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Ganciclovir in the Prevention of Cytomegalovirus Reactivation in Allogeneic Hematopoietic Stem Cell Transplantation: Non-Eliminative Reduction of Viral Load

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ABSTRACT

Objective: Objective: Allogeneic hematopoietic stem cell transplantation (HCT) is used to treat various hematological disorders with a significant risk of treatment-related morbidity and mortality. During long immunosuppressed status, reactivation of cytomegalovirus (CMV) is a challenging complication with its diagnosis, treatment, and toxicity. In our study, we aimed to evaluate the effectiveness of ganciclovir to prophylaxis with valacyclovir in patients who have undergone allogeneic HCT.

Material and Methods: Data of 82 patients were analyzed in a retrospective manner. Patients were grouped as patients receiving valacyclovir alone or ganciclovir plus valacyclovir. CMV-DNA levels were monitored weekly. Reactivation and alterations of viral DNA levels were recorded and compared in both prophylaxis regimes.

Results: Mean age of patients was 44.85 years (19-69 years). The 31 patients were female (37,8%) and 51 were male (62,2%). All recipients were CMV seropositive before allogeneic HCT, and only 2 donors were CMV seronegative (2,4%). Forty-one of the patients received valacyclovir (50%), while 41 received ganciclovir plus valacyclovir (50%). Reactivation was not observed in 32 patients (39%). The 33 of the 41 patients receiving ganciclovir plus valacyclovir and 18 of the 41 patients on valacyclovir alone developed CMV reactivation. Although the inclusion of ganciclovir to valacyclovir was not related with decreased rates of CMV reactivation, the level of CMV DNAemia was relatively lower in patients on ganciclovir plus valacyclovir than in valacyclovir treatment alone.

Conclusion: Inclusion of ganciclovir to valacyclovir in allogeneic HCT patients did not decrease the rate of CMV reactivation, and did not shorten the duration but reduced the degree of CMV DNAemia.

Keywords: Ganciclovir, Cytomegalovirus Reactivation, Allogeneic Hematopoietic Stem Cell Transplantation

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HCT) is used to treat a variety of hematological malignancies and certain non-malignant hematological disorders. The rate of HCTs worldwide is estimated to exceed 40.000 transplants/year.

Cytomegalovirus (CMV) causes a wide spectrum of involvement with various presentations, mainly depending on the host's immune status. In immunocompetent patients, viral replication is limited with T cell-mediated immunity, resulting in latent infection. DNA of the latent virus is detectable in monocytes and dendritic cells, megakaryocytes, and even myeloid progenitors in the bone marrow (1). The secondary disease may be observed later due to reactivation of the latent infection or, less likely, reinfection with a different strain. Reactivation may be observed in immunocompetent patients and patients under immunosuppression, secondary to certain diseases or treatments (2). Introduction to CMV usually happens in the early years of life with an increased prevalence with age, depending on the socioeconomic status as well as inhabitation [3]. In a population-based study from the United States of America, CMV sero-prevalence is reported as 36% in 6-11 year old individuals while reaching 91% in those aged over 80 years [3,4]. As a common infection, the serious disease is not common in immunocompetent patients but is still a major cause of mortality and morbidity in patients who are immunosuppressed due to solid organ and HCT, HIV infection, and immunomodulating treatments towards T cell immunity.

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The most important risk factor for CMV reactivation in patients who have undergone allogenic HCT is the serologic status of the recipient and the donor, particularly in seropositive recipients, the risk of reactivation is reported as 80%. In contrast, in seronegative recipients with a seropositive donor, the risk of primary infection is reported as 30%. Besides the serologic status of the HCT recipient, corticosteroid treatment, T cell depletion with either purine analogues or Alemtuzumab, development of graft versus host disease (GVHD), and the graft source are also suggested as risk factors (5-7).

Manifestations of CMV disease in immunosuppressed patients include fever, hepatitis, pneumonia, upper and lower gastrointestinal diseases, and central nervous system infections, including retinitis and encephalitis. The diagnosis of CMV disease is challenging in HCT recipients due to the clinical signs and symptoms, which may be confused with graft rejection and infections due to other microorganisms. For this diagnostic challenge, it is suggested that every donor and recipient should be surveyed for CMV seropositivity before HCT with a risk estimation of reactivation and later monitored regularly with polymerase chain reaction (PCR) assay (7).

It is important to clarify the definitions of CMV infection and disease, as CMV infection refers to the demonstration of viral antigens or DNA in blood or anybody fluid with or without signs of invasive involvement, but the definition of CMV disease refers to manifestation of disease-related signs and symptoms with alterations in blood count or invasive disease in tissue samples (8). As isolating CMV by culture techniques is not feasible, a pre-emptive approach with the detection of DNAemia and/or primary infection is recommended with conflicts regarding the thresholds of quantitive-DNA values (8). The first first-line agent for pre-emptive treatment and prophylaxis is intravenous ganciclovir. Ganciclovir and Valganciclovir (its oral prodrug) are reported to reduce the risk of reactivation/primary infection without a favorable effect on overall survival (5, 9). Mechanism of action for all antiviral drugs is generally based on the inhibition of DNA synthesis. Myelosuppression is frequently observed in patients receiving ganciclovir. Novel agents, including Brincidofovir, Maribavir, and Letermovir are currently being under investigation with promising results (7).

With the ongoing concerns on CMV reactivation with its diagnostic and therapeutic challenges, we aimed to investigate our patients who have undergone allogeneic HCT with perspectives including epidemiological projection, the risk factors of reactivation, and the effects of prophylactic antivirals on CMV reactivation.

MATERIAL and METHODS

Patient Cohort:

Eighty-two patients who have undergone allogeneic HCT in the Hematology Department of Bahcesehir University between in 2013-2017 were enrolled in the study in a retrospective manner. Underlying hematological malignancy, age, gender, CMV serology of the patient and the donor, the source of HCT, development of CMV DNAemia and/or infection were recorded from the files (**Table 1**).

Conditioning Treatment:

Patients on allogeneic HCT were prepared to receive either myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) regimen before transplantation. MAC regimens included CY/TBI (cyclophosphamide and total body irradiation), Bu4/Cy (Busulfan and cyclophosphamide) or Flu/Bu4 (Busulfan and Fludarabine) while RIC regimens included Flu/Mel (Fludarabin and Melphalan), Flu/Bu2 or 3 (Fludarabin and Busulfan) or Flu/Cy (Fludarabin and Cyclophosphamide).

Antiviral Prophylaxis:

Starting from day minus 7 till day before HCT, all patients received oral Valacyclovir 500 mg once daily (OD) or oral Valacyclovir 500 mg OD plus intravenous ganciclovir 5mg/kg twice daily for seven days and switched to daily dosing afterward. For all patients, prophylaxis was continued until day plus 100, if reactivation was not observed.

Monitorization:

All patients were also monitored for CMV reactivation weekly starting from day +7. CMV DNAemia was analyzed with Anatolia-Bosphore Quantification Assay (Istanbul, Turkey) using a whole blood sample with a linear interval of 60-13.000.000 IU/mL (1IU/mL=1,2 copies/mL).

When asymptomatic CMV DNAemia is detected with two consecutive increased CMV-DNA levels >1000 IU/mL, treatment is given with ganciclovir 5mg/kg/dose every 12 hours for 14 days and then 5 mg/kg/dose daily or oral Valganciclovir 900 mg twice daily or oral Valacyclovir 1000 mg three times daily until 2 consecutive CMV-DNA PCR negativity.

Statistical Analysis

Statistical analysis was performed with IBM SPSS V.20. Descriptive analysis was performed, and median values were calculated and reported for quantitative variables and the percentage was calculated and reported for categorical variables. Comparisons were performed with chi-square test and Mann-Whitney U test depending upon the parametrical and non-parametrical variables. Logistical regression analysis was performed for all significant correlations 95% CI was used to present the statistically significant level of the results. ROC curve analysis was performed for risk estimation.

Ethical approval was obtained from the local ethical committee. Informed written consent forms were obtained from all patients.

RESULTS

General Features

Mean age of patients was 44,85 years (19-69 years). 31 patients were female (37,8%) while 51 were male (62,2%). Primary disease of patients were acute myeloid Leukemia (AML) or myelodysplastic syndrome (MDS) in 35 patients (42,6%), Acute Lymphoblastic Leukemia (ALL) in 24 patients (29,3%), nonHodgkin's Lymphoma (NHL) in 9 patients (11%), multiple myeloma (MM) in 6 patients (7,3%), Aplastic Anemia in 5 patients (6,1%), and Myelofibrosis in 3 patients (3,7%).

The source of HCT was peripheral blood in 77 patients (93,9%) and bone marrow in 5 patients (6,1%) with matching as sibling full match in 63 patients (76,8%), unrelated full match in 7 patients (8,5%), haploidentical mismatch sibling in 5 patients (6,1%), unrelated mismatch in 7 patients (8,5%). 61 patients received myeloablative conditioning treatment (MAC) (74,4%), while 21 patients received reduced intensity conditioning treatment (RIC) (25,6%). The use of a purine analog is observed in 47 patients (57,3%).

Regarding the risk factors of reactivation, all recipients were CMV seropositive before allogeneic HCT. Only 2 donors were CMV seronegative (2,4%). 41 of the patients received Valacyclovir (50%), while 41 received ganciclovir plus Valacyclovir (50%).

Reactivation was not observed in 32 patients (39%) while in 21 patients, reactivation was observed before engraftment (<30 days) (21%), in 20 patients between days 30-60 (24,4%), in 2 patients between days 60-100 (2,4%) and in 7 patients after days 100 (8,5%). Mean value of first observed CMV-DNA positivity is 1518,95 IU/mL (0-43940) while mean maximum value is 574778,1 IU/mL (0-44830).

With the recognition of reactivation, treatment is commenced with ganciclovir in 37 patients (%45,1), Valganciclovir in 3 patients (3,7%) and Valacyclovir in 12 patients (14,6%). In this Valacyclovir alone group, CMV-DNA levels did not trend to increase and were accepted as probable spontaneous resolution. The timing of CMV-DNA negativity is within 14 days of pre-emptive treatment in 6 patients (7,3%) while in 14-28 days in 25 patients (30,5%) and after 28 days in 14 patients (17,1%).

Regarding survival, 15 patients died (18,3%), all after 100 days, 13 patients due to relapse of the primary hematological malignancy and 2 patients died due to causes which were non-hematological and not transplant related.

Comparisons

As all patients were CMV seropositive before allogeneic HCT, donor seropositivity became a major concern regarding activation and the effectiveness of the prophylactic treatment. However, only 2 of the 41 HCT transplants who were on ganciclovir plus Valacyclovir were CMV seronegative, while all donors of 41 HCT patients who were on Valacyclovir alone were CMV seropositive, which suggested that donor CMV serologic status is not a confounding factor (p=0,247).

Our second concern was the primary hematological cancer and its probable effects on CMV reactivation. But two prophylactic treatment groups were similar regarding the primary hematological cancer (p=0,708).

Likewise, conditioning treatment and prophylaxy groups were compared and 32 of the patients on Valacyclovir alone received MAC while 29 of the patients on ganciclovir plus Valacyclovir received MAC (p=0,307) and a homogeneity was preserved in the comparison. 20 of the Valacyclovir alone patients have received purine analogue treatment while 27 patients of ganciclovir plus Valacyclovir group have received purine analogue (p=0,09).

Regarding prophylaxis and CMV reactivation which was the major question to be answered in our study, 33 of the 41 patients who were receiving ganciclovir plus Valacyclovir developed CMV reactivation while only 18 of the 41 patients who were on Valacyclovir alone developed CMV reactivation (p=0,001).

With the recognition of CMV DNAemia and the commencing of pre-emptive treatment, 12 patients in the ganciclovir plus Valacyclovir group developed a CMV-DNA negativity after 28 days of pre-emptive treatment while in the Valacyclovir alone prophylaxy group, 5 of the 18 CMV reactivated patients reached CMV-DNA negativity within 14 days and 10 reached in 14-28 days, and only 2 patients reached CMV-DNA negativity after 28 days (p=0,019).

As CMV reactivation was observed despite the use of combination antivirals, mean initial CMV-DNA level in Valacyclovir alone was 1.865,15 IU/mL (SD 7.171,924) while in ganciclovir plus Valacyclovir it was 1.172,76 (SD 2.898,508) (p<0,05) and maximum CMV-DNA level in Valacyclovir alone group was 1.099.194,29 IU/mL (SD 7000357.926) while in ganciclovir plus Valacyclovir group it was 50.361,90 IU/mL (139.499,392) (p<0,05). Although the inclusion of ganciclovir to Valacyclovir was not related with decreased rates of CMV reactivation, in the combination prophylaxis group during reactivation, level of CMV DNAemia was relatively lower than Valacyclovir alone group.

The source of HSC, prophylaxis type, and CMV reactivation were compared, and it was observed that 4 of the haploidentical HSC have received ganciclovir plus Valacyclovir and all have developed CMV reactivation while in sibling full match and unrelated full match HSCs, distribution was similar regarding prophylaxis and reactivation (p=0,152). Conditioning treatment have not an impact on CMV reactivation alone (p=0,207). As the distribution of conditioning treatments were similar in respect of antiviral prophylaxis, 25 of 29 patients who were on ganciclovir plus Valacyclovir and have received MAC have developed CMV reactivation, while 8 of the 12 patients who have received ganciclovir plus Valacyclovir and RIC developed CMV reactivation, as the difference was observed in the number of the patients, this difference did not reach to a statistical significance (p=0,614).

Likewise, receiving purine analogue was not related with CMV reactivation (p=0,18) and among patients who are receiving purine analogue therapy, 21 of the 27 patients who have received combination antiviral prophylaxy have developed CMV reactivation while 8 of the 20 patients who have received Valacyclovir alone were observed to develop CMV reactivation. Though a higher percentage of patients on combination antiviral prophylaxis has developed CMV reactivation, the relation did not show statistical significance (p=0,19).

Last of all, prophylaxis with Valacyclovir alone or ganciclovir plus Valacyclovir was not related to post-transplantation survival in respect of CMV activation (p=0,249).

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Table 1. Characteristics of Patients on Prophylaxis with Valacyclovir and Ganciclovir plus Valacyclovir

		Valacyclovir Group (n=41)	Ganciclovir+Valacyclovir Group (n=41)	*p* values
Age (mean/years)		48,2 (23-69) (SD:13,085)	41,51 (19-65) (SD:15,017)	>0,05
Gender (F/M)		16/25	15/26	>0,05
Primary Hematological Disease	**AML-MDS**	19 (46,4%)	16 (39%)	>0,05
	ALL	12(29,3%)	12 (29,3%)	
	NHL	4 (9,8%)	5 (12,2%)	
	MM	1 (2,4%)	5 (12,2 %)	
	AA	3 (7,3%)	2 (4,9%)	
	MF	2 (4,9%)	1 (2,4%)	
Conditioning Regimen	**Myelo-ablative**	32 (78%)	29 (70,7%)	>0,05
	Reduced-intensity	9 (22%)	12 (29,3%)	
CMV Reactivation	**None**	24 (58,5%)	8 (19,5%)	<0,05
	<30 days	4 (9,8%)	17 (41,5%)	
	30-60 days	10 (24,4%)	10 (24,4%)	
	60-100 days	1 (2,4%)	1 (2,4%)	
	>100 days	2 (4,9%)	5 (12,2%)	
Pre-transplantation recipient CMV seropositivity		100%	100%	
Donor CMV seropositivity		100%	95,1%	>0,05
Stem Cell Source	**Peripheral Blood**	39 (95,1%)	38 (92,7%)	>0,05
	Bone Marrow	2 (4,9%)	3 (7,3%)	
Transplantation Type	**Full Match Related**	38 (92,7%)	25 (61%)	>0,05
	Full Match Unrelated	2 (4,9%)	5 (12,2%)	
	Haploidentical	1 (2,4%)	4 (9,7%)	
	Unrelated mismatch	none	7 (17,1%)	
Use of Purine Analogue		20 (48,8%)	27 (65,9%)	>0,05
CMV reactivation and Use of Purine Analogue		8 (40%)	21 (77,7%)	
Pre-emptive therapy	**None**	21 (51,2%)	24 (58,5%)	
	Ganciclovir	13 (31,7%)	3 (7,3%)	
	Valacyclovir	7 (17,1%)	5 (12,2%)	
CMV DNA negativity	**0-14 days**	5 patients	1 patient	<0,05
	14-28 days	10 patients	15 patients	
	>28 days	2 patients	12 patients	
Initial CMV DNAemia (mean-IU/mL)		1865,15 (SD 7171,924)	1172,76 (SD 2898,508)	<0,05
Maximum CMV DNAemia (mean-IU/mL)		1099194,29 (SD 7000357,926)	50361,90 (139499,392)	<0,05

DISCUSSION

In relapsed and/or refractory hematological malignancies, HCT is still an undeniable treatment modality. Immunological properties of graft-host interaction may be modified to form a status of balance-unbalance depending on the status of the disease activity. Graft versus leukemia/lymphoma/myeloma effect may be desired in refractory disease. The immunological interaction of graft and host mainly proceeds on T cell mediated immunity, and the entrance of reactivation of a latent CMV may alter the balance leading to increased rates of GVHD, as well as bacterial or fungal infections and both CMV-related or unrelated mortality (5-7). Primary or secondary prophylaxy, pre-emptive treatment with antivirals, specific and nonspecific immunoglobulins, and adoptive specific T cell transfer therapies are reported with conflicting outcomes (7, 10). In a recent meta-analysis regarding antiviral prophylaxis against CMV in allogeneic HCT patients, ganciclovir and Letermovir have been observed as effective agents in terms of advanced surveillance and the use of pre-emptive therapy (13).

As the prevalence of CMV is related with age, socioeconomic status, and region, rates of CMV positivity have been reported over 90% in immunocompetent patient groups (11,12). In our study, all recipients were CMV IgG positive and only 2 donors were seronegative. This may be a reflection of seropositivity in Turkey. The limitation of the donor spectrum leads to the obligation of laying aside the CMV status of the donor and getting prepared for reactivation. In our study group, this limitation led to an unintended yet homogenous comparison between two prophylaxy groups.

Most of the reactivation was observed during the first 100 days, while on close surveillance (21% <30 days and 24,4% between days 30-60) and regardless of the antiviral agent and mean value of a maximum CMV-DNA positivity as 574.778,1 IU/mL which is a significantly high value of CMV replication. Though the inclusion of ganciclovir did not decrease the rates of CMV reactivation, mean initial and maximum CMV-DNA levels were lower in the combination group (p values <0,05).

With lower CMV-DNA levels, the time to reach CMV negativity with the initiation of pre-emptive therapy was not shortened in the combination group as expected.

Regarding the source of HCT, in haploidentical HCT patients all 4 who have received ganciclovir plus Valacyclovir have developed CMV reactivation, but did not developed for one haploidentical who receiving Valacyclovir. However, in the full match related and unrelated HCT patients, reactivation was similar in both prophylaxis groups. Though the number of haploidentical HCT patients is limited, our observation was in favor of not using ganciclovir plus Valacyclovir for CMV reactivation prophylaxis. In a recent meta-analysis, use of acyclovir was observed to be related with less toxicity but also nonsignificant effectivity in CMV prevention, while ganciclovir as related with increased toxicity, effective to prevent CMV reactivation but ineffective in mortality (13). Our study did not observe a decreased rate of CMV reactivation in a combination antiviral group but only a limitation in CMV viremia.

There were certain limitations of our study. First of all, the retrospective nature of our study has limited the equal distribution of the patients, especially haploidentical HCT patients. Though they were all treated with ganciclovir plus Valacyclovir prophylaxy group due to the concern of CMV reactivation in this sensitive group of patients, all have reactivated. The second major limitation of our study was the lack of evidence of CMV infection, which may be attributed to the prophylactic-pre-emptive approach of our clinic.

CONCLUSION

Inclusion of ganciclovir to Valacyclovir in allogeneic HCT patients did not decrease the rate of CMV reactivation, did not shorten the duration of the CMV-DNAemia and did not affect the overall survival. With the perspective of its cost and possible myelosuppressive effects, it may be wise to monitor CMV-DNA routinely and use ganciclovir as a pre-emptive therapy until better antiviral agents like Letermovir are available.

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Author Contributions: OK: Project Design, Data collection, Biochemical Analysis, literature review, **OK:** Manuscript preparation and Revision

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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