Performing a Better Bone Marrow Aspiration



Mayo F. Friedlis, мD^{a,*}, Christopher J. Centeno, мD^b

KEYWORDS

- Bone marrow aspiration Bone marrow aspiration technique
- Bone marrow stem cells Stem cells Image-guided bone marrow aspiration

KEY POINTS

- Bone marrow aspiration (BMA) is the technique used to harvest stem cells for use in regenerative medicine.
- The use of ultrasound or fluoroscopy guidance represents an advance over traditional palpation-guided techniques. BMA combining anesthesia with guidance can improve patient comfort.
- Newer techniques for BMA allows for higher yields of stem cells.
- Patient preparation, equipment, anesthesia, use of guidance, and medical and other considerations for performing BMA are important.

INTRODUCTION

Patients often consider bone marrow aspiration (BMA) to be painful and difficult. Traditionally, BMAs were often performed using palpation-guided techniques that may work well in thin patients but were uncomfortable for most patients and difficult in heavier patients. Additionally, the authors' experience suggests that this procedure is often not performed in a way that maximizes yield. This article helps physicians understand how to obtain the highest possible stem cell yield while reducing patient discomfort.

BASIC SCIENCE OF STEM CELLS

The International Society for Cellular Therapy definition of a mesenchymal stem cell (MSC)¹ includes a cell line that

- · Is plastic adherent
- Expresses CD105, CD73, and CD90 and lacks expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR surface molecules
- Must be capable of trilineage differentiation to osteoblasts, adipocytes, and chondroblasts in vitro

* Corresponding author.

Phys Med Rehabil Clin N Am 27 (2016) 919–939 http://dx.doi.org/10.1016/j.pmr.2016.06.009 1047-9651/16/© 2016 Elsevier Inc. All rights reserved.

^a Stem Cell Arts, 5550 Friendship Blvd, Chevy Chase, MD 20815, USA; ^b Centeno Schultz Clinic, 403 Summit Blvd, Suite 201, Broomfield, CO 80021, USA

E-mail address: mfriedlis@gmail.com

Stem cells are part of the body's natural healing processes. They are responsible for repair of injured tissues in the body on an ongoing basis.² Hence, healing can be enhanced by injecting or surgically placing stem cells into damaged or injured areas.

A recent PubMed search for *mesenchymal stem cells* reveals more than 40,000 publications.³ Some studies have shown that MSCs can heal cartilage, bone, ligament, and tendon.^{4–7} Interventional orthopedics is the use of percutaneous techniques under imaging guidance to deliver MSCs and other orthobiologics to promote healing and avoid the need for surgery. There are early clinical data to suggest that, in the future, many orthopedic conditions that previously required surgical intervention may be treatable with guided placement of MSCs.

Later the authors provide an overview of the types of stem cells available in bone marrow (Fig. 1).

Mesenchymal Stem Cells

MSCs, also known as marrow stromal cells or colony-forming fibroblasts, are multipotent adult stem cells that have shown clinical potential as therapeutic agents in regenerative medicine.^{8–13} They are derived from other mesodermal tissues. Experiments in the 1980s and 1990s demonstrated that local environmental factors cause MSCs to differentiate into different cell types. For example, culturing MSCs with ascorbic acid, inorganic phosphate, or dexamethasone causes them to differentiate into osteoblasts, whereas exposure to transforming growth factor beta causes them to differentiate into chondrocytes.¹¹ More recent research has revealed that MSCs are actually a heterogeneous population of similar cells rather than one distinct cell type.¹⁴

Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are stem cells that are responsible for the production of blood; they are also secondarily involved in muscle repair.¹⁵ In the body they are recruited from the bone marrow when local muscle satellite cells are unable to complete muscle repair.

Endothelial Progenitor Cells

Endothelial progenitor cells are recruited from bone marrow to facilitate vascular homeostasis and neovasculogenesis.¹⁶ This cell type may be useful for reestablishing vascularity in chronically injured musculoskeletal tissues.

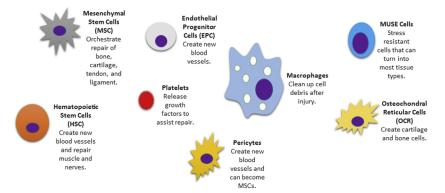


Fig. 1. The bone marrow contains many cells in addition to stem cells to help with orthopedic injuries. The focus of a BMA is to maximize MSC yield. MUSE, multilineage differentiating stress enduring. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

Pericytes

Pericytes reside around blood vessels and are recruited from the bone marrow for neovasculogenesis.¹⁷ Some investigators have suggested that they can differentiate into MSCs when injuries occur.¹⁸

Osteochondral Reticular Cells

These stem cells are concentrated in the metaphysis of long bones but not in the perisinusoidal space. They can differentiate into osteoblasts, chondrocytes, and reticular marrow stromal cells.¹⁹

Multilineage Differentiating Stress-Enduring Cells

Multilineage differentiating stress-enduring cells can differentiate into endoderm, mesoderm, and ectoderm. They are activated by physical stress and act as a reserve cell source. They are also involved in regenerative homeostasis and tissue repair.²⁰

Although bone marrow contains many types of stem and other cells to help orthopedic injuries, the focus of a BMA is to maximize MSC yield.

BASIC SCIENCE OF BONE MARROW AND BONE MARROW ASPIRATION

Drilling into the bone marrow is one of the oldest known medical procedures, with evidence dating back more than 7000 years.²¹ The first attempts to obtain an actual bone marrow sample for diagnostic reasons were independently undertaken by Pianese (Italy) and Wolff (Germany) in 1903.²² The modern Jamshidi needle (**Fig. 2**), first described in 1971,²³ is a commonly used tool for entering the bone marrow cavity.

SAFETY, INDICATIONS, CONTRAINDICATIONS, MEDICATIONS, AND PATIENT PREPARATION

BMA has been performed safely for more than 30 years. A large European Union registry including BMA and bone marrow biopsy shows a serious adverse event



Fig. 2. A Jamshidi needle is one of several types of trocars that can be used in BMA. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

rate of 16 in 27,700 procedures. There was one fatality from pulmonary embolism. Pain at the draw site is the most common adverse event.²⁴

Indications

BMA is indicated for treatment the following conditions:

- Osteoarthritis²⁵
- Tears of ligaments or tendons^{26,27}
- Avascular necrosis⁵

Contraindications

BMA is contraindicated in the following circumstances:

- There is significant anemia; check hematocrit (Hct) if unsure.
- There is local or systemic infection.
- There is active hematologic neoplasm, even if in treatment.
- There is anticoagulant treatment that cannot be stopped for the procedure.
- Patients are unable to be positioned for the procedure.
- There is immune compromise. Patients with immune compromise should not be treated.
- Cancer may be a contraindication. A recent study showed no increased risk of tumor growth when stem cells were used in conjunction with resection of bone cancer and graft placement²⁸
- Rheumatoid disease: The authors have treated patients with rheumatoid disease provided they are not in an acute inflammatory phase. The effects of immunosuppressant or biological therapies for rheumatologic diseases on the efficacy of stem cell treatment are unknown.
- Medications: Some medications must be avoided:
 - Because of its antiinflammatory and antianabolic effects, prednisone is contraindicated for 4 to 6 weeks before treatment.²⁹
 - Statins seem to have a very negative effect on stem cell proliferation and should be avoided at least 1 month before to 1 month after treatment.³⁰
 - Nonsteroidal antiinflammatory medications seem to reduce platelet aggregation and function.³¹ In the experience of one of the authors, they may also reduce MSC proliferation. They should be avoided for 1 week before and for 6 weeks after treatment.

Patient Selection, Body Size, and Positioning

Older patients may have fewer stem cells. They can still be harvested, but a larger volume of bone marrow will be needed to compensate. If properly stimulated, they have been shown to work adequately for tissue repair.³²

Imaging and BMA can be more challenging in certain individuals:

- Body mass index (BMI): BMI, within limits, is not a predictive factor for knee osteoarthritis outcome²⁵ but may be more challenging to perform. Larger body size and habitus increase the amount of excess soft tissue to be penetrated; these cases will require longer trocars, which will increase the opportunity for error and pain. The standard trocar is 3.5 in, and for larger patients a 6-in trocar may be required.
- Imaging is more difficult in larger individuals. Fluoroscopic imaging may be beneficial for guidance in these individuals when compared with ultrasound. The ideal position for performing BMA at the posterior superior iliac crest (PSIS) is prone

because it allows for the proper imaging of the multiple draw sites³³ located in that area. If patients cannot assume this position easily, it is possible to cannulate the anterior superior iliac crest (ASIS). Such cases are best left for experienced practitioners, as the lateral anterior femoral cutaneous nerve is located here.

 Larger patients and those with respiratory problems may have increased difficulty with respiration in the prone position. The authors recommend that new practitioners gain experience before taking on more challenging cases or refer them to experienced practitioners.

How Much Bone Marrow Aspiration Can Be Safely Drawn?

These guidelines are based on the authors' experience and patient size:

- Small woman/child (90–105 lb): no more than 50 mL
- Average woman, small man, or a patient with lower Hct (105–150 Hct): 60 to 70 mL
- Larger man/woman (150-250 lb): up to 120 mL

General Bone Marrow Aspiration Volume Requirements for Different Joints

These guidelines are based on the authors' experience:

- Bilateral knee osteoarthritis (OA): 120 mL
- Unilateral knee OA: 60 to 90 mL
- Medium joint (elbow/transverse tarsal joint axis (TT) ankle): 60 mL
- Small joint/intervertebral disc (IVD): 40 to 60 mL
- Very small joint (finger/foot single): 30 mL one side

PREPARATION FOR BONE MARROW ASPIRATION

Types of Trocars and Differing Techniques

- The bone can be penetrated using either a hand trocar or a commercially available powered driver, such as the one shown in Fig. 8C. The choice of tool will depend on the patients' age and bone density and physician preference. Some physicians may prefer a hand trocar; others will prefer a powered driver.
- Bone hardness changes across the lifecycle and becomes softer with age. A greater effort will be required to penetrate the bone of younger individuals and athletes.
- Hand trocars are recommended for older patients (55 years or older) or those with osteoporosis; use of a driver could result in overpenetration.

Maximizing Mesenchymal Stem Cells

Yield

- To maximize MSC yield, the authors recommend targeting the posterior superior iliac spine (Fig. 3) because it contains more MSCs than other bone aspiration sites like the tibia.³³
- As shown in Fig. 4, the ilium has a thick portion and a thin portion. The thickest part of the ilium is the target.
- Drawing small volumes (5–15 mL) from many sites increases MSC yield; drawing a large volume (more than 10–15 mL) from a single bone site reduces MSC yield.^{34–36}
- MSCs reside in the subcortical areas; pericytes reside around blood vessels. Penetrating the bone marrow space probably dislodges cortical and perivascular pericytes (Fig. 5).

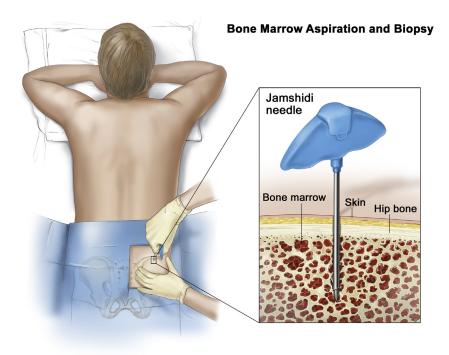


Fig. 3. This image shows a patient lying prone on a table, prepped and draped for BMA from the posterior superior iliac spine. (*Courtesy of* Terese Winslow LLC, Alexandria, Virginia. 2016; with permission.)

- Drawing from more sites maximizes subcortical MSC yield and allows access to pericytes.
- There are other strategies for drawing stem cells. Some practitioners call for going deeper into the marrow and extracting cells at different depths through side port trocars, such as the one shown in Fig. 6.³⁷ There are no published data on the efficacy of this method with regard to improving MSC yield.
- Others practitioners suggest using the same entry position and manipulating the trocar to get different areas of the marrow. Again, no data exist on the relative efficacy of this technique over simply penetrating the cortex at multiple sites (Fig. 7).
- In the authors' experience and based on the peer-reviewed literature cited, using multiple small-volume (5–10 mL) draw sites produces the highest yield.
- MSCs reside in the subcortical areas; pericytes reside around blood vessels.³⁸ Drawing from more sites maximizes subcortical MSC yield and allows access to pericytes.

Equipment and Supplies

The following supplies are required for a 60-mL bone marrow draw (Fig. 8):

- 20 mL 0.5% ropivacaine, 27-gauge 0.5-in skin needle, 22-gauge 3.5-4.0 11/16-in needle
- Sterile, disposable, 11-gauge Jamshidi trocars, 2-gauge, or similar, power drill with bit
- Scalpel blade (if a drill is to be used)

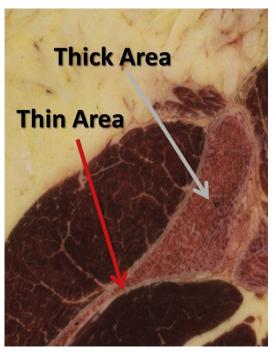


Fig. 4. Cross section of the iliac crest showing thick and thin areas. Penetrating the thin area of the pelvis increases the likelihood of passing through the marrow space. The thick area has a large marrow space with less risk of passing through the marrow-rich area and much higher likelihood of drawing whole marrow. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

- 5 mL syringe with 5000 IU of heparin in normal saline
- Two 30-mL syringes each preloaded with 30,000 IU of heparin (1000 IU of heparin for each milliliter)
- Steri-Strips (3M, St Paul, MN), gauze, tape

Guidance

- Using guidance, either fluoroscopy or ultrasound (US), allows greater precision with anesthesia and placement of the trocar.
- Guidance allows the ilium to be penetrated at predefined intervals to maximize MSC harvest.
- The authors think that using guidance for stem cell aspiration should be the standard of care; the authors discuss advantages and disadvantages of each type next.

Fluoroscopy

- In the authors' experience, fluoroscopy is easier to learn, and it facilitates exact placement of the trocar within the anesthetized area. Bony landmarks identified with the fluoroscope help with targeting specific sites.
- Fluoroscopy is more comfortable for patients, as there is less soft tissue to be penetrated (because of the steeper insertion vector) than with the US approach. Fluoroscopy also makes it easier to manipulate the trocar.

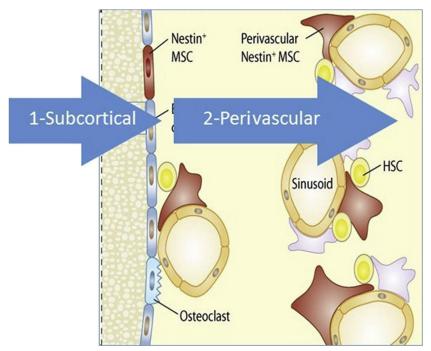


Fig. 5. There are 2 opportunities to dislodge marrow MSCs. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

Ultrasound

- US does not expose patients or the physician to radiation.
- US does not require a large procedural suite. US makes it more challenging to position the trocar within the anesthetized area and to achieve proper depth. Use of a skin marker can help.
- US will require a larger anesthetized area because the trocar will have to travel a longer distance through the soft tissue.

TECHNICAL CONSIDERATIONS

Patient Preparation

- Position patients prone with a pillow under the abdomen to minimize lordosis in the lumbar spine. Sterilely prep and drape the area over the PSIS (Fig. 9).
- For volumes of more than 30 mL of BMA, both left and right iliac crests must be prepped and draped.
- Fig. 10 shows the target sites for BMA.

Anesthesia Notes

Anesthesia dramatically reduces patient discomfort. However, care must be taken in the selection of anesthetics because some are toxic to stem cells. Lidocaine and Marcaine are toxic and should not be used for BMA. Ropivacaine seems to be safe at lower concentrations.³⁹ Although local anesthesia is very effective at preventing discomfort from the bone marrow drilling procedure, some patients may experience pain when BMA is drawn out of the bone marrow cavity.



Fig. 6. BMA needle featuring side ports (only) manufactured by Marrow Cellutions. (*Courtesy of* Ranfac Corp, Avon, MA; with permission.)

Critical Importance of Clot Prevention

- Clots trap stem cells and make them unavailable for their intended use.
- If a commercial closed system is used for preparation, clot formation will not be evident to the operator.
- Heparin, administered through the trocar, is used to prevent clotting before the aspirate is drawn out. The amounts are listed later.
- Preload the syringe to be used for withdrawing the aspirate with heparin. The amounts and volumes for each of these are noted later.

BONE MARROW ASPIRATION STEP-BY-STEP Positioning of Guidance

- With patients positioned on the table, prepped, and draped, position the guidance. Select (A) or (B):
 - (A) Fluoroscopy: Orient the beam of the x-ray 15° ipsilateral oblique. This placement will expand the view of the target sites allowing for an easier approach (Fig. 11).
 - (B) US: Identify the direction of the US head needed to perform the aspiration. A curved low-frequency probe is used (Fig. 12).
 - The target is the thick part of the ilium. On the US screen the thickest part of the ilium appears like a mountain of bone. Cannulate the thick part of this mountain (Fig. 13).



Fig. 7. The single-entry, multiple-direction draw technique. *Arrows*, orientation of the trocar. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

- Use sterile gel and gloves.
- Use a skin marking pen to mark all the different locations that will be needed to accomplish 3 to 5 aspirations (Fig. 14).

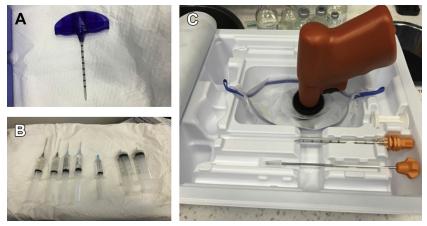


Fig. 8. Basic equipment for BMA: (*A*) Jamshidi needle, (*B*) syringes, and (*C*) Arrow OnControl Driver. (*Courtesy of* Teleflex Inc, Morrisville, NC; with permission.)



Fig. 9. A patient lying prone, prepped for BMA. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)



Fig. 10. Model of the ilium showing the target sites for BMA. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)



Fig. 11. The Ziehm Solo fluoroscope is at 15° ipsilateral to PSIS. (*Courtesy of* Ziehm Imaging, Inc, Orlando, FL; with permission.)

 Start all aspirations from a point lateral on the ilium using a single skin puncture. Align the US head with these preset markings, for both anesthetization and penetration of the cortex (see Fig. 14).

Apply Anesthesia

- Identify a starting point that will allow access to 3 to 5 different sites on the ilium from one location (see Fig. 14).
- Anesthetize with a large skin weal, approximately 1 cm.
- Anesthetize each target using an appropriate length, with a 22- to 25-gauge needle.
- Identify the first target approximately 1 cm from the edge along the posterior surface of the ilium.
- Care should be taken not to perform this procedure too far laterally or inferior to the sacroiliac (SI) joint in order to protect the superior cluneal nerves and the superior gluteal nerve, artery and vein (NAV) bundle (Fig. 15).
- Care should be taken to penetrate the periosteum with anesthesia at each target site.
- Move the needle in a circular fashion to anesthetize an area about 2 cm in diameter. Use about 7 mL of anesthetic for this injection point and the tract leading up to it (Fig. 16).

MSK US-Marrow Draw

Direction of US probe and trocar placement for BMA

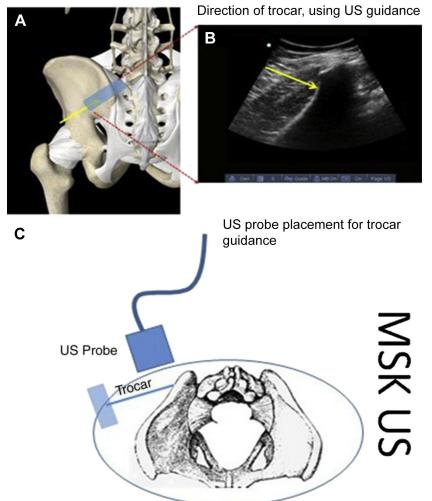


Fig. 12. (*A*) US head orientation to the ilium, (*B*) the view on the US machine of the appropriate approach of anesthetizing needle or trocar, and (*C*) the relationship of the trocar and US head to the pelvis. MSK, musculoskeletal. *Arrows* show orientation of the trocar. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

• The second target (and any subsequent targets) will be about 2 cm from the first one, about 1 cm from the edge of the ilium. Anesthetize as before.

Penetrate the Bone

• Select either a hand trocar or a powered driver. Most cases can be done using a Jamshidi trocar, especially in patients 55 years or older, whose bones may be too



Proper trocar placement to penetrate the thickest part of the lliac crestv

Fig. 13. (A) Cannulate the top part of the mountain (*thick marrow*). (B) Anatomic structure depicted on the US screen. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

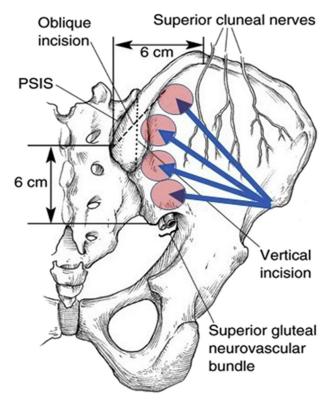


Fig. 14. Draw site targets. One skin site, multiple bone targets. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

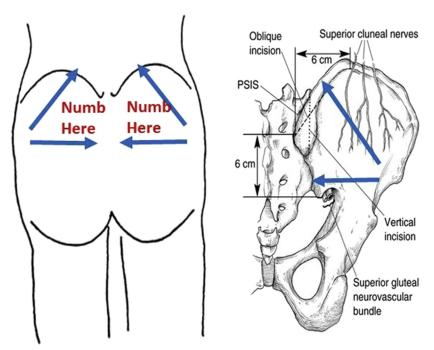


Fig. 15. Target site for anesthesia, relative to the SI joint, the gluteal nerves, and the superior gluteal NAV bundle. (*Courtesy of* Christopher J. Centeno, MD, Broomfield, Colorado.)

osteoporotic to make the drill safe. For younger patients and athletes, a driver will probably make the job much faster and easier.

- A trocar can pierce the skin without a scalpel wound. A commercially available drill bit may not be as easy to introduce and will require a scalpel wound.
 Fig. 17 shows the shape, size, and depth of the scalpel wound.
- Fig. 17A shows the size and shape of the scalpel wound, and Fig. 17B shows the necessary depth of penetration. The pelvis is spherical. The trocar/driver will be on the inside of the sphere pushing out. Drilling on a curved surface is challenging. If the trocar/driver slides slide down into nonanesthetized soft tissue or over the top of the ilium, patients will experience pain and possible complications.
- To prevent errors, approach the ilium perpendicular, or at a right angle, to the surface of the curvature. The exact angle will differ for each of the 3 to 5 penetrations to be performed, as each will be in a different part of the curve. It is a good idea to recheck the direction and make corrections, if needed.
- Tap the trocar with the hand to penetrate the skin. Then advance the trocar or drill through the anesthetized area, down to the bone.
- Patients should feel only the pressure of the trocar or driver but no pain. If patients experience pain, the trocar/driver may be outside the anesthetized area. Reposition the trocar to find the planned location or reapply anesthesia. Query patients to ensure there is no pain.
- To maintain control, advance the trocar with small oscillations of the hand, turning the handle back and forth while applying pressure, rather than rotating it completely. There will be a decrease in resistance when the cortex is pene-trated and the marrow cavity is entered. This depth is the target depth. Patients may feel discomfort when this depth is reached.

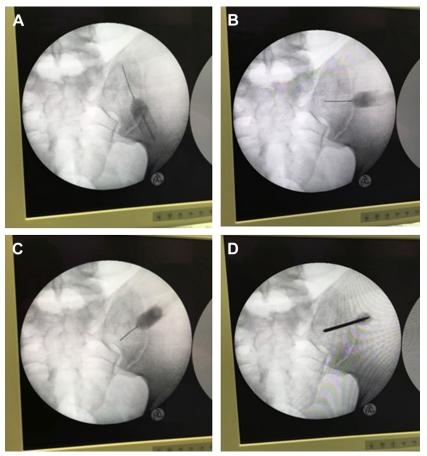


Fig. 16. Ziehm Solo fluoroscope panels (*A*–C) show the 3 target sites being anesthetized using fluoroscopic guidance. (*D*) The trocar placed at the same site that was anesthetized. (*Courtesy of* Ziehm Imaging, Inc, Orlando, FL; with permission.)

- When using a drill, operate it continuously rather than pulsing it. This continuous operation will make it easier to sense, both by the sound of the drill and the resistance, when the marrow cavity is entered.
- When the trocar/driver reaches the marrow cavity, it should be firmly stuck in the bone. If it is loose, continue drilling until the trocar/driver is solidly in the bone. In addition, most commercial trocars and drill bits have a 1-cm marking system that should also help determine how far the tip is advancing.
- For either method, you can tell if the trocar is solidly implanted in bone by performing a tap test. Take one finger and tap on the end of the trocar. If it feels solid, then it is firmly implanted in bone. If it is loose or moves, it needs to be further advanced.
- To prevent clots, remove the inner stylet. Inject approximately 0.3 to 0.5 mL of heparin in normal saline, 500 to 750 IU/mL, through the trocar and into the bone marrow.

Withdraw the Aspirate

• Attach one of the 30-mL syringes preloaded with 30,000 IU of heparin in normal saline (total volume 3-5 mL) and begin withdrawing bone marrow.

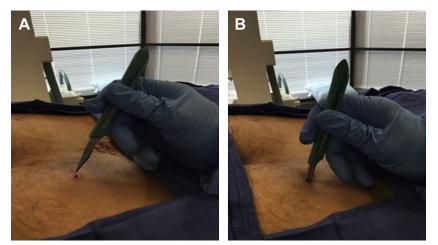


Fig. 17. (A) The size and shape of the scalpel wound. (B) The necessary depth of penetration.

Withdraw 5 to 15 mL from each site. If bone marrow does not flow easily, consider going deeper or redirecting altogether by selecting another site.

- As the BMA meets the heparin, note that the two will not mix. Quickly tap the syringe to force the heparin to mix with the BMA to reduce the chances of clotting.
- Caution: It is rare, but some patients will experience severe pain when marrow aspirate is withdrawn. The pain is brief but can be quite intense and unusual, sometimes mimicking a radiculopathy. It may be helpful to pulse the syringe, pulling gently but in a pulsewise fashion, to slowly extract the BMA out of the marrow cavity.
- Withdraw the trocar carefully by pulling it back, and while still in the tissue using the same skin site (ie, not withdrawing it from the skin) redirect it to the next target.
- After completing 3 sites, remove the 30 mL syringe and cap it. Keeping the syringe in continuous motion for the duration of the procedure may help prevent clotting.
- When all sites are completed and the trocar is withdrawn, put pressure over the wound to assure hemostasis.
- Approach the other side of the ilium in the same fashion.

Provide Aftercare

- Have patients lie on their back with knees bent for 5 minutes to place pressure over the entry wounds and promote hemostasis. Redress the wounds if necessary.
- The authors place a Tegaderm (3M, St Paul, MN) dressing on the wounds and ask patients to keep them dry until the next morning. After that, patients can shower and replace the dressing with a Band-Aid (Johnson & Johnson Consumer Inc, New Brunswick, NJ) and Neosporin (Johnson & Johnson Consumer Inc).
- Opioid medication may be appropriate for postprocedural pain.

Clinical Notes

- In the authors' experience, most patients will not feel the drilling and tolerate the procedure well.
- Some patients will feel discomfort when the trocar/driver reaches the bone cavity and when heparin is injected, so it is best to warn them.

- Some patients will feel pain when bone marrow is withdrawn. For those who feel it, it may be severe. However, it is brief and patient complaints are rare. Communication is key.
- Patients needing a repeat procedure can be scheduled 6 to 12 weeks following the first procedure.

QUICK REFERENCE FOR ULTRASOUND-GUIDED BONE MARROW ASPIRATION

The following guide is provided as a quick reference for use in the office or procedure room.

- 1. Check patients' Hct. It should be more than 30 to 38 (hemoglobin [Hgb]10.0–12.5). If it is lower, target volumes should be adjusted down.
- 2. Prep the low back and pelvis per your clinic protocol.
- 3. Use curved low-frequency US probe with sterile gel and gloves; map out the area to be numbed with a sterile surgical pen. This area should be from just lateral to PSIS to just superior to the bottom one-third of the SI joint.
- 4. Under active US guidance, use a long 25- or 22-gauge needle to numb the area from the skin, soft tissues, and periosteum along the planned BMA sites. Numb the entire area you have outlined with the surgical pen. This should be 10 to 20 mL of anesthetic per side.
- 5. Prepare medications: 5000 IU heparin in a 5-mL syringe with normal saline and as many 30-mL syringes with 30,000 IU heparin each as required for the planned harvest volume.
- 6. On the first side anesthetized, insert the trocar long under the US probe toward the top one-third of the mountain made up by the posterior aspect of the ilium. At the chosen BMA site, put constant forward pressure on the trocar and turn the handle back and forth until it breaks through the cortex.
- 7. Perform a tap test. Once the cortex is engaged and there is a slight loss of resistance, tap the end of the trocar side to side to ensure that it is firmly engaged in bone. If so, inject approximately 500 to 750 IU of heparin into the bone site.
- 8. Place the 30-mL syringe on the trocar and pull back on the plunger. *Flick the syringe as the first BMA enters the syringe to ensure that it mixes with the heparin.*
- 9. Draw 5 to 15 mL at this site and repeat for a total of 3 to 5 sites on the left and 3 to 5 sites on the right, depending on desired final volume.
- 10. The maximum safe volume depends on patient size. In a patient less than 105 lb, limit the withdrawal to 50 mL; in an average man, it should not be more than 90 to 120 mL; and in a large man, it should not be more than 120 to 150 mL.

QUICK REFERENCE FOR FLUOROSCOPE GUIDED BONE MARROW ASPIRATION

The following guide is provided as a quick reference for use in the office or procedure room.

- 11. Check patients' Hct. It should be more than 30 to 38 (Hgb 10.0–12.5). If it is lower, target volumes should be adjusted down.
 - a. Prep the low back and pelvis per your clinic protocol.
 - b. C-arm is anteroposterior with a 15° ipsilateral oblique on the PSIS.
 - c. Under active fluoroscopic guidance, use a long 25- or 22-gauge needle to numb the area from the skin, soft tissues, and periosteum along the planned BMA target sites. Numb the entire target area. This area should require 10 to 20 mL of anesthetic per side.

- d. Draw medications: 5000 IU heparin in a 5-mL syringe with normal saline and as many 30-mL syringes with 30,000 IU heparin each as required for the desired final volume.
- e. Return to the side that was first numbed, and insert the trocar through the skin wheal that was used for anesthesia and advance so that it is positioned at the first chosen target. The trocar enters at approximately a 30° angle. Once a BMA target site is contacted, put constant forward pressure on the trocar and turn the handle back and forth until it breaks through cortex; note the slight loss of resistance.
- f. Perform a tap test. Once the cortex is engaged, tap on the end of the trocar side to side to ensure that it is firmly engaged in bone. If so, then inject approximately 500 to 750 IU of heparin into the bone site through the trocar.
- g. Place the 30-mL syringe on the trocar and pull the plunger back. Flick the syringe as the first BMA enters to ensure that it mixes with the heparin.
- h. Draw 5 to 15 mL at this site and repeat at 3 to 5 sites on the left and 3 to 5 sites on the right depending on desired final volume.
- i. The maximum safe volume depends on patient size. In a patient less than 105 lb, limit withdrawal to 50 mL; in an average male, it should not be more than 90 to 120 mL; and in a large male, it should not be more than 120 to 150 mL.

REFERENCES

- Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315–7.
- 2. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 2013;45(11): e54.
- 3. Pubmed search for "mesenchymal stem cells". Available at: http://www.ncbi.nlm. nih.gov/pubmed/?term=mesenchymal+stem+cells. Accessed April 21, 2016.
- 4. Haleem AM, Singergy AAE, Sabry D, et al. The clinical use of human cultureexpanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. Cartilage 2010;1(4):253–61.
- 5. Hernigou P, Mathieu G, Poignard A, et al. Percutaneous autologous bone-marrow grafting for nonunions. J Bone Joint Surg Am 2006;88(1 Suppl 2):322–7.
- 6. Buda R, Vannini F, Cavallo M, et al. One-step arthroscopic technique for the treatment of osteochondral lesions of the knee with bone-marrow-derived cells: three years results. Musculoskelet Surg 2013;97(2):145–51.
- Schnabel LV, Lynch ME, van der Meulen MCH, et al. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. J Orthop Res 2009;27(10):1392–8.
- 8. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 1976;4(5):267–74.
- 9. Foster T, Puskas B, Mandelbaum B. Platelet-rich plasma: from basic science to clinical applications. Am J Sports Med 2009;37:2259–72.
- Kevy S, Jacobson M. Preparation of growth factors enriched autologous platelet gel. In: Proceedings of the 27th Annual Meeting of Service Biomaterials. St. Paul, MN, April, 2001.
- 11. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 2004;62(4):489–96.

- Pourcho A, Smith J, Wisniewski S, et al. Intraarticular platelet-rich plasma injection in the treatment of knee osteoarthritis: review and recommendations. Am J Phys Med Rehabil 2014;93:S108–21.
- Al-Ajlouni J, Awidi A, Samara O, et al. Safety and efficacy of autologous intraarticular platelet lysates in early and intermediate knee osteoarthrosis in humans: a prospective open-label study. Clin J Sport Med 2015;25(6):524–8. Available at: http://journals.lww.com/cjsportsmed/Fulltext/2015/11000/Safety_ and_Efficacy_of_Autologous_Intra_articular.10.aspx.
- 14. Sun Y, Feng Y, Zhang CQ, et al. The regenerative effect of platelet-rich plasma on healing in large osteochondral defects. Int Orthop 2010;34(4):589–97.
- **15.** Otto A, Collins-Hooper H, Patel K. The origin, molecular regulation and therapeutic potential of myogenic stem cell populations. J Anat 2009;215(5):477–97.
- **16.** Szmitko P, Wang C, Weisel R, et al. Biomarkers of vascular disease linking inflammation to endothelial activation. Circulation 2003;108:2041–8.
- 17. Lamagna C, Bergers G. The bone marrow constitutes a reservoir of pericyte progenitors. J Leukoc Biol 2006;80(4):677–81.
- 18. Caplan AI. All MSCs are pericytes? Cell Stem Cell 2008;3(3):229–30.
- Worthley DL, Churchill M, Compton JT, et al. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. Cell 2015;160(1–2): 269–84.
- Wakao S, Kitada M, Kuroda Y, et al. Multilineage-differentiating stress-enduring (Muse) cells are a primary source of induced pluripotent stem cells in human fibroblasts. Proc Natl Acad Sci U S A 2011;108(24):9875–80.
- 21. Parapia LA. Trepanning or trephines: a history of bone marrow biopsy. Br J Haematol 2007;139(1):14–9.
- 22. Rubinstein M. Aspiration of bone marrow from the iliac crest: comparison of iliac crest and sternal bone marrow studies. J Am Med Assoc 1948;137(15):1281–5.
- 23. Jamshidi K, Swaim WR. Bone marrow biopsy with unaltered architecture: a new biopsy device. J Lab Clin Med 1971;77(2):335–42.
- 24. Bosi A, Bartolozzi B. Safety of bone marrow stem cell donation: a review. Transplant Proc 2010;42(6):2192–4.
- 25. Centeno C, Pitts J, Al-Sayegh H, et al. Efficacy of autologous bone marrow concentrate for knee osteoarthritis with and without adipose graft. BioMed Res Int 2014;2014:1–9.
- 26. Centeno C, Pitts J, Al-Sayegh H, et al. Anterior cruciate ligament tears treated with percutaneous injection of autologous bone marrow nucleated cells: a case series. J Pain Res 2015;8:437–47.
- 27. Centeno C, Al-Sayegh H, Bashir J, et al. A prospective multi-site registry study of a specific protocol of autologous bone marrow concentrate for the treatment of shoulder rotator cuff tears and osteoarthritis. J Pain Res 2015;8:269–76.
- 28. Hernigou P, Flouzat Lachaniette CH, Delambre J, et al. Regenerative therapy with mesenchymal stem cells at the site of malignant primary bone tumour resection: what are the risks of early or late local recurrence? Int Orthop 2014;38(9): 1825–35.
- 29. Wyles CC, Houdek MT, Wyles SP, et al. Differential cytotoxicity of corticosteroids on human mesenchymal stem cells. Clin Orthop Relat Res 2015;473(3):1155–64.
- **30.** Izadpanah R, Schächtele DJ, Pfnür AB, et al. The impact of statins on biological characteristics of stem cells provides a novel explanation for their pleotropic beneficial and adverse clinical effects. Am J Physiol Cell Physiol 2015;309(8): C522–31.

- **31.** Schippinger G, Pruller F, Divjak M, et al. Autologous platelet-rich plasma preparations: influence of nonsteroidal anti-Inflammatory drugs on platelet function. Orthop J Sports Med 2015;3(6). 2325967115588896. p. 3.
- Beane OS, Fonseca VC, Cooper LL, et al. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/ stromal cells. PLoS One 2014;9(12):e115963.
- **33.** Marx RE, Tursun R. A qualitative and quantitative analysis of autologous human multipotent adult stem cells derived from three anatomic areas by marrow aspiration: tibia, anterior ilium, and posterior ilium. Int J Oral Maxillofac Implants 2013;28(5):e290–4.
- Batinić D, Marusić M, Pavletić Z, et al. Relationship between differing volumes of bone marrow aspirates and their cellular composition. Bone Marrow Transplant 1990;6(2):103–7.
- Muschler GF, Boehm C, Easley K. Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume. J Bone Joint Surg Am 1997;79(11):1699–709.
- **36.** Fennema EM, Renard AJS, Leusink A, et al. The effect of bone marrow aspiration strategy on the yield and quality of human mesenchymal stem cells. Acta Orthop 2009;80(5):618–21.
- 37. Scarpone M, Kuebler D, Harrell C. Marrow Cellution Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells.
- **38.** Ehninger A, Trumpp A. The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. J Exp Med 2011;208(3):421–8.
- **39.** Rahnama R, Wang M, Dang AC, et al. Cytotoxicity of local anesthetics on human mesenchymal stem cells. J Bone Jt Surg 2013;95(2):132–7.