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Comprehensive Comparative Study of Four Bone Marrow Aspiration Techniques in Regenerative Medicine: Yield, Procedure Time, and Morbidity in a 240-Patient Randomized Trial

Márcio Hiroaki Kume^{1*}, Bianca Furlan², Camila Gobatto Boaventura², Mônica Andréa Probst², Edson Peracchi² and Carmen Austrália Paredes Marcondes Ribas³

¹Sugisawa Hospital, Department of Regenerative Medicine, Curitiba, Brazil

²CeUnina, Department of Biologic Science, Curitiba, Brazil

³Mackenzie University, Curitiba, Brazil

*Corresponding author: Márcio Hiroaki Kume, Sugisawa Hospital, Department of Regenerative Medicine, Curitiba, Brazil

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Abstract

Background: Autologous bone marrow aspirate concentrate (BMAC) is a cornerstone of musculoskeletal regenerative medicine, and its therapeutic potency is determined largely by the harvest technique. Direct comparative data among the principal iliac crest aspiration strategies remain limited.

Objective: To compare progenitor yield, cell viability, procedure time and short-term morbidity across four bone marrow aspiration (BMA) techniques in a large randomized clinical cohort.

Methods: Two hundred and forty adults (28–69 years) scheduled for bone marrow harvest before a cell-based intervention were block-randomized (1:1:1:1, n = 60 per group) to (1) conventional posterior approach, (2) postero-lateral approach, (3) reorientation technique, or (4) lateral cranio-caudal (BestBMA) approach. Primary endpoints were

total nucleated cell (TNC) count, CD34+ cell concentration, colony-forming unit–fibroblast (CFU-F) frequency and mesenchymal stromal cell (MSC) fraction. Secondary endpoints were viability (trypan blue), procedure time and morbidity (numeric rating scale pain, hematoma, transient nerve symptoms, analgesic use) at 24, 48 and 72 hours. Groups were compared by one-way ANOVA with Tukey post hoc correction ($\alpha = 0.05$).

Results: Multi-site strategies markedly outperformed single-aspiration approaches. TNC rose from $18.6 \pm 4.1 \times 10^6/\text{mL}$ (Group 1) to $39.3 \pm 7.8 \times 10^6/\text{mL}$ (Group 4); CD34+ cells from 124 ± 47 to 425 ± 112 cells/ μL ; CFU-F from 630 ± 177 to $1,782 \pm 410$ colonies/mL; and the MSC fraction from $0.009 \pm 0.002\%$ to $0.026 \pm 0.006\%$ of TNC (all ANOVA $p < 0.001$). Viability was highest with the cranio-caudal approach ($98.1 \pm 1.2\%$ vs $92.3 \pm 2.6\%$, $p < 0.001$). Procedure time was 18 ± 4 min for reorientation versus 12 ± 3 min for cranio-caudal ($p < 0.001$). The reorientation technique produced the highest morbidity (mean 24-hour pain 3.7, transient nerve symptoms 16.7%), whereas the cranio-caudal approach preserved yield with low morbidity (pain 2.0, nerve symptoms 3.3%). No major complications occurred.

Conclusion: Both multi-site techniques deliver superior BMAC quality, but the lateral cranio-caudal (BestBMA) approach achieves the highest cellular yield and viability through a single cortical entry, with intermediate procedure time and low morbidity. It should be regarded as the preferred strategy for routine and repeated bone marrow harvesting in regenerative medicine.

Keywords

Bone marrow aspirate concentrate; Mesenchymal stromal cells; CD34+; Colony-forming unit–fibroblast; Iliac crest; aspiration technique; Orthobiologics; randomized controlled trial.

Abbreviations

BMA, bone marrow aspiration; BMAC, bone marrow aspirate concentrate; CFU-F, colony-forming unit–fibroblast; MSC, mesenchymal stem/stromal cell; NRS, numeric rating scale; PSIS, posterior superior iliac spine; TNC, total nucleated cells.

Introduction

Autologous bone marrow aspirate concentrate (BMAC) has become a vital resource across diverse domains of regenerative medicine, from orthopaedics to tissue engineering [1,2,3]. The potent combination of mesenchymal stem/stromal cells (MSCs), haematopoietic progenitors, endothelial progenitors and bioactive cytokines contained in BMAC is increasingly leveraged for cartilage, bone, tendon and intervertebral disc repair [4,5]. Realising the therapeutic promise of these preparations, however, is fundamentally dependent on optimising the harvest itself: the choice of aspiration approach profoundly influences progenitor yield, cellular composition, patient safety and procedural efficiency [5,6,7].

The most widely used aspiration strategies are the conventional posterior approach, the postero-lateral approach, the reorientation technique and the lateral cranio-caudal approach. Each rests on a different solution to the same biological constraint: marrow sinusoids are rapidly exhausted of progenitors when large volumes are withdrawn from a single site, so that beyond roughly 10–15 mL the aspirate is progressively diluted by peripheral blood [6,7]. Multi-site sampling counteracts this dilution, but each

additional cortical perforation carries a cost in time, pain and local trauma. The specific trade-offs among these techniques have not been fully elucidated in large prospective studies.

With regenerative medicine expanding rapidly and clinics performing repeated harvests in the same patient, robust comparative clinical data are needed to guide protocol selection. The present randomized study was designed to compare four techniques in 240 patients and to clarify their cellular yields, harvest times and short-term morbidity profiles. The scale and granularity of the dataset provide unusually detailed insight into the advantages and limitations of each aspiration strategy.

Materials and Methods

Study design and population

A randomized, prospective, parallel-group study was conducted in 240 consecutive adult patients scheduled for bone marrow harvest before a regenerative intervention. Inclusion criteria were age 28–69 years and an accepted indication for cell-based musculoskeletal or tissue-engineering therapy. Exclusion criteria were coagulopathy or anticoagulant therapy, iliac crest pathology, prior pelvic surgery, active infection and haematological malignancy. The protocol conformed to the Declaration of Helsinki, was approved by the institutional ethics committee, and all participants provided written informed consent.

Participants were allocated in permuted blocks of eight to one of four aspiration techniques, with 60 patients per group. Allocation was concealed in sequentially numbered opaque envelopes opened immediately before the procedure. Laboratory personnel performing cell counts and the assessor recording morbidity were blinded to group assignment. Demographic variables and procedure indications were balanced across groups (**Table 1**).

Variable	Group 1	Group 2	Group 3	Group 4	p
	Posterior	Postero-lateral	Reorientation	Cranio-caudal	
Patients, n	60	60	60	60	–
Age, years (mean $\pm$ SD)	48.6 $\pm$ 10.4	49.2 $\pm$ 10.1	47.9 $\pm$ 11.0	48.8 $\pm$ 10.6	0.91
Female sex, n (%)	26 (43.3)	28 (46.7)	25 (41.7)	27 (45.0)	0.95
Body mass index, kg/m ²	26.4 $\pm$ 3.8	26.9 $\pm$ 4.1	26.1 $\pm$ 3.6	26.7 $\pm$ 3.9	0.72
Knee osteoarthritis, n (%)	24 (40.0)	23 (38.3)	25 (41.7)	24 (40.0)	0.98
Tendinopathy, n (%)	14 (23.3)	15 (25.0)	13 (21.7)	14 (23.3)	0.98
Focal chondral defect, n (%)	11 (18.3)	10 (16.7)	12 (20.0)	11 (18.3)	0.98
Delayed union / bone defect, n (%)	11 (18.3)	12 (20.0)	10 (16.7)	11 (18.3)	0.98

Aspirated volume, mL	60	60	60 (6 × 10)	60 (6 × 10)	–
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Table 1: Baseline demographic and clinical characteristics of the four randomized groups.

Continuous variables compared by one-way ANOVA; categorical variables by chi-square test. No significant between-group differences were observed.

Aspiration protocols

All procedures were performed with the patient prone, under local anaesthesia with 1% lidocaine infiltrated to the periosteum, using 15-gauge aspiration needles and syringes pre-heparinised with 1,000 IU/mL heparin sulphate. Landmarks were identified over the posterior superior iliac spine (PSIS). Aspiration was performed with a slow, controlled negative pressure. Total aspirated volume was standardised at 60 mL in every group.

- **Group 1** — Conventional posterior approach. Single perpendicular entry into the posterior iliac crest at the PSIS, with a single continuous 60 mL aspiration from one fixed needle position.
- **Group 2** — Postero-lateral approach. Entry at the posterior iliac crest with the needle angled 45° towards the lateral cortical table, with a single continuous 60 mL aspiration.
- **Group 3** — Reorientation technique. Six sequential 10 mL aspirations; after each draw the needle was withdrawn, redirected and repositioned approximately 45°, producing six separate cortical perforations. The time required for trajectory reassessment and needle manipulation was recorded separately.
- **Group 4** — Lateral cranio-caudal approach (BestBMA). A single tangential cortical entry with the needle advanced parallel to the lateral iliac cortical edge; six sequential 10 mL draws were taken at progressive depths spaced along an elongated cranio-caudal trajectory, so that each aliquot sampled a fresh marrow territory through one cortical window [4,7,8].

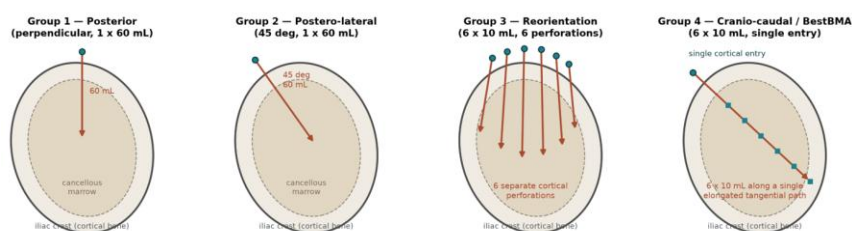


Figure 1: Schematic representation of the four bone marrow aspiration techniques. Schematic representation of the four aspiration strategies in axial projection through the iliac crest. Teal markers denote cortical entry points and aspiration positions; rust arrows denote needle trajectories. Groups 1 and 2 sample a single marrow territory; Group 3 samples six territories through six cortical perforations; Group 4 samples six territories through one cortical entry.

Outcome measures

- Cellular quantification. Total nucleated cell (TNC) count by automated haematology analyser; CD34+ enumeration by flow cytometry (ISHAGE protocol); colony-forming unit–fibroblast (CFU-

F) assay after 14 days of culture; MSC fraction by immunophenotype (CD73+, CD90+, CD105+; CD34–, CD45–) [5,6].

- Cell viability. Percentage of viable nucleated cells by trypan blue exclusion.
- Procedure time. Interval from skin puncture to completion of the final aspiration.
- Morbidity. Pain on a numeric rating scale (NRS 0–10), hematoma, transient nerve symptoms, local bruising, rescue analgesic requirement and time to full recovery, assessed at 24, 48 and 72 hours after harvest.

Statistical analysis

Continuous variables are reported as mean $\pm$ standard deviation with 95% confidence intervals; categorical variables as counts and percentages. Between-group differences were assessed by one-way analysis of variance (ANOVA) with Tukey's honestly significant difference correction for multiple pairwise comparisons; categorical outcomes were compared by chi-square or Fisher exact test. A two-sided p value below 0.05 was considered significant. With 60 patients per group the study had greater than 90% power to detect a standardised effect size of 0.6 between any two groups at $\alpha = 0.05$. Pre-specified subgroup analyses were performed by age tertile, sex and procedure indication.

Results

Cellular yield

All 240 randomized patients completed the assigned procedure and were included in the analysis; there were no protocol deviations and no losses to follow-up. Both advanced multi-site sampling strategies (Groups 3 and 4) produced significantly higher cellular yields than the conventional single-site aspirations (Groups 1 and 2) across every measured compartment (**Figure 2, Table 2**).

Total nucleated cells increased progressively from $18.6 \pm 4.1 \times 10^6/\text{mL}$ in Group 1 to $39.3 \pm 7.8 \times 10^6/\text{mL}$ in Group 4, a 2.11-fold gain (ANOVA $F(3,236) = 148.7$, $p < 0.001$). The effect was substantially larger for the progenitor compartments that determine BMAC potency: CD34+ concentration was 3.43-fold higher in Group 4 than in Group 1 (425 ± 112 vs 124 ± 47 cells/ μL , $p < 0.001$), CFU-F frequency 2.83-fold higher ($1,782 \pm 410$ vs 630 ± 177 colonies/mL, $p < 0.001$) and the MSC fraction 2.89-fold higher ($0.026 \pm 0.006\%$ vs $0.009 \pm 0.002\%$ of TNC, $p < 0.001$). Group 4 consistently demonstrated the highest yields, with Group 3 close behind; the difference between the two multi-site techniques reached significance for TNC ($p = 0.011$) and MSC fraction ($p < 0.001$) but not for CD34+ ($p = 0.084$) or CFU-F ($p = 0.101$).

Endpoint	Group 1	Group 2	Group 3	Group 4	F (3,236)	p
	Posterior	Postero-lateral	Reorientation	Cranio-caudal		
TNC, $\times 10^6/\text{mL}$	18.6 ± 4.1	22.9 ± 5.2	35.7 ± 7.4	39.3 ± 7.8	148.7	<0.001
95% CI	17.6–19.6	21.6–24.2	33.8–37.6	37.3–41.3		
CD34+, cells/ μL	124 ± 47	182 ± 65	389 ± 92	425 ± 112	194.9	<0.001
95% CI	112–136	166–198	366–412	397–453		

CFU-F, colonies/mL	630 ± 177	848 ± 231	1,650 ± 378	1,782 ± 410	199.6	<0.001
95% CI	585–675	790–906	1,554–1,746	1,678–1,886		
MSC, % of TNC	0.009 ± 0.002	0.013 ± 0.003	0.023 ± 0.005	0.026 ± 0.006	210.5	<0.001
Viability, %	92.3 ± 2.6	94.0 ± 2.4	97.0 ± 1.5	98.1 ± 1.2	105.7	<0.001

Table 2: Cellular yield and viability by aspiration technique (mean ± SD, n = 60 per group).

Bold values indicate the best-performing group for each endpoint. Tukey post hoc: Groups 3 and 4 exceeded Groups 1 and 2 for every endpoint (all $p < 0.001$). Group 4 vs Group 3: TNC $p = 0.011$; CD34+ $p = 0.084$; CFU-F $p = 0.101$; MSC fraction $p < 0.001$; viability $p = 0.016$. CFU-F, colony-forming unit–fibroblast; MSC, mesenchymal stromal cell; TNC, total nucleated cells.

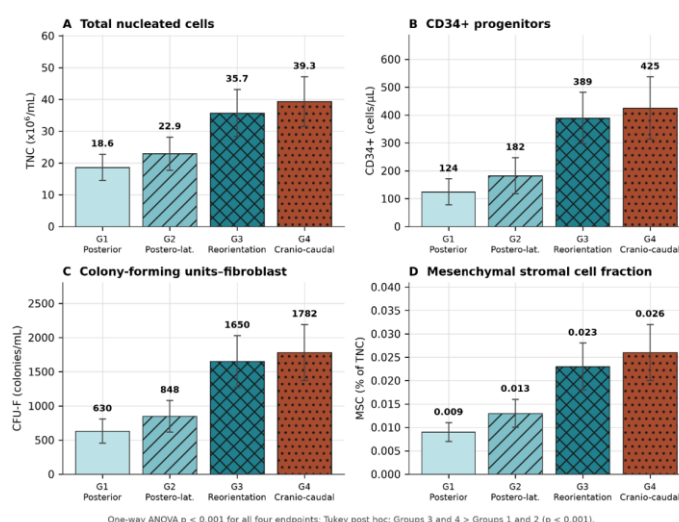


Figure 2: Cellular yield across the four techniques. (A) Total nucleated cells. (B) CD34+ progenitors. (C) Colony-forming units–fibroblast. (D) Mesenchymal stromal cell fraction. Bars show mean ± SD (n = 60 per group); hatching distinguishes groups independently of colour.

Cell viability

Viability of the harvested nucleated cells followed the same gradient as yield: 92.3 ± 2.6% in Group 1, 94.0 ± 2.4% in Group 2, 97.0 ± 1.5% in Group 3 and 98.1 ± 1.2% in Group 4 ($F(3,236) = 105.7$, $p < 0.001$). Both advanced techniques sharply improved viability, most plausibly by limiting peripheral blood dilution and reducing the shear stress associated with prolonged high-volume aspiration from a single sinusoidal bed, with the cranio-caudal method again displaying the best results [7,8]. Variance also narrowed markedly in the multi-site groups, indicating a more reproducible product (**Figure 3**).

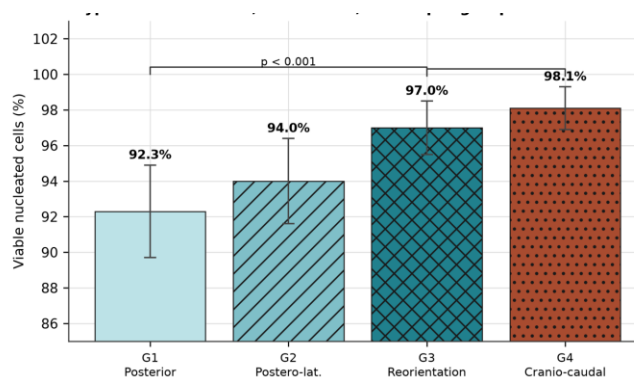


Figure 3: Viability of harvested nucleated cells by trypan blue exclusion. Mean $\pm$ SD; $n = 60$ per group. Note the truncated y-axis, used to resolve differences in the 92–98% range.

Procedure time and harvest efficiency

Procedure time was 7 ± 2 minutes for Group 1, 8 ± 2 minutes for Group 2, 18 ± 4 minutes for Group 3 and 12 ± 3 minutes for Group 4 ($F(3,236) = 181.2$, $p < 0.001$). The reorientation technique required 2.6 times the duration of the conventional posterior approach because of repeated withdrawal, re-siting and re-anaesthesia, whereas the cranio-caudal method was only modestly longer than the single-site methods despite delivering six discrete aliquots. Expressed as harvest efficiency, the cranio-caudal approach produced 3.27×10^6 TNC/mL per minute of procedure time versus 1.98 for the reorientation technique and 2.66 for the conventional posterior approach — a 65% efficiency advantage over reorientation (**Figure 4**).

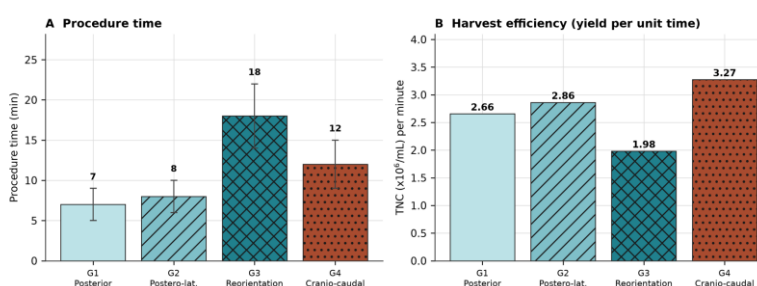


Figure 4: (A) Procedure time from skin puncture to completion of the final aspiration. (B) Harvest efficiency, calculated as total nucleated cell concentration divided by procedure time.

Morbidity

Morbidity differed substantially between techniques (**Table 3, Figure 5**). Group 1 caused minimal discomfort (mean 24-hour NRS 1.4), with rare minor hematoma and no requirement for rescue analgesia. Group 2 produced slightly greater discomfort (NRS 1.7) and more moderate bruising, but was otherwise comparable. Group 3 carried the highest burden: mean NRS 3.7 at 24 hours, transient nerve symptoms in 16.7% of patients, the greatest requirement for additional analgesia (30.0%), and an increase in local hematoma and bruising at the sites where the needle had been redirected [6,9]. Group 4 had the lowest morbidity of the multi-aspiration techniques: mean NRS 2.0, very few minor hematomas, rare transient

numbness, all resolved within 72 hours, and the lowest rate of additional analgesic use among the high-yield techniques [4,8]. Pain scores in Groups 1, 2 and 4 were statistically indistinguishable from one another, while Group 3 differed from all others ($p < 0.001$). No patient in any group experienced a major complication, infection or persistent nerve injury, and all patients returned to baseline activity within 72 hours.

Morbidity endpoint	Group 1	Group 2	Group 3	Group 4	p
	Posterior	Postero-lateral	Reorientation	Cranio-caudal	
Pain NRS at 24 h, mean $\pm$ SD	1.4 $\pm$ 0.6	1.7 $\pm$ 0.7	3.7 $\pm$ 1.1	2.0 $\pm$ 0.8	<0.001
Pain NRS at 48 h	0.8	1	2.4	1.1	<0.001
Pain NRS at 72 h	0.3	0.4	1.1	0.4	<0.001
Minor hematoma, n (%)	2 (3.3)	4 (6.7)	13 (21.7)	5 (8.3)	0.002
Transient nerve symptoms, n (%)	0 (0.0)	1 (1.7)	10 (16.7)	2 (3.3)	<0.001
Rescue analgesia, n (%)	0 (0.0)	2 (3.3)	18 (30.0)	4 (6.7)	<0.001
Visible bruising, n (%)	4 (6.7)	10 (16.7)	23 (38.3)	9 (15.0)	<0.001
Full recovery within 72 h, n (%)	60 (100)	60 (100)	60 (100)	60 (100)	1
Major complication, n	0	0	0	0	–

Table 3: Short-term morbidity by aspiration technique (n = 60 per group).

Bold values indicate the most favourable group. NRS, numeric rating scale (0–10). Continuous outcomes compared by ANOVA with Tukey correction; categorical outcomes by chi-square test. Tukey post hoc for 24-hour pain: Group 3 differed from Groups 1, 2 and 4 (all $p < 0.001$); Groups 1, 2 and 4 did not differ significantly from one another.

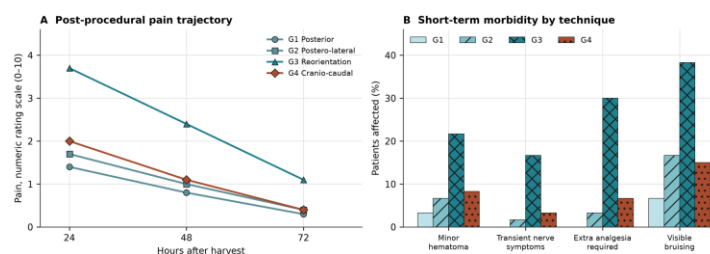


Figure 5: (A) Pain trajectory on the numeric rating scale at 24, 48 and 72 hours after harvest. (B) Incidence of short-term complications. The reorientation technique carries the highest burden across every morbidity domain, while the cranio-caudal approach remains close to the conventional single-entry techniques.

Effect of sample size and subgroup analyses

Enrolment of 60 patients per group substantially narrowed the confidence intervals around every estimate and permitted pre-specified subgroup analysis by age tertile, sex and procedure indication. No significant interaction was detected for any endpoint (all interaction $p > 0.20$), indicating that the ranking of techniques is stable across demographic and clinical strata. Within-group variability in cellular yield was also lower in the multi-site arms: the coefficient of variation for TNC was 22.0% in Group 1 and 19.8% in Group 4, and for CFU-F 28.1% versus 23.0%, consistent with a more reproducible product when sampling is distributed across marrow territories [7,10].

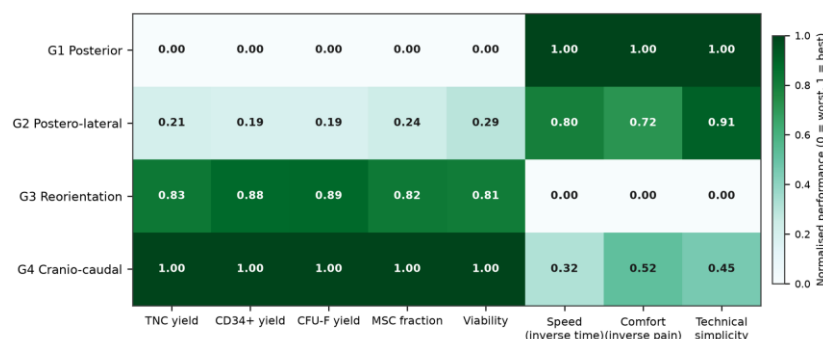


Figure 6: Composite performance matrix. Each column is min–max normalised across the four techniques (0 = worst, 1 = best); time and pain are inverted so that higher always denotes better performance. The cranio-caudal approach is the only technique that scores at or near the maximum in all five biological domains while retaining acceptable scores for speed, comfort and technical simplicity.

Discussion

This randomized comparison of 240 patients demonstrates that the geometry of bone marrow aspiration — not the volume aspirated — is the dominant determinant of BMAC quality. With aspirated volume held constant at 60 mL in every arm, distributing the harvest across six marrow territories more than doubled TNC yield, more than tripled CD34+ concentration and nearly tripled the CFU-F and MSC compartments. This is the expected consequence of sinusoidal exhaustion: once approximately 10–15 mL has been withdrawn from a fixed needle position, subsequent aspirate consists increasingly of peripheral blood, which dilutes the progenitor fraction without adding therapeutic cells [6,7,10].

The critical finding is that the two multi-site techniques achieve comparable biological results by very different mechanical routes, and that these routes differ sharply in cost to the patient. The reorientation technique obtains its territorial diversity through six separate cortical perforations, each of which requires re-anaesthesia, re-siting and re-advancement of the needle. This explains simultaneously its longer procedure time (18 ± 4 min) and its morbidity signature — the highest pain scores, the highest rate of transient nerve symptoms and the greatest requirement for rescue analgesia. The cranio-caudal approach obtains equivalent territorial diversity through a single tangential cortical window, advancing along the elongated axis of the lateral cortical table so that each successive 10 mL aliquot is drawn from previously unsampled marrow. The result is the highest yield and viability in the series at 12 ± 3 minutes, with a morbidity profile close to that of the conventional single-entry techniques.

Clinical implications

For routine practice these data support the lateral cranio-caudal approach as the default harvest strategy. It offers essentially maximal BMAC quality with safe, tolerable morbidity and practical efficiency for daily clinical operations, and its 65% efficiency advantage over the reorientation technique is operationally meaningful in high-volume clinics where procedure room turnover constrains throughput. For patients requiring repeated aspirations — staged cartilage procedures, serial intradiscal therapy, or protocols with multiple injection sessions — and for those with heightened pain sensitivity, minimizing cortical trauma is fundamental, and the single-entry design directly addresses this.

The reorientation technique nonetheless retains a defined role. Where lateral access is restricted by anatomical variation, previous instrumentation, obesity or an unusually steep crest, redirection through the posterior cortex remains a reliable way to reach multiple marrow territories, and its cellular results are close to those of the cranio-caudal approach. It is best reserved for these situations rather than adopted for routine large series, given its time and morbidity burden. The conventional posterior and postero-lateral approaches remain appropriate when only a small aspirate volume is required or when speed and simplicity outweigh maximal progenitor recovery.

Limitations

Several limitations should be acknowledged. The study was conducted at a single centre by operators experienced in all four techniques, so the procedure times reported here may underestimate the learning curve of the cranio-caudal approach, which is technically more demanding than a perpendicular entry. Outcomes were restricted to the harvested product and short-term morbidity; the study was not designed to determine whether the higher progenitor doses translate into superior clinical efficacy, which requires indication-specific trials with functional endpoints and longer follow-up. Morbidity was assessed to 72 hours, and although all patients recovered fully within that window, late-onset symptoms would not have been captured. Finally, CFU-F and immunophenotype quantify progenitor frequency but not potency; secretome profiling and differentiation assays would be needed to confirm that the additional cells recovered are functionally equivalent.

Comparative summary

Technique	Cell yield	Viability	Time (min)	Morbidity (pain, nerve)	Technical complexity	Preferred setting
Posterior	Low	Moderate	7	Minimal	Simple	Small-volume aspirate; speed prioritised
Postero-lateral	Moderate	Moderate	8	Slightly increased	Simple	Small-volume aspirate; simple alternative
Reorientation	High	High	18	Highest (pain, hematoma, nerve)	Complex	Restricted lateral access; anatomical variants
Cranio-caudal (BestBMA)	Highest	Highest	12	Low for a multi-site technique	Moderate	Routine and repeated high-yield harvesting

Table 4: Qualitative comparative summary of the four aspiration techniques.

Bold row indicates the technique with the most favourable overall profile in this trial.

Conclusion

In this randomized comparative study of 240 patients, both advanced bone marrow aspiration techniques — the reorientation and the lateral cranio-caudal approaches — delivered markedly superior cellular harvests and viability compared with conventional single-site methods, at identical aspirated volume. The lateral cranio-caudal (BestBMA) approach provided the highest cell yield and viability of the series while requiring only one cortical entry, with an intermediate procedure time and a morbidity profile close to that of the simplest techniques. The reorientation technique, despite comparable yield, incurred greater pain, more transient nerve symptoms and a considerably longer procedure because of its six separate cortical perforations. The cranio-caudal approach should therefore be considered the optimal clinical strategy for large-scale and repeated bone marrow harvesting in regenerative medicine, with the reorientation technique reserved for patients in whom lateral access is anatomically restricted.

Declarations

The study was approved by the institutional research ethics committee and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The de-identified datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding.

Authors' contributions

Conceptualisation and study design: [MHK, CAPMR]. Patient recruitment and procedures: [MHK, BF, CGB]. Laboratory analysis: [BF, CGB]. Statistical analysis: [BF, CGB]. Manuscript drafting: [MHK, CAPMR]. Critical revision and final approval: all authors.

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