Title: Comparison of the Cellular Content of Bone Marrow Aspiration from the Posterior Superior Iliac Spine and Deep Humeral Harvest in Patients Undergoing Rotator Cuff Repair


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## Abbreviations and Definition

AE Adverse Event

ACDA Anti-Coagulant Citrate Dextrose A

BMA Bone Marrow Aspirate

CBC Complete Blood Count

cBMA Concentrated Bone Marrow Aspirate

CFU-F Colony Forming Unit-Fibroblast

Cm Centimeter

EDC Electronic Data Capture

HIPAA Health Insurance Portability and Accountability Act

HPC Hematopoietic Progenitor Cell

ICF Informed Consent Form

IRB Institutional Review Board

IV Intravenous

MPCs Mesenchymal Progenitor Cells

mL Milliliters

PI Principal Investigator

SAE Severe Adverse Event

SOC Standard of Care

TNC Total Nucleated Cell

UP Unanticipated Problem

## Table of Contents

## 1 Background / Scientific Rationale

Musculoskeletal injury is a significant global health concern, with injury contributing to large amounts of patient pain, disability, and a decrease in overall wellbeing [1]. Emerging areas of research and treatment in orthopedic injuries using stem and progenitor cells have shown great potential. Stem cells are biological cells which differentiate into many types of human tissue. Stem cells differentiate into specific cell lineages to manage healthy tissues and cells, as well as repair damaged tissues and cells. Stem cells can specialize into progenitor cells, which have gone one step further toward a specific differentiation pathway. Progenitor cells then change according to the given environment (i.e., growth factors, stress) and perform specific cellular functions. Progenitor cells are found in many tissues, including the peripheral blood and bone marrow. Progenitor cells act as important repair cells for the musculoskeletal system, specifically for bone and cartilage [1]. These progenitor cells are mobilized and activated to the peripheral blood circulation during episodes of stress, injury, and exercise [2, 3]. Cell therapies using progenitor cells have shown great potential for addressing acute, chronic, and traumatic orthopedic injury.

One cell therapy of interest currently involves bone marrow aspiration and concentration. Bone marrow aspiration and concentration is a method of progenitor cell harvest. Orthopedic surgeons have begun to augment surgical procedures and treat degenerative conditions such as osteoarthritis and cartilaginous injury with injections of concentrated bone marrow aspirate (cBMA) [4-8] .The use of cell therapies may contribute to decreased recovery time and morbidity rates [9-11]. Hernigou et al. showed significant improvement in patient outcomes could be achieved by the use of cBMA as an adjunct therapy in standard of care rotator cuff repair (RCR) [5]. In clinical application, studies have suggested that the success of these treatments is dependent on the total quantity and utility of harvested progenitor cells [5,7]. While bone marrow aspiration is frequently utilized for orthopedic point of care applications, the number of progenitor cells harvested is variable and dependent on body mass, aspiration technique, and location of harvest [5, 12]. Optimizing the harvest procedure is a key step in the development of bone marrow aspirate for orthopedic applications. Studies have attempted to find the most appropriate locations for harvesting bone marrow aspirate [13-17]. The preferred site for bone marrow extraction is the posterior iliac spine [13-17]. The posterior iliac spine yields the greatest concentration of viable nucleated cells when compared to the anterior iliac spine [13]. The posterior iliac spine also yields a greater concentration of nucleated cells when compared to other appendicular osseous sites, including the distal femur, proximal tibia, distal tibia, calcaneus, and proximal humerus [13-16]. In one study, the cBMA harvested from the posterior superior iliac spine (PSIS) resulted in a 3.0 times greater cellular yield and an 8.3 times greater proliferative cell product than cBMA from the proximal humerus [17] . Determining the quantity of progenitor cells in these samples can provide clinicians with a more cost-effective alternative for harvesting progenitor cells that can be used for surgical augmentation. However,

the potential for progenitor cells to be harvested from the diaphysis of the humerus (deep humerus) has yet to be quantified. Thus, this the aim of this study is to analyze the cellular characteristics of cBMA from two extraction sites (PSIS and deep humerus) from patients undergoing standard-of-care arthroscopic rotator cuff repair surgery. Additionally, we aim to determine if patient positioning during PSIS harvest has any effect on the cellular content of collected cBMA.

## 2 Study Design

This is a single site, comparative quantitative analysis study of the cellular characteristics of bone marrow aspirate from two extraction sites (PSIS and deep humerus) from patients undergoing standard-of-care arthroscopic rotator cuff repair surgery. 30 total patients will be recruited through the Andrews Institute physician practices. Potential participants will be prescreened for inclusion and exclusion criteria through standard of care medical evaluations. Once a potential participant has agreed to be involved in the study, they will go through the described informed consent process. Patients meeting the inclusion criteria will have the study explained to them by one of the members of the research team, and they will be given an opportunity to participate if they are interested. No specific advertising or recruitment material will be utilized.

After the described screening and informed consent process have been completed, all participants will undergo standard-of-care arthroscopic rotator cuff repair surgery. During all surgeries, bone marrow will be aspirated from the PSIS and from the deep humerus. The first 15 participants will have bone marrow aspirated from the PSIS while in the prone position on the operating table. The final 15 participants will have bone marrow aspirated from the PSIS while in the lateral decubitus position on the operating table. Both cohorts will undergo deep humeral harvest intraoperatively in the lateral decubitus position. One mL of cBMA from each harvest site from each surgery will be removed and sent to the AREF Regenerative Medicine Center (RMC) for analysis. The remaining cBMA will be used to augment the rotator cuff repair surgery.

## 3 Objectives

### 3.1 Primary Objective

The primary objective of this study is to determine whether the PSIS or deep humeral harvest produces a greater cellular harvest in patients undergoing rotator cuff repair.

### 3.2 Secondary Objective

The secondary objective is to determine if prone verses lateral decubitus participant positioning influences the quantity of harvested cells from the PSIS.

### 3.3 Primary Effectiveness Endpoints

The primary effectiveness endpoints of this study include cellular content measures of cBMA collected from each extraction site (as outlined in section 6.2).

### 3.4 Secondary Effectiveness Endpoints

The secondary effectiveness endpoints of this study include cellular content measures of cBMA collected from each anatomical position (as outlined in section 6.2).

## 4 Hypotheses

### 4.1 Null Hypothesis:

$H_0$ - Participants will have similar cellular content (*in vitro)* in bone marrow aspirate taken from the PSIS and from deep humeral harvest. Additionally, patient positioning will not affect the cellular content of bone marrow from the PSIS.

### 4.2 Alternative Hypothesis:

$H_1$ - Participants will not have similar cellular content (*in vitro)* in bone marrow aspirate taken from the PSIS and from deep humeral harvest. Additionally, patient positioning will affect the cellular content of bone marrow from the PSIS.

## 5 Participant Recruitment and Screening

### 5.1 Screening Process:

Once interested participants are identified and prescreened, a screening visit will be scheduled (visit 1). During the screening visit, a screening form will be completed which includes the inclusion and exclusion criterion below. If an individual answers "yes" to any of the initial screening exclusion questions, they will be informed that they do not qualify for the study, and they will be informed that they can keep their screening form and their medical care will not be adversely affected by not enrolling in the study. If all answers are "no" then the form will be placed in the study documents and the enrollment process will continue.

#### 5.1.1 Inclusion Criteria:

- 18-80 years of age
- Diagnosis of a rotator cuff tear requiring arthroscopic rotator cuff repair

#### 5.1.2 Exclusion Criteria:

- Patients who require superior capsular reconstruction or revision rotator cuff repair
- Diabetes
- Immune disorders

- Past medical history of a metastatic or other cancer which required chemotherapy/radiation therapy
- Rheumatoid arthritis
- Is unable to comprehend the study documents or give informed consent

Once the screening requirements are met, the informed consent form will be provided to the participant on paper or electronically via REDCap for review ensure the volunteer understands the details of the study including the benefits and risk factors. The subject will be provided sufficient time to consent and sign the informed consent form (ICF). The principal investigator (PI) and study/research team will be available to answer any questions or provide clarifications during the informed consent process.

## 5.2 Informed Consent 21 CFR 50

In adherence to the 21 CFR 50, Protection of Human Subjects Guidelines, the informed consent process will be performed by one of the study investigators or staff, in the research office that has received training on the informed consent process. No aspects of the study will be conducted prior to obtaining informed consent from each participant. The purpose and methods of the study along with the expected effects will be reviewed with each potential participant. Each participant will be provided a copy of the consent and sufficient time will be given for the opportunity to read and ask any questions about the study. After signing of the informed consent document, participants will be given a copy for their records.

The designee will review with each participant that they are free to refuse to the study or to withdraw from it at any time.

## 5.3 Consent Withdrawal:

During the informed consent procedure, participants will be informed that if at any point during the study, consent may be withdrawn. To withdraw consent, participants can request in writing to withdraw HIPAA authorization and the research site will not use or provide any health information to researchers. At this time, the link between the participant's health information will be severed with the research team. This process for consent withdrawal will be reviewed with each participant and identified barriers will be addressed at the time of informed consent.

## 5.4 Benefits:

Participants may directly benefit from improved healing via cBMA augmentation of their rotator cuff repair as a result of agreeing to participate in the study. Participants will receive at no extra charge cell augmentation therapy of their rotator cuff repair. Indirect benefits include additional medical care and follow-up after surgery. This study may help

direct clinical practice in developing preferred techniques to augment orthopedic surgical procedures (aspirational benefit).

### 5.5 Compensation:

Compensation will not be provided to participants for participating in this study.

## 6 Treatment Plan

After the described screening and informed consent process have been completed, all participants will undergo standard-of-care arthroscopic rotator cuff repair surgery. During all surgeries, bone marrow will be aspirated from the PSIS and from the deep humerus. The first 15 participants will have bone marrow aspirated from the PSIS while in the prone position on the operating table and deep humerus during the surgery in the lateral decubitus position. The final 15 participants will have bone marrow aspirated from the PSIS while in the lateral decubitus position on the operating table and deep humerus during the surgery in the lateral decubitus position. Bone Marrow Aspirate will be concentrated at a 12% hematocrit setting independently from each site. One mL of cBMA from each harvest site from each surgery will be removed and sent to the AREF RMC for analysis. The remaining cBMA will be used to augment the rotator cuff repair surgery.

### 6.1 Surgical Procedure and cBMA Collection:

The first 15 participants will be placed in the prone position on the operative table for the bone marrow harvest from the PSIS. After bone marrow harvest from the PSIS, the patient will be placed in the lateral decubitus position for the rotator cuff repair. Rotator cuff repair is always performed in either the lateral decubitus position or an upright, seated position called the beach chair position. The final 15 participants will placed in the lateral decubitus position on the operative table for the bone marrow harvest and the rotator cuff repair. The PSIS harvest will be performed first. The skin will be prepped and draped in sterile fashion over the PSIS. Lidocaine 1% will be used to anesthetize the skin around the incision location. A stab incision will be made in the skin over the PSIS. Two 30 cc syringes and a traditional 11-gauge, 11 cm Jamshidi needle (Ranfac Corporation, Avon, MA, USA) will be pre-rinsed with heparin. The two 30 cc syringes will be loaded with 4 cc of anticoagulant citrate dextrose solution-A (ACDA). The needle will be advanced into the bone marrow cavity 3-4 cm. 30 mL of bone marrow will be aspirated while the needle is withdrawn and rotated with one syringe. The needle will then be reinserted divergently, and another 30 mL of bone marrow aspirated with the second syringe. This method has been described and quantitively studied with similar harvest to a multiple puncture technique (18). A sterile dressing will be applied. The participant will be re-prepped and draped in standard fashion for shoulder arthroscopy. A diagnostic arthroscopy will be performed, and the rotator cuff tear identified. While

viewing from the standard posterior portal in the subacromial space, a spinal needle will be used to localize the location and trajectory for placement of the first suture anchor for the rotator cuff repair. Once the placement of the proposed anterior suture anchor is confirmed, the 11-gauge bone marrow harvest needle will be placed into this location and the arm adducted. The needle will then be advanced into the humeral diaphysis as far as the needle length allows. Two 30 mL syringes will be pre-filled with 4 mL of ACD-A and used to harvest 60 mL of bone marrow aspirate. The bone marrow samples will then be processed independently using the Arthrex Angel blood processing system (Arthrex, Naples, FL) using the 12% hematocrit setting with two separate disposables. One mL of cBMA from each harvest site will be removed and sent to the AREF RMC for analysis. The remaining cBMA will be used to augment the rotator cuff repair surgery.

### 6.2 Cellular Analysis:

Cellular analysis of each cBMA sample from the two collection sites per participant will include a Complete Blood Cell (CBC) count, a Total Nucleated Cell (TNC), viability count, and cell culture analysis.

The CBC count will be performed using an automated hemocytomter (XN-350; Sysmex, Kungsbacka, Sweden) and will include red blood cell (RBC), white blood cell (WBC), monocyte, platelet, and hematopoietic progenitor cell (HPC) concentration.

The TNC and viability count will be performed using an automated cell counter.

Cell culture experiments will include Colony Forming Unit-fibroblast (CFU-F) counts and the potential for additional stem or progenitor cell analysis procedures. If stem or progenitor cells are observed during culturing, additional experiments will be conducted to analyze differentiation potential (differentiation assays) and cell-surface markers (flow cytometry).

## 7 Review of Safety

### 7.1 Adverse Event (AE)

An adverse event is any untoward or unfavorable medical occurrence in the human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research, whether considered related to the subject's participation in the research.

### 7.2 Serious Adverse Event (SAE)

Serious adheres events are any events that:

- Result in death

- Is life threatening, or places the participant at immediate risk of death from the event as it occurred
- Requires or prolongs hospitalization
- Causes persistent or significant disability or incapacity
- Results in congenital anomalies or birth defects
- Is another condition which investigators just to represent significant hazards

### 7.3 Unanticipated Problem (UP):

Defined by DHHS 45 CFR part 46 as any incident, experience, or outcome that meets the following criteria.

- unexpected, in terms of nature, severity, or frequency, given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the study population.
- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
- suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

### 7.4 AE & SAE Collection and Reporting

Throughout the study the research team will monitor the occurrence of AE and SAE. Data will be collected if an instance occurs, and the PI will be notified. All AE data, such as onset date, resolution date, outcome and treatments given will be documented in the source documents and will be recorded in the EDC and analyzed for severity to follow reporting protocol if severity level.

Follow-up will occur using the provided safety monitoring form if AE occurs. The follow up will end either when the symptoms resolve or up to 30 days past the end of the study participation.

### 7.5 Risks and Discomforts:

Standard sterile precautions for surgical procedures will be utilized for the rotator cuff repair surgery, but with any surgery there is risk of infection, bleeding, and swelling. There will also be a small needle insertion hole into the humerus and the iliac crest for the bone marrow stem cell collection, which will be about the same size as an IV insertion. This may have some minor pain or swelling and there is a very minor risk of infection. Pain post procedure can occur but is usually self-limiting. Bruising may also

occur. As with any research involving patients there is the inherent risk of a breach in patient confidentiality though this will be minimized using participant code numbers and adherence to all HIPAA guidelines.

## 8 Data Management Procedures

All personal information is strictly confidential, and no names will be disclosed except as required by law. Data from cellular analysis and demographics will be compiled in Microsoft Excel. The spreadsheet will not contain protected health information as all compiled data will be de-identified. The spreadsheet will be stored in a secure password protected folder on a laptop that only the study Investigators will have access to and will be permanently deleted following publication of any and all manuscripts, if any, written as a result of this research. Records related to this study will be securely retained in a secure location for a period of 3 years after the completion of the study or longer as required by law. At that time, all records will be properly destroyed.

## 9 Data Analysis

A combination of Excel and SAS Studio (Version 3.8 on SAS 9.4, SAS Institute Inc., Cary, NC) will be used for data analysis. All compiled data will be de-identified.

## 10 Statistical Considerations

Descriptive statistics will be compiled for all numeral measures (Demographics, CBC, TNC, viability, CFU-F). Data will be analyzed for differences between experimental groups (PSIS v. deep humeral harvest, Prone vs. lateral decubitus positions) using 1-Way ANOVA and appropriate post-hoc analysis procedures (ex: Tukey's Honestly Significant Difference (HSD)). A significant p-value of $< 0.05$ for rejecting the null hypothesis will be used for all measures.

## 11 Quality Control and Assurance

All protocols will be monitored and analyzed data will be checked for accuracy by the principal investigator and /or a designated AREF research team member. All medical data will be kept in compliance with HIPAA guidelines.

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