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Research Article

Laboratory Quantification of Bone Marrow Concentrate Components in Unilateral Versus Bilateral Posterior Superior Iliac Spine Aspiration

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ABSTRACT

Mesenchymal Stem Cells (MSCs) from Bone Marrow Concentrate (BMC) have emerged as a promising treatment for degenerative musculoskeletal pathologies, such as Osteoarthritis (OA). Many aspiration techniques have been described in the literature with little consensus on optimal methodology. This study aimed to compare MSC quantity in unilateral versus bilateral Posterior Superior Iliac Spine (PSIS) bone marrow aspirate concentrations. Patients with unilateral knee OA seeking treatment with intraarticular BMC were recruited and randomized to a unilateral PSIS Bone Marrow Aspiration (BMA) or a bilateral PSIS BMA of equal total volumes. BMA and BMC samples underwent laboratory analysis of Colony Forming Unit-Fibroblasts (CFU-fs) as a marker for MSCs, for quantification of Total Nucleated Cell (TNC) count, and CD-34 positivity, in addition to other metrics. Data from 26 patients were analyzed. Mean total CFU-fs were 1.9 times higher in the bilateral group (n=13) versus the unilateral group (n=13); 42,912 versus 23,038, respectively (p=0.17). The median number of CFU-fs cultured from 1 ml of BMC in the bilateral cohort was 33% higher than the unilateral group (2477 versus 1860 CFU-fs/ml, respectively (p=0.23). Despite the difference in CFU-fs, the TNC counts were similar between the two groups. This descriptive study suggests a lower volume; multisite draw-technique for BMA increases the absolute number of CFU-fs, and therefore the correlated MSC count. Due to the limited statistical power, these data will need to be further evaluated with a larger patient dataset and correlated with patient outcomes data to determine clinical significance.

Keywords: Bone marrow aspiration technique; Bone marrow concentrate; Quantitative analysis; Mesenchymal stem cell

INTRODUCTION

As life expectancy is increasing, musculoskeletal pain has emerged as a leading cause of years lost to disability worldwide [1]. Osteoarthritis (OA) is the most prevalent type of cartilage degenerative disease characterized by a complex interplay of mechanical injury, joint instability, and upregulation of matrix metalloproteinases and inflammatory cytokines, all leading to erosion of cartilage on articular surfaces [2]. These physical changes can lead to alterations in biomechanics and resultant pain, potentially of a debilitating nature. The prevalence of symptomatic knee OA is 6% in people over age 30 in the United States [3], 16% in patients over age 45 years, increases with age, and is more prevalent females [4,5].

Traditional care for pain related to OA has been to abate symptoms via conservative measures, including physical therapy, medications such as Non-Steroidal Anti-Inflammatories (NSAIDs), and corticosteroid injections [6]. With the exception of physical therapy, emerging evidence regarding the side effect profiles and risks associated with NSAIDs [7,8] and corticosteroids [9,10] make traditional conservative management strategies less than ideal for long-term management. As a last resort, surgical options such as partial or total knee arthroplasty can help, though surgery does come with increased risks and up to 12% of patients do not

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experience clinically significant improvement following surgery [11]. Furthermore, one in five patients are dissatisfied with their outcomes following primary total knee arthroplasty [12].

The greater understanding of the pathophysiology underlying OA, improvements in technology, and a push for a paradigm shift in the treatment of degenerative musculoskeletal disorders has led to advances in the use of regenerative medicine over the past two decades. The unifying principle of these types of treatments, particularly for intraarticular therapies, lies in the ability of the injectates to modulate the joint micro-environment via paracrine signaling, decreasing inflammatory cytokines, decreasing catabolic activity of Matrix-Metalloproteinases (MMPs), and direct cell differentiation to positively affect pain and functional outcomes [13-15]. The emergence of regenerative medicine occurred in the 1950s with the use of hypertonic solution to treat ligamentous laxity with resultant pain via a proliferative mechanism of action, known as prolotherapy [16].

More significant advances were made in in the 1980s with the use of Platelet-Rich Plasma (PRP) [17,18] and in the 1990s with Mesenchymal Stem Cells (MSCs) for orthopedic use [19], which can be harvested from bone marrow, adipose tissue, chondral tissue, and synovium [13]. Over time, the term orthobiologics has developed as an overarching term to describe these substances with biological activity that can be injected to induce improvements in musculoskeletal microarchitecture and inflammatory tissue profiles [20]. These therapies have the potential to fill the gap between traditional conservative care and surgery by offering treatments with the potential for disease modification, rather than purely symptom management.

The therapeutic potential of MCSs harvested via Bone Marrow Aspiration (BMA) and centrifuged to create Bone Marrow Concentrate (BMC), has become of great interest in the literature for treatment of OA. BMC has been shown to have anti-inflammatory, immunomodulatory, anti-apoptotic, antibacterial, and chondrogenic properties [21]. Another mechanism theorized to provide clinical benefit is the ability of bone marrow derived MSCs to differentiate into bone, cartilage, muscle, and tendon [22]. Paracrine signaling of growth factor and cytokines, such as Transforming Growth Factor Beta (TGF-ß), Vascular Endothelial Growth Factor (VEGF), and Fibroblast Growth Factor (FGF), may also play a role in modulating tissue repair and micro-environment modulation [23,24]. In addition, BMC has been shown to have higher concentrations of Interleukin-1 Receptor Antagonist (IL-1Ra) than those found in other bone marrow and blood preparations, which may contribute to additional anti-inflammatory properties [25].

Though MSCs are located in many tissues throughout the body, to be compliant with homologous use [26] and for ease of access, BMA/BMC are among the most commonly utilized source of MSCs in the United States. Based on previous studies, it is generally accepted that the highest yield of MSCs can be obtained from ilium [27,28] and therefore the Posterior Superior Iliac Spine (PSIS) is a common harvest site. Regarding dose, higher number of MSCs has been associated with improved outcomes [29], and specifically a TNC count of greater than 400 million cells was shown to yield significantly improved outcomes in following intra-articular knee injection with BMC [30]. However, there is a lack of consensus on optimal aspiration technique in the literature. Trabecular bone contains a variety of niches within which many different cell types live. MSCs are most commonly found in the subcortical endosteal region, as well as perivascularly in the deeper marrow space (Figure 1) [31,32].

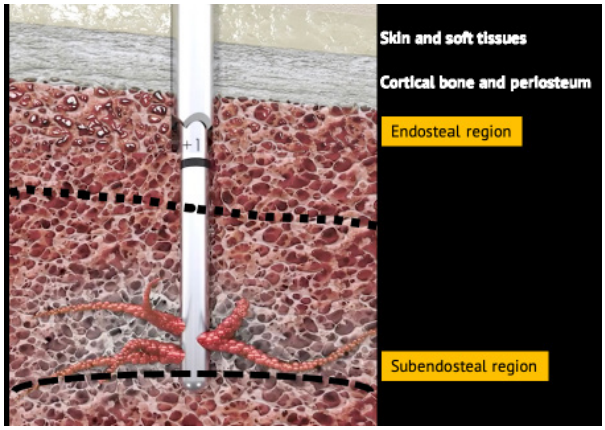

Figure 1: Schematic diagram highlighting the regions within the bone marrow cavity. The highest concentrations of MSCs are found in the subcortical endosteal region, as well as perivascularly in the deeper marrow space.

Several studies have previously shown that small volume, rapid draws of bone marrow during aspiration yields the highest quality aspirations [33-39] but determination of precise volume needed and syringe size vary [40]. Furthermore, one study suggests that a smaller syringe size (10 cc) results in a higher number of MSCs due to the negative pressure created that pulls the MSCs from the stroma when compared with a 50 cc syringe aspirating the same volume [36]. This has not been reproduced in other studies and there is some suggestion that similar cells counts are obtained when using 30 cc syringes compared with 10 cc sizes [35]. In addition, a recent study by Oliver et al on a small number of patients showed no differences in CFU-fs in multisite BMA versus single site BMA of the same volume [41]. In order to add to the body of literature to promote better understanding of optimal bone marrow aspiration technique, we aimed to compare the total number of CFU-fs obtained via low-volume bilateral PSIS aspiration with high volume BMA extraction from unilateral PSIS draws. Based on the most common locations of MSCs within trabecular bone marrow and previously published literature, our hypothesis was that bilateral draws of lower volumes would yield higher numbers of total CFUs than unilateral, higher volume draws.

The secondary aim of this study was to measure differences in pain and patient reported functional outcomes between the two groups after unilateral intra-articular BMC injection for knee osteoarthritis. This clinical outcomes data remains in the data-collection phase and will be reported at a later date.

MATERIALS AND METHODS

Patient selection and recruitment

Patients who were seeking treatment for knee osteoarthritis with bone marrow concentrate were recruited from a single, academic sports medicine center. Approval was obtained by the university's Institutional Review Board. To meet inclusion criteria, patients were required to be between the ages of 18 and 70, have unilateral symptomatic knee OA, and have cognitive capacity to provide informed consent. Patients with cortisone injection into the affected joint within 6 weeks, NSAIDs within one week of the planned BMC procedure, a history of bleeding disorders or inflammatory joint disease, a surgical intervention on the affected

or contralateral joint within 3 months, infection of the joints within 6 months, and those with an active systemic infection or active malignancy were excluded from the study. Pregnant and breast-feeding patients were also excluded.

Laboratory analysis

All BMA and BMC preparations were prepared following the laboratory instructions and specimen was shipped for analysis to an independent, FDA and Good Laboratory Practice accredited laboratory (Biosciences Research Associates, Inc. 767 cc Concord Ave, Cambridge, MA, 02138). Samples were analyzed within 24 hours of collection.

Quantification of platelets and red blood cells

Complete Blood Counts (CBCs) were performed using a 3-part differential hematology analyzer to quantify the platelets, RBCs, and calculated HCT. CBCs were measured according to the BSR TM-076 Coulter Ac-T diff 2 Hematology Analyzer. TNCs counts were performed using a Beckman Coulter AcT diff2 hematology analyzer (Beckman Coulter, Brea, CA) for baseline samples and BM concentrates. Cell counts were performed in open sample mode according to the manufacturer's and laboratories standard procedures. Prior to sample cell counts, the analyzer passed all system setups, calibration and daily quality control testing.

Flow cytometry

Samples for flow cytometry were prepared and analyzed as recommended by the International Society for Hematotherapy and Graft Engineering. TNCs were incubated with PE anti-human CD34 and anti-human CD45 Alexa Fluor 647. To validate the specificity of the CD34 antibody, a control sample was also prepared with an isotype control. Stained samples were protected from light and analyzed using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) immediately following processing.

The CD34 positive population, implemented as a Hematopoietic Stem Cell (HSC) marker, determined using a single platform methodology, was defined as. the CD45 'dim' and CD34 'bright' population. Cell viability was assessed by dye exclusion of 7-AAD solution. The 7-AAD negative population was reported as a percentage of viable cells. Spectral compensation between fluorescent channels was set using beads labeled with the respective fluorophores for corresponding channels. (PE Anti-human CD34, PE IgG1 k Isotype Ctrl, Lysing Buffer, Cell Viability Solution – BD Biosciences, San Jose, CA; Anti-human CD45 Alexa Fluor 647–BioLegend, San Diego, CA; Counting Beads–Spherotech, Lake Forest, IL).

Colony-Forming Units-Fibroblasts (CFU-f)

Samples were adjusted to a density of 2×10^6 nucleated cells per ml and cultured with supplemented mesenchymal stem cell growth media (Stem Cell Technologies, Cambridge, MA) at 37°C in 5% CO_2. Following 10-14 days of incubation, non-adherent cells were removed by washing with PBS. Adherent cells were stained with Giemsa stain at room temperature (Ricca Chemical Company, Arlington, TX). Excess stain was washed away with distilled water.

Colonies containing more than 50 cells with fibroblast morphology were counted using a Nikon Diaphot 300 microscope and reported as CFU-f per ml of sample. Isolation and expansion of MSCs were quantitatively and qualitatively assessed between testing and control culture conditions using two tailed t-tests.

Study design

The patients were randomized to undergo either a bilateral or unilateral BMA from the PSIS. The randomization was achieved by a #1 or #2 on the BMC kit that was selected by the study coordinator. Both unilateral and bilateral draws were performed using a posterior approach with the Aspire™ bone marrow harvesting system (EmCyte Corporation, Ft. Myers, FL 33916).

All procedures were performed by a single, board-certified sports medicine physician at an academic center. For the unilateral aspiration technique, a subcutaneous tissue tract to the periosteum was injected with a combination of 1% lidocaine and 0.25% bupivacaine. For the bilateral aspiration group, local anesthesia was again administered in the same fashion in a bilateral distribution. Once local anesthesia was obtained, a single cutaneous entry site was used to access a total of three sites at least 1 cm apart into one PSIS for the unilateral cohort and a total of six sites, three at each PSIS, for the bilateral cohort via ultrasound-guided trocar placement. A surgical mallet was used to tap the introducer and trocar just beneath the bone cortex at each aspiration site.

Once in place, the introducer canula was left seated in the subcortical bone marrow and the trocar was removed. The aspiration needle with a blunt tip and 3 lateral side holes was introduced through the introducer canula in the marrow cavity and the appropriate volume of bone marrow was aspirated per the randomization and study protocol. The aspiration needle was then replaced by the trocar which was then redirected under identical technique to the remaining sites.

For the unilateral group, just over 20 cc was aspirated from each site using 10 cc syringes pre-rinsed with heparin at a dose of 2000 IU/ml and a quick draw technique with ninety-degree rotation of the bevel every 2-3 cc for a total of 61 cc of volume. The identical technique was performed for the bilateral group but with three sites on the left and three sites on the right accessed with just over 10 cc of bone marrow aspirated from each, equaling 30.5 cc on each side and 61 cc total, once again with 10 cc syringes pre-rinsed with heparin.

A total of 61 cc of bone marrow was aspirated from each patient in both groups. One cc per patient was removed from the total volume to be sent for laboratory analysis. The remaining 60 cc of BMA was centrifuged at bedside with an EmCyte GenesisCS Pure BMC®-60 ml 2015 kit (product number BC60-PURE) (EmCyte Corporation, Fort Myers, Fl 33916). A buffy coat of 8-9 cc BMC was obtained for each patient. 1 cc of BMC was reserved for laboratory analysis. The remaining 7-8 cc of BMC was injected intraarticularly into the patient's affected knee using a 1.5 inch 22 gauge needle under ultrasound-guidance from a medial approach. For each patient, 1 cc of BMA and 1 cc of BMC were then sent for analysis.

In addition to the quantitative analysis above, included patients underwent baseline pain and functional surveys. Ongoing follow-up surveys were administered at one month, two months, 6 months, 12 months, and 24 months to assess overall outcomes and

any significant associations with the results of laboratory analysis. This data collection is ongoing and will be reported at a later date. Patient data including gender, age, BMI, medical comorbidities, medications, smoking status, prior treatment, Kellgren-Lawrence knee OA grade, and presence or absence of effusion was collected from chart review.

Statistical analysis

TNC and CFU/ml of unilateral and bilateral groups were compared with the Wilcoxon rank-sum test. Age of unilateral and bilateral patients was compared with a two-sided two-sample equal-variance t-test. The Spearman rank correlation coefficient was used to determine the association between CFU/ml and age. Because the data for CFU/ml were not normally distributed, a natural log transformation was performed prior to linear regression of loge CFU/ml on age (predictor) for each study group (unilateral or bilateral). For each study group, the adjusted mean for CFU/ml is the mean CFU/ml obtained by evaluating the regression model at the mean age of the two study groups. After regression analysis, the CFU/ml mean, and its 95% confidence interval were back transformed to the original scale and reported as the geometric mean with 95% confidence interval. Similarly, back transformation of the difference between the mean of log transformed CFU/ml among unilateral patients and bilateral patients yielded the ratio of the geometric means. Confidence intervals for the Geometric Mean Ratio (GMR) were computed by back transforming the 95% confidence bounds for the mean difference. A GMR of 1.0 suggests no treatment effect.

RESULTS

Thirty-five patients were recruited for the study. Of these, one patient withdrew from the study. The remaining thirty-four patient samples were analyzed. No CFUs were detected in seven samples and an eighth was excluded due to excessive clotting preventing complete analysis (Figure 2).

Of the remaining 26 samples, half were in the unilateral group (n=13) and the other half in the bilateral group (n=13). Patient demographics varied between groups. The mean age was slightly lower in the unilateral cohort at 51.8 years old versus 58.2 years old in the bilateral cohort. The majority of patients in the bilateral group were male (84.6%) and there were more female patients in the unilateral group (61.5%). Mean BMI was in the obese classification at 30.0 (± 5.7) for the unilateral group and in the overweight classification at 28.4 (± 3.6) in the bilateral group (Table 1).

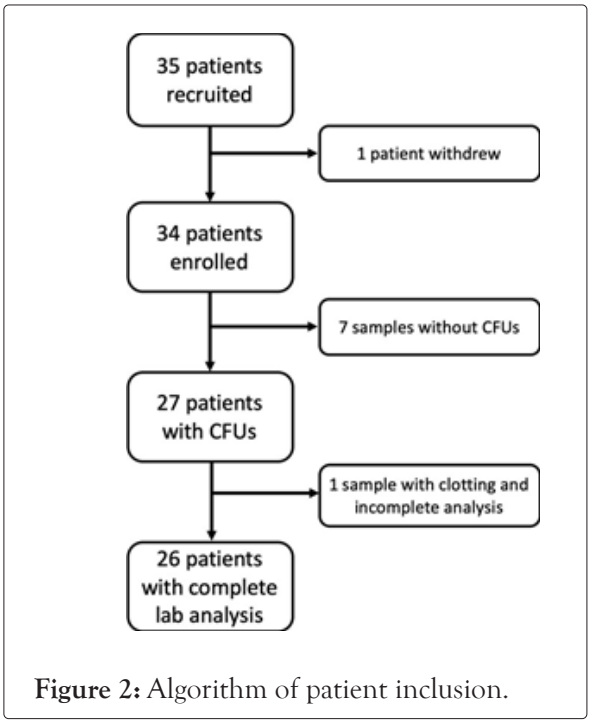

Figure 2: Algorithm of patient inclusion.

Table 1: Age was slightly higher in the bilateral group. [a]: Patients in the unilateral group were primarily female, while those in the bilateral group were mostly male. SD: Standard deviation; BMI: Body mass index. [b]: One BMI missing from bilateral group.

Patient demographics[a]		
	Unilateral (N=13)	**Bilateral (N=13)**
Female (%)	8/13 (61.5%)	2/13 (15.4%)
Mean age, years (SD)	51.8 (± 9.8)	58.2 (± 5.8)
Mean BMI	30.0 (± 5.7)	28.4 (± 3.6)[b]

Individual and mean TNC, platelets count, and CFU-fs corrected for injectate volume are reported for both unilateral and bilateral cohorts in Table 2

Table 2: Individual BMA, BMC, TNC, and CFU data per ml. Adjusted for injectate volume for the 13 unilateral patients and 13 bilateral patients with calculated means. The bold-italic cells indicate outlier samples with CFU of >50,000/ml. [a] :Patient TNC, PLT, and CFU total cell counts are corrected for sample volume, for available total deliverable cell counts in the BMA, unilateral, and bilateral BMC injectates. TNC: Total Nucleated Cells; PLT: Platelet; CFU: Colony-Forming Unit; BMA: Bone Marrow Aspirate; BMC: Bone Marrow Concentrate

Unilateral BMA			Bilateral BMA			Injectate volume (ml)	Unilateral BMC					Injectate volume (ml)	Bilateral BMC				
× 10^6	× 10^6		× 10^6	× 10^6			× 10^6	× 10^6		× 10^6			× 10^6	× 10^6	× 10^6	× 10^6	
TNC /ml	PLT /ml	CFU /ml	TNC /ml	PLT /ml	CFU /ml		Total TNC	Total PLT	Total CFU	TNC /ml	CFU /ml		Total TNC	Total PLT	Total CFU	TNC /ml	CFU /ml
18.4	107	175	29.1	93	116	7	656	4,627	2,625	93.7	375	7	1,035	3,108	7,245	148	1,035
24.1	65	169	23.7	48	277	8	776	4,272	20,696	97	2,587	7	834	2,709	24,178	119	3,454
17.3	88	426	10	62	100	8	1,022	5,232	33,400	128	4,175	7	857	3,276	11,998	122	1,714
19.9	69	2,388	59.9	76	5,181	8	723	4,904	***88,232***	90.4	11,029	8	1,829	3,576	***1,37,160***	229	17,145
56	182	196	14.1	107	266	8	1,654	10,056	14,880	207	1,860	7	795	2,688	***94,626***	114	13,518
16.9	56	254	32	51	576	7	511	3,066	7,154	73	1,022	8	1,093	3,056	29,504	137	3,688
35.5	154	1,349	28.3	134	396	8	1,322	6,336	***57,488***	165	7,186	8	979	5,832	***1,01,840***	122	12,730
27.8	76	195	12.5	94	2,025	9	1,192	5,580	5,958	132	662	8	1,148	5,352	***94,072***	144	11,759
32.1	93	546	12.8	46	704	8	890	3,136	10,672	111	1,334	7	444	3,948	14,644	63.4	2,092
29.3	94	703	47.9	91	1,149	7	633	5,026	25,312	90.4	3,616	7	1,576	6,272	17,339	225	2,477
25.6	76	179	15	136	105	7.5	1,142	7,260	6,848	152	913	7	496	7,280	4,963	70.9	709
20.2	99	505	60.8	118	2,006	7	872	7,434	16,569	125	2,367	8	1,529	11,592	9,176	191	1,147
25.1	106	251	17.5	75	438	8.5	1,073	5,185	9,656	126	1,136	8	529	3,416	11,116	75.6	1,588
Mean/ml			**Mean/ml**			**Mean**	**Mean Total Deliverable Cells in Injectate[a]**			**Mean/ml**		**Mean**	**Mean Total Deliverable Cells in Injectate[a]**			**Mean/ml**	
							× 10^6	× 10^6					× 10^6	× 10^6			
							Total TNC	**Total PLT**	**Total CFU**				**Total TNC**	**Total PLT**	**Total CFU**		
26.8	**97**	**564**	**28**	**88**	**1,026**	**8.8**	**959**	**5,547**	**23,038**	**122**	**2,943**	**8.5**	**1,011**	**4,777**	**42,912**	**135**	**5,620**

Though bilateral patients were on average about six years older than the unilateral patients, no statistically significant difference was identified (p=0.07). When performing descriptive statistics by group, the median number of CFU-fs cultured from 1 ml of BMC in the bilateral cohort (n=13) was 33% higher in the bilateral group compared to the unilateral group (median=2477 and 1860 respectively, median difference 617, p=0.23).

Median TNC counts were similar between the two study groups, with 979 × 10^6 cells for the bilateral cohort versus 890 × 10^6 cells in the unilateral group (p=0.91). The mean total CFU-fs corrected for sample volume were 1.9 times higher in the bilateral group versus the unilateral group, with a mean of 42,912 versus 23,038, respectively (p=0.17). Mean TNC counts did not have as much variation, with the bilateral count being only 1.06 times higher than the unilateral (1011 × 10^6/ml for the bilateral group versus 959 × 10^6/ml, respectively, p=0.72). When evaluating mean CD34+ cell counts in the bone marrow concentrate, the values were similar in the bilateral and unilateral draws (49 × 10^6/ml versus 39 × 10^6, respectively, p=0.5) (Table 3).

Table 3: Mean and median values by unilateral and bilateral cohorts, including ratios among groups. Age was compared using a two-sided two-sample equal-variance t-test. Cell counts were compared using the Wilcoxon rank-sum test. SD: Standard deviation; CI: Confidence interval; P25: 25th percentile; P75: 75th percentile.

Descriptive statistics by group[a]					
	Unilateral		Bilateral		
Outcome	**N**	**Mean ± SD (95% CI)**	**N**	**Mean (95% CI)**	**Bilat/Unilat**	**P-value**
Age	13	52.3 ± 9.6 (47.0-57.6)	13	58.1 ± 6.1 (54.7-61.4)		0.07
Total NC	13	959 ± 319 (784-1,134)	13	1,011 ± 429 (775-1,247)	1.1	0.72
CD34	13	38.8 ± 13.5 (31.4- 46.3)	12	48.8 ± 50.2 (20.1-77.4)	1.26	0.5
Total CFU	13	23,038 ± 24,526 (9,549-36,526)	13	42,912 ± 46,050 (17,586-68,239)	1.86	0.17
CFU/ml	13	2,943 ± 3,061 (1,260-4,627)	13	5,620 ± 5,851 (2,402-8838)	1.91	0.14
Outcome	**N**	**Median (P25; P75)**	**N**	**Median (P25; P75)**	**Bilat/Unilat**	**value**
Total NC × 10^6	13	890 (723; 1142)	13	979 (795; 1148)	1.1	0.91
CFU/ml	13	1860 (1022; 3616)	13	2,477 (1,588; 11,759)	1.33	0.23

We observed about a 0.5 loge higher response for CFU/ml in the bilateral group (mean=7.560 for the unilateral group or approximately 1920 CFU/ml count after back transformation of the log 7.560 value and 8.098 (3288 CFU/ml) for the bilateral group. These means (1920 and 3288) are referred to as the geometric means. The age adjusted geometric mean CFU/ml was 3381 for bilateral patients and 1866 for unilateral patients, demonstrating a 1.8-fold higher CFU-f/ml in bilateral compared to unilateral patients as geometric mean ratio (GMR) (95% CI: 0.7-4.7, p=0.21) (Table 4). There was no correlation between age and CFU/ml for the entire study group (Spearman rank correlation coefficient=0.10, p=0.62).

Table 4: A GMR of 1.0 suggests no treatment effect. [a]: Loge CFU/ml, Means and 95% Confidence intervals by Study group, age-adjusted and back-transformed to obtain Geometric Mean Ratio (GMR). CFU: Colony Forming Unit; CI: Confidence Interval.

Regression analysis[a]				
Unilateral		Bilateral		
Outcome	Age adjusted Model Based Mean (95% CI)	Age Adjusted Model Based Mean (95% CI)	Geometric Mean Ratio (95% CI)	P-value
CFU/ml (ln) back-transformed	1,866 (1,034-3,368)	3,381 (1,776-6,435)	1.812 (0.706-4.649)	0.205

The Spearman rank correlation coefficient was 0.025 and 0.31 respectively for unilateral and bilateral patients. Though no statistical difference was seen regarding age effect on CFU-f count, there does appear to be a decline in CFU-f count with age in the bilateral group, but not the unilateral group (Figure 3).

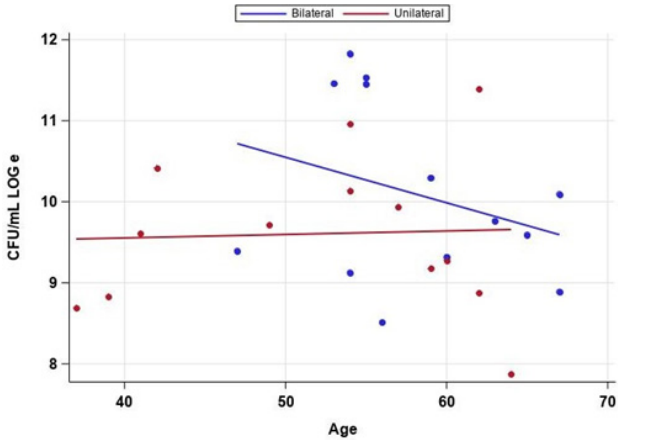

Figure 3: CFU count versus age. The Spearman rank correlation coefficient was used to determine the association between CFU/mL and age. Spearman rho is 0.025 and -0.31 respectively for unilateral and bilateral patients.

There was a subgroup of patients with counts greater than 50,000 CFU-fs per 7-8 cc BMC. When further examining the six patients with higher CFU-f counts, five patients were male. The one female patient had the lowest count at 57,000 cells. The remainder of CFU-f counts ranged from 88,232 to 137,160. The two lowest values, 57,000 and 88,232, were among the unilateral draw group. Review of characteristics among the 7 patients who did not yield any CFU-Fs revealed that 6 of 7 patients were female. Three of 7 patients were taking estrogen analogues and one was taking tamoxifen. This is in contrast to the 26 patients with CFUs, only one of whom had exogenous hormonal modulation with a testosterone transdermal system.

DISCUSSION

With the increased utilization of orthobiologics for musculoskeletal conditions, much remains unanswered. The specific components of various injectates leading to therapeutic benefit have not been conclusively elucidated, however basic science and clinical research does suggest that MSCs are one of the likely many factors contributing to positive outcomes [29]. Both CFU-fs and TNC for BMC demonstrate a dose-response for therapeutic benefit. Patients who underwent percutaneous bone marrow concentrate grafting for treatment of non-union tibial fractures had superior outcomes with injection of greater than 1500 CFU-f/cm^3 compared to those with injection of a lower number. For knee arthritis, BMC with TNC>400 × 10^6 is demonstrated to be optimal for superior pain and functional outcomes [30]. Therefore, it is theorized that we should work to optimize bone marrow aspiration techniques such that the highest yield of TNCs and MSCs, of CFU-fs, are obtained.

This study supports previous data demonstrating that a multisite bone marrow aspirations of lower volumes result in higher concentration of CFU-Fs [34,36-38]. Due to a few patient outliers causing a variation in median versus mean cell counts (1.3-fold increase versus 1.9-fold increase), a logarithmic transformation was applied prior to performing a regression analysis to establish a GMR. This statistical analysis demonstrated a more accurate value of a 1.8-fold increase in age-adjusted CFU/ml counts in bilateral versus unilateral patients, which is close to that of the means for the initial, non-corrected counts. Theoretically, the lower number of CFU-fs in the unilateral group is due to increased peripheral blood accumulation that occurs in higher volume bone marrow aspirations. This is inconsistent with the findings presented by Oliver et al., in which performing multisite draws demonstrated no statistical increase in CFU counts when compared to a singles-site draw at multiple depths using the same type of trocar [41]. One reason for this potential difference is that in the Oliver study, the multisite draw included a single draw from each site at a depth of 2 cm, which may have missed the subcortical MSCs [31]. In Oliver's study, a low TNC counts with only a doubling of relative concentration of cells from BMA to BMC were also noted [42], however this may has been attributed to the Arthrex system selectively removing neutrophils [43]. Furthermore, these differences may also be explained by the different design characteristics of the bone marrow harvesting needle. In our study, we utilized a harvesting needle with a blunt tip and only 3 side holes. In the Oliver study the traditional Jamshidi needle was used, employing an open distal tip, facilitating preferential marrow aspiration from the deeper marrow regions and therefore diluting the aspirate with RBCs.

Many factors have been shown to contribute to optimal bone marrow aspiration to attain higher numbers of MSCs. One factor is the bone marrow architecture and niche environments containing the highest concentration of MSCs [31]. Another factor is fluid dynamics and negative pressure and pulls technique that assist with cellular liberation from bone and perivascular structures [33,36]. It does appear that CFU-fs are higher per ml in the bilateral group in the present study than has been published in other literature for optimal draw technique. There are several possible reasons for this. First, the method of culture could have resulted in heterogenous samples that are not directly comparable [44]. It is also possible

that the type of trocar used allowed for greater extraction of MSCs due to the presence of side ports versus the more commonly used Jamshidi needle, however data definitely demonstrating this is lacking.

It should also be noted that though higher TNC counts have correlated with better outcomes in some limited studies, the exact component(s) present in BMC with biological activity contributing to positive therapeutic outcomes have yet to be fully elucidated. As has been previously demonstrated, MSCs quantity within bone marrow has been shown to range between 0.01-0.0001% of the total mononuclear cell composition [45,46]. In the present study, though there is nearly a two-fold increase in CFU-fs per ml in the bilateral group compared to the unilateral group, TNC count was the same between groups. This result underlies the fact that doing bedside TNC counts prior to procedures is not reflectively of how many MSCs the patient will receive. Whether this ultimately affects outcomes from these procedures is still unknown, as both culture-expanded MSCs [47,48] and BMC have demonstrated benefit for knee OA [30,49,50] despite having vastly different cellular compositions. Continued study into the specific therapeutic efficacy of the various components of BMC is needed. It is our hope that our clinical outcomes data to follow will help shed some light in this area.

This study does have several limitations. Primarily, this is a descriptive pilot study and therefore was underpowered to determine statistical significance. In addition, a peripheral blood sample was not performed at the time of BMA to extrapolate bone marrow TNC versus the peripheral blood component of BMA. This would have been helpful in further characterizing the qualitative differences accounting for the similar TNC counts between groups as compared to the larger differences in CFU-f counts. Though the present study was not large enough to identify a statistical difference in the number of CFU-fs between the groups, it is possible that the difference is nonetheless clinically and biologically significant. Furthermore, it does appear that there is an age-dependent decrease in the number of CFU-fs in the bilateral draw group compared with that of the unilateral group. Reasons for this are unclear and will require a larger study with greater statistical power to confirm this finding. In addition, the patients with significantly higher cell counts were mostly male while the majority of patients who did not yield any CFU-f growth on culture were female. It is difficult to draw conclusions from these trends due to the low sample size and asymmetric randomization of genders within the groups. However, the potential differences among men and women, as well has effects of exogenous hormonal modulation, warrants further investigation within the field of musculoskeletal orthobiologic therapies.

CONCLUSION

This study further supports previous evidence that bilateral bone marrow aspiration using a higher number of lower volume draws from the PSIS yields a higher number of CFU-fs. Despite the fact that TNC counts were the same between unilateral and bilateral groups, there was a 1.8-fold increase in CFU-fs in the bilateral group compared with the unilateral group. There also appeared to be an age-related decline in CFU/ml in the bilateral group that was not observed in the unilateral group, but once again the study was underpowered to identify statistical significance. Due to the low number of patients, further studies with a larger population are needed to fully validate these findings.

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