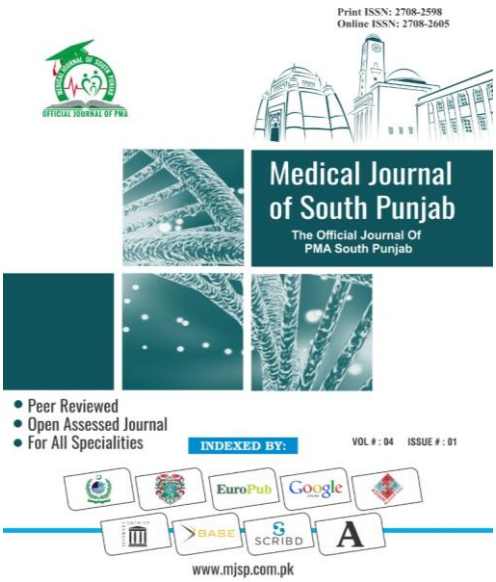
ISSN (E): 2708-2601
ISSN (P): 2708-2598

Medical Journal of South Punjab
Article DOI:10.61581/MJSP.VOL05/01/13
Volume 5, Issue 1, 2024



Correlation of CD3+ T cells infusion dose with Acute Graft Versus Host Disease (GVHD) in patients undergoing Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

Publication History

Received: Nov, 20 2023 Revised: Nov 11, 2023
Accepted: Dec 20, 2024 Published: Mar 30, 2024

Authors and Affiliation:

Muhammad Afzal[1*], Jahanzeb ur Rehman[2], Asghar Ali Kerio[3], Raheel Iftikhar[4], Uzma Rahim[5], Sahla Riaz[6]

[1-6]Armed Forces Bone Marrow Transplant Center, Rawalpindi, Pakistan

[*]Corresponding Author Email: Afzalkhan16@gmail.com

Conflict of Interest:

Author(s) declared no conflict of interest.

Acknowledgment:

No Funding received.

Citation: Afzal M, Rehman UJ, Kerio AA, Iftikhar R, Rahim U, Riaz S. Correlation of CD3+ T cells infusion dose with Acute Graft Versus Host Disease (GVHD) in patients undergoing Allogeneic Hematopoietic Stem Cell Transplantation (HSCT). Medical Journal of South Puniab. 2024 March 30: 5(1):66-72.

Please scan me to access online.

An official publication of
Medteach Private Limited, Multan, Pakistan.
Email: farman@mjsp.com.pk, Website: https://mjsp.com.pk/index.php/mjsp

Medical Journal of South Punjab
Volume 5, Issue 1, 2024; pp: 66-72
Original Article

Correlation of CD3+ T cells infusion dose with Acute Graft Versus Host Disease (GVHD) in patients undergoing Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

Muhammad Afzal[1*], Jahanzeb ur Rehman[2], Asghar Ali Kerio[3], Raheel Iftikhar[4], Uzma Rahim[5], Sahla Riaz[6]
[1-6]Armed Forces Bone Marrow Transplant Center, Rawalpindi, Pakistan
[*]Corresponding Author Email: Afzalkhan16@gmail.com

ABSTRACT

Objective: *To determine correlation of CD3dose with Acute Graft Versus Host Disease (GVHD) in patients undergoing HSCT.*

Methods: *This prospective study enrolled a total of n=124HSCT patients at the Armed Forces Bone Marrow Transplant Center in Rawalpindi between February 2021 and October 2022. Total n=124 patients were enrolled. All patients were followed for 100 days post allogenic stem cell transplant. Quantitative variables, such as recipient's and donor's age, Total nucleated cells, CD3+ T cells and CD34 dose. Qualitative variables, including the recipient's and donor's age, gender, gender mismatches, underlying disease, type of transplant, source of graft, CMV infection, type of GVHD prophylaxis, incidence of stage and grade of GVHD.*

Results: *Median age was 10years. Eighty patients were male and n=40were females. The primary source of graft was bone marrow harvest (81.3%) followed by a combination of bone BMH and peripheral blood stem cells (16.9%), and only (PBSC) (1.6%). Median dose of TNC was 5.10, CD34 dose 4.07and CD3+ T cells dose was 3.7. Twenty six (21%).patients developed acute graft versus host disease. Grade II in 61.8 %, grade III in 30.8 %while grade IV in 7.7% patients. Those patients with TNC dose ≥8x10^8/kg hadfrequent aGVHD which showed a statistical significance. CD34 and CD3 cells dose didn't show any statistically significant correlation with development of aGVHD .Eight (6.4%) patients died in our study. OS was 93.5% and DFS was 91.6% with GRFS of 78.7%.*

Conclusion: *Our findings suggest that CD3+ T cell dose may not be an independent predictor of aGVHD in our patient population where donors are sibling donors in majority of cases.Further larger-scale studies are required to provide a more comprehensive understanding of the role of CD3+ T cells doseand other intricate factors influencing development of aGVHD in allogeneic HSCT in our population.*

Keywords: *Hematopoietic stem cell transplant, Acutegraft versus host disease, CD3+ T cells dose, Total Nucleated Cells*

1. INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for a malignant and non-malignant hematologic illnesses.[1]aGVHD is a leading cause of non-relapse death, and it can cause long-term morbidity.[2] HLA mismatching is regarded as the major risk factor of aGVHD.Other risk includes recipient-donor gender mismatch, female to male donor, donor age, type of GVHD prophylaxis, graft source, CD34/TNC dose.[3] Data on CD3+T cells dose in graft and its association with the occurrence of aGVHD is still evolving. [4]

CD3+T cells surface marker plays role in the immune system, in the recognition and targeting of foreign cells or tissues.[5] Hypothetically in context of allogenic HSCT, the dose of CD3+ T-cells infused to the recipient can have a profound influence in the incidence and severity of aGVHD.[6]

Studies have shown that patients who receive higher CD3+ T cells dose during transplantation are more likely to develop aGVHD.[7] Saad et al reported CD3+T cells dose of $<30 \times 10^7$/kg was associated with reduced risk of acute GVHD.[8] However, finding the optimal CD3 dose that balances the risk of aGVHD with the desired graft-versus-leukemia (GVL) effect, remains a challenge .[9]

In this article, we present the findings of single- transplant center study, with an aim to evaluate the correlation between CD3+T cellsdose and aGVHD.

2. METHODOLOGY

This prospective study enrolled a total of n=124HSCT patients at the Armed Forces Bone Marrow Transplant Center in Rawalpindi between February 2021 and October 2022. All patients were followed for 100 days post HSCT. Patients who had a primary graft failure or underwent a second HSCT were not eligible.The institutional review board formallyapproved the study. Informed consent was obtained from the legal guardians of all participants.

Data collection included variables i.e age, gender of the patients and donors, gender mismatch, the use of Thymoglobulin (ATG), the source and dose of infused stem cells (including CD3+T cells, CD34+, and TNC), aGVHD stage and grade,aGVHDprophylaxis, mucositis, and CMV reactivation.

Conditioning regimens employed in the study included myeloablative (MAC), reduced intensity (RIC), and non-myeloablative (NMA) regimens.Gluck berg-Seattle criteria was used to grade skin, gut and liver GVHD.Treatment of aGVHD was used as perseverity criteria. Spanning from topical steroids to weight based systemic immune suppression treatments in severe cases.

The collected data was analyzed using (SPSS) version 26. Quantitative variables, such as recipient's and donor's age, TNC dose, CD3+ T cells dose, CD34 dose, were assessed for their mean and standard deviation. Qualitative variables, including the recipient's and donor's gender, gender mismatch, underlying disease, type of transplant, source of graft, CMV infection, GVHD prophylaxis, and stages and grades of aGVHD, were analyzed for frequency and percentages. The chi-square and t-tests were used to determine the significance of various variables. Variables with p-values less than 0.05 were deemed statistically significant.

The Kaplan-Meier plot was used to calculate overall survival (OS), disease-free survival (DFS), and graft-versus-host disease-free and relapse-free survival (GRFS).

3. RESULTS

Theoverall number of participants was 124. Median age were 10. The main source of graft was BMH (81.3%) followed by BMH+PBSC (16.9%) and then PBSC only (1.6%). Median TNC dose was

5.10x10^8/kg, CD34 count was 4.07x10^6/kg and CD3+T cells dose was 3.74x 10^7/kg. Patient and transplant characteristics are given under

Table 01

Table 1: Patients and donor demography			
		Frequency	**Percent**
Recipient Gender	Male	84	67.7
	Female	40	32.3
Donor Gender	Male	69	55.6
	Female	55	44.4
Disease	AA	17	13.7
	AML	11	8.9
	B-ALL	10	8.1
	BSS	01	0.8
	BTM	48	38.7
	CML	02	1.6
	CML-ALL	02	1.6
	FA	04	3.2
	GT	01	0.8
	MDS	09	7.3
	Osteopetrosis	01	0.8
	PID(CGD=1, HLH=3, SCID=1 and Jobs=1)	06	4.8
	PNH	05	4.0
	Sideroblastic Anemia	01	0.7
	T-ALL	06	4.8
Types of Transplant	Fully matched	118	95.2
	Haploidentical match	6	4.8
Type of Graft	BMH	101	81.3
	BMH+PBSC	21	17.2
	PBSC	02	01.5
GVHD Prophylaxis CSA CSA+MMF CSA+MTX CSA+MTX+MMF CSA+MMF+PTCy		 15 14 82 08 05	 12.1 11.3 66.1 6.4 04
TG Yes No		 108 16	 87.1 12.9

Ninety patients developed mucositis. Grade(1) in(n=23,18.5%),Grade(2) in (n=441,33.1%),Grade(3) in(n=21,16.9%) and Grade(4)in (n=5,4%). CMV reactivation was observed in 53.2%.

Twenty six (21%) patients developed aGVHD. Mean day for onset of acute GVHD was Day +34. Frequency of organs involvement was in order of skin, gut followed by liver.

Details of aGHVD with stages and grades are given in

Table:2.

Table:2 aGVHD , its grades and different stages			
		Frequency	Percent
aGVHD	Yes	26	21
	No	98	79
Acute skin GVHD	Stage I	5	35.7
	Stage II	4	28.6
	Stage III	3	21.4
	Stage IV	2	14.3
Acute Gut GVHD	Stage I	5	35.7
	Stage II	4	28.6
	Stage III	3	21.4
	Stage IV	2	14.3
Acute Hepatic GVHD	Stage I	4	36.4
	Stage II	3	27.3
	Stage III	3	27.3
	Stage IV	1	9
Grades of acute GVHD	Grade II	16	61.5
	GradeIII	8	30.8
	Grade IV	2	7.7

aGVHDcompletely resolved in 21 (80.8%)whereas in 5 (19.2%) patientsGVHD persisted beyond +100 days of observation. Complete resolution of aGVHD occurred by mean day+73. Patients who received TNC dose ≥8x10^8/kg experienced more frequent aGVHDas compared to patients receiving TNC dose <8x10^8/kg (p-value=0.002). CD34 and CD3+T cells dose didn't show any statistically significant correlation with aGVHD(p-value=0.06).

Correlations of aGVHD with other variables are given in **Table 3**.

Table 3: Correlations of aGVHD with different variables

	aGVHD		
	Yes	No	P-Value
	n (%)	n (%)	
Donor Gender			
Male	10 (14.5)	59 (85.5)	
Female	16 (29.1)	39 (70.9)	
			0.047
Female to Male Donor	11 (8.9)	27 (21.8)	**0.147**
Relation with Recipient			
Sister	14 (23.7)	35 (59.3)	
Brother	10 (15.2)	56 (84.8)	
Mother	2 (40)	3 (60)	
Father	0(0)	4(100)	
			0.157
Conditioning			
MAC	23 (23.7)	74 (76.3)	
NMA	3 (14.2)	18 (85.8)	
RIC	0 (0)	6 (100)	
			0.273
Type of Transplant			
Allo	24 (20.3)	94 (79.7)	
Haplo	2 (33.3)	4 (66.7)	
			0.446
GVHD Prophylaxis			
CSA	2 (13.3)	13 (86.7)	
CSA+MTX	15(18.2)	67(81.7)	
CSA+MMF	5(35.7)	9(64.3)	
CSA+MTX+MMF	4 (50)	4 (50)	
CSA+MMF+PTCy	0	5(100)	
			0.082
TG administration	23 (88.5)	3 (11.5)	
			0.815

Total 8(6.4%) patients died in our observation period. Most common cause of death was secondary graft failurefollowed by disease relapse, veno-occlusive disease and transplant associated thrombotic microangiopathy. OS was 93.5% with DFS of91.6% while GRFS was 78.7%.

Figure 1: Overall Survival

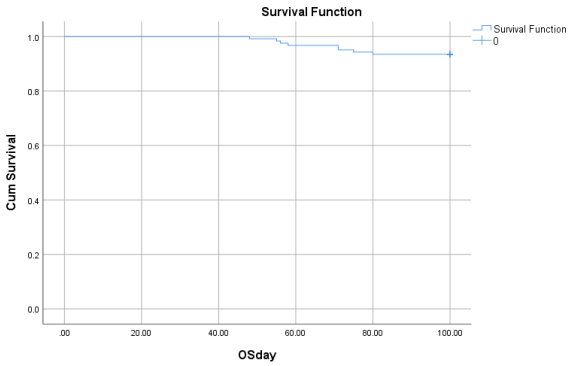


Figure 2: Disease Free Survival

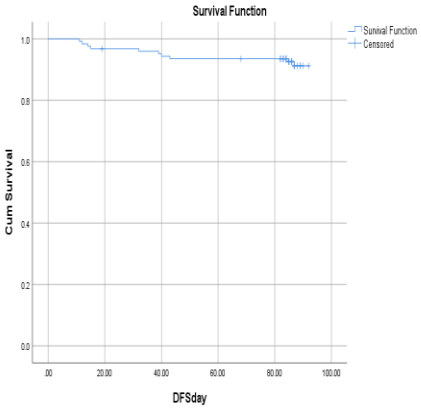


Figure 3: Graft versus Host Disease Relapse Free Survival

4. DISCUSSION

HSCTis therapeutic approach for a wide range ofbenign and malignant hematologic disorders. The decision to pursue HSCT is influenced by several factors, including diagnosis, disease stage, patient's performance status, and the availability of suitable donor.However,

HSCT is associated with inherent risks of morbidity and mortality. Major contributors of morbidity and mortality in post HSCT patients are opportunistic infections, organ toxicity and Graft vs Host disease.[10]

A subset of Tcells plays a significant role in mediating aGvHD. CD3+ T cells is responsible for adaptive immune responses however when donor T cells identify host tissues as foreign antigens this can lead to tissue injury by mounting an immune response against them manifest asaGVHD.[11] Within the CD3 subset, there are two major categories: alpha/beta (αβ) T cells and gamma/delta (γδ) T cells. α/β T cells are implicated in adaptive immune responses and are often responsible for GVHD whereas γ/δ T cells are part of the innate immune system and do not contribute to aGvHD. Strategies that selectively deplete α/β T cells while sparing γ/δ T cells have shown promise in reducing GVHD risk, improving immune reconstitution and potentially enhancing the graft-versus-tumor (GVT) effect.[12]

Research in the role of specific CD3 subsets like α/β**,** γ/δ**,** CD4+ and CD8+ T-cells in the incidence of acute GVHD is ongoing process.In Pakistan we do not havethe technological sophistication to carry out the same. However, we want to see the impact of CD3+ T cells innocuous relation to aGVHD.[13]Achieving the delicate balance between graft-versus-leukemia effects and GVHD mitigation remains a central challenge in HSCT.[14]

Our study included a total of n=124 patients. The primary source of graft was bone marrow harvest (81.3%), followed by a combination of bone marrow harvest and peripheral blood stem cells (BMH+PBSC) (16.9%), and only (PBSC) (1.6%). Similar to our results Furey et al from Colambia reported BMH as the primary source of graft, followed by PBSC.[15]In our study median dose of TNC was $5.10x10^8$/kg, CD34 $4.07x10^6$/kg and CD3+ $3.74x\ 10^7$/kg. Halahleh et al from Jordan reported a median dose of TNC, CD34+ and CD3+ T cells as 7×10^8/kg , 7.2×10^6/kg, 19.5×10^7/kg respectively.[16] Higher CD3 +T cells dose in this cohort is likely due to stem cells collection from peripheral blood while in our study main source of stem cells was bone marrow.

Thirty-seven (29.8%) patients developed aGVHD(grade II-IV). When stratifying aGVHD with various risk factors, donor gender, donor relation, GVHD prophylaxis, conditioning and transplant type didn't show statistically significant correlation with incidence of aGVHD. In contrast to our study Zaidman et al, reported an incidence of aGVHD of (29.1%)with statistically significant incidence of aGVHD related to gender mismatch, donor type, stem cells source, however unlike our study Zaidman et aldid not show any statistical significance of GVHD prophylaxis (CSA+MMF).[17] In contrast to Rombergers and colleague[18] study that showed no substantial correlation between TNC dosage and aGVHD , our study showed that patients who received TNC dose $\geq 8\ x\ 10^8$/kg experienced a higher incidence of aGVHD and this difference was statistically significant (p-value=0.000).This discrepancy might be attributed to the fact that Remberger's study utilized a highest TNC dose of 3.2x10^8/kg.CD3+ T cells dose in our study didn't show any statistically significant correlation with aGVHD. This could be attributed to use of sibling donors in our study besides use of ATG/TG being employed as prophylaxis. In parallel to our observation, Saad et al use a cutoff value of CD3+ T-cell doses of (14×10^7/kg in MSD; 15×10^7/kg in MUD) for PBSC, and they did not find a significant influence on incidence of aGVHD.[19]Mussetti et al reported 234 subjects haplo HSCT showed that graft CD3+ T-cell dose was directly related to incidence of all-grade aGVHD and cGVHD.[20]This effects is explained by the

greater degree of HLA disparity between Haploidentical donors.

5. CONCLUSION

Our findings suggest that CD3+ T cell dose may not be an independent predictor of aGVHD in our patient population where donors are sibling donors in majority of cases. Further larger-scale studies are required to provide a more comprehensive understanding of the role of CD3+ T cells dose and other intricate factors influencing development of aGVHD in allogeneic HSCT in our population.

REFERENCES

1. de Dreuzy E, Bhukhai K, Leboulch P, Payen E. Current and future alternative therapies for beta-thalassemia major. Biomed J. 2016 Feb 1;39(1):24-38.
2. Bohannon L, Tang H, Page K, Ren Y, Decreased Mortality in 1-Year Survivors of Umbilical Cord Blood Transplant vs. Matched Related or Matched Unrelated Donor Transplant in Patients with Hematologic Malignancies. Transplant Cellular Therapy. 2021 Aug 1;27(8):669-e1
3. Ghobadi A, Milton DR, Gowda L, Rondon. HLA-DP mismatch and CMV reactivation increase risk of aGVHD independently in recipients of allogeneic stem cell transplant. Current Res Translational Med. 2019 May 1;67(2):51-5
4. Przepiorka D, Smith TL, Folloder J, Khouri I, Ueno NT, Mehra R et al. Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. Blood ASH, 1999 Aug 15;94(4):1465-70.
5. Adusei KM, Ngo TB, Sadtler K. T lymphocytes as critical mediators in tissue regeneration, fibrosis, and the foreign body response. Acta Biomaterialia. 2021 Oct 1;133:17-33
6. Zhang Y, Guo C, Sun C, Chen Y, Zhu H, Xi J et al. High proportions of CD3+ T cells in grafts delayed lymphocyte recovery and reduced overall survival in haploidentical peripheral blood stem cell transplantation. Molecular Clin Oncol. 2020 Jun 1;12(6):574-80.
7. Yao D, Li B, Chu X. Association between CD34+ and CD3+ T-cells in allogeneic grafts and acute graft-versus-host disease in children undergoing allogeneic hematopoietic stem cell transplantation: A single-center study. Transplant Immunol. 2023 Apr 1;77:101779
8. Saad A, Visweshwar N, Sehbai A, Cumpston A, Watkins K, Buckhalter R et al. Correlation of CD3 and CD34 cell dose with incidence of acute GVHD in myeloablative stem cell transplantation. JCO. 2006 Jun 20;24(18_suppl):6553-.
9. Hsiao M, Martynova A, Yaghmour G, Foss C. Investigating the Relationship between CD34+ and CD3+ Cell Doses, One-Year Graft-Versus-Relapse-Free-Survival, Graft-Versus-Host Disease, and Overall Survival in Haploidentical Hematopoietic Stem Cell Transplantation: A Single Center Experience. Blood. 2019 Nov 13;134:2051.
10. Bertaina A, Pitisci A, Sinibaldi M, Algeri M. T cell-depleted and T cell-replete HLA-haploidentical stem cell transplantation for non-malignant disorders. Cur Hematol Malign Reports. 2017 Feb;12:68-78.
11. Nagasawa M, Mitsuiki N, Yanagimachi M, Yamamoto M, Fukuda T, Miura O et al. Utility of novel T-cell-specific extracellular

vesicles in monitoring and evaluation of acute GVHD. Intern J Hematol. 2021 Jun;113:910-20.
12. Saad A, Lamb LS. Ex vivo T-cell depletion in allogeneic hematopoietic stem cell transplant: past, present and future. Bone Marrow Transplant. 2017 Sep;52(9):1241-8.
13. Shah RM, Elfeky R, Nademi Z, Qasim W, Amrolia P, Chiesa R et al. T-cell receptor αβ+ and CD19+ cell–depleted haploidentical and mismatched hematopoietic stem cell transplantation in primary immune deficiency. J Allergy Clin Immunoly. 2018 Apr 1;141(4):1417-26.
14. Perko R, Kang G, Sunkara A, Leung W, Thomas PG, Dallas MH. Gamma delta T cell reconstitution is associated with fewer infections and improved event-free survival after hematopoietic stem cell transplantation for pediatric leukemia. Biol Blood Marrow Transplant. 2015 Jan 1;21(1):130-6.
15. Furey A, Rastogi S, Prince R, Jin Z, Smilow E, Briamonte C et al. Bone marrow harvest in pediatric sibling donors: role of granulocyte colony-stimulating factor priming and CD34+ cell dose. Biol Blood Marrow Transplant. 2018 Feb 1;24(2):324-9
16. Halahleh K, Mustafa R, Sarhan D, Al Rimawi D, Abdelkhaleq H, Muradi I et al. The Impact of Graft CD3+ T-Cell Dose on the Outcome of T-Cell Replete Human Leukocyte Antigen-Mismatched Allogeneic Hematopoietic Peripheral Blood Stem Cells Transplantation. J Hematol. 2023 Feb 25;12(1):27-36.
17. Zaidman I, Even-Or E, Aharoni E, Averbuch D, Dinur-Schejter Y, NaserEddin A et al. Risk and promise: an 11-year, single-center retrospective study of severe acute GVHD in pediatric patients undergoing allogeneic HSCT for nonmalignant diseases. Frontiers Pediatr. 2023 May 26;11:1194891.
18. Remberger M, Törlén J, Ringdén O, Engström M, Watz E, Uhlin M et al. Effect of total nucleated and CD34+ cell dose on outcome after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2015 May 1;21(5):889-93
19. Saad A, Lamb L, Wang T, Hemmer MT, Spellman S, Couriel D, Alousi A, et al. Impact of T cell dose on outcome of T cell-replete HLA-matched allogeneic peripheral blood stem cell transplantation. Biol Blood Marrow Transplant. 2019;25(9):1875-1883.
20. Mussetti A, De Philippis C, Carniti C, Bastos-Oreiro M, Gayoso J, Cieri N et al. CD3+ graft cell count influence on chronic GVHD in haploidentical allogeneic transplantation using post-transplant cyclophosphamide. Bone Marrow Transplant. 2018;53(12):1522-1531.