Journal of Regenerative Medicine

Research Article

A SCITECHNOL JOURNAL

Comparative Analysis of Different Needle Techniques for Bone Marrow Harvest

Peter W Kyriakides[1], Gregory E. Lutz[2,3], Christopher Kyriakides[2], Mohammed M. Sharaf[2], Christopher Lutz[2,3], Jennifer Soloman[2], Emma Higgins[4], Francesca C. Noviello[4], Richard Abiuso[4], Daniel Kuebler[4]*

[1]Department of Orthopedic Surgery, NYU Langone Hospital – Long Island, Mineola, New York, USA

[2]Department of Physiatry, Regenerative SportsCare Institute, New York, NY, USA

[3]Department Physiatry, Hospital for Special Surgery, New York, NY, USA

[4]Department of Biology, Franciscan University of Steubenville, Steubenville, Ohio, USA

***Corresponding author:** Daniel Kuebler, Department of Biology, Franciscan University of Steubenville, Steubenville, USA, E-mail: dkuebler@franciscan.edu

Citation: Kyriakides PW, Lutz GE, Kyriakides C, Sharaf MM, Lutz C, et al. (2024) Comparative Analysis of Different Needle Techniques for Bone Marrow Harvest. J Regen Med 13:4.

Received: 30-May-2024, Manuscript No. JRGM-24-134994, **Editor assigned:** 01-Jun-2024, PreQC No. JRGM-24-134994 (PQ), **Reviewed:** 14-Jun-2024, QC No. JRGM-24-134994, **Revised:** 18-Jun-2024, Manuscript No. JRGM-24-134994 (R), **Published:** 25-Jun-2024, DOI:10.4172/2325-9620.1000315

Abstract

Background: Biologic therapies, including the use of autologous bone marrow-based preparations, have demonstrated promise for the treatment of painful musculoskeletal pathologies. Historically, the primary therapeutic cell of interest within bone for musculoskeletal applications is the Mesenchymal Signaling Cell (MSC). As interest in these treatments has increased, further study of harvest techniques and laboratory quantification of aspirates is needed. The aim of this pilot study was to quantify and Compare Total Nucleated Cell Counts (TNCs), MSCs (as measured by fibroblast colony-forming units, CFU-fs), and CD34+ cells in small-volume aspirates using two different commercially available bone marrow harvesting needles in the same patient.

Methods: Twenty-nine patients undergoing elective bone-marrow-based procedures had a bone marrow aspiration of 5 cc using a single port 11-gauge bone marrow biopsy needle (SP side) on one posterior superior iliac crest (PSIS) and another 5 cc aspiration on the contralateral side using a multi-port 11 gauge bone marrow biopsy (MP side). Small samples from these aspirations were sent to an independent lab for TNC, CFU, and CD34+ cell comparative analysis. Wilcoxon matched-pairs signed rank tests were used for comparison of differences between groups.

Results: Of the 29 patients included in this pilot study, 7 patients were female and 22 were male and the mean age of the patients was 57.9 years with a range from 24 to 81 years. Mean age was 57.9 years (range 24 to 81 years). The mean TNC count for the MP side, 46.4 x 10^6 ± 4.6 x 10^6 per mL, was significantly higher ($p < 0.001$) than the mean TNC count for the SP side, 33.8 x 10^6 ± 4.51 x 10^6 per mL. In addition, the mean CFU-f count for the MP side, 4469 ± 583 per mL, was significantly higher ($p < 0.0001$) than the mean CFU-f count for the SP side, 2676 ± 626 per mL. Of the 29 patients studied, 23 (79.3%) had higher CFU-f levels in the MP aspirate as compared with the SP aspirate. CFU-fs for both the SP and MP sides demonstrated a strong correlation with TNC, with Spearman's Correlation Coefficient of 0.718 and 0.749, respectively. The levels of CD34+ cells were analyzed in 21 of the 29 patients and no significant difference ($p = 0.1193$) was seen between the two aspiration techniques.

Conclusions: In this pilot study, two thirds of patients had a substantially higher CFU-fs/mL yield from a MP aspiration when compared to the SP side. This suggests that using a multiport bone marrow biopsy needle may provide an increased yield of MSCs present within an initial, small volume, 5 cc bone marrow aspiration from the PSIS when compared with a SP low volume aspiration. This could have clinical implications for targets requiring only a small volume of bone marrow for treatment and may eliminate the need for concentration of aspirate for these applications, thereby potentially decreasing procedural time and risk of contamination. In addition, the mean CFU-f/mL levels of both aspirates were higher than previously reported minimal CFU-f/mL levels associated for clinical efficacy in prior studies. Further study is needed to validate these findings and determine clinical significance.

Keywords

Bone Marrow Aspirates (BMAs), Orthobiologics, Regenerative, Harvest

Introduction

Bone Marrow Aspirates (BMAs) and Bone Marrow Concentrates (BMCs) are increasingly being used to treat a variety of musculoskeletal pathologies. BMAs are drawn directly from the bone marrow, and centrifugation of BMAs generates BMCs that are concentrated with desired cell contents for injection. Physicians rely on manufacturers for accurate cell concentrations in order to choose the best bone marrow harvesting systems for procedures to initiate recovery [1, 2]. Studies that have examined the efficacy of different BMA and BMC preps in treating osteoarthritis, tendinopathies, nonunion, and vertebral disc problems have shown mixed results [3-10]. A major inconsistency between these studies is the heterogeneity in the bone marrow draw techniques and bone marrow concentration systems and the frequent lack of characterization of the final product [3,11,12,13]. As a result, it is difficult to determine the efficacy of BMA and BMC treatments due to inconsistency between bone marrow harvesting and/or processing techniques.

There are a wide variety of factors that influence the efficacy of bone marrow draws, including the size of the collection syringe, needle design, speed of aspiration, volume of aspiration, and aspiration technique [14,15,16,17,18,11,19]. Previous research has shown that low-volume bone marrow draws contain higher concentrations of

progenitor cells as measured by the number of CFU-fs or the number of progenitor cells than larger-volume draws because high-volume bone marrow draws get diluted with red blood cells [14,16,17,18]. Furthermore, combining multi-site small-volume draws seems to yield higher levels of progenitor cells as compared to single-site high-volume draws [14,16,17,18]. Because high-volume draws have lower concentrations of progenitor cells due to collection of significant volumes of peripheral blood, it has become common practice to concentrate bone marrow aspirates using a variety of centrifugation systems [1]. The variability in these systems, however, can also contribute to the heterogeneity in outcomes of BMC treatments in published studies [20].

There are a variety of orthopedic treatment sites that require only small volumes of BMA or BMC injections, such as knee joints, hip joints, and vertebral discs [17]. In fact, a number of studies have found that clinically relevant amounts of MSCs can be extracted in low-volume draws [14,16,17,18]. Therefore, identifying the optimal aspiration technique that can produce 5-10 mL aspirates with high levels of progenitor cells would be ideal in such cases. This technique would eliminate the centrifugation step because centrifugation and other separation techniques have been shown to cause a loss of progenitor cells, immune cells, and/or platelets from the final bone marrow concentrate [20].

While low-volume draws can extract high levels of MSCs, there have been few studies that examine the effects of the aspiration needle on the contents of these draws. To address this question, the present study compared the contents of low-volume BMAs extracted using a Marrow Cellution™ (MC) needle system with the contents of low-volume aspirates extracted using the standard Jamshidi needle. The aim of this pilot study was to quantify and compare Total Nucleated Cell Counts (TNCC) and MSCs (as measured by fibroblast colony-forming units, CFU-fs) in small volume aspirates generated using these two different commercially available bone marrow harvesting needles contralaterally on the same patient.

Methods

TNC Counts: To determine TNC counts, each BMAC sample was diluted 1:20 in Hank's Balanced Salt Solution (HBSS). The diluted samples were mixed with AO/PI dye (Nexcelom Bioscience) in a 1:1 ratio and cells were counted using a Nexcelom Cellometer Vision fluorescent cell counter. Each sample was counted twice to determine the concentration of live nucleated cells.

CFU-f Counts: To determine CFU-f counts, a small, undiluted volume of each BMAC sample was added to a T-25 tissue culture vial with 5 mL of fresh 10% FBS MSC Growth Media. Samples were cultured under normal conditions of 37°C and 5% CO_2 for three days to allow cells to adhere to the vial. After three days, non-adherent cells were removed with repeated washes of HBSS. The adherent cells were cultured for an additional nine days, with media changes occurring every three days. After twelve days of incubation, cell colonies were stained with 0.5% crystal violet solution in methanol. Each colony that consisted of more than 100 cells was counted as a CFU-f.

CD34+ Cell Counts: CD34+ cell counts in each BMAC sample were determined using an established ISHAGE protocol. 50 µl of each BMAC sample was mixed with 45 µl of cell staining buffer, 2 µl of FITC CD45+ antibody, and 2 µl of CD34+ antibody. Each sample mixture was incubated in the dark at RT for 20 min. After incubation, 1.4 mL of red cell lysis buffer was added to each sample mixture. CD34+ cell counts were analyzed with an Accuri flow cytometer using gates established following the ISHAGE protocol for CD34+ cell analysis in bone marrow.

Patient Selection: This study involved the prospective analysis of bone marrow aspiration extractions from 29 patients between the months of December 2020 and January 2023. It was decided that this study qualified for exempt review, and retroactive patient consent was not required. All research and analyses were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its amendments. In terms of limitations, patient data was included for analysis given the following conditions: weight is greater than 110 lbs, non-pregnant, self-reported healthy, free of COVID-19, cold, and flu symptoms, and reports no history of prior infection within two weeks.

Bone Marrow Aspirate Harvesting Process: Bone marrow aspirate was harvested using two different needle systems, Jamshidi and Marrow Cellution, both of which required that patients were under sedation in sterile conditions.

Jamshidi: The tip of the T-Handle Jamshidi biopsy needle was inserted into the posterior iliac crest of each patient with firm pressure and slight alternating clockwise and counterclockwise rotating movements until 3–4 cm deep in the iliac crest. Then, the sharp trocar was removed, the syringe was connected, and 5 mL of bone marrow aspirate was harvested under vacuum, resulting in 6 mL of solution (5 mL bone marrow aspirate and 1 mL heparin solution) [11]. Finally, the needle was removed.

Marrow Cellution: The tip of the Marrow Cellution biopsy needle was inserted into the posterior iliac crest of each patient with firm pressure and slight alternating clockwise and counterclockwise rotating movements until 5-6 cm deep in the iliac crest. Then, the trocar was removed, the needle was connected to a guide sleeve by a rotating mechanism, and the needle was inserted into the device and rotated clockwise. Once the needle was atop the iliac crest, the syringe was reconnected, and 1 mL of bone marrow aspirate was drawn. The grip was rotated 360° counterclockwise four times, and 1 mL of bone marrow aspirate was harvested under vacuum after each rotation. Each 360° counterclockwise rotation corresponded to lifting the aspiration needle by approximately 0.5 cm, thus ensuring a standardized aspiration technique. In total, the syringe contained a 6 mL of solution (5mL bone marrow aspirate and 1 mL heparin solution) [16]. Finally, the needle was removed.

Data Analysis: The data was analyzed for normality using the Shapiro-Wilk test and the TNC, CFU and CD34+ data was found to be non-normally distributed. A Wilcoxon matched-pairs signed rank test (non-parametric) was used to compare the differences in TNCs, CFU-fs, and CD34+ cells between the MP and SP aspirates. All group data except age are presented as mean ± 95% confidence interval (CI) with $p < 0.01$ being used to indicate a statistically significant difference between groups. Age data is presented as mean ± the standard deviation. Correlation analysis was done by calculating the Spearman's rank correlation coefficient. All data analysis was performed using Prism 9.0 (Graphpad Software).

Results

A total of twenty-nine subjects undergoing elective regenerative medicine procedures involving bilateral hip bone marrow aspirations were included in this study. The average patient age (range) was 57.9 ± 15.5 (24 to 81) years, with 24.1% of subjects being female and 75.9% male. The data for individual patients can be found in **(Table 1).**

Table 1: Comparison of low volume aspirates harvested using the Marrow Cellutions needle (MP) or the traditional Jamshidi needle (SP). Both the TNC counts and the CFU-f count were significant higher in the MP samples ($p < 0.05$). Averages represent the mean ± the Standard Error of the Mean (SEM).

	Age	Sex	TNCs (10^6 per mL)		CFU-fs (per mL)		CD34+ (per mL)	
Patient			MP	SP	MP	SP	MP	SP
1	66	M	41.6	29.6	7193	3071	664,680	314,430
2	81	F	33.5	29	3483	2352	519,756	661,222
3	78	F	30.8	16.4	799	963	635,710	241,258
4	62	M	21.8	43	314	1620	201,622	528,971
5	52	F	40.8	27.4	2307	1045	567,857	394,777
6	58	M	24.8	20.3	1001	1756	223,270	205,660
7	38	M	43.4	40	6779	86	749,644	380,606
8	62	M	28.8	16.7	695	222	338,710	198,940
9	39	M	43.5	41.1	6016	3934	779,490	715,000
10	57	M	34	16	5651	2728	407,070	156,220
11	64	M	19.8	25.2	2734	2834	198,711	308,200
12	58	M	33.6	18.6	4889	1994	379,622	169,978
13	56	M	50.2	34.9	5389	3055	466,044	365,841
14	50	F	38.4	13.3	6195	435	195,133	24,533
15	47	M	54.0	62.6	6000	5940	1,090,422	1,274,689
16	65	M	108	109	12,665	17,220	1,386,867	1,304,762
17	78	F	56.7	23.5	3054	798	625,828	296,648
18	78	M	14.6	13.9	1144	1668	115,917	205,089
19	26	M	54.4	79.3	7220	6555	639,133	1,259,436
20	50	M	110	68	11,330	6665	1,377,956	1,285,711
21	75	M	44.0	26.5	3232	1870	438,311	263,444
22	63	F	97.8	41.4	5720	2050		
23	68	M	35.0	7.6	5911	68		
24	66	M	39.4	8.3	641	134		
25	42	M	30.0	11.9	2189	26		
26	63	F	47.6	23.1	2546	365		
27	63	M	18.3	14.3	1339	806		
28	24	M	77	46.2	5005	3945		
29	49	M	72.8	74	8170	3390		
Average	57.9		46.3	33.8	4469	2676	571,512	502,639
SEM	15.5		4.64	4.51	583	626	78,310	91,430

Total Nucleated Cell Counts

When comparing low-volume aspirates extracted using a traditional single-port (SP) needle with those extracted using the multiport (MP) MarrowCellution needle in the same patient, significantly higher levels of TNCs were found in the MP aspirates [$p = 0.0006$; (**Figure 1**). The MP draw contained an average of 46.4 x 10^6 ± 4.6 x 10^6 TNCs/mL vs the SP draw, which contained an average of 33.8 x 10^6 ± 4.51 x 10^6 per mL. The comparisons for individual patients can be seen in **(Table 1)**.Of the patients examined, higher TNCs were found in the MP aspirate in 23 of the 29 cases.

Colony Forming Unit-Fibroblast (CFU-f) Counts

The level of CFU-fs in an aspirate are known to correlate with the level of progenitor cells and have been found to be a marker of clinical efficacy of bone marrow aspirates. [21] In the case of these aspirates, significantly higher levels of CFU-fs were found in the MP aspirates vs the SP aspirates [$p = 0.0002$; (**Figure 2**)]. The MP draw contained an average of 4469 ± 583 CFU-fs/mL while the SP draw contained an average of 2676 ± 626 per mL. The comparisons for individual patients can be seen in **(Table 1)**. Of the patients examined, higher CFU-f were found in the MP aspirate in 23 of the 29 cases. In addition, there were six cases in which the aspirate with the highest CFU count did not correspond to the aspirate with the highest TNC count **(Table 1)**.

CD34+ Cell Counts

The level of CD34+ cells is correlated with the quality of the bone marrow aspirate as higher levels indicate lower levels of peripheral blood contamination. In this study, the levels of CD34+ cells were quantified in 21 of the 29 subjects. No significant difference was seen between the two techniques regarding the level of CD34+ cells in the aspirates [$p = 0.1193$; (**Figure 3**). The MP draw contained an average of 571,512 ± 78,310 CD34+ cells/mL while the SP draw, which contained an average of 502,639 ± 91,430 per mL. The comparisons for individual patients can be seen in **(Table 1)**. While there was no significant difference in CD34+ cell counts between the two draws, the MP draws exhibited higher CD34+ cell counts in 15 of the 21 patients.

Correlations Between TNCs and CFU-fs

Aspirates with higher TNC levels tended to exhibit higher CFU-f levels (**Figure 4**). This was the case with both the MP aspirates and the SP aspirates. TNC levels were significantly correlated with CFU-f levels in MP aspirates ($p < 0.0001$) with a Spearman's correlation

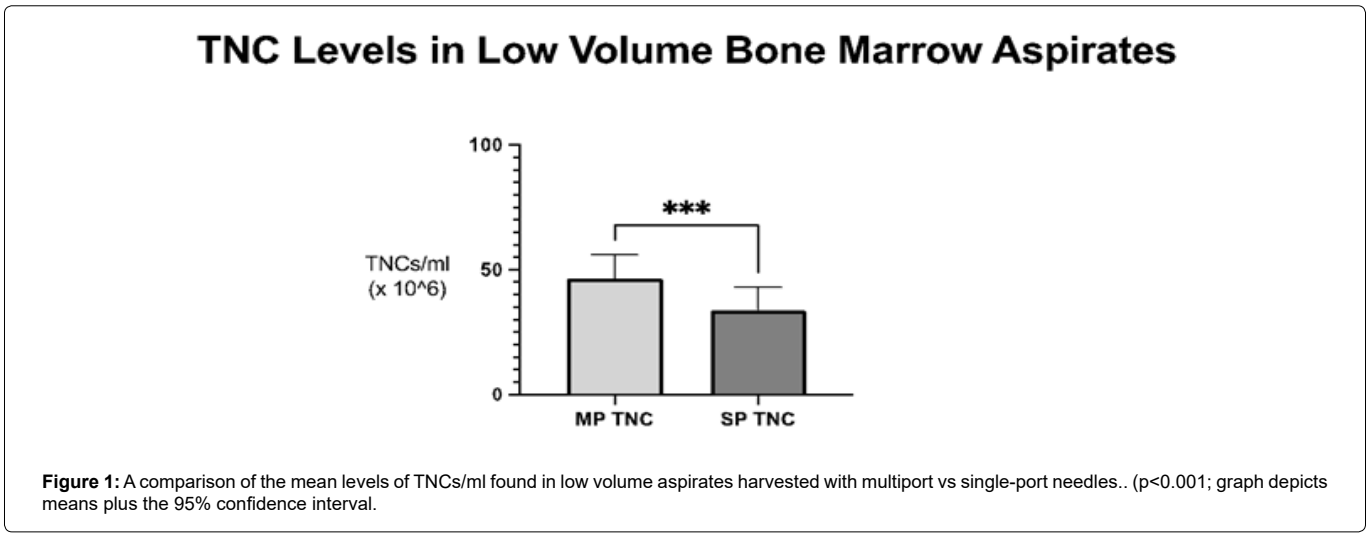

Figure 1: A comparison of the mean levels of TNCs/ml found in low volume aspirates harvested with multiport vs single-port needles.. (p<0.001; graph depicts means plus the 95% confidence interval.

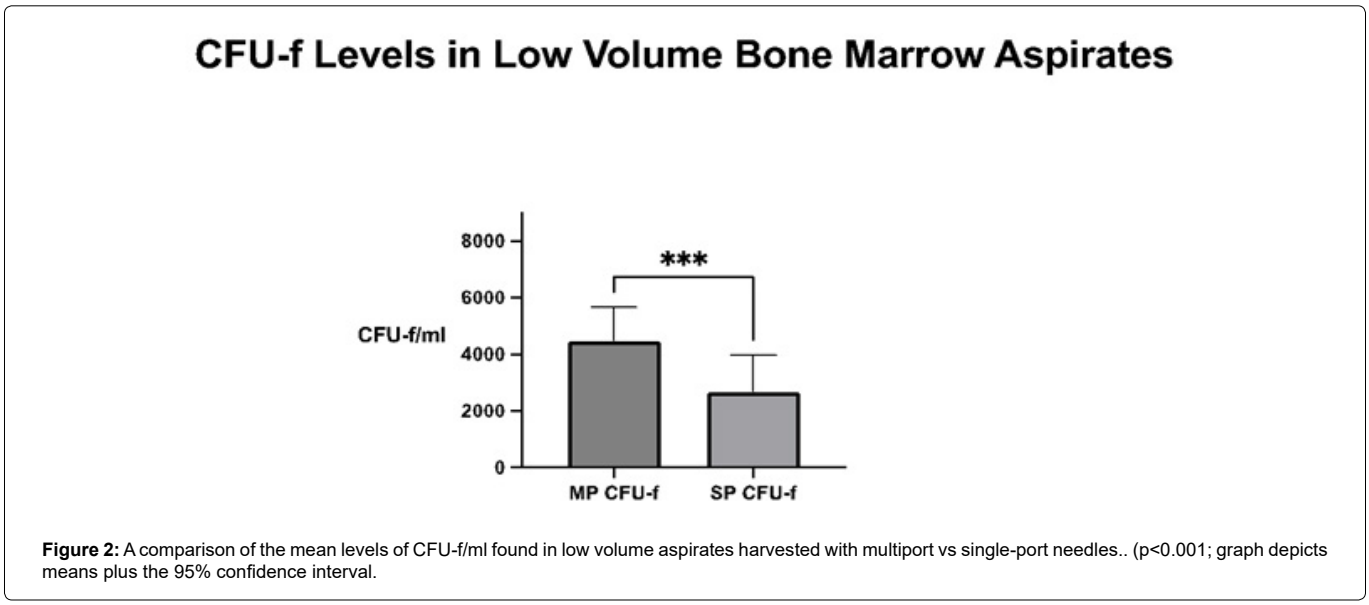

Figure 2: A comparison of the mean levels of CFU-f/ml found in low volume aspirates harvested with multiport vs single-port needles.. (p<0.001; graph depicts means plus the 95% confidence interval.

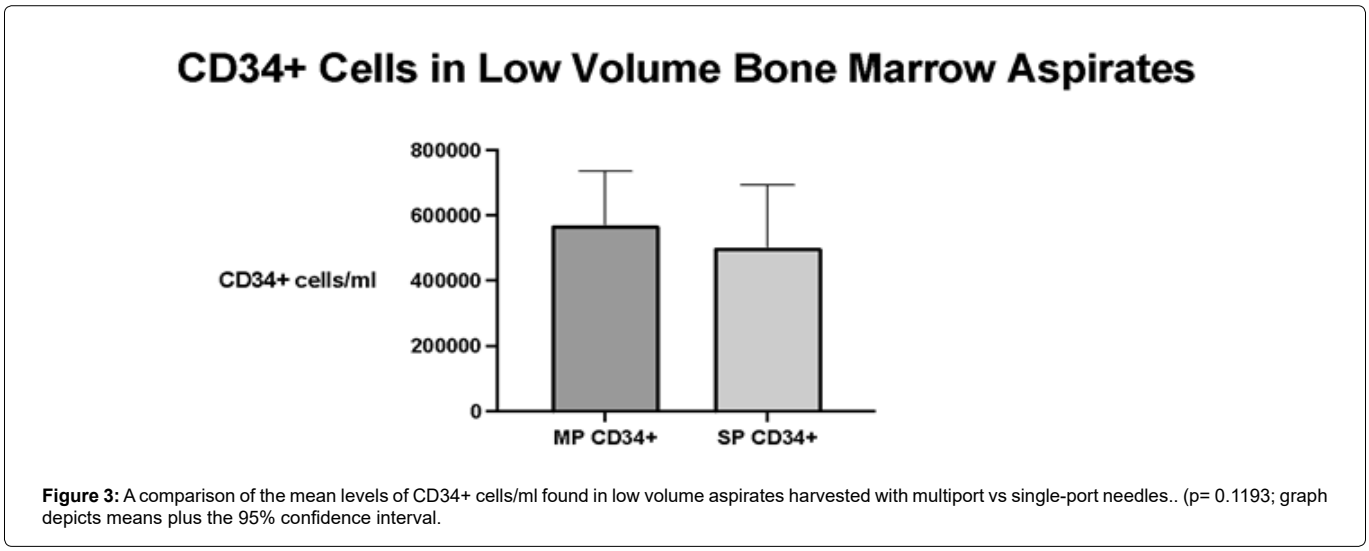

Figure 3: A comparison of the mean levels of CD34+ cells/ml found in low volume aspirates harvested with multiport vs single-port needles.. (p= 0.1193; graph depicts means plus the 95% confidence interval.

coefficient of 0.7071. Likewise, in SP aspirates the TNC levels were significantly correlated with CFU-f levels ($p < 0.0001$) with a Spearman's correlation coefficient of 0.7759.

Correlations Between TNCs and CD34+ Cells

Aspirates with higher TNC levels tended to exhibit higher CD34+ levels (**Figure 5**). This was the case with both the MP aspirates and the SP aspirates. TNC levels were significantly correlated with CD34+ cell levels in MP aspirates ($p < 0.0001$) with a Spearman's rank correlation coefficient of 0.7909. Likewise, in SP aspirates the TNC levels were significantly correlated with CD34+ levels ($p < 0.0001$) with a Spearman's rank correlation coefficient of 0.9455.

Correlations Between TNCs and CD34+ Cells

Aspirates with higher CD34+ cell levels tended to exhibit higher CFU-f levels (**Figure 6**). In this case, a modest correlation was found with both the MP aspirates and the SP aspirates. CD34+ cell levels were significantly correlated with CFU-f levels in MP aspirates ($p < 0.0001$) with a Spearman's rank correlation coefficient of 0.6519. Likewise, in SP aspirates the CD34+ cell levels were significantly correlated with CFU-f levels ($p < 0.0001$) with a Spearman's rank correlation coefficient of 0.6208.

Correlations Between Age and Bone Marrow Aspirate Parameters

There was a small inverse correlation between the age of the patient and the number of CFU-fs in the MP aspirate that was significant (p = Interestingly, age did not significantly correlate with any measurement parameter of the aspirate ($p > 0.01$). TNCs, CFU-fs and CD34+ cells did not significantly increase or decrease with the age of the subject, regardless of the aspiration needle used. There was a small inverse correlation (Spearman's rank correlation coefficient of -0.3917) between the age of the patient and the number of CFU-fs in the MP aspirate. However, the p value ($p = 0.0356$) indicated that this weak correlation was not statistically significant.

The levels of CFU-f/mL found here are higher than those reported for centrifugation systems including Harvest (1270/mL) and Arteriocyte Magellan (514/mL). Finally, the levels of CFU-f/mL found in the MarrowCellutions low volume aspirate is higher than that found when larger volumes of bone marrow are drawn using the MC needle and then concentrated via centrifugation (**Figure 4**).

Discussion

Our data supports the use of a multiport needle with rotational depth change interval aspiration to obtain maximal MSC, TNC, and CFU-f concentrations in BMA.

Our data shows a statistically significant difference in TNC levels between needle systems. The multiport needle delivered $46.4 \times 10^6 \pm 4.6 \times 10^6$ TNCs/ml, while the single port needle delivered $33.8 \times 10^6 \pm 4.51 \times 10^6$ TNCs/ml, and higher TNC levels were reported from the MP needle in 23 out of the 29 surveyed patients.

Furthermore, there was an additional statistically significant difference in CFU-fs delivered per milliliter. The multiport draw delivered 4469 ± 583 CFU-fs/ml, while the single-port draw delivered 2676 ± 626 CFU-fs/per ml. Similarly to TNC counts, 23 out of 29 patients displayed higher CFU-fs from the multiport needle system.

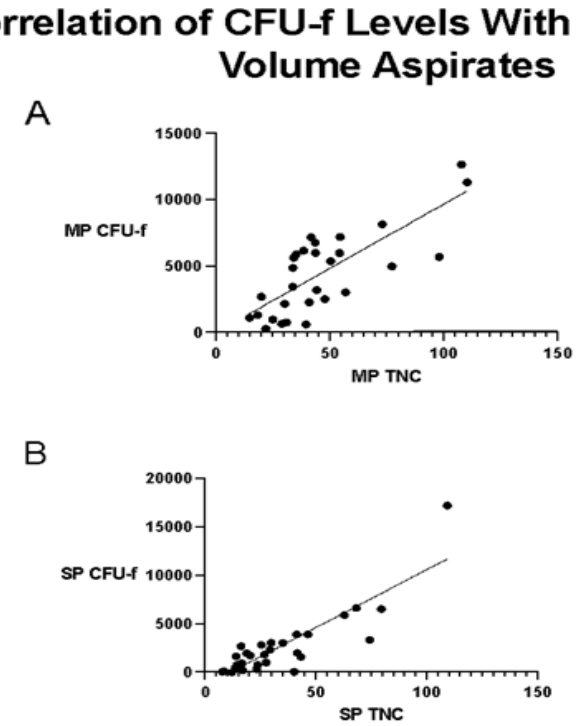

Figure 4: Correlation of TNCs/ml with CFU-fs/ml in low volume aspirates harvested with multiport vs single- port needles. A) TNC levels were significantly correlated with CFU-f levels in MP aspirates ($p< 0.0001$) with a Spearman's correlation coefficient of 0.7071. B) TNC levels were significantly correlated with CFU-f levels ($p< 0.0001$) in SP aspirates with a Spearman's correlation coefficient of 0.7759.

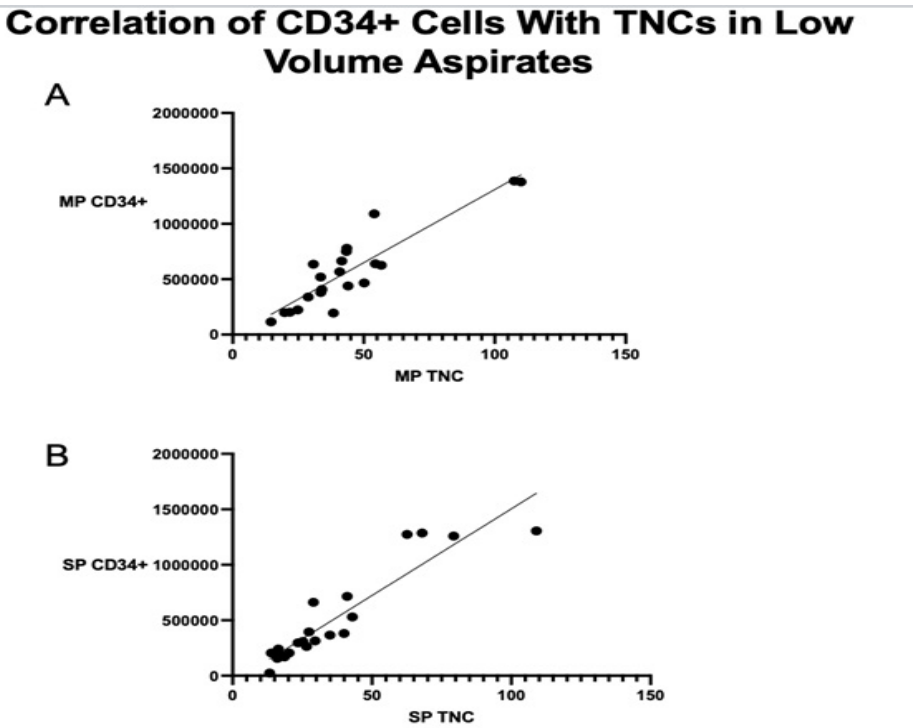

Figure 5: Correlation of TNCs/ml with the number of CD34+ cells/ml in low volume aspirates harvested with multiport vs single- port needles. A) TNC levels were significantly correlated with CD34+levels in MP aspirates ($p< 0.0001$) with a Spearman's correlation coefficient of 0.7908. B) TNC levels were significantly correlated with CD34+levels ($p< 0.0001$) in SP aspirates with a Spearman's correlation coefficient of 0.9455.

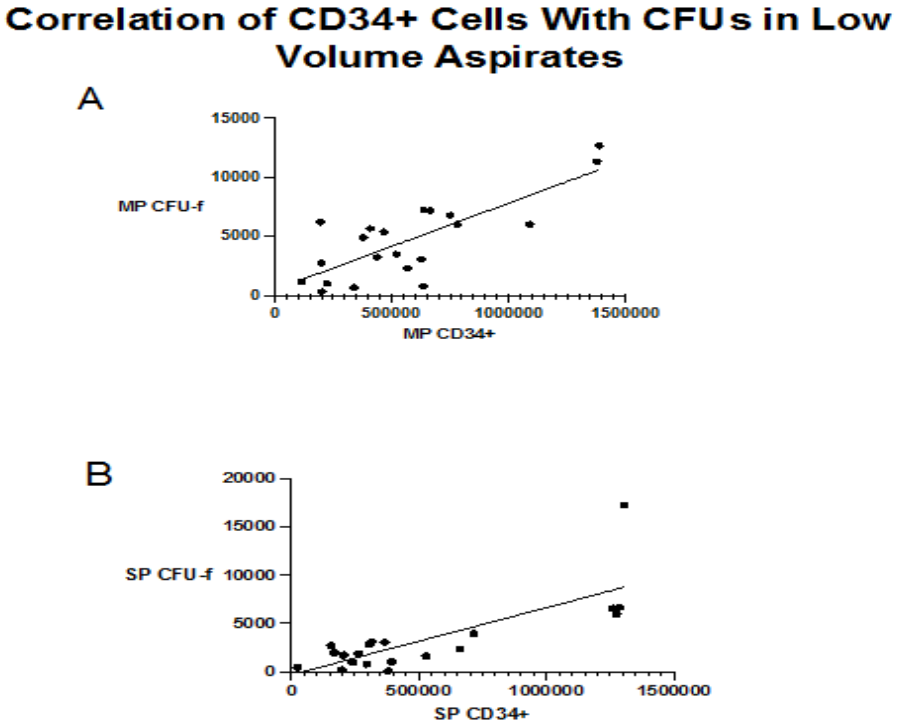

Figure 6: Correlation of CFU-fs/ml with the number of CD34+ cells/ml in low volume aspirates harvested with multiport vs single- port needles. A) TNC levels were significantly correlated with CD34+levels in MP aspirates ($p< 0.0001$) with a Spearman's correlation coefficient of 0.6519. B) TNC levels were significantly correlated with CD34+levels ($p< 0.0001$) in SP aspirates with a Spearman's correlation coefficient of 0.6208.

Alternatively, both systems displayed similar CD-34+ cell counts, with the multiport needle drawing slightly higher numbers on average. The multiport needle contained 571,512 ± 78,310 CD34+ cells/ml, while the SP draw contained an average of 502,639 ± 91,430 CD34+ cells/ml. There was no statistically significant difference displayed between the two systems.

There was a positive correlation between TNC and CFU-f counts. A Spearman's correlation test found that the multiport system delivered a Spearman's correlation coefficient of 0.7071, while the single port system maintained a correlation coefficient of 0.7759.

Previous Studies Comparing Single and Multiport Systems

In a 2021 study comparing the two different systems using a 10-milliliter draw as opposed to a 5-milliliter draw, Feddahi et al found that the single-port system produced more CFU-fs per mL than the multiport system, with the multiport producing 3717 ± 5556 CFU-fs per mL, and the single-port system producing 4305 ± 5507 CFU-fs per mL [11]. A possible explanation for this discrepancy in results could be the larger draw, as there is evidence that states that a smaller volume aspirate is more advantageous [14,16,17,18].

Advantageous Use of Low Volume Aspirate

Hernigou et al stated that a larger negative pressure is needed to aspirate the MSCs in bone marrow properly. As such, aspirate should only fill about 10-20% of the syringe [13]. There is a common misconception that larger draws are required to maximize the number of MSCs pulled from the marrow, but drawing past the 10-20% indicated amount causes a larger draw of peripheral blood where there is a significantly lower concentration of MSCs, therefore diluting the total accumulated MSCs in the final aspirate. As shown by our data, there is a clear advantage in aspirating lower volumes to maximize cell counts.

CFU-f as a Potential Indicator of Better Patient Outcomes

Fibroblast colony forming units are essential to the healing process associated with BM procedures. A possible correlation exists between patient outcomes and the presence of CFU-fs [21]. In producing more CFU-fs, these cells serve as a precursor for differentiation into cells frequently used within BM procedures [22]. Therefore, maximizing CFU-f counts could improve clinical patient outcomes [23].

Relationship between TNC Count and Efficient Aspirate Harvest

TNC count can serve as an indicator of efficiency in harvesting aspirate. Unlike CD34+ and CFU-fs, where maximizing these values improves healing potential, rapid TNC increases indicate that cells are being drawn from peripheral blood [13]. This further supports the conclusion that a lower volume injection could be more advantageous for harvesting undiluted aspirate. Therefore, TNCs can be utilized as an indicator of peripheral blood aspiration, which may be particularly helpful in larger volume draws. Finding the optimal draw volume to ensure a lack of diluted aspirate with optimal TNC, CFU-f, and CD34+ cell counts is critical in providing better patient outcomes and improving aspiration efficiency.

Indications of CD34+ Cell Counts for BMA Concentrations

Previously, it was thought that CD34+ and CFU-fs are correlated in that CD34+ cells could potentially originate from CFU-fs. However, several studies have shown that CD34+ cells appear in higher concentrations at lower volume draws, even when CFU-fs have not increased in the same comparison [21]. This means that CD34+ cells reside in limited quantities per aliquot, showing that CD34+ levels may also benefit from smaller volumes. Additionally, like CFU-fs, increased levels of CD34+ cells have been shown to lead to better patient outcomes [22].

Conclusion

Our data supports the use of smaller volume aspirations utilizing a multiport system to optimize the quality of bone marrow aspirations and regenerative potential. Potential limitations include a lack of uniformity of harvesting procedure amongst physicians, which can increase variation in reported cell counts. Further studies are needed to determine the clinical impact of these changes and to determine if concentration is still required with optimal aspiration parameters and needle design. A potential improvement in determining clinical impact would be to apply patient-based surveys such as the FRI or NASS over a prolonged period to determine long-term outcomes associated with the procedure.

Author Contributions

GL, CL, CK, DK, and MS were responsible for study design and drafting of the manuscript. GL, CL, and CK were responsible for the bone marrow harvesting and DK and EH were responsible for the bone marrow lab analysis. DK was responsible for statistical analysis and GL, CL, CK, MS, FN, and RA were responsible for manuscript preparation. All authors have given their final approval of the manuscript to be published.

Declaration of Interests: None

References

1. I El-Jawhari J, Ilas D, Jones W, Cuthbert R, Jones E, et al. (2020) Enrichment and preserved functionality of multipotential stromal cells in bone marrow concentrate processed by vertical centrifugation. Eur Cell Mater; 40:58-73.
2. Kim GB, Seo MS, Park WT, Lee GW (2020). Bone marrow aspirate concentrate: its uses in osteoarthritis. Int J Mol Sci; 21(9): 3224.
3. Awad ME, Hussein KA, Helwa I, Abdelsamid MF, Aguilar-Perez A, et al (2019). Meta-analysis and evidence base for the efficacy of autologous bone marrow mesenchymal stem cells in knee cartilage repair: methodological guidelines and quality assessment. Stem Cells Int; 2019.
4. A. Cavallo C, Boffa A, Andriolo L, Silva S, Grigolo B, et al (2021) Bone marrow concentrate injections for the treatment of osteoarthritis: evidence from preclinical findings to the clinical application. Int Orthop; 45(2):525-538
5. Colberg RE, Jurado Velez JA, Walsh KP, Fleisig G (2020) Short-term outcomes after pure bone marrow aspirate injection for severe knee osteoarthritis: a case series. Regen Med; 15(7):1851-1859.
6. Gupta PK, Das AK, Chullikana A, Majumdar AS (2012) Mesenchymal stem cells for cartilage repair in osteoarthritis. Stem Cell Res Ther; 3(4):25.
7. Hernigou PH, Poignard A, Beaujean F, Rouard H (2005) Percutaneous autologous bone-marrow grafting for nonunions: influence of the number and concentration of progenitor cells. J Bone Joint Surg Am; 87(7):1430-7.
8. Hernigou P, Beaujean F (2002) Treatment of osteonecrosis with autologous bone marrow grafting. Clin Orthop Relat Res; (405):14-23.
9. Shapiro SA, Kazmerchak SE, Heckman MG, Zubair AC, O'Connor MI (2017) A prospective, single-blind, placebo-controlled trial of bone marrow aspirate concentrate for knee osteoarthritis.Am J Sports Med; 45(1):82-90.
10. Desai P, Hasan SM, Zambrana L, Hegde V, Saleh A, et al. (2015). Bone mesenchymal stem cells with growth factors successfully treat nonunions and delayed unions. HSS J; 11(2):104-111.
11. Feddahi N, Herten M, Tassemeier T, Rekasi H, Hackel A, et al. (2021) Does needle design affect the regenerative potential of bone marrow aspirate? An in vitro study. Life (Basel); 11(8):748.

12. Hegde V, Shonuga O, Ellis S, Fragomen A, Kennedy J, et al (2014) A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated nonequivalency in progenitor cell number and concentration. J Orthop Trauma; 28(10):591-598.

13. McLain RF, Fleming JE, Boehm CA, Muschler GF (2005) Aspiration of osteoprogenitor cells for augmenting spinal fusion: comparison of progenitor cell concentrations from the vertebral body and iliac crest. J Bone Joint Surg Am; 87(12):2655-2661.

14. Hernigou P, Homma Y, Flouzat Lachaniette CH, Poignard A, Allain J, et al (2013) Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. Int Orthop; 37(11):2279-87.

15. Grønkjær M, Hasselgren CF, Østergaard AS, Johansen P, Korup J, et al (2016) Bone marrow aspiration: a randomized controlled trial assessing the quality of bone marrow specimens using slow and rapid aspiration techniques and evaluating pain intensity. Acta Haematol; 135(2):81-87.

16. Batinić D, Marusić M, Pavletić Z, Bogdanić V, Uzarević B, et al (1990). Relationship between differing volumes of bone marrow aspirates and their cellular composition. Bone Marrow Transplant; 6(2):103-107.

17. Kuebler D, Schnee A, Moore L, Kouri J, McLaughlin A, et al. (2022) Short-term efficacy of using a novel low-volume bone marrow aspiration technique to treat knee osteoarthritis: A retrospective cohort study. Stem Cells Int; 2022:5394441.

18. Muschler GF, Boehm C, Easley K (1997) Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume. J Bone Joint Surg Am; 79(11):1699-709.

19. Jäger M, Hernigou P, Zilkens C, Herten M, Fischer J, et al (2010) Cell therapy in bone-healing disorders. Orthop Rev (Pavia); 2(2):e20.

20. Pösel C, Möller K, Fröhlich W, Schulz I, Boltze J, et al (2012) Density gradient centrifugation compromises bone marrow mononuclear cell yield. PLoS One; 7(12):e50293.

21. Scarpone M, Kuebler D, Chambers A, De Filippo CM, Amatuzio M, et al. (2019) Isolation of clinically relevant concentrations of bone marrow mesenchymal stem cells without centrifugation. J Transl Med; 17(1):10.

22. Kuznetsov SA, Mankani MH, Bianco P, Robey PG (2009) Enumeration of the colony-forming units–fibroblast from mouse and human bone marrow in normal and pathological conditions. Stem Cell Res; 2(1):83-94.

23. Belk JW, Lim JJ, Keeter C, McCulloch PC, Houck DA, et al. (2023) Patients with knee osteoarthritis who receive platelet-rich plasma or bone-marrow aspirate concentrate injections have better outcomes than patients who receive hyaluronic acid: Systematic review and meta-analysis. Arthroscopy; 39(7):1714-1734.