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Article

Human Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients. A single center experience

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Abstract

*Background***:** Herperviruses remain latent after primary infection and may reactivate under immunosuppressive conditions. Our study is focused on Cytomegalovirus (CMV) in hematopoietic stem cell transplantation (HSCT) recipients, as this virus may be a major cause of post-HSCT complications. The study was designed to evaluate the incidence of CMV DNAemia and its associated factors in autologous (auto) and allogeneic (allo) HSCT recipients. In addition, estimate the necessity for regular monitoring of CMV DNAemia in auto-HSCT recipients.
*Methods***:** This prospective study included forty HSCT patients at the Bone marrow transplant unit. CMV DNA in plasma was detected by PCR weekly in allo-HSCT recipients from post-transplantation week 2 until day 70 post-HSCT. For auto-HSCT recipients, PCR was done at weeks 2,4,6 and whenever CMV was clinically suspected. *Results*: CMV DNA was detected in 13/40 (32.5%) of HSCT recipients at a median of 6 weeks post-HSCT. CMV reactivation occurred in 3/12 (25%) of allo- HSCT recipients, and 10/28 (35.7%) of auto-HSCT recipients (p=0.716). No significant

association was noted for age, gender, underlying disease, conditioning regimen, pre-HSCT CMV serology with CMV reactivation. Acute graft versus host disease (aGVHD) occurred in (66.7%) of CMV positive allo-patients (p=0.05).CMV was asymptomatic in (90%) of auto-HSCT recipients. *Conclusion*: CMV DNAemia is common after HSCT. CMV DNA routine monitoring and preemptive treatment is essential in allo-HSCT recipients as they are more prone to symptomatic CMV disease. We propose that serial post-transplant PCR monitoring in auto-HSCT is not necessary in the absence of clinical manifestations or pathologic evidence suggestive for CMV disease, supporting the protocol followed at the bone marrow transplant unit.

Keywords: Cytomegalovirus, hematopoietic stem cell transplantation, autologous, allogeneic, CMV reactivation.

Introduction

Hematopoietic stem cell transplantation (HSCT) is now a standard treatment for hematological diseases that have no other curative alternative. (1) Herpesviruses are among the most common opportunistic viral infections causing complications and even fatalities in HSCT recipients. (2) Herpes viral infections in humans usually start at an early age.(1) Latency is the hallmark of herpes viruses, and reactivation under immunosuppression may lead to clinical disease. (3) Herpes simplex virus (HSV) and varicella zoster virus (VZV) infections have become relatively less common in HSCT recipients in the setting of antiviral prophylaxis with acyclovir. (4) Cytomegalovirus (CMV) is the most frequently studied virus in transplantation. (1)Thus, we focused our study on CMV reactivation in HSCT recipients.

CMV is classified as the beta human herpesvirus type 5 (HHV-5), with a high rate of seropositivity among the population. (5) Seropositive individuals are very likely to have CMV in the latent form. (6) CMV reactivation after HSCT has a wide spectrum ranging from asymptomatic DNAemia to CMV end-organ diseases such as gastroenteritis, pneumonitis, hepatitis, retinitis, and encephalitis. (7),(8)CMV can also indirectly cause graft failure or immunosuppression increasing the risk of bacterial and fungal infections and its association with graft-versus-host disease (GVHD). (8) Polymerase chain reaction (PCR) is the most sensitive method for detecting actively replicating CMV in HSCT recipients.(5) The incidence of CMV disease has been decreased by routine monitoring and preemptive treatment of CMV infection. (9) Due to the adverse drug reactions associated with antiviral drugs, including bone marrow suppression, pre-emptive management is the approach most commonly used in current clinical practice.(10) However, the regimens of PCR monitoring and preemptive therapy for CMV are not standardized in autologous transplantation.(11)

The study aimed to evaluate the incidence of CMV DNAemia and its associated factors in autologous (auto) and allogeneic (allo) HSCT recipients. In addition, estimate the necessity for regular monitoring of CMV DNAemia in auto-HSCT recipients.

Materials and Methods

Patients

A prospective cohort study was carried out at the Bone marrow transplant unit in Alexandria University, Egypt, from January 2020 till June 2022. All patients admitted to the unit were included in the study. A total of 40 patients were observed.

According to the policies of the transplant unit, the stem cell source received by all patients was peripheral blood stem cell transplant (PBSCT). None of them received bone marrow (BM) harvest or cord blood transplant (CBT). All patients received myeloablative conditioning (MAC) that was chosen according to the age of the patient, the medical history and underlying hematological disease. All allo-HSCT recipients received graft from HLA matched related donors. Methotrexate was received by allo-HSCT recipients for prophylaxis against GVHD with or without Cyclosporin A. All patients received Acyclovir as antiviral prophylaxis, Levofloxacin as antibacterial prophylaxis, Sulfamethoxazole-Trimethoprim for Toxoplasma gondii and Pneumocystis jirovecii prophylaxis, and Fluconazole for antifungal prophylaxis. No prophylaxis for CMV was administered, but preemptive treatment with valganciclovir was given for allo-HSCT recipients if CMV DNA was detected by real time PCR. The following was obtained from patient's records before transplantation: HLA typing, renal function, liver function, echocardiography, serological tests including CMV IgG and IgM for recipients and donors, Epstein–Barr virus (EBV) IgG and IgM, hepatitis C virus (HCV) antibody, hepatitis B virus (HBV) surface antigen, Human immunodeficiency virus (HIV) antibody. Patients were monitored after HSCT for development of aGVHD and CMV related symptoms.

Ethics

Written informed consent was taken from each patient regarding participation in the study. The study protocol (number 0201316) was approved by the Ethics Committee, faculty of Medicine, Alexandria University.

Sample collection

A blood sample was withdrawn from each patient weekly in allo-HSCT recipients starting from post-transplantation week 2 until day 70 post-HSCT. For auto-HSCT recipients, samples were collected at post-transplantation weeks 2,4,6 and if any symptoms related to CMV were suspected. Plasma was separated and stored in sterile eppendorf tubes at -70°C.

CMV DNA Monitoring

DNA extraction was done from 200 µl of plasma according to manufacturer's instructions using (Thermo Fisher Scientific GeneJET Viral DNA and RNA Purification Kit #K0821, Vilnius, Lithuania). (11) A 25 µl PCR reaction mixture was prepared using 12.5 µl Maxima SYBR Green/ROX qPCR Master Mix, 1 µl of each of the forward and reverse primers, DNA extract made up to 25 µl with water. The sequence of forward CMV primer used was 5' GCGGTGGTTGCCCAACAGGA3' and the reverse CMV primer was 5' ACGACCCGTGGTCATCTTTA 3'.(12) The designed primers amplify a target of 94 bp fragment

from the CMV "UL55" genes that code for glycoprotein B. Checking the specificity of the primers was done by BLAST search using the database (http://blast.ncbi.nlm.nih.gov).(13)

The thermal cycling profile started with 2 minutes at 50ºC and 10 minutes at 95 °C followed by 40 cycles of denaturation (15 seconds at 95 °C), annealing (30 seconds at 55 °C), and extension (30 seconds at 72 °C). This was followed by melting point analysis. The program was run in the thermal cycler (Rotor- Gene Q MDx). Cycle threshold (Ct) for positive CMV DNA samples was less than 40, and melting temperature (Tm) was 76 ± 2 ºC.

CMV viral load was quantitated using a standard curve that was generated in the quantitative PCR assay using a known concentration of CMV DNA that was diluted in serial dilutions from 10^5 to 10^1 copies of CMV genome. The sensitivity of this assay was 80 copies of CMV genome/mL of plasma.

Statistical analysis

IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp) was used for data analysis.(14) Qualitative data were described using number and percent. To verify the normality of distribution, Shapiro-Wilk test was used. Quantitative data were described using range, mean, standard deviation, median and interquartile range (IQR). Chi-square test was used for categorical variables, to compare between different groups. Fisher's Exact or Monte Carlo correction for chi-square when more than 20% of the cells have expected count less than 5. Student t-test for normally distributed quantitative variables, to compare between two studied groups. Statistically significant values were considered at *P* values <0.05.

Results

Demographics and patients' characteristics

The study included 40 HSCT recipients with mean age 40.47 ± 14.69 years. Males versus females were 62.5% versus 37.5%, respectively. Allo-HSCT was received by 12 patients; for treatment of acute leukemia (n=11) and severe aplastic anaemia (n=1). Auto-HSCT was received by 28 patients; for treatment of multiple myeloma (n=17) and lymphoma (n=11). Conditioning regimens received were: Busulfan plus Cyclophosphamide for acute leukemia, Cyclophosphamide plus Fludarabine for severe aplastic anemia, BEAM protocol for lymphoma, and Melphalan for multiple myeloma. CMV IgG was positive in 95% of cases. All allo-HSCT recipients and their donors (100%) were CMV IgG positive (D+, R+), while 26/28 (92.9%) of auto-HSCT recipients were CMV IgG positive before transplantation. Patients' characteristics are summarized in table (1).

Table (1). Characteristics of hematopoietic stem cell transplant recipients (n=40)

	HSCT recipients (n = 40)	
	No.	**%**
Gender		
Male	25	62.5%
Female	15	37.5%
Age (years)		
Min. – Max.	19.0 – 72.0	
Mean ± SD.	40.47 ± 14.69	
Median (IQR)	39.50 (27.5 - 55)	
Type of graft		
Allogeneic	12	30
Autologous	28	70
Source of stem cells		
PSCT	40	100.0
BM harvest	0	0.0
CBT	0	0.0
Underlying haematologic disease:		
AML	7	17.5
ALL	3	7.5
Biphenotypic acute leukemia	1	2.5
Aplastic anaemia	1	2.5
HL	7	17.5
NHL	4	10
Multiple Myeloma	17	42.5
HLA disparity **Matched related**	12	100.0
Matched unrelated	0	0.0
Mismatched related	0	0.0
Conditioning regimen **MAC** **Busulfan + Cyclophosphamide**	11	27.5
Cyclophosphamide+Fludarabine	1	2.5
BEAM protocol	11	27.5
Melphalan	17	42.5
RIC	0	0.0
GVHD prophylaxis	12	100.0

Methotrexate ± Cyclosporine A		
Anti-viral prophylaxis **Acyclovir**	40	100.0
CMV IgG positive	38	95.0

Abbreviations: **HSCT:** hematopoietic stem cell transplant, **PSCT:** Peripheral stem cell transplant, **BM:** bone marrow, **CBT:** cord blood transplant, **AML:** acute myeloid leukemia, **ALL:** acute lymphocytic leukemia, **HL:** Hodgkin's lymphoma, **NHL:** non Hodgkin's lymphoma, **MAC:** myeloablative conditioning, **RIC:** reduced intensity conditioning, **GVHD:** graft versus host disease, **CMV:** Cytomegalovirus

Detection of CMV in plasma by real time PCR

Thirteen out of 40 HSCT recipients (32.5%) were positive for CMV DNA in plasma after HSCT. CMV DNAemia occurred in 3/12 (25%) of allo-HSCT recipients, and 10/28 (35.7%) of auto-HSCT recipients (χ^2=0.440, p=0.716).

Twelve out of the 13 CMV DNA positive HSCT recipients (92.3%) had only one positive sample, while one (7.7%) had 2 positive CMV DNA samples.

Around 85% of the CMV positive patients had an onset of CMV reactivation between post-transplantation week 4 and 6. The median time of CMV DNA detection was 6 weeks post-transplantation. The relation between CMV DNAemia and time after HSCT in our studied patients is shown in **figure (1)**.

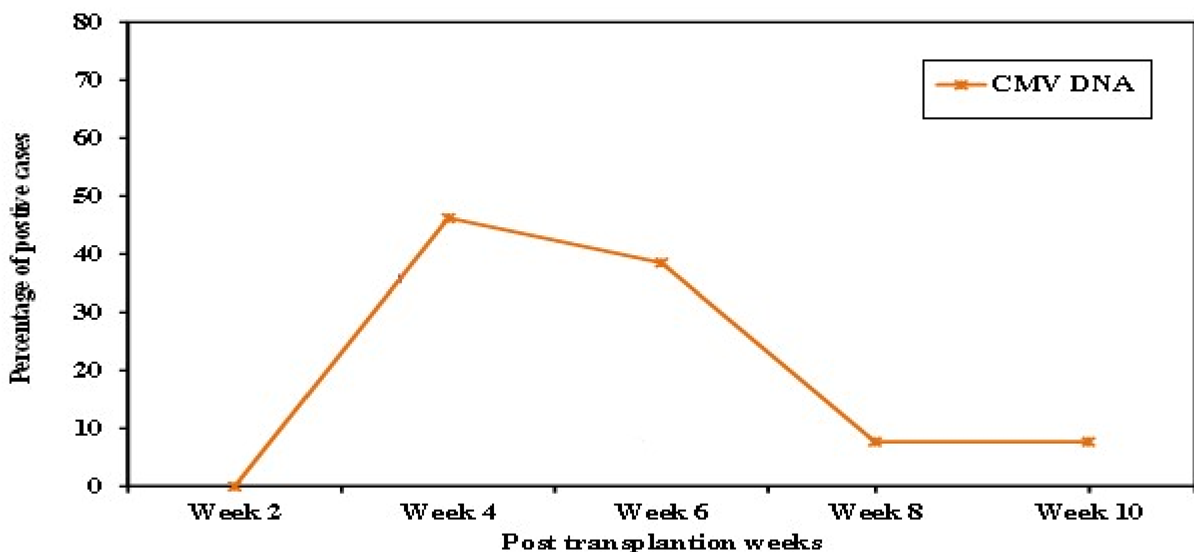

Figure (1). **The relation between CMV DNAemia and time after hematopoietic stem cell transplant in the studied population (n=40).**

CMV viral load

Quantitation of CMV viral load in patients with positive CMV DNAemia showed a range of CMV DNA from 85 to 22,600 copies/mL of plasma. The median of CMV copies in plasma was 896 copies/ml.

Factors affecting CMV reactivation

No statistically significant association was noted for age, gender, underlying disease, conditioning regimen, pre-HSCT CMV IgG serological status with CMV reactivation. Statistically significant association was found for aGVHD with CMV reactivation (p=0.05). (Table 2 and 3).

Table (2). Factors associated with Cytomegalovirus DNAemia in hematopoietic stem cell transplant recipients.

	Patients negative for CMV DNAemia (n= 27)		Patients positive for CMV DNAemia (n= 13)		Test of significance	p
	No.	%	No.	%		
Age (years)						
Min. – Max.	19.0 – 65.0		21.0 – 72.0			
Mean ± SD.	39.93 ± 14.60		41.62 ± 15.40		t= 0.046	0.963
Median (IQR)	38.0 (27.5 – 53.5)		41.0 (32.0 – 55.0)			
Sex						
Male	17	63.0	8	61.5	χ^2=0.008	^{FE}p=1.000
Female	10	37.0	5	38.5		
Underlying haematologic disease:						
AML	6	22.2	1	7.7	χ^2=1.283	^{FE}p=0.393
ALL	2	7.4	1	7.7	χ^2=0.001	^{FE}p=1.000
Biphenotypic acute leukemia	1	3.7	0	0.0	χ^2=0.494	^{FE}p=1.000
Aplastic anaemia	0	0.0	1	7.7	χ^2=2.130	^{FE}p=0.325
HL	6	22.2	1	7.7	χ^2=1.283	^{FE}p=0.393
NHL	3	11.1	1	7.7	χ^2=0.114	^{FE}p=1.000
Multiple Myeloma	9	33.3	8	61.5	χ^2=2.857	0.091
Conditioning regimen						
MAC						
Busulfan + Cysclophosphamide	9	33.3	2	15.4	χ^2=1.418	^{FE}p=0.286
Cyslophosphamide+Fludarabine	0	0.0	1	7.7	χ^2=2.130	^{FE}p=0.325
BEAM protocol	9	33.3	2	15.4	χ^2=1.418	^{FE}p=0.286
Melphalan	9	33.3	8	61.5	χ^2=2.857	0.091
CMV IgG positive	25	92.6	13	100	χ^2=0.294	^{FE}p=1.000

Abbreviations: **AML:** acute myeloid leukemia, **ALL:** acute lymphocytic leukemia, **HL:** Hodgkin's lymphoma, **NHL:** non Hodgkin's lymphoma, **MAC:** myeloablative conditioning, **RIC:** reduced intensity conditioning, **GVHD:** graft versus host disease, **CMV:** Cytomegalovirus

χ^2: **Chi square test**, FE: **Fisher Exact,** t: **Student t- test**

p: p value for comparing between **Patients negative for CMV DNAemia and Patients positive for CMV DNAemia.**

Table (3). Relation between Cytomegalovirus DNAemia and acute graft versus host disease in allogeneic hematopoietic stem cell transplant recipients (n=12).

	CMV DNA positive allo-HSCT(n=3)	CMV DNA negative allo-HSCT (n=9)	*P*
aGVHD (n=3)	2 (66.7%)	1(11.1%)	X^2_{MC}= 2.7 P= 0.05*
No aGVHD (n=9)	1 (33.3)	8(88.9%)	
Total (n=12)	3(100%)	9 (100%)	

Abbreviations: **CMV:** Cytomegalovirus**,** **Allo:** allogeneic, **HSCT:** hematopoietic stem cell transplant**,** a**GVHD:** acute graft versus host disease.

X^2_{MC} = Monte Carlo test value

*: Statistically significant at $p \leq 0.05$

CMV-associated symptoms and outcomes

Three allo-HSCT recipients (25%) had gastro-intestinal symptoms highly suspicious of aGVHD during follow-up. The 3 patients received corticosteroid therapy for control of intestinal aGVHD. About 2-3 weeks later, CMV DNAemia was positive in two cases of them, one patient at post-transplantation week 8 and the other at post-transplantation week 10, with viral load 300 and 140 copies/mL respectively. The third allo-HSCT recipient who was positive for CMV DNA (85 copies/ml) at post-transplantation week 6, didn't develop aGVHD. Preemptive treatment with valganciclovir was given for the 3 CMV DNA positive allo-HSCT recipients, and the PCR test was negative within 2 weeks.

One auto-HSCT recipient was positive for CMV DNA at post-transplantation week 4. The patient had history of delayed neutrophil engraftment (day 24 post-HSCT). However, by the time CMV DNA was detected during the study, the neutrophil engraftment had occurred and the patient didn't receive specific treatment for CMV at the time of CMV DNAemia. The rest (90%) of the CMV DNA positive auto-HSCT recipients (n=9) had asymptomatic reactivation, and the viremia resolved spontaneously within 2 weeks without receiving specific treatment.

None of the HSCT recipients in our study developed manifestations of CMV disease during the follow-up period.

Discussion

CMV reactivation remains a threat after HSCT. Routine monitoring of CMV DNA in allo-HSCT recipients and preemptive management is the most commonly used approach in current clinical practice. The role of routine monitoring and preemptive therapy for CMV are still controversial in auto-HSCT. The current study was carried out to determine the incidence of CMV

DNAemia among allo and auto-HSCT recipients, possible factors associated with CMV DNAemia and estimate the necessity for regular monitoring of CMV DNAemia in auto-HSCT recipients.

In the present study, 32.5% of HSCT recipients were positive for CMV DNA in plasma. CMV DNAemia occurred in 25% of allo- HSCT recipients, and 35.7% of auto-HSCT recipients. The difference between both groups was not statistically significant (p=0.716). This is interesting, as the reconstitution of the immune system is known to be more rapid in auto-HSCT than in allo-HSCT. Our findings may be explained by the uneven number of patients between the two groups, and that the frequent monitoring for auto-HSCT recipients may have yielded higher rates of detection of asymptomatic reactivation compared to that detected in studies that only perform PCR if CMV infection is suspected.

Rojas-Rechy MH et al.(2022) consistently, detected CMV DNAemia in auto-HSCT (6.7%) more than in allo-HSCT recipients (5%), with no significant difference. (1) Also, *Piukovics K et al.(2017)* detected similar results (33.3%) among auto-HSCT recipients. (15) In contrast, *Payandeh M et al.(2021)* demonstrated CMV DNAemia with significantly higher rates among allo-HSCT (60%) than auto-HSCT recipients (6%). (16)

Some studies reported higher incidences (35%, 60%, 69%) among allo-HSCT, (17),(16),(18) and (38.8%, 42.2%) among auto-HSCT recipients. (19),(20)Other studies reported lower rates (13.2%,22%) among allo-HSCT recipients,(21), (22) and (11%, 29.4%) among auto-HSCT recipients. (23),(24)

Different results may be explained by the use of different assays for detection of CMV reactivation, variations in the type of samples used (whole blood versus plasma), differences in studied populations regarding underlying hematologic diseases and stem cell source used (whether PBSCT, BM harvest or CBT). Various studies have displayed high rates of CMV reactivation among allo-HSCT recipients and attributed this to receiving T cell-depleted grafts, graft from HLA mismatched donors or difference in CMV serostatus between donors and recipients.(25),(22),(26) None of allo-HSCT recipients in our study experienced the fore-mentioned conditions.

In our study, the median time of onset of CMV DNAemia was 6 weeks after HSCT. Similar results were reported by *Crocchiolo R et al.(2016)* and *Shan Li et al.(2022)* where the median of CMV detection was 40 days.(27),(28) This was in agreement with *Peres RM et al.(2010)* who demonstrated the highest probability for CMV reactivation at 44.4 days post-HSCT. (6) Also, *Lodding IP et al. (2018)* detected CMV DNA in HSCT recipients at a median of 48 days.(29) On the other hand, a wide range was illustrated by *Luo XH et al.(2021)* and *Jang JE et al.(2012)* who detected CMV DNAemia at a median of 33 and 81 days, respectively.(30),(31)

No statistically significant differences were noticed between CMV PCR positive and negative patients regarding age, sex, underlying hematologic disease and conditioning regimen. This was consistent with *Schetelig J et al. (2003), Valadkhani B et al.(2016), Metwally MA et al. (2021), Yeh TJ et al.(2022),*and *Vallejo M et al.(2022)* (32),(33),(34),(26),(35)

Different findings were detected by *Marchesi F et al.(2015)* who reported that higher age showed significant association with risk of CMV reactivation after transplantation.(23) Regarding the received conditioning regimen, *Mardani M et al.(2020)* found a significant decrease in CMV reactivation in patients who received busulfan plus cyclophosphamide and busulfan plus fludarabine.(36) *Piukovics K et al.(2017)* demonstrated that Melphalan was significantly associated with CMV DNA positive rates.(15) All the patients in our study received myeloablative

conditioning. Some studies identified myeloblative conditioning as a risk for CMV reactivation compared to reduced-intensity conditioning.(37)

Regarding CMV serostatus, all allo-HSCT recipients and their donors in our study were CMV IgG positive (D+, R+). Out of the 40 HSCT recipients included in the study, only 2 auto-HSCT recipients were CMV IgG negative pre-HSCT. All HSCT recipients who developed CMV DNAemia were CMV seropositive. Thus, a statistically significant association for CMV serostatus and CMV reactivation couldn't be detected. This was in agreement with *Nakano N et al.(2014)* and *Vallejo M et al.(2022)*. (35),(37) In contrast, several studies have detected that CMV seropositive recipient was significantly associated with CMV reactivation. (23) ,(32) Some studies also identified D+/R+ CMV serostatus as a minor risk factor for reactivation of CMV (38), (39) and D−/R+ as a major risk factor for CMV reactivation(6), (26), (38)Thus, this factor should be considered during donor selections for an optimal post-HSCT outcome.(6)

In the present study, out of the 3 CMV DNA positive allo-HSCT recipients, 2(66.7%) developed symptoms of intestinal aGVHD before CMV DNAemia was detected by 2-3 weeks. The relation was statistically significant (p=0.05). Our results agreed with different studies which demonstrated that aGVHD was correlated with higher rates of CMV reactivation, (21),(32),(35), (39) and some of them considered aGVHD as a major risk factor for CMV reactivation. (8) ,(38) Steroids received by these patients for treatment of aGVHD may have inhibited the immune system by suppressing T-cell activation and may be a cause of CMV reactivation as supposed in some studies. (37), (39),(40) CMV reactivation may trigger GVHD and on the other side, immunosuppression for GVHD may elevate the risk for CMV, a vicious circle. (41) However, other studies as *Nakano N et al.(2014)* and *Yeh TJ et al. (2022)* didn't find a significant impact for aGVHD on CMV reactivation. (26) ,(37)

Meanwhile, a point that should be highlighted is that CMV can infect the gastrointestinal tract (GIT) producing ulcers that can be seen on endoscopy, but may be confused with other disorders as GVHD.(42)Diagnosing CMV GIT disease depends on finding CMV in biopsy by culture or histology and may occur with the absence of CMV in blood, even by PCR. (41) CMV and GVHD frequently occur concomitantly, which makes the assessment of the contribution of each of them in the symptomatology difficult.(42)

In the present study, 90% of CMV DNA positive auto-HSCT recipients had asymptomatic reactivation that resolved spontaneously without specific treatment. This was in agreement with Piukovics K et al.(2017) who detected asymptomatic reactivation among 81% of auto-HSCT recipients. (15) Also, *Rossini F et al.* (2005) demonstrated that in most auto-HSCT cases, CMV infection was asymptomatic and cleared spontaneously, (24) which was also supported in other studies.(20) Previous reports identified the risks for CMV disease after auto-HSCT as CD341 selection, receiving corticosteroids with high doses, and receiving conditioning with total-body irradiation or fludarabine or alemtuzumab. (42) Auto-HSCT recipients having these risk factors should receive preemptive CMV therapy. (43) None of those patients were found in our study. As the probability for symptomatic infection is greater after allo-HSCT, (44) in our study and according to the unit's protocol, all allo CMV DNA positive patients received preemptive therapy regardless of the viral load, which was effective in preventing the development of symptoms.

Limitations of our study include the small number of patients enrolled during the study period, as this was affected by the lockdown during the COVID-19 pandemic.

Conclusion

As demonstrated in the present study, CMV DNAemia is common after HSCT. CMV reactivation is significantly associated with aGVHD. CMV DNA routine monitoring and preemptive treatment is essential in allo-HSCT recipients. We propose that serial post-transplant PCR monitoring in auto-HSCT recipients is not necessary in the absence of risks factors or clinical manifestations suggestive for CMV infection, supporting the protocol followed at the bone marrow transplant unit.

Conflict of interest

None

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