Bone Marrow Aspirate Concentrate Harvesting and Processing Technique

Jorge Chahla, M.D., Sandeep Mannava, M.D., Ph.D., Mark E. Cinque, B.S., Andrew G. Geeslin, M.D., David Codina, M.D., and Robert F. LaPrade, M.D., Ph.D.

Abstract: Bone marrow obtained by iliac crest aspiration is a common source for harvesting mesenchymal stem cells, other progenitor cells, and associated cytokine/growth factors. Recent studies have reported good to excellent outcomes with the use of bone marrow aspirate concentrate (BMAC) for pain relief in the treatment of focal chondral lesions and osteoarthritis of the knee. However, the harvesting and processing technique are crucial to achieve satisfactory results. Several studies have examined outcomes after BMAC injection, with encouraging results, but there is a lack of consensus in terms of the frequency of injection, the amount of BMAC that is injected, and the timing of BMAC injections. The purpose of this Technical Note was to describe a standardized bone marrow aspiration harvesting technique and processing method.

Bone marrow obtained by iliac crest aspiration is a common source for harvesting mesenchymal stem cells, other progenitor cells, and associated cytokine/growth factors. Because the use of bone marrow aspirate concentrate (BMAC) is currently approved by the United States Food and Drug Administration, it represents one of the few means for acquiring progenitor cells and growth factors for subsequent injection.1-4

After density gradient centrifugation to remove red blood cells, granulocytes, immature myeloid precursors, and platelets, progenitor cells account for a small population within the bone marrow (0.001% to 0.01%).5-7 However, a high concentration of growth factors, including platelet-derived growth factor, transforming growth factor-β, and bone morphogenetic proteins 2 and 7, which are reported to have anabolic and anti-inflammatory effects,8-10 are present in BMAC. Of note, it has been reported that BMAC has a considerable concentration of interleukin-1 receptor antagonist (IL-1RA).11 This molecule inhibits IL-1 catabolism and therefore may be responsible for the beneficial symptomatic pain relief with this biologic approach.12

A recent systematic review reported good to excellent outcomes with the use of BMAC for the treatment of focal chondral defects and mild to moderate osteoarthritis (OA) of the knee with a relatively safe profile.13 However, harvesting and processing techniques are vital to achieve optimal molecular concentrations and of both stem cells and growth factors in order to ultimately achieve satisfactory results. The purpose of this Technical Note was to describe the bone marrow aspiration technique and processing method.

Patient Positioning

The patient can be positioned either supine or prone. In the supine position, BMAC is harvested from the anterior superior iliac spine or the iliac crest. In the prone position, BMAC is harvested from the posterior superior iliac crest region. When the patient is positioned prone, it is important that care is taken to ensure that all bony pressure points and areas of potential nerve compression are adequately padded. Monitored anesthesia (conscious sedation), local anesthesia, or general anesthesia can be used for this BMAC harvest procedure, we prefer positioning the patient in the prone position with light sedation monitored by an anesthesiologist for access to the posterior superior iliac crest region.

References:

1. Smith & Nephew, Ossur (consultancy fees), Health East, Norway and NIH R-13 grant for biomaterials (grants/ grants pending); Arthrex, Smith & Nephew, and Ossur (royalties); and Arthrex, Smith & Nephew, and Ossur (patents).

2. Received October 8, 2016; accepted October 24, 2016.

3. Address correspondence to Robert F. LaPrade, M.D., Ph.D., Steadman Philippon Research Institute, The Steadman Clinic, 181 West Meadow Drive, Suite 400, Vail, CO 81657, U.S.A. E-mail: rlaprade@thesteadmanclinic.com

4. © 2016 by the Arthroscopy Association of North America 2212-6287/16/$36.00

5. http://dx.doi.org/10.1016j.eats.2016.10.024
crest. After palpation of the bony landmarks, the procedural site is sterilely prepared and widely draped to ensure an adequate field. The BMAC aspiration kit is opened and then the battery-powered BMAC aspiration device is sterilely draped (Fig 1).

Bone Marrow Aspiration

A bone marrow aspiration kit (MarrowStim; Biomet Biologics, Warsaw, IN) is used for bone marrow aspiration (Fig 2).

First the bony landmarks of the posterior iliac crest and sacroiliac joint are palpated (Fig 3). The skin is then injected down to and including the periosteum with 1% lidocaine without epinephrine. Then, the bone marrow aspiration trochar and needle are percutaneously inserted through the skin and subcutaneous tissues until it reaches the posterior iliac crest. Then, manual pressure is used to position the bone marrow aspiration trochar against the dense cortical bone, attempting to center it over the middle of the posterior crest cortical walls. The trajectory of the needle should be parallel to the iliac crest, or perpendicular to the ASIS or PSIS, depending on the harvest site used (Fig 3). A battery-powered power instrument is then used to drill the trochar and needle into the medullary cavity of the posterior iliac crest (Fig 4).

After the trochar is inserted into the posterior iliac crest but prior to aspiration, 1 mL of heparin (1,000 U/mL) should be preloaded into the syringe. Preloading the needles avoids clot formation and coagulation, which can diminish the ultimate yield from the aspiration. Approximately 60 mL of bone marrow is aspirated, which requires the use of two 30-mL syringes (Fig 5). At the conclusion of BMAC harvesting, a sterile dressing is applied to the harvest site (Fig 6).

Processing of the Bone Marrow Aspirate

The bone marrow aspirate (BMA) sample must be processed after it is harvested. BMA is filtered through a 200-μm mesh filter into 50-mL conical tubes. Then, 1 to 1.5 mL of the filtered BMA is pipetted into a 2-mL microcentrifuge tube for hemanalysis and the sample complete blood count with differential is automatically recorded. Subsequently, 60 to 90 mL of BMA is transferred into 2 × 50-mL conical tubes, and initially centrifuged at 2,400 rpm for 10 minutes. After completion of this process, the buffy coat layer and platelet-poor plasma layer are extracted from the conical tube and discarded. The red blood cell layers are combined into 1 × 50-mL conical tube for second centrifugation (3,400 rpm for 6 minutes). Finally, the BMAC/white cell pellet is resuspended in platelet poor plasma, hemanalysis is performed and complete blood count with differential is recorded (including monocyte count) to assess the final product to inject.

Application of BMAC

BMAC can be sterilely injected into a variety of locations, including focal cartilage defects, arthritic joints, and the femoral head after core decompression.13,14 The use of fluoroscopy, ultrasonography, or arthroscopy can help guide localization of the BMAC injection to the desired location of the joint.

The advantages and disadvantages of the described BMAC technique are reported in Table 1. A summary of the process of BMAC harvesting, processing, and application is displayed in Table 2.

Video 1 outlines the entire described technique, beginning with patient positioning, and concludes with intra-articular injection of BMAC.
Discussion

A stepwise technique for BMAC harvest and injection is reported in this Technical Note. Several recent clinical studies have reported on the extraction, processing, and clinical applications of BMAC in orthopaedic settings. Most recent studies have reported aspirating 60 mL of BMAC for their clinical applications. However, other studies have reported using volumes as low as 30 mL and as high as 120 mL. In the present Technical Note, 60 mL of BMAC was aspirated and later processed. In regard to BMAC processing, studies have used centrifugation, followed by BMAC activation with batroxobin enzyme. The technique presented herein uses centrifugation but does not involve BMAC activation. Recent studies have also examined the quality of tissue repair after BMAC application.

Despite promising initial clinical studies, the optimal use for BMAC in certain orthopaedic conditions has not been identified. Moreover, studies have been limited in identifying the component of BMAC responsible for its desired clinical effects. Cassano and Fortier reported that BMAC has a significant amount of monocytes and IL-1RA. It was hypothesized that IL-1RA may be responsible for the beneficial effects of the BMAC. Others have postulated that the beneficial effects of BMAC are related to the mesenchymal stem cell (MSC) content. The number of MSCs in BMAC, although relatively small in quantity, varies depending on the site of harvest and patient sex and age. Lavasani et al. postulated that the therapeutic effects of MSCs were mediated by secreted factors. However, the mechanism by which MSCs exert their effect is still unclear.

Use of BMAC in patients with focal chondral defects and/or OA in the knee has been scarcely delineated. Three recent studies showed BMAC to be moderately effective in the treatment of OA. These studies used different outcome scores and treatment protocols, making inter-study analysis difficult. One of these studies, by Hauser and Orlofsky, reported improved symptoms and quality of life scores after injection of 2 to 6 injections of BMAC at 2- to 3-month intervals. This study highlights a potential treatment regimen of 2 to 6 injections over a 3-month period for the treatment of patients with OA. Furthermore, BMAC application has

Fig 3. Patient placed in the prone position: (A) palpation of the left posterior superior iliac spine (PSIS), (B) infiltration of local anesthetic in the trajectory of the harvesting, and (C) percutaneous insertion of the bone marrow aspiration (BMA) trochar parallel to the iliac crest.

Fig 4. A steriley draped power instrument being used to advance the trochar through the dense cortical bone (left PSIS) into the medullary cavity of the posterior iliac crest. Needle trajectory should be perpendicular to the PSIS. (PSIS, posterior superior iliac spine.)

Fig 5. Aspiration of bone marrow on a left posterior superior iliac spine; the bone marrow aspiration needle is inserted into the cancellous bone of the iliac crest after penetrating the cortical bone using a power drill and the sample is obtained.
been shown to be more effective for patients with lesions that are of less than Kellgren-Lawrence grade 2 than for patients with advanced OA (Kellgren-Lawrence grade 4),21,24 which may indicate a temporal relationship between BMAC administration and effect size. Taken together, these studies indicate that there is interplay between the frequency, amount, and timing of BMAC injections to observe positive effects in patients with OA.

The majority of studies reporting on BMAC application in patients with focal cartilage defects have reported good outcomes.21,24,25 Some studies have used microfracture or scaffolds in addition to BMAC application,26,27 whereas other studies used scaffolds without microfracture.18 There is currently a lack of a consensus regarding the use of a scaffold or augmentation with BMAC injections. However, given the positive results of the aforementioned studies, further investigation is warranted. In patients with focal chondral defects, Gobbi et al. reported superior outcomes for patients younger than 45 years of age, with smaller chondral lesion size and with fewer lesions.10 This study adds to the existing literature in that there may be a subset of focal chondral defect patients who obtain the greatest benefit from BMAC injections.

Identifying the ideal number of BMAC treatments, the volume of treatment, and the timing of injections for BMAC has not been well characterized. Patients with focal chondral defects who received a single BMAC injection have been reported to have improved outcomes.9-11 In patients with OA, improved outcomes after BMAC injections have been reported; however, these studies used a variable number of treatments and had limited follow-up intervals.21,24,25 There is also a paucity of literature addressing the optimal augmentation method for BMAC application. Platelet-rich plasma has emerged as a promising augmentation method given its positive healing effects in degenerative knee pathologies.28 However, more studies are needed to elucidate the effects of platelet-rich plasma augmentation on BMAC effectiveness.

The safety of the MSC injected with BMAC is of the upmost importance and remains an issue that requires further study. The primary theoretical concern is that these cells may further divide into unwanted oncologic cell lineages.29 Self-limited pain and swelling were the most commonly reported adverse events at the injection site. Further studies are needed to allow for development of a comprehensive BMAC safety profile.

In conclusion, a stepwise approach to BMAC harvest, concentration, and subsequent injection is presented. Further study is necessary to clarify the number, volume, and timing of injections for BMAC treatment for specific knee pathologies. Moreover, standardization of the techniques of obtaining and processing BMAC aspirate is needed. The clinical role for BMAC is noted and we
encourage the further study of BMAC injections in patients with advanced osteochondral defects and/or OA.

References