem cells; Platelet-rich plasma

1. Introduction

Osteoarthritis (OA) of the knee joint is a progressive disease leading to cartilage damage, pain, and soft tissue contracture which can ultimately lead to loss of function [1-4]. Currently, there are no therapies that can definitively modify the course of the disease, and many times prevention strategies are employed in an effort to delay or avoid the need for total knee arthroplasty (TKA). Multiple orthobiologics and synthetic injectables have been studied as it pertains to the nonoperative treatment of knee joint OA, and these include, but are not limited to, platelet-rich plasma (PRP), autologous conditioned plasma (ACP), bone marrow aspirate concentrate (BMAC), mesenchymal stem cells (MSCs), corticosteroids, and hyaluronic acid (HA) [5-7]. BMAC is an orthobiologic that has been of considerable interest in recent years. It can be administered as intraarticular injections, peri-operatively to specific anatomical structures, in combination with other orthobiologics, or as adjuncts to other treatments [8]. In July 2020, the FDA updated its guidance on the use of human cells, tissues, and cellular and tissue-based products (HCT/P) [9]. These guidelines state that BMAC is not considered HCT/P as long as it is minimally manipulated, not combined with other substances, and used for homologous purposes. Therefore, BMAC is advantageous in research and clinical practice since it does not require premarket approval by the FDA. Based on this, BMAC is a potentially promising treatment aimed at disease modification and tissue regeneration in the setting of knee OA; however, its use has yet to be optimized and standardized.

The purpose with this review is to provide a summary of the characteristics of BMAC from a regenerative medicine perspective as it pertains to OA. We will additionally review the most current preclinical and clinical results when utilizing BMAC for the treatment of OA of the knee.

2. Background of Bone Marrow Aspirate Concentrate

When used for knee pathology, autologous bone marrow is most commonly harvested from the iliac crest [10]. After centrifugation, the components are concentrated into three layers [11]: plasma, red blood cells, and “buffy coat” with mononucleated cells (leukocytes, MSCs, hematopoietic stem cells, and platelets). The buffy coat is used for the BMAC injection and has the potential to increase tissue healing through anti-inflammatory, angiogenic and immunomodulatory properties [12].

Treatment with BMAC involves the delivery of a combination of several important factors, including MSCs, growth factors, and IL-1ra, however, the effects of BMAC are still not fully understood [13-15]. MSCs are multipotent cells and constitute about 0.001-0.01% of the cells in the bone marrow [16]. Although a low percentage, these cells are thought to play a valuable role in cartilage healing. Their

capability of self-renewal and differentiation into various tissues, including muscle, cartilage, and bone has been emphasized [16, 17]. However, this theory lacks evidence in the literature, and their capacity to signal the surrounding tissue to secrete factors that reduce inflammation and stimulate tissue regeneration has been suggested to be of more importance [18, 19]. The MSCs have an immunosuppressive effect by adjusting the activation of natural killer cells, macrophages and lymphocytes [20-25]. Additionally, they may recruit more cells to the injury site or degenerative cartilage through homing of hematopoietic stem cells that become available for proliferation and differentiation into mature cartilage [15, 26, 27].

Besides MSCs, BMAC contains significant amounts of platelets, growth factors, and cytokines that may stimulate tissue regeneration [12, 28, 29]. The growth factors platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-ß), and vascular endothelial growth factor (VEGF) are all present in concentrated levels and have positive effects on cartilage healing through stimulation of MSC proliferation, chondrogenesis, or inhibition of chondrocyte apoptosis and inflammation [28]. These growth factors are diminished in OA [4, 30].

Activation of pro-inflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) is widely known to play significant roles in OA progression [31, 32]. Despite the presence of high levels of leukocytes and increased concentrations of pro-inflammatory cytokines [12, 29], a significant amount of the interleukin-1 receptor antagonist (IL-1ra) results in an overall inhibitory effect on IL-1ß mediated inflammation and matrix degradation, and is thought to be responsible for the important anti-inflammatory effect and pain relief of BMAC [12, 29, 33, 34]. This, in combination with its regenerative capability via multipotent MSCs and growth factors, allows BMAC to act as a viable candidate for disease modification in OA [30, 32, 35].

Harvesting and processing of BMAC is a simple, single-stage procedure which after minimal manipulation offers immediately available cell concentrate without the need for cell culture expansion [10, 11]. Additionally, the percutaneous procedure and the transplantation of autologous cells have been reported to be safe with few complications and no risk for allogeneic disease transmission [10, 32, 36]. Disadvantages include mainly the risk of pain and swelling after the procedure [32].

Unfortunately, the different processing techniques and variable application protocols result in wide heterogeneity between studies [32, 36]. Several commercial kits for BMAC preparation are available, however, their capability to concentrate bone marrow and the resulting composition of factors are inconsistent [12, 36]. Additionally, variations in bone marrow with patient age and sex suggest thoughtful patient selection for this treatment [37]. Standardized administration of BMAC with agreement on number, volume, and timing of treatments is also desired [36, 38].

3. Preclinical Studies

The ability of bone marrow products to inhibit the progression of OA has been studied in some animal models. Singh et al. injected a single dose of MSCs or saline in 20 osteoarthritic rabbit knees [39]. At 20 weeks, the MSCs group demonstrated better gross morphology, normal thickness and cell distribution, and less severe signs of OA on radiographs compared to the saline group. Song et al. compared one injection of MSCs, cultured MSCs, or saline in 18 sheep [40]. After 8 weeks, they found improved cartilage regeneration and lower grade of OA, in addition to increased cartilage matrix synthesis and reduced inflammation in both cellular groups compared to the saline group. The results were best for the cultured MSCs group. Interestingly, in a rabbit model, Desando et al. reported that BMAC or cultured MSCs combined with hyaluronic acid resulted in increased homing of cells to cartilage in osteoarthritic areas and, furthermore, the best repair of cartilage and meniscus was achieved with the use of BMAC compared to cultured MSCs [41]. Since BMAC, unlike cultured MSCs, is a single-stage low-risk procedure that does not require premarket approval by the FDA, BMAC may be a suitable choice for cell transplantation [40].

BMAC has also been compared to PRP. In a goat model with knee OA, Wang et al. randomized 24 individuals to receive injections of BMAC, platelet-rich plasma (PRP), or saline every four weeks on three occasions, with one group not receiving injections [42]. After 6 weeks, the BMAC group demonstrated better gross morphology, delayed degenerative changes, and better preservation of chondrocytes and ECM content compared to the PRP group. This was thought to be related to the lower levels of inflammatory cytokines, especially IL-1ß, found in BMAC compared to PRP.

Desando et al. studied BMAC combined with a scaffold in a sheep model [43]. They assessed the effects of hyaluronan-based scaffold, scaffold with BMAC, and scaffold with expanded MSC in 20 osteoarthritic knees. After 12 weeks, both the BMAC and MSCs group demonstrated better meniscus regeneration, with reduced inflammation in the meniscus, cartilage, and synovial membrane. Strikingly, the BMAC group demonstrated more favorable results than the MSCs group which was thought to be related to decreased levels of IL-1ß in BMAC, similar to Wang et al.

Although the preclinical studies demonstrate promising results with few side effects, a systematic review by Cavallo et al. concluded that more studies are needed to support the use of BMAC in patients with OA of the knee in clinical practice [44].

4. Clinical Implications of Bone Marrow Aspirate Concentrate for Osteoarthritis of the Knee

The transition to clinical trials has been fraught with the same concerns as those observed in preclinical studies- promising results and minimal side effects, however, heterogeneity in reporting, methodology, and follow-up. In the previously mentioned systematic review by Cavallo et al., 18 studies reporting on the current clinical evidence of BMAC for treatment of OA in various joints were summarized [44]. They noted that while most studies

documented improvement in pain and function, there was significant heterogeneity, short follow-up and overall poor methodology. Other reviews have been performed and found similar results-positive short- and mid-term clinical outcomes in the setting of varying preparations and administrations [8, 36, 45, 46]. Specifically from Cavallo et al's review, a placebo-blinded randomized control trial demonstrated no difference between BMAC and saline intraarticular injections [47]. They injected patients with bilateral knee OA-one knee with saline and another with BMAC and found no difference in 25 patients with an average age of 60 years. Conversely, Centeno et al. compared intraarticular BMAC injections (in combination with a platelet product) with exercise therapy in knee OA patients and at 24 months of follow-up, found that the BMAC injections yielded better results [48]. Another highlighted study by Hernigou et al. includes a comparative study of subchondral BMAC injections versus TKA [49]. At an average of 12 years of follow-up, subchondral BMAC injections resulted in similar outcomes compared with TKA in younger patients with knee OA secondary to corticosteroid-related osteonecrosis [44, 49].

There has been speculation as to whether multiple BMAC injections can prove to be more efficacious than a single injection. Shaw et al. reported that outcomes after a 4^{th} treatment of BMAC in 15 patients with hip or knee OA were better than at baseline, and that improvements were made with each subsequent treatment [50]. In addition to the number of injections, a dose-response effect may be evident with BMAC as Centeno et al. suggested that improvements in outcomes were seen with increasing concentrations of cells [51].

This brings up the potential concerns with current BMAC processing-the variability in cell concentrations, preparation, analyzing techniques, and reporting. Wells et al. demonstrated that iliac crest samples that were analyzed using flow cytometry, enzyme-linked immunosorbent assays and colony forming unit (CFU) assays demonstrated wide ranges in total nucleated cells, MSC concentrations, CFUs, and IL-1ra concentrations [52]. Additionally, Doyle et al. performed a review of preclinical studies between 2014 and 2019 that investigated the use of BMAC for OA [53]. They found that a moderate number of cells (40×10^6) were identified as most likely to achieve optimal responses in patients with Kellgren scores of at least 2. However, improvements were also reported with concentrations as low as 24×10^6 and 100×10^6.

With regards to preparation, Dragoo et al. attempted to evaluate the consistency of cell yield and concentration increase from baseline for leukocytes, platelets, CD34+ cells, and CFU-fibroblasts between 3 different BMAC preparation devices [54]. They found that the Harvest system (Terumo BCT Japan, Inc., Tokyo, Japan) concentrated leukocytes more consistently than the Arthrex system (Arthrex, Naples, FL), but noted no other differences. In 2019, Gaul et al. compared the published data on the BMAC devices from Arteriocyte (ISTO Biologics, Hopkinton, MA), Arthrex (Arthrex, Naples, FL), Celling Biosciences (Austin, TX), EmCyte (EmCyte Corporation, Fort Myers, FL), Exactech (Gainesville,

FL), ISTO Tech (St. Louis, MO), Harvest Tech/Terumo BCT (Terumo BCT Japan, Inc., Tokyo, Japan), and Zimmer/BIOMET (Warsaw, IN) [55]. They found significant differences in the features and centrifugation patterns in the systems; specifically, not all systems used universal kits that allowed processing of different volumes of BMAC, and only the Arthrex system allowed selection of final hematocrit. Additionally, there was no standardized reporting method to describe biologic potency. They recommended further standardization to allow more accurate clinical outcomes reporting when using different preparation systems.

Piuzzi et al performed a systematic review of 46 studies with clinical trials for the use of BMAC in musculoskeletal conditions assessing the preparation, use, and reporting of BMAC [56]. None of the studies provided a comprehensive, clear description of the preparation protocol that could be reproduced, and only 30% provided quantitative metrics of the composition of the BMAC. Similarly, Murray et al evaluated 48 research studies looking at similar parameters as Piuzzi et al and found considerable deficiencies in reports of BMAC preparation and composition [57]. They found that no studies presented adequate, reproducible protocols or characterizations of their BMAC formulations.

In an attempt to standardize preparation and concentration, a reproducible technique has been described by Chahla et al. who evaluated the efficacy of 1, 20 or 50 million bone marrow MSCs in a phase I/IIa trial [10, 58]. They found that while all patients had significant overall improvements, the 50 million dose achieved clinically relevant improvements across most patient recorded outcome measures including the Knee Injury and Osteoarthritis Outcome Score (KOOS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores. Additionally, cartilage catabolic biomarkers and MRI synovitis were significantly lower when higher doses of MSCs were used, although the contrast-enhanced MRI for cartilage morphology (Whole Organ MRI Scores [WORMS]) and collagen content (T2 Scores) were unchanged from baseline. All patients had lower numbers of monocytes, macrophages, and interleukin-12 (IL-12) in their synovial fluid after MSC injection.

This failure to show radiologic improvement on MRI has been evident in other studies [59]. In the study by Shapiro et al., the T2 quantitative mapping that was performed after BMAC and saline injections showed no significant changes as a result of treatment at 1-year follow-up. They concluded that the mechanisms that led to pain relief were unclear as there was also no difference in Visual Analog Scale (VAS) and Osteoarthritis Research Society International Intermittent and Constant Osteoarthritis Pain (ICOAP) scores.

There has also been debate about the harvesting technique and how it relates to sample preparation and characteristics. Oliver et al. found that a single-insertion technique can produce final cellular concentrations and culture results that are no different than a multiple-insertion technique [60]. Additionally, the single-insertion technique is significantly less painful during and after the procedure. Conversely, Peters et al. reported that multiple advancements of up

to 4 passes of the aspirate needle resulted in a higher concentration at the time of collection [61].

Additional considerations with BMAC include its applicability to the severity of knee degeneration as well as its concomitant use with other orthobiologics. As would be expected, the current literature suggests that the severity of pre-existing OA has an effect on outcomes after BMAC treatment, with more severe degenerative changes demonstrating poorer results [62, 63]. Finally, when considering combining BMAC with other regenerative products, such as adipose tissue, combined injections did not pose any additional benefits when compared to BMAC alone [64, 65]. Estrada et al compared treatment with BMAC, PRP, and adipose-derived MSC injections in a total of 89 patients with OA [66]. They found that statistical improvement was observed in the three groups at all time points during the follow-up period of 1 year. However, their methods were biased in that treatment groups were allocated according OA severity as defined by Kellgren-Lawrence scores: PRP (stage I), BMAC (stage II), or adipose-derived MSC (stage III). These are in contrast to the study mentioned above by Centeno et al [48] where BMAC and PRP injections resulted improved outcomes compared to exercise alone, however, a study group of BMAC without PRP was not utilized in this study.

5. Clinical Applications on the Horizon

Several of the preclinical and clinical studies outlined have provided a foundation for future research into BMAC applications. The molecular data and pre-clinical trials have shown promise for BMAC both as an anti-inflammatory agent and for its regenerative properties for cartilage restoration. The current evidentiary gap is in showing high quality tissue regeneration in human trials. In fact, at the time of this publication, there are more systematic reviews and review articles than human randomized clinical trials involving BMAC.

Additional studies are required to examine stem cell differentiation in greater detail to evaluate the true regenerative properties of BMAC, especially in attempts to restore hyaline cartilage [61]. Novel synthetic tissue engineering with the use of nanotechnology has been suggested to provide a more consistent adhesion and proliferation matrix for MSCs in BMAC [7]. Further studies are underway looking at high definition T2 cartilage mapping similar to Shapiro et al. after BMAC treatment both in isolation and in conjunction with augmented cartilage restoration procedures such as matrix-induce autologous chondrocyte implantation (MACI) and osteochondral allograft transplantation [7, 56, 61]. Additionally, clinical trials are underway at our institution evaluating the efficacy of BMAC compared to PRP and placebo in the setting of anterior cruciate ligament (ACL) reconstruction and meniscus repair procedures in attempts to enhance healing, decrease post-operative inflammation, and ultimately prevent osteoarthritis progression after acute knee injuries. Finally, more robust patient reported outcomes studies are needed to assess positive improvements in function and pain relief after BMAC treatments and to correlate this on both high quality imaging studies and at the molecular level. Through more strict and enhanced harvest and preparation models, standardized study protocols, and

larger patient cohorts, this can be achieved and ultimately passed on to benefit our patient population.

6. Conclusions

BMAC demonstrates promising orthobiologic properties as it pertains to the nonoperative management of knee joint OA due to its source of MSCs and regenerative growth factors, in addition to its anti-inflammatory effects. However, the clinical application of BMAC is limited by heterogeneity of the data in both preclinical and clinical studies as it pertains to the preparation and administration. Future research should be directed at more universal and standardized clinical application so that reproducible data and outcomes can be achieved.

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