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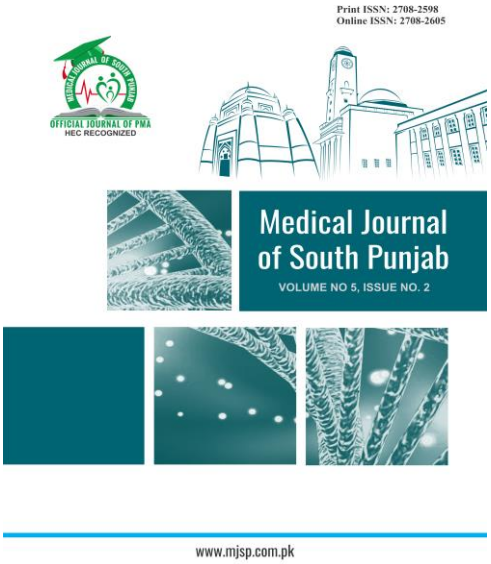

Cytomegalovirus Reactivation and its Effect on Graft Function in Post Hematopoietic Stem Cell Transplant (HSCT) Patients

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Original Article

Cytomegalovirus reactivation and its effect on graft function in post hematopoietic stem cell transplant (HSCT) patients

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ABSTRACT

Objective: *To evaluate the effect of Cytomegalovirus reactivation on graft function and to study the factors contributing to primary graft failure in patients underwent hematopoietic stem cell transplant.*

Methods: *We conducted a prospective study at Armed Forces Bone Marrow Transplant centre between Jan 2022 to July 2023.This study included 80 patients of all age groups and both genders, suffering from various hematological diseases. Patients with primary graft failure were excluded. CMV reactivation was monitored during two distinct time frames post-transplantation (initial 100 days and day +100 to day +180).*

Results: *Among the study group, 60% of patients experienced CMV reactivation, and 22.5% who received antiviral treatment developed myelosuppression. CMV reactivation at day +90 had statistically significant correlation(p=0.031) with graft function.18(22.5%) had myelosuppression after CMV treatment with (p value= 0.010). The CD34 dose had a statistically significant correlation with graft function on Day 30 and Day 60. Chimerism analysis at day +60 had significant correlations with CMV reactivation(p=0.042).*

Conclusion: *In conclusion, our study underscores the significance of CMV reactivation as a common complication post-HSCT, with implications for graft function. We observed a substantial myelosuppressive effect associated with antiviral treatment. The study highlights the importance of CD34 dose in graft function assessment. While further research is needed, our findings emphasize the need for vigilant monitoring and tailored interventions to improve outcomes in post-HSCT patients.*

Keywords: *CMV Reactivation, Donor Chimerism, Graft Function, Hematopoietic stem cell transplant*

1. INTRODUCTION

Hematopoietic stem cell transplant (HSCT) is effective treatment for a number of benign and malignant hematological disorders[1].Human cytomegalovirus (HCMV) reactivation, is the most common viral complication after HSCT and is associated with an increased risk of non-relapse mortality (NRM) resulting from multisystem organ dysfunction to myelosuppression from poor graft function[2].

Poor graft function (PGF) is a life threateningcomplicationafterHSCT resulting in highmorbidity and mortality.[3] The incidence of secondary poor graft function (sPGF)ranges from 5%[4] to 27% [5]and is associated with a high mortality. The initial engraftment of neutrophils usually occurs in the first 2 weeks, followed by platelet engraftment that usuallyoccurs3–4 weeks after HSCT.[6] Various risk factors contributing to poor graft function have been mentioned in literature and includes low total CD34 dose,CMVreactivation[4] Ebstein bar virus(EBV) reactivation, conditioning regimen comprised of Fludarabine and Cyclophosphamide ,haplo HSCT and acute GVHD.[7]

CMV reactivation can directly or indirectly by the myelosuppressive effects of antiviral agents impact the function of the transplanted graft, potentially leading to concurrent bacterial and/or fungal infections[8].Poor graft function after HSCT contributes to high post-transplant morbidity, mortality and cost.

The purpose of this study is to evaluate the effect of CMV reactivation on graft function and to better understand the factors contributing to PGF in patients who underwent HSCT. To the best of our knowledge, no such study has been conducted in our country so far.

2. METHODOLOGY

Between Jan 2022 and July 2023, we conducted a prospective study involving a cohort of 80 consecutive patients who had undergone allogenic bone marrow transplant (BMT) at the Armed Forces Bone Marrow Transplant Center/National Institute of Blood and Marrow Transplant (AFBMTC/NIBMT). This cohort encompassed patients of all age groups and both genders, suffering from various benign and malignant hematological conditions. However, patients experiencing primary graft failure were excluded from this study.

Ethical approval for the study protocol was obtained from the Institute's Ethical Review Board and Research Department, and informed consent was acquired from either the patients or their guardians.

To evaluate the impact of CMV reactivation on graft function, we conducted CMV DNA polymerase chain reaction (PCR) testing during two distinct time frames post-transplantation. The first phase covered the initial 100 days following transplantation, and the second phase spanned from day +100 to day +180. Those displaying a CMV quantitative PCR viral load exceeding 2000 copies/mL underwent treatment with either Valganciclovir or Ganciclovir, following a pre-emptive approach. The administration of antiviral treatment was halted after two consecutive negative PCR analyses.

All data were recorded and analyzed using SPSS version 25. Categorical variables such as gender, CMV reactivation antiviral treatment, acute graft-versus-host disease (GVHD), steroid administration, conditioning regimen, use of antithymocyte globulin (ATG), GVHD prophylaxis, ABO mismatch, gender mismatch, and graft function were expressed as frequencies and percentages. Statistical significance was assessed using the chi-square test and Kruskal-Wallis test. Univariate analysis was conducted to evaluate the significance of different variables, with a p-value of less than 0.05 considered statistically significant. Overall survival (OS) was defined as the survival status at the time of the last follow-up, while disease-free survival (DFS) indicated survival without relapse or rejection. The probabilities of OS and DFS were determined using the Kaplan-Meier method.

3. RESULTS

The study group comprised 80 patients enrolled consecutively, including 55 males and 25 females (2.2:1). The median age was 13±11.4 years (range: 1 to 53 years).62(77.5%) patients underwent allo-HSCT and 18 (22.5%) had haplo identical HSCT. Among 80 patients 15(18.8%) had major ABO mismatch and 13(16.3%) had minor ABO mismatch. Among 22 malignant disorders, n=18(81.8%)were taken to transplant in CR1 and n=4(18.2%) were in CR2. In 50(62.5%) cases ATG and TG (n=22, 27.5%) were added in conditioning protocols respectively. The median dose of TG/ATG was 10mg/kg.Patient and donor characteristics and transplant details are summarized in Table 1.Median TNC dose was $5.02x10^8$/kg and CD34 count was $5.05x10^6$/kg. The median

time for neutrophil engraftment was 13.5 days and for platelets engraftment was 24 days.

Table 1: Patient, donor and transplant characteristics

Characteristics	N(%)
Gender	
Male	55 (68.8%)
Female	25 (31.8%)
Donor Relationship	
Brother	41(51.2%)
Sister	28 (35%)
Father	05(6.3%)
Mother	5(6.3%)
Cousin	1(1.3%)
Gender Mismatch	
Yes	40 (50%)
No	40(50%)
Underlying Diagnosis	
Aplastic Anemia	24(31.25%)
Beta-thalassemia Major	23(28.7%)
Acute leukemia (ALL+AML+CML-BP+MPAL)	18(21.4%)
PNH	5(6.3%)
Inherited Bone Marrow Failure(FA,CAMT)	4(5.0%)
MDS	4(5.0%)
HLH	2(2.5%)
Type conditioning	
MAC	42(52.5%)
NMA	20(25%)
RIC with PTCy	9(11.3%)
MAC with PTCy	9(11.3%)
GVHD Prophylaxis	
CSA	15 (18.8%)
CSA+MMF	20 (20.5%)
CSA+MTX	45 (56%)
Source of Stem Cells	
BMH	58(72.5%)
BMH+PBSC	20(25%)
PBSC	2(2.5%)

In three (3.75%) patients stem cells boost was given for secondary poor graft function and only one (1.25%) received DLI after early relapse of acute leukemia.25(31.3%) had developed acute GVHD. 48(60%) experienced CMV reactivation. The median time and copies of CMV reactivation was 34.50 days and 4396 copies/ml respectively. 33(68.75%) received antiviral treatment for CMV reactivation (valganciclovir in 32 and IV ganciclovir followed by oral valganciclovir in 1). The median duration of antiviral therapy was 21 days. 18(22.5%) had myelosuppression after CMV treatment. CMV treatment had statistically significant correlation with myelosuppression(p value= 0.010).Graft and Chimerism details are given below in table 2. Median of blood counts on day 30 ,60 ,90 ,120 and 180 respectively are given below in table 03.

The Kruskal-Wallis test revealed that the CD34 dose had a statistically significant correlation with Day 30 graft function ($p = 0.001$). In contrast, the TNC dose did not exhibit a statistically significant correlation with Day 30 graft function ($p = 0.546$).The Kruskal-Wallis test indicated that neither the neutrophil engraftment day

(p = 0.063) nor the platelet engraftment day (p = 0.299) exhibited a statistically significant correlation with Day 30 graft function.In the analysis of blood counts (WBC, ANC, Hb, and Platelets) on Day 30, day 60, day 90 and day 180 respectively in relation to graft function , it was observed that there was no statistically significant correlation between these blood counts and graft function except platelet counts exhibited a statistically significant correlation with day 60 graft (p value=0.004). The Kruskal-Wallis test indicated that the CD34 dose had a statistically significant correlation with Day 60 graft function (p = 0.036). However, the TNC dose did not exhibit a statistically significant correlation with Day 60 graft function (p = 0.437). However on the the analysis of blood counts (WBC, ANC, Hb, Platelets), CD34 dose and TNC dose in relation to Day 90 and Day 180 graft function, it was observed that none of these factors exhibited a strong correlation with Day 90 and Day 180 graft function. Comparison of CMV reactivation with graft function and Chimerism at different time points are given below in table.

Out of the total 80 patients, 7 patients died with a mortality rate of 8.75%. Most common cause of death was secondary poor graft function associated with infections leading to neutropenic sepsis. Only one patient died because of relapsed disease (14.2%).

The overall survival (OS) rate for the study group was determined to be 91.3%, indicating that the majority of patients survived. The disease-free survival (DFS) rate was calculated to be 91.3 %.

Table 2: Graft and chimerisim details

Day 30 Graft (n,%)	Day 30 Chimerism (n,%)	Day 60 Graft (n,%)	Day 60 Chimerism (n,%)	Day 90 Graft (n,%)	Day 90 Chimerism (n,%)	Day 180 Graft (n,%)	Day 180 Chimerism (n,%)
Adequate graft function (n= 36, 45%)	Complete (n=52, 65%)	Adequate graft function n=32, 40%)	Complete (n=42, 52.5%)	Adequate graft function (n=29, 36.25%)	Complete(n= 50, 62.5%	Adequate graft function (n=43, 53.75%)	Complete n=63, 78.75%)
BM Not done(adequate) (n=36, 45%)	Mixed in CD3 (n=7, 8.75%)	BM Not done (adequate) (n= 40, 50%)	Mixed(n=7, 8.75%)	BM Not done(adequate) (n=41, 51.25%)	Mixed (n=6, 7.5%)	BM Not done (adequate) (n=29,36 .25%)	Mixed (n= 4, 05%)
Marrow in relapse (n=1, 1.25%)	Mixed in WB n=4, 05%)	N.Ap(n=2, 2.5%)	N.Ap (n=2, 2.5%)	N.Ap(n = 4, 05%)	N.Ap n=3, 3.75%)	N.Ap (n=7, 8.75%)	N.Ap (n=7, 8.75%)
Poor graft function (n=4, 05%)	Mixed in WB, CD3 (n=9, 11.25%)	Poor graft function (n=5,6.25%)	Not done (n=29, 36.25%)	Not done (n=1, 1.25%)	Not done (n= 21,26.25%)	PRCA (n=1, 1.25%)	Not done (n=6, 7.5%)
PRCA (n=3, 3.75%)	NA (n=2, 2.5%)	PRCA (n=1, 1.25%)		Poor graft function (n=5, 6.25%)			

Table: 3 Details of blood counts

Blood Counts	Day 30	Day 60	Day 90	Day 120	Day 180
WBC	4.3	4.7	5.05	6.2	6.4

ANC	2.35	2.5	2.5	3.25	3.2
Hb	10.0	10.0	10.2	10.70	11.0
PLTS	88.50	149	155	185	177

TABLE 4: Comparison of cmv reactivation with graft function at different time points

Time Point	Graft	CMV Reactivation (Yes)	CMV Reactivation (No)	p-valu
Day 3(	Adequate graft function	25	11	**0.105**
	BM Not done(adequate)	17	19	
	Poor graft function	3	1	
	PRCA	3	0	
	Marrow in relapse	0	1	
Day 60	Adequate graft function	24	8	**0.077**
	BM Not done(adequate)	19	21	
	Poor graft function	3	2	
	PRCA	0	1	
	N.Ap	2	0	
Day 90	Adequate graft	21	8	**0.031**
	Function			
	BM Not done(adequate)	18	23	
	Poor graft function	5	0	
	N.Ap	3	1	
	Not done	1	0	
Day 18	Adequate graft function	29	14	**0.0**
	BM Not done(adequate)	13	16	
	PRCA	0	1	
	N.Ap	6	1	

Table 5: Comparison of cmv reactivation with chimerism at different time points

Time Point	Graft	CMV Reactivatio (Yes)	CMV Reactivatio (No)	p-val
Day 3	Adequate graft function	25	11	**0.105**
	BM Not done(adequate)	17	19	
	Poor graft function	3	1	
	PRCA	3	0	
	Marrow in relapse	0	1	
Day 6	Adequate graft function	24	8	**0.077**
	BM Not done(adequate)	19	21	
	Poor graft function	3	2	
	PRCA	0	1	
	N.Ap	2	0	
Day 9	Adequate graft	21	8	**0.031**
	Function			
	BM Not done(adequate)	18	23	
	Poor graft function	5	0	
	N.Ap	3	1	
	Not done	1	0	
Day 1	Adequate graft function	29	14	**0.066**
	BM Not done(adequate)	13	16	
	PRCA	0	1	
	N.Ap	6	1	

Figure 01: Overall survival

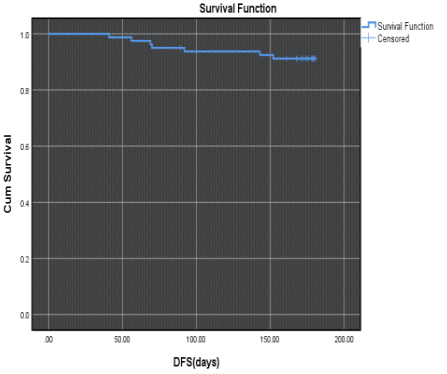
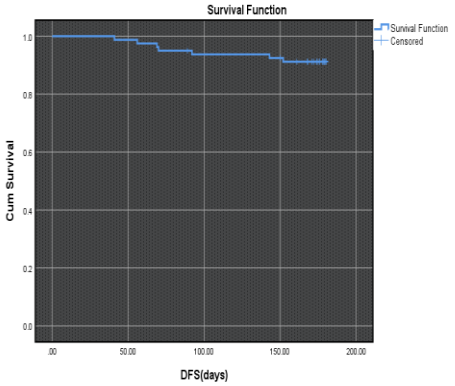


Figure 2: Disease free survival



4. DISCUSSION

Poor graft function is a serious complication resulting in high mortality rate post HSCT patients.CMVreactivation post HSCT has variable clinical manifestations ranging from mild CMV viremia to grave complications like poor graft function.Graft function post HSCT is assessed by various means like peripheral blood counts, BM cellularity and donor chimerisim. Multiple risk factors pre and post transplant contribute to graft function comprising of conditioning drugs, radiation, CMV serostatus,major blood group disparity CMV and EBV reactivation ,low total CD34 dose and acute GVHD.[9-10].

A recently published metaanalysis from 69 different studies has reported the median incidence of 7% for poor graft function.[11] Our study demonstrated a significant myelosuppressive effect(p value=0.010) caused by valganciclovir which was evident as most of these patients had counts supported by GCSF,RCC and erythropoietin stimulating agents or platelets. Only 33 out of 48 patients who had CMV reactivation received antiviral therapy and out of thsese,18(22.5%) had myelosuppression after treatment.The median time for CMV reactivation and treatment duration in our study population was 35 and 21 days respectively however we had not established the dosage effect on cytopenias.A study by M.Takahata et al has reported the incidence of neutropenia (15-33%,p=0.26) and thrombocytopenia(15-39%,p=0.14)[12].

Another important conclusion from similar study was the establishment of almost equal efficacy of low dose valganciclovir in clearance of CMV viremia as of standard dose. Out of 38 patients in this study population 18(47%) were given 900 mg twice a day valganciclovir and 20(53%) received <900mg dose. A study from China by (Yang Xiao et al) in post HSCT patients over a follow up period of 7 months has demonstrated the effect of increasing age (median age of study population: 28 years), ABO incompatabilityand early CMV reactivation in 30 days post HSCT as

significant risk factors for PGF.[13] The major transplant indications in this study was acute leukemias and small number of patients with aplastic anemia and thalassemia, which was however reversed in our study where majority of patients were of aplastic anemia(n=24,31.25%) followed by beta-thalassemia major(n=23,28.7%).

A study conducted by Yu-Qian Sun et al on 490 patients at Peking University People`s hospital in first 100 days post transplant period has established a significant correlation of low than median total CD34 dose(2.64x10^6/kg),(p=0.019)and CMV reactivation (p=0.003)as risk factor for sPGF[14].The median time to neutrophil and platelet engraftments were 13 and 14 days respectively which was similar for neutrophil engraftment in our patients, however they had a slight delayed engraftment of platelets(median=24 days) Similar finding was observed in our study showing significant correlation of CD34 dose on day 30(p=0.001) and day 60 graft function(p=0.036),however no significant correlation with TNC dose .

Majority of patients with poor graft function in our study responded to supportive measurements like GCSF and RCC support. Nevertheless 3 patients required stem cell boost without further conditioning which resulted in improvement of graft function on follow up. In a study by Evengy Klyuncnikov et al,32 patients with poor graft function were given CD34 selected stem cell boost without prior conditioning and hematological improvement was observed in 81% of patients.[15] Considering ABO incompatibility, recipients with major ABO mismatch has more episodes of immune induced hemolysis and delayed engraftment.[16-17-18] Previous studies showed that use of Fludarabine in had significant impact on incidence of secondary poor graft function and mixed donor chimerisim.[19-20] Contrary to previous studies we could not establish impact of age , HLA disparity , ABO mismatch and acute GVHD impact on graft function.

5. CONCLUSION

In conclusion, our study underscores the significance of CMV reactivation as a common complication post-HSCT, with implications for graft function. We observed a substantial myelosuppressive effect associated with antiviral treatment. The study highlights the importance of CD34 dose and platelet counts in graft function assessment. While further research is needed, our findings emphasize the need for vigilant monitoring and tailored interventions

to improve outcomes in post-HSCT patients.

One limitation this Limitation of the study: One limitation of this study is the relatively small sample size.
Additionally, this study was conducted at a single center, which may limit the generalizability of the findings.

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