

## **Age and Sex Influence the Mesenchymal Stem Cell Composition of Bone Marrow Aspirate Concentrate (BMAC)**

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### **INTRODUCTION**

Bone marrow aspirate concentrate (BMAC) use is exponentially expanding, in part due to the fact it constitutes one of the few methods of delivering stem cells along with several anti-inflammatory growth factors. Although numerous trials have reported that BMAC effectively concentrates hematopoietic progenitor cells (HPCs), limited data are available on the ability of BMAC to concentrate mesenchymal stem cells (MSCs), given that these cells comprise as little as 0.01% of bone marrow aspirate (BMA). In addition, the impact of demographics factors on mesenchymal stem cell concentrations is unknown.

### **PURPOSE**

The purpose of this study was to quantify the change in mesenchymal stem cell concentration change between BMA and BMAC. Additionally, to determine differences in mesenchymal stem cell concentrations in BMA versus BMAC based on age, sex and BMI. We hypothesized that BMAC will have a significantly higher number of mesenchymal stem cells compared to BMA. Also, that older adults and those with higher BMI will have significantly reduced MSC concentrations in BMA, and thus in BMC. Sex will have an insignificant relationship with BMA and BMAC MSC concentrations.

### **METHODS**

BMA was harvested from the non-dominant iliac crest of 36 healthy patients (20 female) undergoing partial meniscectomy, anterior cruciate ligament (ACL) reconstruction or rotator cuff repair (RCR) using an Arthrex Angel BMC Kit (Arthrex Inc., Naples, FL, USA). Exclusion criteria included revision procedures, worker's compensation patients, and various medical comorbidities (e.g. cancer, autoimmune conditions, diabetes). Demographic factors including age, sex and BMI were collected via chart review. With respect to age, the cohort was broken into tertiles, with a 'young' group (24-38 years old; n=12), 'middle' group (39-56 years old; n=12) and 'old' group (56-72 years old; n=12). Once harvested, BMA was immediately processed into BMAC to a hematocrit of 7 by using the Arthrex Angel cPRP & Bone Marrow Processing System. Processed BMAC was stained for mesenchymal stem cell (MSC) surface markers (CD90, CD105 and CD73) within 12 hours of collection using a Human MSC Analysis Kit (BD Biosciences, San Jose, CA, USA). Flow cytometry was conducted using TruCount tubes (BD Biosciences, San Jose, CA) to calculate absolute counts and concentration of MSCs. MSCs

were defined as cell that were CD45-, CD90+, CD105+ and CD73+, using FlowJo v9.9 software (FlowJo Inc., USA). Statistical analysis was conducted using R v3.4.1 (R Foundation for Statistical Computing, Vienna, Austria). Paired Student's t-tests and Chi-squared tests were run to compare mean differences between MSC concentration values and age, sex and BMI for continuous and categorical variables, respectively.

## **RESULTS**

Of the 36 healthy patients, average  $\pm$  SD BMI was  $27.52 \pm 4.70$  with average age  $50.41 \pm 13.71$ . An average of  $46.57 \pm 9.57$  ml of BMA was drawn, yielding  $3.08 \pm 1.23$  ml of BMAC. The average concentration of CD45-CD90+CD105+CD73+ cells in BMA was  $2.51 \pm 1.21$  MSC/ul, whereas in BMAC the average concentration was  $14.89 \pm 3.52$  MSC/ul. The concentration of CD45-CD90+CD105+CD73+ cells between BMAC and BMA increased by 5.93 on average ( $P < 0.001$ ). Significant mean differences were observed between the 'young' and 'old' age groups in terms of mean BMA concentration ( $3.075$  vs.  $1.57$  BMA MSC/ul,  $P < 0.001$ ) and mean BMAC concentration ( $12.40$  vs.  $20.93$  BMAC MSC/ul,  $P < 0.001$ ). BMAC:BMA concentration ratio differed significantly between each age group, with the 'old' group experiencing the most concentration post-processing ( $23.63$  vs.  $13.78$  vs.  $7.02$ ,  $P < 0.001$ ). Men and women had significant different mean BMA concentrations ( $2.17$  vs.  $2.69$  MSC/ul,  $P = 0.001$ ), BMC concentrations ( $12.70$  vs.  $17.39$  MSC/ul,  $P < 0.001$ ) and BMAC:BMA MSC concentration ratios ( $21.54$  vs.  $10.03$ ,  $P < 0.001$ ). No significant relationship was found between BMI and BMA concentration ( $P = 0.46$ ) or BMAC concentration ( $P = 0.42$ ).

## **CONCLUSION**

The main findings of our study were that BMAC processing significantly concentrates MSCs in BMA. Importantly, increasing age and sex both exert an effect on MSC concentration of BMA and BMAC. Both age and sex must be considered and compensated for when delivering stem cells to patients in research and clinical settings.