Methodology

Regenerative Medicine

Bone marrow aspiration for regenerative orthopedic intervention: technique with ultrasound guidance for needle placement

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Aim: We review relevant anatomy of the iliac crest, and describe an interventional technique to maximize harvesting of desired progenitor cells with ultrasound to guide safe trochar placement. **Materials & methods:** We validated the technique on both sides of the pelvis in four human cadavers. **Results:** Using ultrasound guidance, 32 BMA needles were placed in a safe zone along various portions of the iliac crest. **Conclusion:** Ultrasound guidance can improve accuracy of bone marrow aspirations form the iliac crest. Mastery of this procedure will facilitate cell harvest and aid in patient safety when procuring mesenchymal stem cells from a bone marrow source.

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In current clinical practice, bone marrow continues to be the most common used substrate of progenitor cells. Aspirated bone marrow for orthopedic intervention was first proposed in 1986 for osseous nonunion of the tibia [1–5], based on the known osteogenic potential of mesenchymal stem cells contained within the stroma of bone marrow [4–8]. Hernigou and Beaujean [9] subsequently reported on the treatment of osteonecrosis of the femoral head and other osseous nonunions as an adjunct to orthopedic surgical treatment of both conditions. Later techniques evolved to concentrate larger volumes of harvested marrow using either a collection bag and gravity or density gradient centrifugation [10–12]. Bone marrow aspiration and concentration (BMAC) has in subsequent years, been proposed for use in a number of regenerative treatment strategies [13] and is increasingly performed by musculoskeletal interventionists for degenerative joint diseases and tendinopathies and as a continued adjunct to orthopedic surgeries. The suggested benefits of BMAC include the presence of both osteogenic and chondrogenic progenitor cells and the capability for paracrine signaling, immunomodulation and anti-inflammatory signaling [14–16]. As a result of this proposed regenerative potential and subsequent widespread patient demand, the BMAC procedure is being performed with increasing frequency by interventionists without classical training in the aspiration of bone marrow. Despite being a well-tolerated procedure, bone marrow aspiration (BMA) is not without its risks and reported complications including vascular, neuronal, ligamentous, muscular and osseous injury [17–25].

Aim

We review the relevant anatomy of the iliac crest as it pertains to BMA, describe an interventional technique to maximize harvesting of desired progenitor cells along with the use of ultrasound to guide safe trochar placement, and finally validate the technique in a human cadaver model.

Osteology

Prior to considering BMA, it is imperative to first recognize the osseous anatomy of the pelvis. In the adult human, the ilium, ischium and pubic bones fuse to form the pelvis bone, or *os coxae*, Latin for hip. All three components

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contribute portions to the acetabulum. The three bones have also been called innominates due to the fact that they do not resemble much and thus could not be named.

Anatomy of the ilium

The *ilium*, Latin for lower abdomen, forms the largest portion of the pelvis. The upper ilium is flat and fan-shaped and may be referred to as *ala*. Its superior margin, or iliac crest, is the thickened superior-most border of the *os coxae*, palpable as a ridge at the top of the pelvis. It averages 1 cm in width through most of the crest [26,27] with an inner lip that faces medially and an outer lip that faces laterally toward the gluteal surface. Gluteal lines on the lateral surface include the anterior, posterior and inferior lines and function as muscle origins for the gluteus minimus, medius and maximus, respectively. The inner lip gives rise to the iliac fossa, a broad, medial surface of the bone which caves in slightly. On their medial surface, the iliac alae articulate with the sacrum at the auricular surface to form the sacroiliac joints. There are four iliac spines per side, the anterior superior and inferior (ASIS, AIIS) and the posterior superior and inferior iliac spines (PSIS, PIIS) which serve as muscle and ligament attachment sites. The iliac tubercle which lies toward the ASIS iliac spine is a rough surface off the crest which projects laterally while the iliac tuberosity is a rough area posteriorly, medially to the crest and PSIS. Both are ligament and muscle attachment sites.

Though BMA commonly takes place at sites along the crest, it is important to consider other osseous elements of the pelvis to prevent complications. This includes the greater sciatic notch, which is a wide opening inferior and anterior to the PIIS through which important soft tissue and neurovascular structures pass. Figure 1 illustrates these important osseous structures.

Materials & methods

Bone marrow harvesting

It is important to recognize that the procedures described below are best learned under direct supervision by practitioners with extensive experience in BMA.

Hernigou *et al.* [15] proposed the anterior iliac crest when harvesting intraoperatively, as patients would otherwise be positioned supine. If the patient is lateral decubitus, either the ASIS or PSIS may be utilized with trochars employed in a direction parallel to the operating room table [28,29]. Harvesting may also involve either unilateral or bilateral harvesting from the iliac crest beginning at the PSIS when the patient is comfortably lying prone. Limited evidence exists to support the PSIS as a greater source of mesenchymal stem cells [28,30]. Practitioners may wish to premedicate patients with a low dose of valium or other light sedative to minimize anxiety and discomfort during the procedure [17,19].

For palpation-guided aspirations and physical examination techniques, the authors employ a standard technique using the base of the long finger placed over the superior border of natal cleft followed by abduction of the index and ring fingers to land the finger tips over the bilateral PSIS of most adults, both male and female. This is demonstrated in Figure 2. Once the PSIS and iliac crest are localized, the skin, subcutaneous tissues, gluteal musculature and periosteum are anesthetized with 1% lidocaine without epinephrine making sure to conclude with tapping on the targeted bony landmarks with an 18-gauge needle. This tapping technique serves to both anesthetize the periosteum and map out the medial and lateral borders of the iliac crest suitable for harvest.

Both the anesthetization of the periosteum and the placement of the aspiration needle may be performed under image guidance. Commonly employed techniques include fluoroscopy, computed tomography (CT) guidance or ultrasound guidance [31–34]. Ultrasound has been demonstrated to have similar or improved accuracy in image guidance of injections involving the musculoskeletal system, including those around the hip and pelvis similar to the imaging required for injection of the iliac crest [35–39]. An additional advantage of ultrasound over both CT and fluoroscopy is that neither the patient nor practitioner are exposed to ionizing radiation [40]. If employing ultrasound guidance for anesthetization or BMA needle placement, the probe is initially placed transversely or in the axial plane over the PSIS after using the palpation-guided technique listed to find the most likely location. Musculoskeletal ultrasound commonly uses the bony cortical margins as a "home base" or signature landmark through which to define the point of origin for translating the ultrasound transducer proximally, distally, medially and laterally. When starting from the transverse view of the PSIS, the appearance is one of a rounded hyperechoic bony structure, and the most superficial of all the cortical structures in the region of the posterior pelvis. In our experience, there is a wide variability in both the width and shape of the bony *signature* (Figure 3). The iliac tuberosity will be adjacent to the PSIS and can add variability to the bony appearance. Confirmation of this location can be achieved by

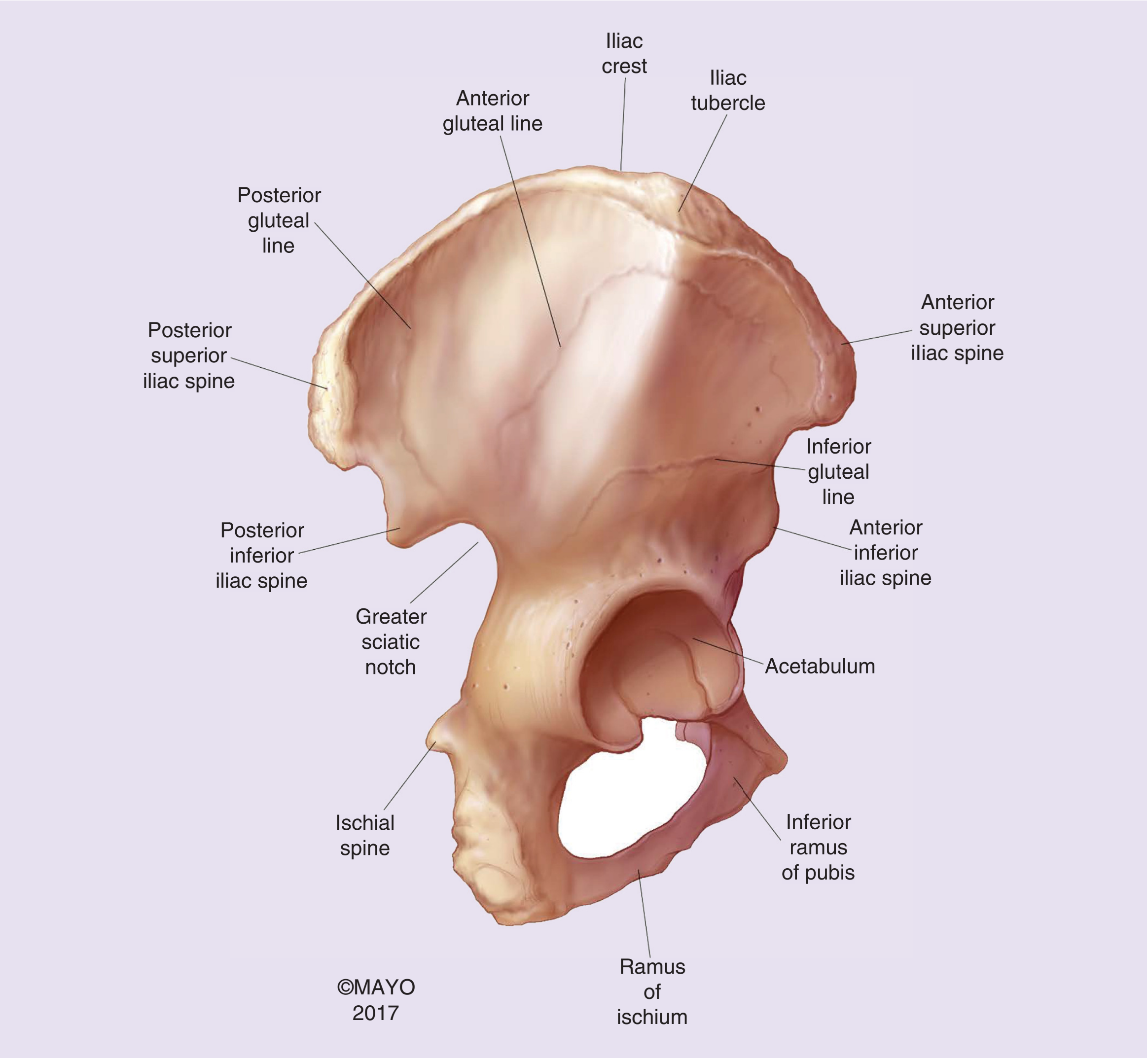

Figure 1. Osseous anatomy of the human adult hemi-pelvis with relevant landmarks. Presented with permission from Mayo Foundation for Medical Education and Research; all rights reserved.

Figure 2. Photograph of hand and finger placement with superimposed pelvic anatomy demonstrating appropriate localization of posterior superior iliac spine surface anatomy. Presented with permission from Mayo Foundation for Medical Education and Research; all rights reserved.

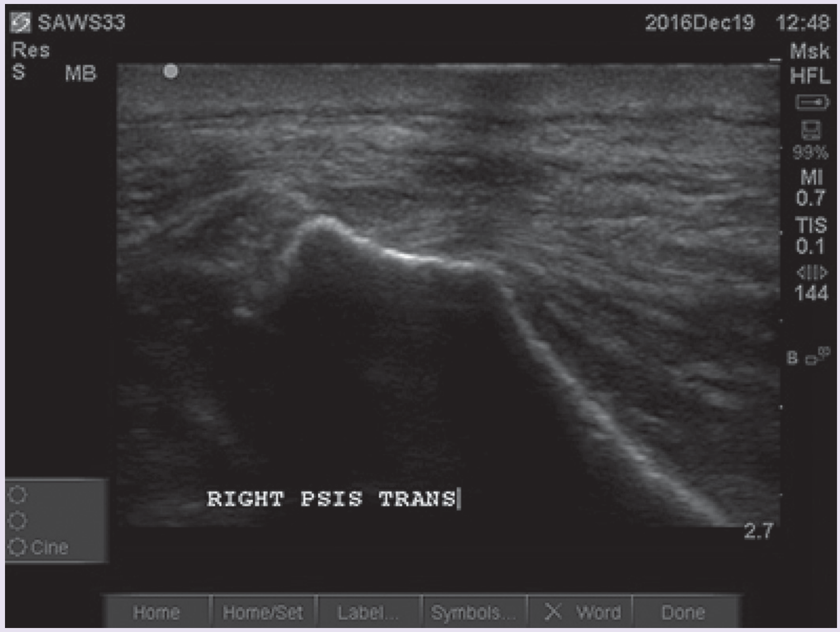

Figure 3. Sonographic appearance of the right posterior superior iliac spine in transverse orientation (or axial plane) after using the palpation-guided technique listed to find the most likely location. Variability in the cortical appearance or *signature* of the PSIS will exist. PSIS: Posterior superior iliac spine.

translating the probe both proximally and distally along the crest observing both the most superficial depth and broad appearance of the cortical bone drop off and then transition to a deeper and narrower cortical structure in both proximal and distal directions. Once comfortable with the location of the PSIS, the probe is swept medial to view the sacroiliac joint and then back to the home base position to confirm localization of the iliac crest. Probe translation in the caudad direction will cause the PSIS hyperechoic appearance to drop off in favor of the deeper PIIS; translation in the cephalad direction reveals the thinner, more laterally positioned iliac crest. The probe is rotated obliquely approximately 45° to get a long axis view of the iliac crest (Figure 4). Subsequent translation in an anterolateral and posteromedial direction allows for determination of suitable angles for needle placement. Due to the iliac crest angling laterally in the cephalad direction, we recommend translating the probe in a medial direction while in the long axis view of the crest until the hyperechoic cortical margin is lost, followed by a similar pass laterally at which point the cortex of the crest reappears and then drops off again. This allows the examiner to define both the inner (medial) plate and the outer (lateral) lip of the crest. If performing the procedure with ultrasound assistance only, the central portion of the iliac crest may be marked at this appropriate site. Continuous ultrasound guidance requires the BMA needle to be introduced into the skin at this stage while visualizing the needle tip and shaft down to the target location on the ilium, as described below.

Once comfortable with the margins of the iliac crest, and after appropriate aseptic technique, a 1-cm stab incision over the most cephalad portion of the PSIS is performed so as to permit passage of low gauge instruments through the skin. The first pass with a trephine BMA needle is made at a steep angle, quite possibly requiring a gel standoff or heel-toe maneuver to achieve a more desirable angle of insonation (Figure 5). At this point, we advocate for a 90° rotation of the probe to obtain an orthogonal view of the iliac crest and needle out-of-plane view once the BMA needle contacts bone. This out-of-plane orthogonal view helps the sonographer localize the BMA needle centered between the inner and outer crest (Figure 6).

Centered between the inner and outer lips of the crest, the BMA needle is then advanced with pressure and an alternating clockwise and counterclockwise rotation until firmly seated within the bone marrow space, approximately 1-cm deep, and the first pass aspiration is performed. Either a mallet or a drill may be employed for easier cortical bone penetration [29], reduced time and physical strain on the practitioner [41,42], though doing so eliminates the beneficial localization within the segment of bone that is provided by the fine motor feedback when

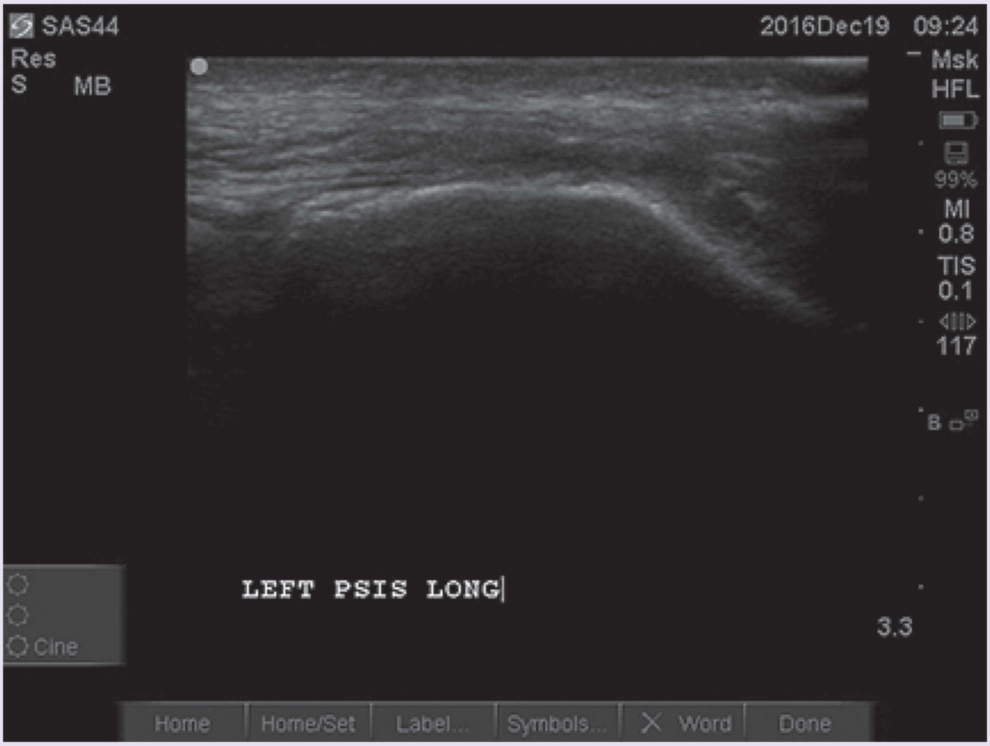

Figure 4. Long axis view of the iliac crest. The transducer is positioned centrally over the thin cortical margins of the iliac crest cephalad (left side of image) and laterally while the cortical margins of the PSIS appear just medially and caudad (right side of image) in the sagittal oblique plane. PSIS: Posterior superior iliac spine.

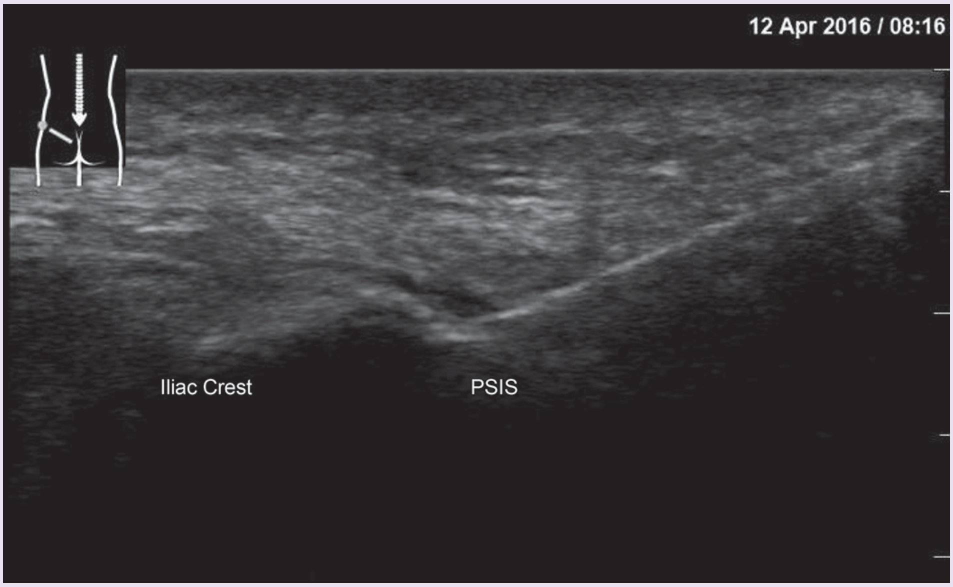

Figure 5. Sonographic long axis view of the bone marrow aspiration needle, in-plane, advancing to the hyperechoic bony iliac crest and posterior superior iliac spine.

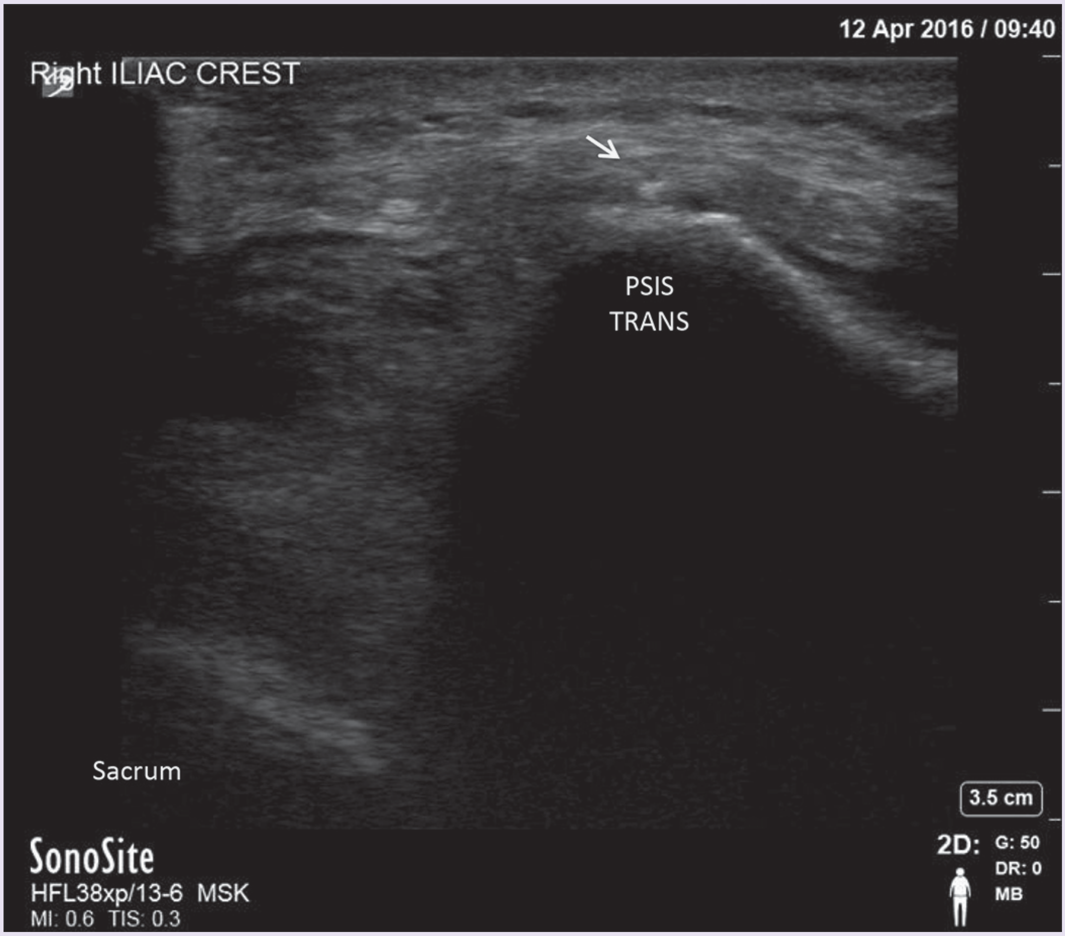

Figure 6. Transverse view of the bone marrow aspiration needle, out-of-plane, at the iliac crest for confirmation of central placement between the inner and outer lips. Arrow denotes hyperechoic cross-section of the needle tip.

the aspiration needle insertion is advanced by hand [43]. Additionally, little is reported in the scientific literature regarding the quality and viability of progenitor cells aspirated with drill assistance [43]. The authors will occasionally employ a mallet for initial penetration of robust bone, with light oral sedation required to prevent anxiety from the mallet tapping sensation. We employ a single bevel or diamond-tipped, cannulated aspiration needle with multiple side holes using 45° rotations at differing depths, to maximize progenitor cell yield, though this procedure has yet to be verified in controlled scientific studies [32].

What has been studied is the proportion of marrow aspiration volume containing maximum regenerative therapeutic potential. The first 2–5 ml show the most mononuclear cells, with all further volume aspiration likely to contain a varying amount of peripheral blood dilution [44–48]. For this reason, we keep individual aspirations under 8 ml. Additional attempts to maximize progenitor yield have introduced differing bevel angles and bore numbers, again without clear evidence of benefit [49,50]. Appropriate anticoagulants are employed per different manufacturer standard operating procedures to prevent clotting of the aspirated marrow, for example coating marrow aspiration instruments with heparin.

After an initial 5–8 ml of marrow is recovered, the BMA needle is withdrawn to the level of the skin, and then readvanced at an angle that will achieve a new cortical entry point 1 cm proximal and lateral to the previous entry point along the iliac crest. Hernigou *et al.* [27] divided the ilium into six zones from ASIS to PSIS 24 cm along the crest, each 4 cm apart for the purpose of defining areas of the ilium wide enough for suitable passage of a 3 mm aspiration needle through the center of the plate. The PSIS delineates zone 6 [27] and was deemed to be a safe zone whereas the adjacent zones 4 and 5 were demonstrated to be thinner regions of the iliac wing, only 3 mm

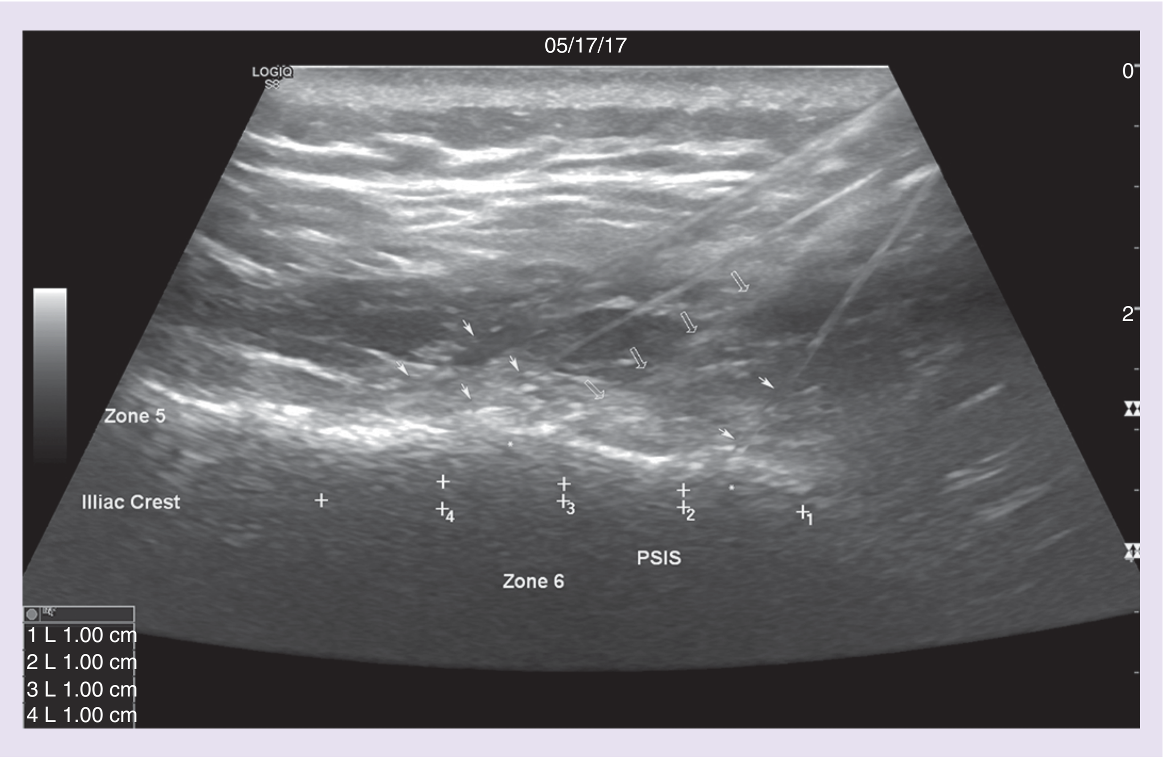

Figure 7. Composite representative schematic of 4 sonographic needle passages along the iliac crest. Zone 6, containing the PSIS, is deemed to be a safe zone for passage of the BMA needle and can be divided into four needle passages each approximately 1 cm apart (arrows).
BMA: Bone marrow aspiration; PSIS: Posterior superior iliac spine.

in thickness at some points. For this reason, unless using CT or fluoroscopic guidance, we advocate limiting the aspiration needle insertion on the iliac crest to a maximum of 4 cm from the PSIS. Four passes along the long axis of zone 6 can be made 1 cm apart, needle in plane (Figure 7).

Because small volume aspirations with lower volume syringes have been shown to be beneficial [45–47], it has been proposed that a 5 cm or greater initial iliac wing penetration can produce multiple small volume aspirations with repeated withdrawal of the needle by 1-cm increments [51]. The concern with this technique is maximal depth iliac wing penetrations, when attempted at oblique angles parallel to the alae may increase the risk of complication in inexperienced hands, especially without fluoroscopic guidance [29]. The authors propose multiple separate shallower penetrations along the iliac crest to access different channels within the marrow, thus requiring less depth of penetration, and possibly reducing the risk of complication of penetrating through either the medial or lateral plates.

If greater volume is desired, a subsequent single deeper pass of 1 cm within each of the four passes is proposed [51]. The BMA needle is restilletted and advanced an additional 1 cm while rotating the bevel 45–90° to access different channels within the marrow. Thereafter a second aspiration of 5–8 ml can be taken. Such a technique has previously yielded median mononuclear cell counts of 80 million, hematopoietic stem cell counts of 4.62 million and mesenchymal stem cell counts of 34,000 from a total of 52 ml using small volume aspirations of 5–8 ml each [52]. Typically in the BMAC procedure, the marrow harvested is then filtered of bone spicules, fat and cellular debris prior to centrifugation and subsequent concentration [11–13]. This process has been described previously [29,52,53].

Cadaver validation

The ultrasound-guided BMA needle technique described above was validated in a cadaver model using four fresh, previously frozen cadaver pelvises. After a 1-cm stab incision with an 11-blade scalpel, a commercially available BMA needle was employed under ultrasound guidance to four sequential locations on each of the eight iliac crests (4 pelvises, 2 crests each), followed by direct injection with blue latex dye through the needle into each puncture.

Table 1. Cadaver dissection and iliac localization of bone marrow aspiration needle and blue latex.

Cadaver	PSIS	Central crest	Outer crest	Inner crest
Pelvis 1, left	1	3		
Pelvis 1, right	1	3		
Pelvis 2, left	2	1	1	
Pelvis 2, right			4	
Pelvis 3, left	1	2		1
Pelvis 3, right	2		2	
Pelvis 4, left	2		2	
Pelvis 4, right	1	2	1	
Total	10	11	10	1

PSIS: Posterior superior iliac spine.

Figure 8. Left hemipelvis dissection with four blue latex needle penetrations demarcated. Two cephalad penetrations on the outer lip of the iliac crest and two penetrations along the PSIS. Colored pins demarcate the superficial iliac crest (green), PSIS (white), sacroiliac joint (yellow), posterior inferior iliac spine above the sciatic notch (blue) and sacrum (red). Blue marker delineates the posterior gluteal line and a bone marrow aspiration needle is anchored within the contralateral PSIS for surface anatomy comparison. PSIS: Posterior superior iliac spine.

After the first cortical penetration, attempts were made in each case to withdraw the initial needle passage into the subcutaneous tissue and reorient the subsequent three needle passages approximately 1 cm apart. A limited dissection was then performed to document the location of each needle penetration as confirmed by the blue latex.

Results

The first pelvis was from a thin female cadaver. Three BMA penetrations were placed linearly along the central portion of the iliac crest of both the left and right sides of the pelvis, with the fourth placed in line with the first three at the PSIS. Pelvis 2 was from an average-sized male cadaver. Two passages on the left side were placed on the PSIS, with the other two placed along the outer crest, the most cranial of which was placed just lateral to the lateral lip of the crest. On the right side of Pelvis 2, all four penetrations were placed on the outer lip of the crest. Pelvis 3 was from a slender female. Of the passages on the left side of the pelvis, one was placed on the PSIS, one on the crest just cephalad to the PSIS, one medially along the inner plate and the final placed proximal and cephalad along the crest. On the right side of Pelvis 3, two penetrations were placed side by side on the PSIS and two more cephalad, lateral to the crest. Pelvis 4 was from an average-sized male cadaver. On the left side of this pelvis, two needle punctures were placed along the PSIS, and the other two were more cepahalad on the outer lip of the iliac crest. On the right side, one puncture was placed on the PSIS, two were central along the crest and one was on the outer lip. In total, 10 needles were placed on the PSIS, 11 were placed more cephalad along the central iliac crest, 10 were placed along the outer crest and a single needle placed along the inner crest. Table 1 summarizes all 32 BMA needle placements and Figure 8 shows a left hemipelvis dissection with needle placement and relevant anatomical landmarks demarcated.

Discussion

Bone Marrow Aspiration has been proposed in regenerative treatment strategies with increasing frequency. The marrow aspirations for BMAC, are being performed by practitioners of varied specialization. This includes hematologists, registered nurse practitioners, orthopedists, sports medicine physicians, radiologists, physiatrists and interventional pain specialists, among others [10,17,54]. A number of methods for aspirating marrow from different anatomic locations exist, some of which utilize image guidance, while others are palpation-guided. While reports of fluoroscopic and CT-guided bone marrow exist within the scientific literature, little is written regarding ultrasound-guided techniques or their validation. Here, we have discussed anatomic considerations and reported an ultrasound-guided marrow technique for safe use in BMA procedures for those with training and experience in musculoskeletal ultrasound while also attempting to validate the technique. Our results support the use of ultrasound imaging for needle or trochar guidance to aspirate bone marrow at the PSIS and iliac crest.

A few variables regarding BMA under ultrasound guidance warrant mention. The PSIS and iliac crest are common locations for marrow harvest. Safe zones for aspiration have been reported [27], and we demonstrate that image guidance can assist with aspiration needle placement within the desirable Zone 6. While anterior–posterior and lateral applications of fluoroscopic imaging can assist with parallel needle placement, fluoroscopy is not always present in locations used for BMA, and comes at some cost of radiation exposure to patient and practitioner. Ultrasound is portable, inexpensive and free of radiation, but sound waves cannot penetrate bone. For this reason, once the BMA needle is placed on the desired location of the ilium, the postpenetration depth and needle localization cannot be assessed. Additionally, as depth increases, such as in cases of overweight and obese patients, lower sound wave frequencies are required, and visualization of both needle and target decline. We advocate for a detailed understanding of the iliac anatomy to help with the angulation of the needle penetration, as presented in the osseous anatomical considerations above.

We placed 32 BMA needle passes along the PSISs and crests of four cadaver pelvises. All 32 passes were placed in areas safe from structures at increased risk of complication. Twenty-one of these 32 were centrally located along the crest or PSIS while 11 were on either the inner or outer lip of the crest. The likelihood of placing the aspiration needle on the central crest is increased by the orthogonal rotation of the ultrasound transducer. Without such a practice, the possibility of slight transducer drift to either the inner or outer lip and plate is increased without changing the sonographic appearance of the bony cortex. Nevertheless, the outer lip and plate of the iliac crest are still deemed safe for marrow harvest and have even been advocated as a preferred location for aspiration [29,32]. It should be noted that this only applies to initial needle placement at shallow to intermediate depths. Some sources advocate for greater depths of needle penetration to perform multiple aspirations [27,31]. However, even with accurate initial needle placement, the driven angle of the aspiration needle, if incorrect, can lead to breech of either the inner or outer plate at depths >2 cm. If desiring to aspirate from depths >2 cm, the initial placement should be toward the inner lip and angled laterally. Such an approach can be undertaken with the practitioner standing on the contralateral side of the table, though marrow harvest from the contralateral side is difficult when two practitioners harvest from two iliac crests simultaneously. Because of concern for penetration of either medial or lateral tables at angles not entirely parallel to the ilium orientation, we have chosen not to perform needle aspirations at maximal depths. Additionally, given the results of our needle placements on eight PSISs of four pelvises, and the subsequent dissections, individual practitioners may wish to place more than one pass of the BMA needle side by side 1 cm apart on the PSIS in a horizontal placement rather than a vertical placement as the PSIS is the widest platform to place the needle under ultrasound guidance.

It bears mentioning that BMA from the ilium can be performed from a number of approaches and through several techniques [26,27,29,30,32,53]. We describe only one such technique, and individual practitioners should be comfortable with whatever technique best suits the clinical condition they are treating and the environment in which the procedure is being performed. Additional limitations of our description include the size of the cadavers used to test the technique. Of the four cadavers, two were of a smaller body habitus, while two were of average size. Ultrasound guided aspirations of the iliac crest in larger body habitus individuals are likely to be more technically challenging, and will require additional validation studies. Despite this, the emphasis of this technique is on the appearance of the bony signature of the PSIS and iliac crest making visualization of the adjacent soft tissues somewhat less important for marrow harvesting provided localization of safe bony anatomy. Additionally, the wide variability in both the width and shape of the cortical bone anatomy necessitates a thorough preprocedural scan to map out the margins of the iliac crest in their entirety. The decision to take multiple draws at multiple depths through a single needle passage as opposed to multiple small volume aspirations from different needle passages is still debated within the field of regenerative medicine and may also influence the selection of locations along the crest.

Conclusion

Regardless of specific BMA technique, it is likely that applications of BMA for regenerative medicine will increase in the coming years, be they for same day usage, such as BMAC, or initial harvest for autologous and allogeneic culture expanded techniques. Ultrasound guidance to the iliac crest and posterior iliac spine is safe and feasible to image the relevant and important osseous anatomy. Mastery of this procedure will facilitate cell harvest and aid in patient safety when procuring mesenchymal stem cells from a bone marrow source.

Summary points

- Due to increased interest in regenerative medicine procedures and the utility of mesenchymal stem cells, bone marrow aspiration (BMA) is being increasingly performed by practitioners without classical training in the appropriate technique.
- We describe the osseous anatomy of the relevant structures about the ilium.
- A number of approaches to the BMA and the iliac crest have been described.
- We detail a BMA interventional technique making use of ultrasound guidance to safely guide BMA needles to safe zones along the iliac crest.
- We tested this ultrasound guided BMA technique by injecting blue latex dye into four osseous penetrations of eight cadaver hemipelvises.
- All 32 passes were placed in areas safe from structures at increased risk of complication.

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