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ULTRASOUND-GUIDED POSTERIOR ILIAC CREST ASPIRATION

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Abstract

Background: Bone Marrow Aspirate (BMA) contains growth factors and signaling entities essential for local cellular repair. BMA and concentrated BMA injections (BMC) are used for orthopedic procedures, such as in treating cartilaginous and bony defects. Several techniques exist for harvesting BMA. A technique termed ultrasound-guided posterior iliac crest aspiration (UPICA) is of special interest. This case series was undertaken to determine if practitioners can effectively perform the UPICA technique in a clinical setting.
Methods: In accordance with the UPICA technique, ultrasound guidance was used to localize the posterior iliac crest. Following local anesthesia, aspiration was performed using 10 mL syringes at multiple depths. The periosteum was entered once. A needle was advanced 0.5 cm with each new depth. At each penetration level, 10 mL was drawn before rotating 90 degrees and aspirating an additional 10 mL. Following aspiration at various depths, approximately 60 mL of BMA was obtained. The inclusion criteria for this study were patients with diagnosed glenohumeral osteoarthritis, knee osteoarthritis, hip osteoarthritis, or rotator cuff tears whose condition had failed to improve with conservative care. The UPICA technique was performed in each case by the senior author (BG).
Results: For the 10 cases, the average patient age was 72 (SD 7.6; range 58–81) years old. The sample included 8 males and 2 females. The mean total nucleated cell count (TNCC)/mL BMA was 9.7E+06 (SD 2.9E+06), while the TNCC/mL bone marrow concentrate (BMC) was 43.9E+06 (SD 29.5E+06), a 4.3 (SD 2.3)-fold increase in concentration. The mean cell colony forming units-fibroblasts (CFU-f)/mL BMA was 520 (SD 155) with a mean CFU-f/mL BMC of 4899 (SD 2887) resulting in a 9.6 (SD 4.6)-fold increase in concentration. The viability for the BMA was 97.5 (SD 2.3) %, and the BMC was 94.9 (SD 4.55) %.
Conclusions: Utilizing the UPICA technique, similar yields for the BMC CFU-f/mL counts were obtained to those reported previously by other clinicians. Additionally, the results demonstrated the ability of the bone marrow concentration system to successfully concentrate the BMA. Based on these findings, the research team endorses the UPICA technique as a viable method for bone marrow aspiration.

Keywords: *Bone marrow aspirate*; *Posterior iliac crest*; *Ultrasound-guided bone marrow aspiration*

BACKGROUND

The optimal biological environment to promote tissue healing contains signaling and growth factors and a biological scaffold. Bone marrow aspirate (BMA) provides a source of growth factors. Reasons for utilizing BMA include its relative ease of harvest, low morbidity, and inexpensive cost.[1] Other signaling entities, including platelets and mesenchymal stem cells (MSCs), are also found in the BMA.

MSCs may have specific signaling properties.[1] The quantity of MSCs injected into the injured tissue may influence clinical outcomes; therefore, assessing the quantity of MSCs within the BMA may be beneficial. A decline in the number and functional capacity of MSCs has been shown to occur with advancing age.[2] BMA offers a low percentage of MSCs, with only 0.001 to 0.01% of all nucleated cells in BMA being MSCs.[1] As a result, the BMA commonly undergoes centrifugation to increase the concentration of MSCs and allow for a smaller injection volume at the injured site. Although the concentrated bone marrow aspirate (BMC) has a higher concentration of MSCs, the BMA maintains a higher number of hematopoietic cells, mainly red blood cells.[3] BMA and BMC supply growth factors and signaling effects needed for local cellular repair.[1]

BMA and BMC injections are being utilized to enhance orthopedic procedures, notably in treating cartilaginous lesions, bony defects, and tendinous injuries.[1,4,5] Several techniques for harvesting BMA have been published.[3] BMA can be harvested from multiple sites, resulting in varied amounts of MSCs. Studies have shown that the greatest yield comes from the posterior iliac crest.[6] Multiple harvesting sites and small syringes have resulted in increased yields.[7]

Buford has popularized an indirect technique called ultrasound-guided posterior iliac crest aspiration (UPICA) and has authored a course manual that details the procedure.[8,9] The research team was unaware of any independent verification of the efficacy of this procedure. This technique uses ultrasound guidance to localize the posterior iliac crest. Then, following local anesthesia, aspiration is performed using 10 mL syringes at multiple depths. This procedure can be done under local anesthesia with minimal discomfort. It entails entering through the periosteum once. Infiltration of blood and red blood cells (RBCs) into the specimen is limited by withdrawing samples while advancing forward rather than initially penetrating deep and aspirating during withdrawal. There is adequate depth to advance 0.5 cm and aspirate 20 mL at each level to obtain approximately 60 mL of BMA. At each penetration level, 10 mL can be drawn before rotating 90 degrees and aspirating from a different site. Hernigou has shown a minimal distance of 5.6 cm from the posterior iliac crest to the sciatic notch, which is considered the "safe zone" for the procedure. The practitioner will likely avoid significant neurovascular structures by remaining within the safe zone.[10]

This case series was undertaken to determine if other practitioners in a clinic setting can effectively perform the procedure outlined in the course manual. The research team aimed to determine whether the procedure outlined by Buford would produce a similar number of MSCs when performed by other practitioners. Cell colony forming units-fibroblasts (CFU-f) were used as a surrogate for functional MSCs. Several authors have published their total nucleated cell counts (TNCC) and CFU-f values in clinical studies.[11]

METHODS

Patient Selection

The inclusion criteria for this study were patients with diagnosed glenohumeral osteoarthritis, knee osteoarthritis, hip osteoarthritis, or rotator cuff tears whose condition had failed to improve with conservative care. All patients knew surgical options and completed the appropriate counseling and consent forms. Exclusion criteria were active malignancy, present long-term anticoagulation that could not be stopped for two weeks, NSAID use two weeks before the procedure, or systemic infections.

Posterior Iliac Crest Aspiration UPICA Technique

All procedures were performed by the senior author (BG). With the patient placed in a prone position, the inferior portion of the posterior iliac crest was identified by ultrasound utilizing an L14-4 high-frequency linear probe (SonImage HS1, Konica Minolta, Tokyo, Japan) and landmarks delineated using an indirect method of bone marrow aspiration. The area of harvest was prepared with Hibiclens skin cleanser (Mölnlycke, Gothenburg, Sweden), and anesthetized with 1% lidocaine (Hospira, Inc., Lake Forest, Illinois, USA) without epinephrine down to and including the periosteum, titrating up to 20 mL of local anesthetic as needed. The needle

was kept in place for localization. After waiting a minimum of 5 minutes for the local anesthesia to act, a sterile field was prepared. A small skin incision was made with a # 11 blade. Next, an 11-gauge 6-inch Jamshidi™ needle (Ranfac Corp., Avon, Massachusetts, USA) was advanced through the incision and down to the periosteum.

Using manual force, the needle was advanced past the periosteum and into the iliac crest approximately 2 cm and verified on the needle distance markings. The trocar was removed, and 6–8 mL of bone marrow was aspirated into a 10 mL syringe pre-coated with between 0.5 and 1 mL of heparin (concentration 10,000 units/mL, McKesson Corp., Irving, TX, USA). The amount of BMA harvested at each site varied based on the rate of return and patient discomfort. The harvest needle was then rotated 90 degrees, and the physician attempted to aspirate another 6–8 mL into another 10 mL syringe with 1 mL of the heparin mixture. This resulted in 12–16 mL of BMA from this level mixed with 1–2 mL of heparin solution. The trocar was replaced, and the needle was advanced by 0.5 cm. After removing the trocar, the physician aspirated using a similar technique. This was repeated after another advancement of 0.5 cm. This procedure was repeated until obtaining approximately 60 mL of BMA with the heparin solution.

The trocar was reinserted after the final aspiration, and the harvesting needle was manually removed. Direct pressure was applied for 5 minutes after the procedure to maintain hemostasis. The area was then dressed with a small pressure dressing. Most patients had mild discomfort during the procedure that resolved when the procedure was completed. No infections or complications were reported at one-month and six-month follow-up visits. Only one patient was lost to follow-up at the six-month visit. However, this patient returned at the one-year mark and reported no signs of infection or complications at the procedure site.

The collected 60 mL BMA was mixed thoroughly before drawing off 1 mL for laboratory analysis. The remaining 59 mL was filtered, then placed in the ART BMC concentration system for concentration by centrifugation (Celling Biosciences, Austin, TX, USA). The 1 mL pre-concentration sample and a 1 mL post-concentration sample were sent for laboratory analysis by the Celling Biosciences Laboratory.

Statistical analysis was performed using Excel (Microsoft Corp., Redmond, WA, USA). Calculations of descriptive statistics (means, standard deviations, minimums, maximums) were performed for each set of values. A paired t-test was chosen to compare the TNCC/mL and CFU-f/mL values for the BMA and BMC samples after a normality test revealed normal distributions for each set of values. The Jarque-Bera test was utilized to determine the normality of each distribution. For each set of values, this test revealed a p-value greater than 0.05 indicating insufficient evidence to conclude that the datasets did not follow a normal distribution. Thus, assuming a normal distribution for each set of values, the paired t-test was chosen to evaluate differences in means between the two groups of values.[12]

RESULTS

For the 10 cases completed, the average patient age was 72 (SD 7.6; range 58–81). The sample included 8 males and 2 females. The mean TNCC/mL BMA was 9.7E+06 (SD 2.9E+06), while the TNCC/mL BMC was 43.9E+06 (SD 29.5E+06), a 4.3 (SD 2.3)-fold increase in concentration. See Table 1. Viability for the BMA was 97.5 (SD 2.3) %. See Table 1. The mean CFU-f/mL BMA was 520 (SD 155) with a mean CFU-f/mL BMC of 4,899 (SD 2,887), resulting in a 9.6 (SD 6.3)-fold concentration. Viability for the BMC was 94.9 (SD 4.6) %. See Table 3.

DISCUSSION

Several outliers may be indicative of variable patient intrinsic factors and cell viability.[13,14] Studies have shown that multiple sites and lower draws per site result in better yields. The number of syringes and the fact that the initial 60cc large collecting syringe was coated with several mL of heparin accounted for our usual collection being 54 mL of BMA and 6 mL of heparin. The patient's tolerance to aspiration speed may have also affected the counts.

Table 1. Comparison of TNCC/mL BMA to TNCC/mL BMC

Patient	Age (years)	TNCC/mL BMA	TNCC/mL BMC	Baseline Increase	Viability %
1	58	10,329,000	103,343,333	10	98
2	74	13,275,294	47,232,857	3.6	99
3	76	10,428,000	34,256,667	3.3	96
4	72	12,879,000	54,107,778	4.2	99
5	75	8,977,500	25,620,000	2.9	99
6	81	4,615,333	15,122,667	3.3	92
7	78	6,771,571	20,788,571	3.1	99
8	60	10,434,909	31,655,333	3	99
9	77	12,563,222	85,620,000	6.8	96
10	69	7,058,882	21,827,647	3.1	98
Mean	72 (SD 7.6)	9,733,271 (SD 2,876,504)	43,957,485 (SD 29,469,170)	4.3 (SD 2.3)	97.5 (SD 2.3)

Table 2. Comparison of TNCC/mL BMA to TNCC/mL BMC

Variable	Mean (SD)		*t*-statistic (df)	*P*-value[a]
TNCC/mL	BMA 9,733,271 (2,876,504)	BMC 43,957,485 (2,9469,170)	–3.895 (9)	<0.005

[a]*Paired t-test*

Table 3. Comparison of CFU-f/mL BMA to CFU-f/mL BMC

Patient	Age	CFU-f/mL BMA	CFU-f/mL BMC	Baseline Increase	Viability %
1	58	409	10,541	25.8	99
2	74	829	6,214	7.5	99
3	76	698	5,040	7.2	98
4	72	521	5,711	11	99
5	75	497	2,898	5.8	95
6	81	405	1,781	4.4	91
7	78	352	2,818	8	94
8	60	498	3,735	7.5	85
9	77	625	8,391	13.4	97
10	69	366	1,868	5.1	92
Mean	72 (SD 7.6)	520 (SD 155)	4,900 (SD 2,887)	9.57 (SD 6.3)	94.9 (SD 4.6)

Buford reviews his own Cell Analysis Results in his course manual, showing average cell viability for BMA and BMC of 97.5%. Our BMA viability was also 97.5%; however, the BMC viability was 94.9%. The concentration process or overnight shipping from Florida to the Celling Biosciences Laboratory in Texas may have diminished sample viability.

Buford had a 6.0-fold concentration in both TNCC/mL and CFU-f/mL. Our data revealed a 4.3-fold concentration in TNCC/mL and a 9.6-fold concentration in CFU-f/mL.

Buford published a TNCC/mL BMA of 2.0E+07 compared to our 0.9E+07. Fennema et al., showed great variability in their concentration of nucleated cells.[13] However, the CFU-f/mL BMC values were

Table 4. Comparison of CFU-f/mL BMA to CFU-f/mL BMC

Variable	Mean (SD)		*t*-statistic (df)	*P* -value[a]
CFU-f/mL	BMA 520 (155)	BMC 4,900 (2,887)	–4.883 (9)	<0.005

[a]*Paired t-test*

Table 5. Comparison with Buford/Published Protocol Values

Variable	TNC/mL (BMA)	TNC/mL (BMC)	Fold Concentrated	CFU-F/mL (BMA)	CFU-F/mL (BMC)	Fold Increased
Buford	2.00E+07	1.19E+08	6	870	4907	6
Garcia	9.70E+06	4.39E+07	4.3	520	4900	9.57

almost identical, with values of 4,907 (Buford) versus our 4,899. This discrepancy was accounted for by the larger fold increase experienced with concentration of the CFU-f/mL. There does not appear to be a clear correlation between TNCC/mL and CFU-f/mL. The CFU-f/mL data is unavailable during the procedure because the colony-forming units develop over two weeks. However, CFU-f/mL counts may yield a more accurate picture of the eventual clinical outcome.[15] Future investigation should be focused on developing a more thorough understanding of the correlation between CFU-f/mL counts and clinical outcomes.

CONCLUSIONS

Utilizing the UPICA technique, similar yields for the BMC CFU-f/mL counts were obtained to those reported previously by other clinicians. Additionally, our results demonstrated the ability of the bone marrow concentration system to successfully concentrate the BMA. Based on these findings, the research team endorses the UPICA technique as a viable method for bone marrow aspiration.

AUTHORS' CONTRIBUTIONS

NO was responsible for the statistical analysis of data, revising the manuscript for important intellectual content, editing the manuscript, and final approval of the version to be published.

BG was responsible for the study conception and design, acquisition and analysis of data, drafting and editing the manuscript, and final approval of the version to be published.

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