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ORIGINAL ARTICLE

Cytomegalovirus Reactivation as a Risk Factor for All-Cause Mortality in Children Undergoing Hematopoietic Stem Cell Transplantation: Experience Over Two Decades from a Tertiary Referral Center in India

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Abstract The aim of the study was to analyse the burden of cytomegalovirus (CMV) disease in children undergoing hematopoietic stem cell transplantation (HSCT) and its correlation with all-cause mortality. We performed a retrospective study in children up to 18 years of age who underwent allogeneic HSCT between February 2002 to December 2021 in the pediatric blood and marrow transplantation unit. A total of 1035 patients were included where five hundred forty-three (52.4%) patients underwent matched family donor (MFD) HSCT, 213 (20.5%) underwent matched unrelated donor (MUD) HSCT; 279 (26.9%) underwent haploidentical HSCT (T cell replete in 213 and T cell depleted in 66 patients). CMV reactivation was documented in 258 (24.9% patients). CMV was seen in 39 (7.2%) MFD, 77 (36.1%) MUD, 106 T cell replete (49.7%) and 36 T cell depleted (54.5%) transplants. CMV reactivation was predominantly documented in those where donor and recipient were positive (D + /R +) for CMV serostatus (77%)) prior to HSCT. Overall mortality rate was significantly higher in the CMV positive group (103/258, 39.9%), as compared to the CMV negative group (152/777, 19.6%) (p value = 0.0001). CMV was the direct cause of death in 13/1035 children (1.2%). GvHD as a cause of death was found to be significantly higher among those with CMV (n = 32) as compared to those without CMV (n = 14) (35.6 versus 9%, p value = 0.0001). The incidence of CMV reactivation was noted in 25% of HSCT recipients, and predominantly in haploidentical HSCTs. CMV reactivation was shown to significantly impact all-cause mortality and there was a significantly increased risk of mortality due to GvHD among those with CMV reactivation.

Keywords CMV · HSCT · All-cause mortality · Haploidentical · GVHD

Abbreviations

HSCT	Hematopoietic stem cell transplantation
GVHD	Graft versus host disease
MUD	Matched unrelated donor
MFD	Matched family donor
MAC	Myeloablative conditioning
RIC	Reduced intensity conditioning
CMV	Cytomegalovirus
ATG	Anti-thymocyte globulin
OS	Overall survival
IEI	Inborn errors of immunity

Introduction

Cytomegalovirus (CMV) is a DNA virus which is a member of the Herpesviridae family. The prevalence of this infection is high worldwide, ranging between 60 and 100% [1]. The prevalence of CMV demonstrated by IgG positivity in our population is as high as 95–97% [2]. Primary CMV infection is mostly asymptomatic and self-limiting in nature in immunocompetent hosts and is characterized by persistence of

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the virus in a latent form in the body. However, CMV infection in the immunocompromised host, such as in patients undergoing allogeneic HSCT can be a cause of significant morbidity and mortality.

CMV has both direct and indirect effects on the body. Direct effects are caused by viral proliferation in various host tissues leading to manifestations such as gastroenteritis, pneumonitis, hepatitis, retinitis, encephalitis, etc. On the other hand, increased risk of graft rejection and dysfunction, GvHD, opportunistic infections and malignancies are a sequela of the indirect effects of the virus due to persistent low-level replication in the body [3]. Activation of the immune system by viral proteins predisposes to graft rejection and acute or chronic GvHD, whereas immunosuppressive effects of CMV in turn lead to an increased risk of other opportunistic fungal and bacterial infections. Boeckh and Nichols demonstrated a higher incidence of acute GvHD in CMV-seropositive recipients after allogeneic HSCT than seronegative ones [4]. CMV is associated with GvHD and rejection; on the other hand, both rejection and GvHD promote CMV replication.

Despite several advances in the field of HSCT, CMV reactivation post-transplant remains a significant factor influencing transplant outcomes, especially in a developing country like India, where the majority of patients and donors are CMV seropositive. The factors predisposing to CMV reactivation include pre-transplant donor and recipient serological status, the degree of human leucocyte antigen match, the presence of GvHD, the degree and duration of immunosuppression, the conditioning regimen, and the use of T cell-depleted grafts [5, 6]. With the progressive increase in the number of alternate donor transplants being performed, the burden of CMV infection post HSCT has increased manifold. Reported CMV reactivation rates after HSCT are variable, ranging between 30 and 70% and is associated with a higher non-relapse mortality rate (relative risk (RR), 1.61–1.95) [7].

This study aimed to analyze the burden of CMV reactivation in patients undergoing allogeneic HSCT at our center and to study its correlation with all-cause mortality. In addition, we analyzed the impact of CMV reactivation on all-cause mortality and overall survival (OS).

Patients and Methods

This study was a single-center retrospective study conducted at the Blood and Marrow Transplantation Unit at Apollo Specialty Cancer Hospital, Chennai. We included all children up to 18 years of age who underwent allogeneic HSCT between February 2002 to December 2021. We obtained institutional ethics committee approval before the study. We collected the data through a retrospective review of patient charts and medical records. The data analyzed included the incidence of CMV reactivation, its correlation with donor and graft type, source of stem cells, underlying diagnosis, and acute and chronic GvHD.

CMV Monitoring Protocol

CMV viral load was monitored in blood using real time quantitative DNA PCR analysis (Argene SA assay, Biomerieux) every week starting from the time of engraftment until day + 100 for all children undergoing MUD and haploidentical HSCT. Based on a previous study conducted at our center [8], patients undergoing MFD HSCTs were monitored for CMV only if they met any of the following criteria—underlying diagnosis of inborn errors of immunity, presence of GvHD, or when they received granulocyte transfusions. Beyond day + 100, CMV monitoring was individualized and was performed in those patients with GvHD requiring prolonged immunosuppression. CMV viremia greater than 1000 copies/ml was considered to be positive, and pre-emptive treatment was started with ganciclovir or valganciclovir.

Definitions

Acute GvHD was diagnosed and graded according to Glucksberg criteria [8] and chronic GvHD was diagnosed according to the National Institutes of Health consensus criteria [9]. All-cause mortality was defined as death due to any cause. Overall Survival (OS) was calculated as the time from transplantation to death due to any reason.

Statistical Analysis

The continuous variables were represented as means ± SDs and as medians if they were usually or nonnormally distributed, respectively. The comparison of typically, nonnormally, categorical variables was performed by independent sample *t*-test, Mann–Whitney test, chi-square test, or Fisher exact test, respectively. Kaplan–Meier curve was drawn to assess the survival pattern. Data validation and analysis were carried out by SPSS version 25.0 (SPSS, Inc., Chicago, IL). P values < 0.05 were considered statistically significant.

Results

We included 1035 patients in the study, with a median age of 6 years (0.1–18 years). The study group comprised 647 males and 388 females (M: F ratio 1.66:1). Table 1 highlights the details of the patient demographics, disease, and HSCT data.

Table 1 Demographic, disease, patient and HSCT data in the cohort $n = 1035$

Patient characteristics	Number
Total no. of patients	1035
Baseline diagnosis	
Benign	784 (75.7%)
Haemoglobinopathies	430
Inborn errors of immunity	179
Acquired aplastic anemia	42
Inherited bone marrow failure syndrome	93
Metabolic	31
Others	9
Malignant	251 (24.3%)
ALL	154
AML	57
ALCL	2
CML	5
MDS	12
Neuroblastoma	5
Non-Hodgkin's lymphoma	5
Hodgkin's lymphoma	6
JMML	5
Type of HSCT	
MFD	543 (52.4%)
MUD	213 (20.5%)
Haploidentical	279 (26.9%)
T-cell depleted	66
T-cell replete	213

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *ALCL* anaplastic large cell lymphoma, *CML* chronic myeloid leukemia, *MDS* myelodysplastic syndrome, *JMML* Juvenile myelomonocytic leukemia

CMV Reactivation

CMV reactivation was documented in 258/1035 (24.9%) patients. Among the 258 children with CMV reactivation, the incidence was highest among those with haploidentical donor – 142 (51%), followed by 77 (36.1%) patients in the MUD group and 39 (7.2%) in the MFD group (p-value < 0.0001). Incidence of CMV reactivation was 216/745 (28.9%) among those receiving peripheral blood, 18/201 (9%) in those receiving bone marrow, and 22/45 (48.8%) in those receiving cord as stem cell source (p-value < 0.0001). There was no significant difference between T replete –106 (49.7%), and T deplete—36 (54.5%) haploidentical HSCT (p-value 0.49). Table 2 highlights the association of CMV reactivation with demographic and HSCT parameters.

On subset analysis in 205 children who underwent alternate donor HSCT including MUD and haploidentical HSCT, CMV reactivation was documented in 84/205 (40%) children. Among the 84 children, donor recipient serostatus for CMV prior to HSCT was noted to be D + R + in 65/84 (77%) children followed by D + R− in 13/84 (15%).

Relation of CMV Reactivation with All-Cause Mortality

There was a total of 255 deaths (24.6%). The overall mortality rate was significantly higher in the CMV positive group compared to the CMV negative group (39.9 versus 19.6%, p-value = 0.0001). Among the 103 mortalities in the CMV positive group, only 13 (12.6%) deaths were attributed to CMV disease, of which 9 (69.2%) were in patients diagnosed with inborn errors of immunity. Figure 1 highlights our finding that GvHD as a cause of death was significantly higher among those with CMV infection (31 versus 10%, p-value = 0.0001). The mean survival time was 13.7 years (range 13.1–14.4) in the CMV negative group versus 9.2 years (range 7.5–11) in the CMV positive group (p-value = 0.0001) (Fig. 2).

Discussion

In our study, the overall CMV infection rate post HSCT in the entire cohort was 25%. The risk factors for CMV reactivation were the source of stem cells (peripheral blood and cord), the type of donor (MUD and haploidentical), and the presence of acute and chronic GvHD. In addition, CMV was associated with increased overall mortality but low attributable mortality.

In a previous study done at our center, we documented CMV reactivation in 3% of children undergoing MFD transplants, 33.3% undergoing MUD HSCT using peripheral blood as stem cell source, 17.4% undergoing unrelated donor cord blood HSCT and 36.5% undergoing mismatched or haploidentical grafts ($P = < 0.0001$) [10]. Other studies from India have reported variable CMV reactivation rates of 43.8% [11] and 9.56% [12] post HSCT. Hugo Sousa [13] observed CMV infection in 60.3% of their patients undergoing HSCT in Portugal, with an increased risk in those undergoing mismatched related or unrelated donor transplants. However, they found no significant association between stem cell sources and the presence of GVHD.

Broers et al. [14] noted CMV seropositivity to be a significant adverse risk factor for transplant-related morbidity and mortality (TRM) despite the very efficient prevention of CMV disease by preemptive therapy. Patients with CMV seropositivity experienced an increased incidence of acute GvHD, which was associated with increased TRM and decreased OS. GvHD itself and the drugs used to treat it both cause immunosuppression. On the other hand, CMV can lead to the production of inflammatory cytokines, which

Table 2 Correlation of CMV reactivation with demographic and HSCT parameters in the cohort $n = 1035$

Characteristics		CMV negative	CMV positive	Chi square	*P* value
Sex	Male	482	165	0.3047	0.58
	Female	295	93		
Stem cell source	Bone marrow	183	18	47.9233	< 0.00001
	PBSC	529	216		
	Cord	23	22		
	Combined	42	22		
Type of donor	MFD	504	39	206.86	< 0.00001
	MUD	136	77		
	T cell depleted Haplo	30	36		
	T cell replete Haplo	107	106		
Diagnosis	Benign	587	197	0.0691	0.79
	Malignant	190	61		
Acute GVHD	Present	259	138	33.2765	< 0.00001
	Absent	518	120		
Chronic GVHD	Present	212	101	12.9205	0.000325
	Absent	565	157		

PBSC peripheral blood stem cells, *MFD* matched family donor, *MUD* matched unrelated donor, *Haplo* Haploidentical, *GVHD* graft versus host disease

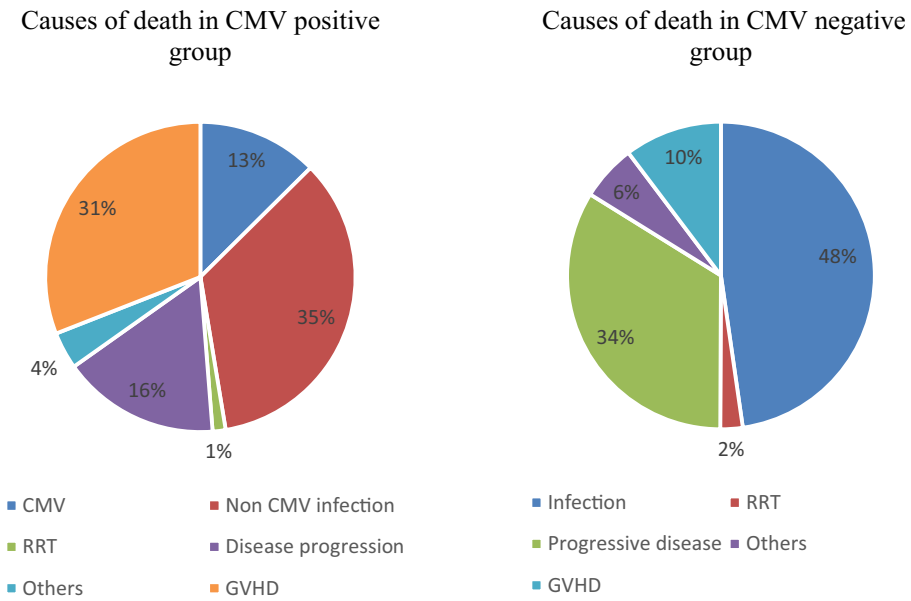

Fig. 1 Comparison of causes of death in relation to CMV reactivation

play a role in the initiation of GVHD [15]. Jain et al. [16] and Meet et al. [11] both found a significant relationship between CMV reactivation and GvHD.

Studies have shown CMV reactivation to be associated with decreased OS [13]. In addition, any level of CMV viremia in seropositive patients had a significant impact on all-cause mortality and non-relapse mortality despite the use of pre-emptive therapy [17]. However, Meet et al. [11] found no differences in the OS of patients with or without CMV reactivation in their study.

The fact that CMV replication is associated with mortality is the principal reason to advocate the use of prophylaxis against CMV infection after allogeneic HSCT. Pre-emptive therapy has no effect on the indirect effects of CMV caused by persistent low-level viremia in the body [18]. Letermovir is an important advance, breaking the paradigm of preemptive therapy with the shift to prophylaxis. Letermovir led

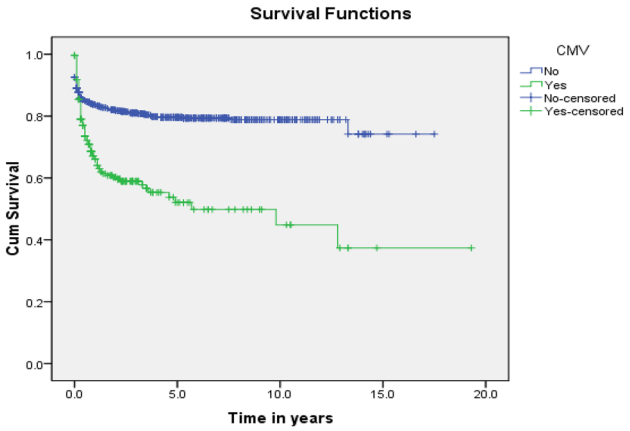

Fig. 2 Kaplan Meier survival curve analysis depicting mean survival time of 13.7 years (range 13.1–14.4) in the CMV negative group, versus 9.2 years (range 7.5–11) in the CMV positive group (p value = 0.0001)

to lower all-cause mortality in CMV seropositive patients at high risk of reactivation. However, pediatric data is still scarce about this drug [19], and we need to wait for more information regarding its efficacy and safety in children before it can be incorporated into clinical practice.

Conclusion

Patients undergoing haploidentical and MUD transplants are at high risk of CMV reactivation, highlighting the importance of regular surveillance and early pre-emptive therapy in these patients. Patients with underlying inborn errors of immunity are a vulnerable group at high risk of CMV disease. CMV reactivation is associated with an increased risk of GvHD and transplant-related morbidity and mortality. Pre-emptive therapy may not be sufficient to mitigate the indirect effects of CMV reactivation. Hence, prophylaxis may be the way forward to decrease transplant-related mortality and improve survival.

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Author Contributions SC, RU, RR: conceptualized the study and wrote the manuscript; SKM, VVS: data analysis; KG, SD: data collection; VK: revised manuscript critically for important intellectual content, IJ: proofreading. All authors approved the final version of the manuscript and are accountable for all aspects related to the study.

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Declarations

Conflict of interest There are no conflicts of interest.

Ethics Clearance Institutional Ethics Committee, Apollo Hospital, Chennai; No. ASH-C-S-OIO/06-22 dated June 25, 2022.

References

1. Staras SA, Dollard SC, Radford KW et al (2006) Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. Clin Infect Dis 43(9):1143–1151. https://doi.org/10.1086/508173. Epub 2006 Oct 2 PMID: 17029132
2. Devasia AJ, Mammen S, Korula A, et al. (2018) A low incidence of cytomegalo virus infection following allogeneic hematopoietic stem cell transplantation despite a high seroprevalence. Indian J Hematol Blood Transfus 34(4):636–642. https://doi.org/10.1007/s12288-018-0960-y. Epub 2018 Apr 16. PMID: 30369733; PMCID: PMC6186215.
3. Freeman RB Jr (2009) The "indirect" effects of cytomegalovirus infection. Am J Transplant 9(11):2453–2458. https://doi.org/10.1111/j.1600-6143.2009.02824.x. PMID: 19843027
4. Boeckh M, Nichols WG (2004) The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. Blood 103(6):2003–2008. https://doi.org/10.1182/blood-2003-10-3616. Epub 2003 Nov 26 PMID: 14644993
5. El-Cheikh J, Devillier R, Crocchiolo R et al (2013) Impact of pretransplant donor and recipient cytomegalovirus serostatus on outcome for multiple myeloma patients undergoing reduced intensity conditioning allogeneic stem cell transplantation. Mediterr J Hematol Infect Dis. 5(1):e2013026. https://doi.org/10.4084/MJHID.2013.026. PMID: 23667724; PMCID: PMC3647712.
6. George B, Kerridge IH, Gilroy N et al (2012) A risk score for early cytomegalovirus reactivation after allogeneic stem cell transplantation identifies low-, intermediate-, and high-risk groups: reactivation risk is increased by graft-versus-host disease only in the intermediate-risk group. Transpl Infect Dis 14(2):141–148. https://doi.org/10.1111/j.1399-3062.2011.00706.x. Epub 2012 Jan 29 PMID: 22283838
7. Cho SY, Lee DG, Kim HJ (2019) Cytomegalovirus infections after hematopoietic stem cell transplantation: current status and future immunotherapy. Int J Mol Sci 20(11):2666. https://doi.org/10.3390/ijms20112666.PMID:31151230;PMCID:PMC6600658
8. Przepiorka D, Weisdorf D, Martin P et al (1995) 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant 15(6):825–828. PMID: 7581076
9. Vigorito AC, Campregher PV, Storer BE et al (2009) National Institutes of Health. Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. Blood 114(3):702–708. https://doi.org/10.1182/blood-2009-03-208983. Epub 2009 May 21. PMID: 19470693; PMCID: PMC2713471.
10. Uppuluri R, Subburaj D, Jayaraman D et al (2017) Cytomegalovirus reactivation posthematopoietic stem cell transplantation (HSCT) and type of graft: a step toward rationalizing CMV testing and positively impacting the economics of HSCT in developing countries. Pediatr Blood Cancer 64(11). https://doi.org/10.1002/pbc.26639. Epub 2017 May 22. PMID: 28544502.

11. Kumar M, Roychowdhury M, Kumar J et al (2018) Cytomegalovirus reactivation and disease amongst patients with allogeneic haematopoietic stem cell transplantation in Eastern India: epidemiology, outcome and healthcare cost. Indian J Med Microbiol 36(1):49–53. https://doi.org/10.4103/ijmm.IJMM_17_269. (PMID: 29735826)
12. Sharma SK, Kumar S, Agrawal N et al (2013) Cytomegalovirus reactivation following hematopoietic stem cell transplantation. J Infect Dev Ctries 7(12):1003–1007. https://doi.org/10.3855/jidc.2947. PMID: 24334950
13. Sousa H, Boutolleau D, Ribeiro J et al (2014) Cytomegalovirus infection in patients who underwent allogeneic hematopoietic stem cell transplantation in Portugal: a 5-year retrospective review. Biol Blood Marrow Transplant 20(12):1958–1967. https://doi.org/10.1016/j.bbmt.2014.08.010. Epub 2014 Aug 17 PMID: 25139217
14. Broers AE, van Der Holt R, van Esser JW et al (2000) Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. Blood 95(7):2240–2245. PMID 10733491
15. Grefte A, van der Giessen M, van Son W, The TH (1993) Circulating cytomegalovirus (CMV)-infected endothelial cells in patients with an active CMV infection. J Infect Dis 167(2):270–277. https://doi.org/10.1093/infdis/167.2.270. (PMID: 8380609)
16. Jaing TH, Chang TY, Chen SH et al (2019) Factors associated with cytomegalovirus infection in children undergoing allogeneic hematopoietic stem-cell transplantation. Medicine (Baltimore) 98(4):e14172. https://doi.org/10.1097/MD.0000000000014172. PMID: 30681583; PMCID: PMC6358375.
17. Green ML, Leisenring W, Xie H et al (2016) Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study. Lancet Haematol 3(3):e119–e127. https://doi.org/10.1016/S2352-3026(15)00289-6. Epub 2016 Feb 20. PMID: 26947200; PMCID: PMC4914379.
18. Styczyński J (2020) Prophylaxis vs. preemptive therapy in prevention of CMV infection: new insight on prophylactic strategy after allogeneic hematopoietic cell transplantation. Acta Haematol Polonica 51(1):17–23. https://doi.org/10.2478/ahp-2020-0005
19. Cheng CN, Li SS, Yeh YH et al (2022) Letermovir prophylaxis for cytomegalovirus reactivation in children who underwent hematopoietic stem cell transplantation: a single-institute experience in Taiwan. J Microbiol Immunol Infect 55(2):323–327. https://doi.org/10.1016/j.jmii.2022.01.002. Epub 2022 Feb 23 PMID: 35241378