Comparison of Steady-State Bone Marrow and GCSF-Primed Stem Cell Sources Used in Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Objective: To compare donor graft characteristics and clinical outcomes in recipients of allogeneic heamatopoietic stem cell transplantation (HSCT) using GCSF primed bone marrow (GBM) and steady-state bone marrow (SBM) as stem cell sources.

Study Design: Observational study.

Place and Duration of the Study: Department of Clinical Haematology, Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan, from August 2018 to October 2020.

Methodology: Eighty patients undergoing allogeneic HSCT were analysed. Among these, forty each received GBM and SBM from HLA identical siblings. Graft characteristics, such as total nucleated cells, CD34+ cell yield; clinical outcomes such as neutrophil and platelet engraftment, primary and secondary graft failure (GF), as well as the frequency of acute and chronic graft *versus* host disease (GvHD), were recorded and compared using the t-test, with significance at $p < 0.05$.

Results: A statistically significant difference was observed in CD34+ dose with median dose 7.68 (p=0.002) but not in TNC dose with meadin dose 5 (p=0.86). Neutrophil engraftment occurred much more quickly with median of 13.43 days in the GBM than SBM group (p=0.025). While no statistically significant difference (p=0.89) in platelet engraftment was reported in both SBM and GBM. At the same time, patients with both GBM and SBM transplants showed a comparable ratio of acute to chronic GvHD and primary to secondary GF.

Conclusion: GBM is associated with better CD34+ stem cell yield and quicker neutrophil engraftment in clinical outcomes.

Key Words: *Granulocyte colony-stimulating factor, Bone marrow, Hematopoietic stem cell transplantation.*

How to cite this article: Nadeem HM, Ali A, Shahbaz N, Ghafoor T, Khattak TA, Chaudary QUN. Comparison of Steady-State Bone Marrow and GCSF-Primed Stem Cell Sources Used in Allogeneic Hematopoietic Stem Cell Transplantation. *J Coll Physicians Surg Pak* 2024; **34(03)**:355-359.

INTRODUCTION

The source of haematopoietic stem cells (HSCs) for allogeneic stem cell transplantation has been changing throughout the previous decades, from the cord blood and bone marrow (BM), peripheral stem cells (PBSC) moblilised by combination with BM and primed bone marrow (GBM).[1] Currently, the most common source of allogeneic stem cells is PBSCs mobilised with G-CSF. Many malignant and non-malignant illnesses can be treated by allogeneic haematopoetic stem cell transplantation HSCT.

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Received: April 07, 2023; Revised: October 14, 2023; Accepted: October 26, 2023
DOI: https://doi.org/10.29271/jcpsp.2024.03.355

According to the Center for International Blood and Marrow Transplantation Research (CIBMTR), PBSC accounted for up to 80% of donations among people under the age of 20 years, and BM accounted for up to 15% of stem cell sources worldwide between 2007 and 2011.[2] Over the past 20 years, PBSCs have replaced BM as the main source of stem cells due to their rapid engraftment and viability.[3]

Steady-state PBSCs and steady-state bone marrow (SBM) have the same potential for engraftment in autologous transplants.[4,5] Additional research has revealed that G-CSF-primed bone marrow (GBM) may be safely given to patients undergoing autologous transplantation and can produce engraftment equivalent to that of GCF mobilised PBSC. It also outperforms unprimed BM in terms of success. The effects of various transplant preparative regimens and previous chemotherapy make autologous transplantation a poor model system for determining the viability of this concept.[6,7]

In the allogeneic transplant context, G-PBSCs offer some advantages mainly due to the larger cell dose compared to SBM and progenitor cells that are slightly more differentiated than BM,

that result in rapid platelet and neutrophil engraftment with rapid immune reconstitution.[8,9] However, disease-free survival (DFS), transplant-related mortality (TRM), and overall survival (OS) are similar for both sources. Although both acute and chronic graft-versus-host disease (GvHD) depend on HSC sources, their effect on survival, severity, susceptibility to immunotherapy, disease type, and graft-versus-leukemia (GVL) effect.[10-13]

Patients receiving amount of HSCs and other immune cells, such as cytotoxic T cells and natural killers is crucial because it affects both the onset and severity of GvHD, the most serious side effect of allo-HSCT, at one hand while on the other hand, they also determine the success or failure of the transplantation through GVL.[14-16] Until now, there has been no published data on the comparison of various sources of stem cells used in HSCT in Pakistan. The rationale of the present study was to compare GBM as a source of stem cells with SBM in allogeneic bone marrow transplant, and its effect on stem cell dose, engraftment, GvHD, GF, DFS, and OS. The objective of this study was to compare donor graft characteristics and clinical outcomes in recipients of allogeneic HSCT using GBM and SBM as stem cell sources.

METHODOLOGY

This prospective observational study inducted patients undergoing allogeneic bone marrow transplants (BMT) at the Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan, from August 2018 to October 2020. The analysis excluded patients of haploidentical HSCT, second transplants, and patients receiving PBSC alone or PBSC plus BMH. The final analysis comprised 80 patients, 40 each received SBM and GBM. Age, gender, diagnosis with risk categories, ABO, and gender mismatch are among the data variable collected. Informed consent was obtained from each volunteer prior to BMT and approved by the Institutional Ethical Research Board (IRB/FCPS-011/AFBMTC/Approved/20).

All donors and recipients were HLA-typed using PCR-SSP (according to the user manual) for both HLA class I and II alleles. Patients underwent myeloablative (MAC), non-myeloablative (NMA) or reduced intensity (RIC), conditioning regimens according to their status and the transplant procedures. Antithymocyte globulin (ATG), Cyclosporin, and/or Metho-trexate were utilised for GvHD prophylaxis. G-CSF 10 ug/kg subcutaneous injection was given to donors for four days to the GBM arm (from Day 3 to Day 0). BM was extracted on the day of a transplant from posterior and lateral iliac crests with 15-20 ml/kg of donor body weight under general anaesthesia. Since GBM has high TNC due to high neutrophil count, the authors corrected the TNC according to the following formula: Corrected TNC = TNC x 45 /neutrophil% in BMH. This correction was based on the mean neutrophil count obtained in unprimed BMH that was found to be 45%. Using flow cytometry on a BD FASC Caliber flow cytometer, CD 34+ count in the collected bone marrow sample was evaluated. The donors were closely watched for any untoward effects till complete recovery.

Data were analysed using SPSS version 25.0 software. Mean with standard deviation for numerical variables were calculated and tested using independent samples. Frequency and percentage were determined for classification variables. Paired t-test was used to evaluate the effect of GCSF priming on platelet and neutrophil transplantation, primary and secondary graft failure, and acute and chronic GVHD. A t-test value of ≤0.05 was considered statistically significant

RESULTS

A total of 80 patients were included. Demographic characteristics of the recruited donors and recipients for BMT are given in Table I. G-CSF priming before the harvest was done in 50% of cases, while the other half received unprimed marrow. The association of priming with stem cell yield, engraftment, graft failure, and GvHD is given in Tables I, II, and III, respectively. Table II presents BMT donors' CD34+ and TNC counts in SBM and GBM. The median corrected TNC dose obtained from primed marrow was 5.00x 10^8/kg (range = 2.96 - 6.64). The median corrected TNC dose for unprimed marrow was 4.98 x 10^8/kg (range = 2.72 - 8.8). The findings were not statistically significant between the groups (p=0.86) (Table II). The median CD34+ dose from primed and unprimed marrow was 7.68 x 10^6/kg (range 2.96 - 6.64) and 4.5 x 10^6/kg (range 1.53 – 12.6), respectively. CD34 yields from primed marrow were significantly higher than unprimed marrow (p=0.001, Table II).

Neutrophil engraftment occurred in all patients. The median time to neutrophil engraftment was 12.5 days (10 - 16) in recipients of primed marrow and 13.0 days (11 - 21) post-transplant in unprimed marrow recipients. Neutrophil engraftment occurred significantly earlier in primed marrow recipients (p=0.003) (Table III). Platelet engraftment was achieved at a median of day 19 (10 - 40 days) in primed marrow and day 22 (14 - 64 days) in unprimed marrow. The difference between the groups was statistically significant (p=0.002, Table III). Of the 2 patients who failed to engraft platelets despite achieving neutrophil engraftment, one died on Day 32 due to sepsis. The other experienced secondary graft failure on Day 60.

With a median follow-up of 1-year, secondary graft failure occurred in four patients, 1 of whom received primed marrow and three unprimed. The sample size is too small to draw a meaningful association between secondary graft failure and marrow priming.

GvHD occurred in 37 of 79 patients (46%), out of them, 26 patients had acute, 5 patients had chronic, and six patients developed both acute and chronic GvHD. Skin is the most common organ, 22 patients had isolated skin GvHD, and another 4 patients had combined skin and intestinal GvHD. Isolated liver and gut aGvHD affect 1 patient each. The difference between primed and unprimed marrow and the incidence of acute GvHD, 37% (n=15) *vs.* 42% (n=17), was not statistically significant (p=0.58).

Table I: Demographics characteristics of the recruited donor and recipient for BMT.

	Age			Gender		Weight	
	Median age	Minimun (years)	Maximum (years)	Female (n)	Male (n)	Female (kg)	Male (kg)
Recipient (n=80)	8	0.7	42	58 (72.5%)	22 (27.5%)	28	37
Donor (n=80)	11.5	4	42	21 (26.3%)	59 (73.7%)	38.8	37

Table II: Graft characteristics comprising TNC and Cd34+ dose in SBM and GBM grafts.

	TNC dose (10^8/kg)					CD34+ (10^6/kg)					t-test
	Mean	S. D	Median	Min	Max	Mean	S. D	Median	Min	Max	
GBM (primed) (n=40)	4.76	±1.2	5	2.72	7.6	9.22	±5.57	7.68	2.48	24.1	0.001
SBM (unprimed) (n=40)	4.77	±0.79	4.98	2.96	6.64	5.40	±2.67	4.5	2	12.68	

Table III: Clinical outcomes comprising neutrophil and platelet engraftment in SBM and GMB transplanted patients.

	Neutrophil Engraftment						Platelet Engraftment					
	Mean	Median	Mode	SD	N	p-value	Mean	Median	Mode	SD	N	t-test
GBM	13.435	13	13	±3.17	40	0.003	20.05	19	19	±6.47	40	0.002
SBM	12.425	12.5	13	±1.33	39		24.71	21	20	±13.3	38	

DISCUSSION

The CD34+ count was substantially greater in primed donors than in unprimed donors in the current investigation, however, there was no discernible difference in TNC yield between primed and unprimed bone marrow collection. In G-CSF primed bone marrow, early neutrophil engraftment was detected, but this effect was not found for platelet recovery. There was no difference in the occurrence of primary and secondary graft failure, acute or chronic GvHD, or either. The study involved 80 patients, with 40 in each group, and a significant multicentre trial is required to draw firm conclusions about the efficacy of primed bone marrow.

According to Schmitz *et al*., G-BM includes high CD34+ cells than SBM, which is evident from the quicker neutrophil and platelet engraftment. G-CSF-mobilised PBSC is virtually equivalent to G-BM in neutrophil and platelet engraftment to a lesser extent.[17] The present results are in agreement as more CD34+ cells and quicker neutrophil engraftment were observed in GBM.

Faster neutrophil and platelet engraftment was seen in comparing GBM and SBM patients. However, no changes in the incidence of aGvHD were observed in the previous studies. Isola *et al*. compared 112 patients as historical controls receiving SBM with 17 patients who received GBM grafts. The study found accelerated neutrophil and platelet engraftment in the GBM group.[18] This study is in concurrence with the present study as faster neutrophil engraftment in GBM and similar GvHD in both SBM and GBM groups were observed. Couban *et al*. used bone marrow using G-CSF with dose 10 ug/kg per day for four days and observed neutrophils and platelets fast recruitment in GBM (n=29) compared to historical transplant SBM (n = 20).[19] Ji *et al*. used two G-CSF priming protocols, 10 ug/kg per day for two days and 3-4 ug/kg per day G-CSF for seven days, and reported quicker neutrophil and platelet engraftment with GBM grafts receiving 7 days protocol compared to 2 days protocol. CD34+ counts were high among 7-day protocol.[20] While Ji *et al*. observed a lower cGvHD in 2-day G-CSF protocol compared with 7 days. In another study by Ji *et al*.[21], both SBM and GBM-treated patients, had a similar rate of cGvHD. Ostronoff *et al*. compared the outcomes of 38 patients who received GBM (5 g/kg per day G-CSF for five days) and BM with historical patient data, showing that the GBM grafts had quicker neutrophil engraftment and equivalent platelet engraftment,[22] while in the current study faster neutrophil engraftment in GBM, while similar platelet engraftment in both GBM and SBM groups were observed. Similar to this study's RESULTS, aGvHD and cGvHD patterns in both GBM and SBM groups were observed by Ostronoff *et al*.

CONCLUSION

GCSF-primed bone marrow offers advantages in terms of CD34+ stem cell yield and faster neutrophil engraftment in allogeneic HSCT. However, it does not significantly impact total nucleated cell yield, platelet engraftment, or the incidence of GvHD or graft failure. Further research is needed for definitive conclusion.

ETHICAL APPROVAL:

Ethical approval for this research was obtained from the Armed Forces Bone Marrow Transplant Centre in Rawalpindi. The approval reference number is IRB-011/AFBMTC/Approval/2020.

PATIENTS' CONSENT:

Informed consent have been obtained from the patients whose data are included in the study.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

HMN: The acquisition, drafting the work, and accountable for

all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
NS: Conception and Design of the work.
AA: Revising it critically for important intellectual content, analysis, and interpretation of data for the work.
TG: Revising it critically for important intellectual content.
TAK: Data collection.
QNC: Final approval of the version to be published.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Howard CA, Fernandez-Vina MA, Appelbaum FR, Confer DL, Devine SM, Horowitz MM, *et al*. Recommendations for donor human leukocyte antigen assessment and matching for allogeneic stem cell transplantation: Consensus opinion of the blood and marrow transplant clinical trials network (BMT CTN). *Biol Blood Marrow Transplant* 2015; **21(1)**:4-7. doi: 10.1016/j.bbmt.2014.09.017.
2. Muffly L, Pasquini MC, Martens M, Brazauskas R, Zhu X, Adekola K, *et al*. Increasing use of allogeneic hematopoietic cell transplantation in patients aged 70 years and older in the United States. *Blood* 2017 31; **130(9)**:1156-64. doi: 10.1182/blood-2017-03-772368.
3. Holtick U, Albrecht M, Chemnitz JM, Theurich S, Skoetz N, Scheid C, *et al*. Bone marrow versus peripheral blood allogeneic haematopoietic stem cell transplantation for haematological malignancies in adults. *Cochrane Database Syst Rev* 2014; **2014(4)**:CD010189. doi: 10.1002/14651858.CD010189.pub2.
4. Kessinger A, Armitage JO, Landmark JD, Smith DM, Weisenburger DD. Autologous peripheral hematopoietic stem cell transplantation restores hematopoietic function following marrow ablative therapy. *Blood* 1988; **71(3)**:723-7. doi: 10.1182/blood.V71.3.723.723.
5. Lobo F, Kessinger A, Landmark JD, Smith DM, Weisenburger DD, Wigton RS, *et al.* Addition of peripheral blood stem cells collected without mobilization techniques to transplanted autologous bone marrow did not hasten marrow recovery following myeloablative therapy. *Bone Marrow Transplant* 1991; **8(5)**:389-92.
6. Damiani D, Fanin R, Silvestri F, Grimaz S, Infanti L, Geromin A, *et al*. Randomized trial of autologous filgrastim-primed bone marrow transplantation versus filgrastim-mobilized peripheral blood stem cell transplantation in lymphoma patients. *Blood* 1997; **90(1)**:36-42. doi:/10.1182/blood.V90.1.36.
7. Lowenthal RM, Sullivan SA, Parker N, Marsden KA. G-CSF-primed bone marrow cells for autologous transplantation. *Lancet* 1996; **347(9008)**:1125. doi: 10.1016/s0140-6736(96)90327-x.
8. Sheridan WP, Fox RM, Begley CG, Maher D, McGrath KM, Juttner CA, *et al*. Effect of peripheral-blood progenitor cells mobilised by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy. *Lancet* 1992; **339(8794)**:640-4. doi: 10.1016/0140-6736(92)90795-5.
9. Bensinger WI, Weaver CH, Appelbaum FR, Rowley S, Demirer T, Sanders J, *et al*. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. *Stem Cells* 1996; **14(1)**:90-105. doi: 10.1002/stem.140090.
10. Nagler A, Labopin M, Shimoni A, Niederwieser D, Mufti GJ, Zander AR, *et al*. Mobilized peripheral blood stem cells compared with bone marrow as the stem cell source for unrelated donor allogeneic transplantation with reduced-intensity conditioning in patients with acute myeloid leukemia in complete remission: An analysis from the acute leukemia working party of the european group for blood and marrow transplantation. *Biol Blood Marrow Transplant* 2012; **18(9)**:1422-9. doi: 10.1016/j.bbmt.2012.02.013.
11. Eapen M, Logan BR, Confer DL, Haagenson M, Wagner JE, Weisdorf DJ, *et al*. Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. *Biol Blood Marrow Transplant* 2007; **13(12)**:1461-8. doi: 10.1016/j.bbmt.2007.08.006.
12. Pasquini MC. Impact of graft-versus-host disease on survival. *Best Pract Res Clin Haematol* 2008; **21(2)**: 193-204. doi: 10.1016/j.beha.2008.02.011.
13. Ringden O, Labopin M, Beelen DW, Volin L, Ehninger G, Finke J, *et al*. Bone marrow or peripheral blood stem cell transplantation from unrelated donors in adult patients with acute myeloid leukaemia, an acute leukaemia working party analysis in 2262 patients. *J Intern Med* 2012; **272(5)**:472-83. doi: 10.1111/j.1365-2796.2012.02547.x.
14. Niederwieser D, Lange T, Cross M, Basara N, Al-Ali H. Reduced intensity conditioning (RIC) haematopoietic cell transplants in elderly patients with AML. *Best Pract Res Clin Haematol* 2006; **19(4)**:825-38. doi: 10.1016/j.beha.2006.06.007.
15. Kröger N, Bacher U, Bader P, Böttcher S, Borowitz MJ, Dreger P, *et al*. NCI first international workshop on the biology, prevention, and treatment of relapse after allogeneic hematopoietic stem cell transplantation: Report from the committee on disease-specific methods and strategies for monitoring relapse following allogeneic stem cell transplantation. Part I: Methods, acute leukemias, and myelodysplastic syndromes. *Biol Blood Marrow Transplant* 2010; **16(9)**:1187-211. doi: 10.1016/j.bbmt.2010.06.008.
16. Baron F, Little MT, Storb R. Kinetics of engraftment following allogeneic hematopoietic cell transplantation with reduced-intensity or nonmyeloablative conditioning. *Blood Rev* 2005; **19(3)**:153-64. doi: 10.1016/j.blre.2004.06.003.
17. Schmitz N, Eapen M, Horowitz MM, Zhang MJ, Klein JP, Rizzo JD, *et al*. Long-term outcome of patients given transplants of mobilized blood or bone marrow: A report from the international bone marrow transplant registry and the European group for blood and marrow transplantation. *Blood* 2006; **108(13)**:4288-90. doi: 10.1182/blood-2006-05-024042.

18. Isola L, Scigliano E, Skerrett D, Fruchtman S. Long term follow-up after allogeneic G-CSF primed BMT. *Biol Blood Marrow Transplant* 2000; **6(4A)**:428-33. doi: 10.1016/s1083-8791(00)70034-6.

19. Couban S, Messner HA, Andreou P, Egan B, Price S, Tinker L, *et al*. Bone marrow mobilized with granulocyte colony-stimulating factor in related allogeneic transplant recipients: A study of 29 patients. *Biol Blood Marrow Transplant* 2000; **6(4A)**:422-7. doi: 10.1016/s1083-8791(00)70033-4.

20. Ji S, Chen H, Wang H, Ma J, Pan S, Xue M, *et al*. Low incidence of severe aGVHD and accelerating hemopoietic reconstitution in allo-BMT using lenograstim stimulated BM cells. *Chin Med J (Engl)* 2001; **114(2)**:191-5.

21. Ji SQ, Chen HR, Wang HX, Yan HM, Pan SP, Xun CQ. Comparison of outcome of allogeneic bone marrow transplantation with and without granulocyte colony-stimulating factor (lenograstim) donor-marrow priming in patients with chronic myelogenous leukemia. *Biol Blood Marrow Transplant* 2002; **8(5)**:261-7. doi: 10.1053/bbmt.2002.v8.pm12064363.

22. Ostronoff M, Ostronoff F, Maior PS, Matias C, Calixto R, Sucupira A, *et al*. Pilot study of allogeneic G-CSF–Stimulated bone marrow transplantation: Harvest, engraftment, and graft-versus-host disease. *Biol Blood Marrow Transplant* 2006; **12(7)**:729-33. doi: 10.1016/j.bbmt.2006.03.006.