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ARTICLE

Clinical outcomes of allogeneic hematopoietic stem cell transplant recipients developing Cytomegalovirus DNAemia prior to engraftment

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Abstract

There is limited information on the impact of CMV DNAemia episodes developing prior to engraftment (pre-CMV DNAemia) on clinical outcomes following allogeneic hematopoietic stem cell transplantation (allo-HSCT). This issue was addressed in the current retrospective multicenter study including 878 patients. All participant centers used preemptive antiviral therapy strategies for prevention of CMV disease. CMV DNA load in blood was monitored by real-time PCR assays. A total of 144 patients (cumulative incidence 16.5%, 95% CI, 14%–19%) had an episode of pre-CMV DNAemia at a median of 10 days after allo-HSCT. Patients who developed pre-CMV DNAemia had a significantly higher ($P = < 0.001$) probability of recurrent episodes (50%) than those who experienced post-CMV DNAemia (32.9%); Nevertheless, the incidence of CMV disease was comparable ($P = 0.52$). Cumulative incidences of overall mortality (OM) and non-relapse mortality (NRM) at 1-year after allo-HSCT were 32% (95% CI, 29–35%) and 23% (95% CI 20–26%), respectively. The risk of OM and NRM in adjusted models appeared comparable in patients developing a single episode of CMV DNAemia, regardless of whether it occurred before or after engraftment, in patients with pre- and post-engraftment CMV DNAemia episodes or in those without CMV DNAemia.

Introduction

The incidence of Cytomegalovirus (CMV) end-organ disease following allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been dramatically reduced due to the efficacy of antiviral therapy preventative strategies [1]. Nevertheless, CMV DNAemia, which is exceedingly common, has been associated with increased overall and non-relapse mortality (OM and NRM, respectively) in this setting [2–5]. Letermovir has been approved by regulatory agencies for prophylaxis of CMV infection in adult CMV-seropositive allo-HSCT recipients. Letermovir treatment decreases the risk of clinically significant CMV infection and OM through week 24 when compared to placebo [6]. Letermovir can be administered at any time point between the day of transplant and day 28 after allo-HSCT in the absence of CMV DNAemia.

CMV DNAemia developing prior to engraftment (pre-CMV DNAemia), which usually occur between the third and fourth week after allo-HSCT, may conceivably have a different course from that emerging after engraftment once patients have begun to expand donor-derived T cells (post-CMV DNAemia). There is limited information on the impact of CMV pre-DNAemia on clinical outcomes. Previous studies found no association between pre-CMV DNAemia an increased risk of CMV disease or mortality [7, 8]; these studies, however, were limited by their single center design and scarce number of events of interest. Here, we conducted a retrospective multicenter, noninterventional study to further address this issue.

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Patients and methods

Study design and data collection

The current study enrolled 898 adult patients undergoing T-cell replete allo-HSCT at 20 different centers in Spain from September 2014 to December 2015 (Registry of the Working group on Infectious and Non-Infectious Complications of the GETH-Spanish Hematopoietic Transplantation and Cell Therapy Group-). A total of 218 adult patients who received an unmanipulated allo-HSCT at the Clinical University Hospital of Valencia from March 2010 to May 2019 (excluding patients recruited in the GETH registry) were also included. Out of the 1,116 patients, 238 patients were excluded from the study for one or more of the following reasons: lack of CMV DNA PCR results ($n = 81$), D and R CMV-seronegative status ($n = 89$), use of anti-CMV prophylaxis with (val)ganciclovir, foscarnet or letermovir ($n = 68$). Finally, the cohort consisted of 878 patients (Table 1) from 19 centers. Median age of patients at allo-HSCT was 53 years (range, 18–72). Clinical outcomes of interest included CMV DNAemia developing either before (pre-CMV DNAemia) or after (post-CMV DNAemia) engraftment, recurrent CMV DNAemia, CMV disease, OM and NRM through day 365 after transplantation. The study was approved by the Research Ethics Committees of the participating centers.

CMV DNA monitoring and management of CMV DNAemia

All centers used preemptive antiviral therapy (PET) strategies for prevention of CMV disease. Monitoring of CMV DNA load in blood was performed by commercial real-time PCRs at most centers (Table 2). Monitoring was conducted once a week through day +100 and at each scheduled visit thereafter. Patients at high risk for recurrences were also monitored on a weekly basis [1]. Patients with CMV DNAemia developing at any time point were monitored at least once a week until clearance. CMV surveillance began one or two weeks before allo-HSCT at eight out of 19 centers (42%). CMV DNA levels prompting PET are detailed in Table 2. In center 9, PET was initiated when plasma CMV DNA levels were above 1500 IU/ml or when the CMV DNA doubling time (dt) was ≤2 days, whichever occurred first [9]. (Val)ganciclovir or foscarnet at conventional doses were used for PET [1].

CMV DNA doubling time

The CMV DNA dt was calculated using the first 2 PCR positive results in the absence of antiviral treatment for analysis, as previously described [10].

Table 1 Demographic and clinical characteristics of allogeneic hematopoietic stem cell transplant recipients included in the study.

Factor	no. (%)
Total	878
Sex (Male/Female)	512 (58)/366 (42)
Age (≥53/<53)	441 (50.2)/437 (49.8)
Underlying disease	
Acute Leukemia	406 (46.2)
Chronic Leukemia	49 (5.6)
Hodgkin's Lymphoma	57 (6.5)
Non-Hodgkin's Lymphoma	111 (12.6)
Multiple Myeloma	55 (6.3)
Myelodysplastic syndrome	110 (12.5)
Myelofibrosis	16 (1.8)
Other hematological diseases	74 (8.4)
Stem cell source	
PB	763 (86.9)
UCB	25 (2.8)
BM	90 (10.3)
HCT-CI	
≥3	272 (31)
1–2	284 (32.3)
0	275 (31.3)
Missing values	47 (5.4)
Allograft type	
MMD	121 (13.8)
Haploidentical	175 (19.9)
MUD	235 (26.8)
MRD	347 (39.5)
CMV serostatus	
D + /R +	507 (57.7)
D − /R +	300 (34.2)
D + /R −	71 (8.1)
Conditioning regimen	
Myeloablative/ Reduced intensity	361 (41.1)/517 (58.9)
Containing ATG,	131 (14.9)
GvHD prophylaxis	
Based on Cyclosporine A	391 (44.5)
Based on Tacrolimus	436 (49.7)
Based on mTOR inhibitors	51 (5.8)
aGvHD prophylaxis containing Cyclophosphamide	233 (26.5)
CMV disease	31 (3.5)
aGvHD	
0	519 (59.1)
I	55 (6.3)
II	201 (22.9)
III-IV	103 (11.7)

aGvHD acute Graft versus Host Disease, *ATG* anti-Thymocyte Globulin, *BM* bone marrow, *CMV* cytomegalovirus; *D* donor, *HCT-CI* HCT-CI, comorbidity index, *MMD* HLA-mismatched from related or unrelated donors, *MRD* matched related, *MUD* matched unrelated, *PB* peripheral blood, *R* recipient, *UCB* umbilical cord transplantation.

Definitions

CMV DNAemia was defined as detection of CMV DNA in one or more blood specimens. Recurrent CMV DNAemia

episodes were those occurring >15 after clearance of the previous one. Diagnosis and grading of aGvHD was done as previously detailed [11]. CMV disease was diagnosed according to published criteria [12]. The hematopoietic cell transplantation co-morbidity index (HCT-CI) was calculated as previously reported [13]. Engraftment was defined as absolute neutrophil count ≥500/mm^3 on 3 consecutive days, the first of which being time of engraftment.

Statistical analysis

Cumulative incidences were assessed using the statistical software R (http://www.r-project.org/). OM was the total number of deaths from any cause. NRM was the total number of deaths in the absence of relapse or underlying disease progression. The causes of death were established as previously indicated [14]. Death and relapse were categorized as competing events for the cumulative incidence of CMV DNAemia. The Chi-squared test was used for frequency comparisons. Differences between medians were compared using the Mann–Whitney U-test and the Kruskal–Wallis test, when appropriate. Two-sided P values < 0.05 were deemed to be significant. Cox proportional hazards regression models were used to assess the potential risk factors for the occurrence of CMV DNAemia, OM and NRM. CMV DNAemia and aGvHD were treated as time-dependent variables. For multivariate analyses, only variables with parameter estimates showing a P value ≤ 0.10 in the univariate analyses were included. The latter statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

Occurrence of CMV DNAemia and CMV disease in the cohort

Out of 878 patients, 566 developed one ($n = 355$) or more ($n = 211$) episodes of CMV DNAemia through day +365 after allo-HSCT (cumulative incidence, 64%; 95% CI 61–67%). First episodes were detected at a median of 34 days after transplantation (range, −9 days to 354). Engraftment was achieved in 855 patients (97.4%) at a median of 19 days after allo-HSCT (range, 7–59 days).

In total, 144 of the 566 episodes were detected prior to engraftment (cumulative incidence, 16.5%; 95% CI, 14–19%), at a median of 10 days (range, −9 to 59) after allo-HSCT, of which 24 developed during conditioning before stem cell infusion. PET was administered to 92 out of 144 patients with pre-CMV DNAemia (63.9%) and 300 out of 422 (71.1%) patients with post-CMV DNAemia ($P = 0.12$). Times to occurrence of pre-CMV DNAemia episodes (either treated with PET or self-resolving) in allo-HSCT recipients are shown in Fig. 1.

(Val)ganciclovir was used in monotherapy in 49 and 75% of pre- and post-CMV DNAemia episodes ($P = <0.001$). Foscarnet was prescribed alone in 19.6% and 14.3% of pre- and post-CMV DNAemia episodes ($P = 0.25$). Both drugs were given (sequentially) in the remaining episodes.

Among the 92 patients with pre-CMV DNAemia who underwent PET, 35 (42.7%) were treated before neutrophil's engraftment, whereas PET was delayed until neutrophil engraftment in 47 patients (57.3%). This information was unavailable for the remaining 10 patients.

Recurrences were more frequent ($P = <0.001$) in patients with pre-CMV DNAemia (72 out of 144; 50%) than in those who experienced post-CMV DNAemia (139 out of 423; 32.9%). Nevertheless, PET was used comparably ($P = 0.11$) for recurrent CMV DNAemia that followed either pre-CMV DNAemia (50%) or post-CMV DNAemia (62%).

There were 31 cases of CMV end-organ CMV disease ($n = 21$ gastrointestinal, $n = 6$ pneumonitis; $n = 2$ encephalitis; $n = 1$ hepatitis and $n = 1$ retinitis) in our cohort (cumulative incidence, 3.5%; 95% CI 2–5%) that developed at a median of 72 days after transplantation (range, 2–224). Of these, 9 occurred in patients with pre-CMV DNAemia at a median of 103 days (2–158), 21 in patients with post-CMV DNAemia (median, 72 days; range, 27 to 224 days), and one in a patient without CMV DNAemia (day 44); Thus, the cumulative incidence of CMV disease was 7% (95% CI 3–13) and 5% (95% CI 3–8) in patients with pre-CMV and post-CMV DNAemia episodes, respectively ($P = 0.52$).

Baseline risk factors of pre-engraftment CMV DNAemia

Factors independently associated with increased risk of pre-CMV DNAemia were recipient CMV seropositivity, inclusion of post-transplant cyclophosphamide in aGvHD prophylaxis regimen and use of the Abbott PCR assay for CMV DNA monitoring (Table 3).

Kinetics of CMV DNAemia by episode type

The CMV DNA dt could be calculated for 82 episodes of pre-CMV DNAemia (56.9%) and 268 of post-CMV DNAemia (64%). The CMV DNA dt was comparable ($P = 0.13$) in pre-CMV DNAemia (median, 1.75 days; range, 0.44–6.42) and post-CMV DNAemia episodes (median, 2.0 days; range 0.23–6.11).

Table 2 Real-time PCR assays used for CMV DNA monitoring in the blood compartment at participating centers.

Center code number	Number of patients	Blood specimen	CMV DNA cut-off used for initiation of PET (IU/ml)	Real-time PCR assay[a]
1	73	Plasma	600	COBAS Ampliprep/COBAS Taqman CMV Test (Roche Diagnostics)
2	64	Plasma	910/(455 in UCB)	COBAS Ampliprep/COBAS Taqman CMV Test
3	60	Whole blood	910	COBAS Ampliprep/COBAS Taqman CMV Test
7	55	Plasma	400	COBAS Ampliprep/COBAS Taqman CMV Test
5	50	Plasma	660	COBAS Ampliprep/COBAS Taqman CMV Test
4	32	Plasma	150	COBAS Ampliprep/COBAS Taqman CMV Test
8	41	Plasma	400/(136 in UCB)	COBAS Ampliprep/COBAS Taqman CMV Test
11	31	Plasma	1,000/(136 in UCB)	COBAS Ampliprep/COBAS Taqman CMV Test
12	30	Plasma	136	COBAS Ampliprep/COBAS Taqman CMV Test
18	22	Plasma	136	COBAS Ampliprep/COBAS Taqman CMV Test
19	23	Plasma	136	COBAS Ampliprep/COBAS Taqman CMV Test
20	17	Plasma	455	COBAS Ampliprep/COBAS Taqman CMV Test
9	245	Plasma	1500	Abbott RealTi*me* CMV PCR Kit (Abbott Diagnostics)
14	20	Plasma	780	Abbott RealTi*me* CMV PCR Kit
6	31	Plasma	500 (two consecutive tests)	RealStar CMV PCR Kit (Altona Diagnostics)
17	29	Plasma	455	Artus LightCycler CMV Quantitative Kit (Roche Diagnostics)
15	25	Plasma	308	CMV R-GENE® (Biomèrieux)
10	10	Whole blood	1000	CMV R-GENE®
13	20	Whole blood	1000 copies/ml/(500 copies/ml in UCB)	"In-house" Real-time PCR[b]

CMV cytomegalovirus, *PET* preemptive antiviral therapy, *UCB* umbilical cord blood.

[a]The limit of detection of the assays in plasma specimens are as follows (according to the respective manufacturer): COBAS Taqman CMV Test, 136 IU/ml; Abbott RealTi*me* CMV PCR Kit, 30.5 IU/ml; RealStar CMV PCR Kit, 41 IU/ml; CMV R-GENE®, 65 IU/ml; Artus LightCycler CMV Quantitative Kit, 250 IU/ml.

[b]The limit of detection was not disclosed.

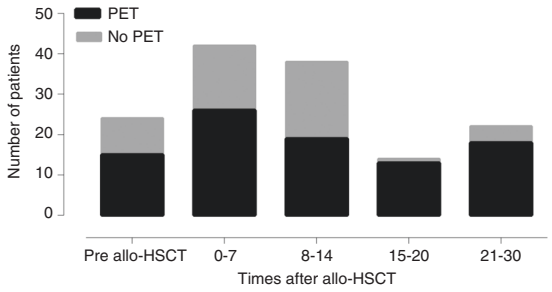

Fig. 1 Time to detection of pre-engraftmet CMV DNAemia. The number of episodes of pre-engraftment CMV DNAemia that either underwent preemptive antiviral therapy (PET) or resolved spontaneously (No PET) occurring prior to or after allo-HSCT is shown.

Pre-engraftment CMV DNAemia and mortality

In total, 279 patients died during the study period (mortality rate, 32%; 95% CI 29–35), at a median of 134 days after allo-HSCT (range, 2–365 days). Deaths were attributed to relapse ($n = 75$), aGvHD ($n = 64$), infection ($n = 55$) or other causes ($n = 85$). The 1-year cumulative incidence of NRM was 23% (95% CI 20–26%).

No difference ($P = 0.10$) was found regarding the cause of death between patients with pre-CMV DNAemia, post-CMV DNAemia and no CMV DNAemia (not shown).

As shown in Table 4, occurrence of pre-CMV DNAemia, considered as a qualitative variable, was not found to increase risk of OM or NRM. Similar data were obtained after excluding CMV D + /R− patients (not shown).

Moreover, OM and NRM risk in adjusted models appeared to be comparable in patients developing a single episode of CMV DNAemia within the study period, regardless of whether it occurred before (HR, 0.92; 95% CI, 0.53–1.60; $P = 0.76$ for OM and HR,0.81; 95% CI,0.41–1.58; $P = 0.54$) or after engraftment (HR, 0.83; 95% CI, 0.55–1.25; $P = 0.38$ for OM and HR, 0.78; 95% CI, 0.48–1.26; $P = 0.31$ for NRM), in patients with pre and post-CMV DNAemia episodes (HR, 0.87; 95% CI, 0.50–1.51; $P = 0.62$ for OM and HR, 0.66; 95% CI, 0.33–1.32; $P = 0.24$) or in those without CMV DNAemia throughout the study period (reference).

Factors independently associated with an increased risk of OM through day +365 in this series (Table 4) were HCT-CI score ≥3, UCB allograft, occurrence of CMV disease and grades III-IV aGvHD, for OM. Factors independently associated with increased risk of NRM were the use of antithymocyte globulin during the conditioning regimen, receipt of an UCB allograft and grades III-IV aGvHD (Table 4).

Finally, aGvHD developed at a comparable frequency ($P = 0.79$) in patients experiencing a single episode of pre-CMV DNAemia (43.1%), in those displaying post-CMV DNAemia (46.7%), or in those exhibiting pre-CMV and post-CMV DNAemia (48.6%).

Discussion

In this multicenter study we investigated the potential impact of pre-CMV DNAemia episodes on several clinical outcomes in allo-HSCT recipients. The data in the current study largely replicated those previously reported by one of the participating centers [7], and are summarized as follows. First, cumulative incidence of pre-CMV DNAemia episodes in this series was around 16%, a similar figure to the previously reported one (19%) [7], but higher than that found (6.5%) by Martin et al. [8] Differences can most likely be explained by use of a variety of real-time PCR assays displaying a wide range of LODs in the current and Martin's studies as opposed to a single highly-sensitive PCR method in the former one [7]. In support of this view, use of the Abbott PCR assay (the most sensitive PCR assay across those used in this study) for CMV DNAemia monitoring was independently associated with increased risk of pre-CMV DNAemia. Baseline factors associated with occurrence of pre-CMV DNAemia episodes were recipient CMV seropositivity, in line with a previous report, [8] and use of post-transplant cyclophosphamide as aGvHD prophylaxis, this latter observation warranting confirmation in further series.

Second, the kinetics of CMV replication, as inferred by the CMV DNA dt, in pre-CMV DNAemia episodes appeared similar to that in post-CMV DNAemia episodes. The above parameter, in contrast to single CMV DNA load values, permits comparison between episodes monitored by different PCR assays, given their co-linearity across the entire range of viral load quantitation [15, 16]. It was assumed that plasma CMV DNA dt reflects the rate of CMV replication in organ and tissues, which is debatable.

Third, recurrent CMV DNAemia occurred at a higher frequency in patients with pre-CMV DNAemia than in those who experienced post-CMV DNAemia, yet the number of recurrences receiving PET was comparable. Delay in acquisition of protective CMV-specific T-cell responses in the former patients could account for this finding [17]; Nevertheless, arguing against this hypothesis, no difference in the incidence of CMV disease was noted across patients with pre or post-CMV DNAemia. In line with this latter finding, Martin et al. [8] reported no cases of CMV disease in their series. Moreover, in a very size-limited series, the frequency of peripheral blood CMV IE-1/pp65-specific IFN-γ-producing $CD8^+$ T cells by day 30+ after allo-HSCT was found to be similar between groups [7].

Fourth, in a previous study including only patients from the GETH registry we failed to show an impact on mortality of CMV DNAemia occurring within the first year after allo-HSCT [18]. Here, pre-CMV DNAemia was also not found to be associated with an increased risk of OM or NRM when compared to that of post-CMV DNAemia or

Table 3 Risk factors for pre-engraftment CMV DNAemia.

Factor	Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*
Type of PCR						
Amplicor/Ampliprep CMV PCR (Roche)	0.56	0.33–0.98	0.04	0.54	0.31–0.94	0.03
Abbott RealTime PCR Kit	1.95	1.17–3.24	0.01	1.77	1.05–2.96	0.03
Other	1 (ref)			1 (ref)		
Sex						
Male vs Female	1.09	0.78–1.52	0.61			
Age (≥53 years vs <53 years)[a]	1.09	0.79–1.51	0.60			
Underlying disease						
Acute Leukemia	1.21	0.66–2.23	0.75			
Chronic Leukemia	0.75	0.28–2.00	0.57			
Hodgkin's Lymphoma	0.86	0.35–2.09	0.73			
Non-Hodgkin's lymphoma	0.86	0.42–1.87	0.75			
Multiple Myeloma	1.40	0.63–3.12	0.41			
Myelodysplastic syndrome	0.44	0.18–1.07	0.07			
Myelofibrosis	2.04	0.72–5.79	0.18			
Other hematological diseases	1 (ref)					
HCT-CI						
>3	1.08	0.71–1.63	0.72			
1–2	1.07	0.71–1.62	0.74			
0	1 (ref)					
Stem cell source						
PB	0.78	0.32–1.91	0.59			
BM	1.09	0.41–2.93	0.86			
UCB	1 (ref)					
Allograft type						
MMD	1.24	0.75–2.08	0.40			
Haploidentical	1.34	0.86–2.09	0.19			
MUD	1.27	0.84–1.92	0.26			
MRD	1 (ref)					
CMV serostatus						
D + /R +	2.42	0.98–5.96	0.06	**2.80**	**1.13–6.91**	**0.03**
D − /R +	**2.85**	**1.14–7.12**	**0.03**	**3.12**	**1.25–7.79**	**0.02**
D + /R −	1 (ref)			1 (ref)		
Conditioning regimen						
Myeloablative vs Reduced intensity	0.99	0.71–1.38	0.94			
Containing ATG (Yes vs. No)	1.33	0.87–2.02	0.19			
Use of post-transplant Cyclophosphamide as a part of aGvHD prophylaxis (Yes vs. No)	1.78	1.27–2.50	0.001	1.45	1.06–2.12	0.02

aGvHD acute Graft versus Host Disease, *ATG* anti-Thymocyte Globulin, *BM* bone marrow, *CMV* Cytomegalovirus; *D* donor, *HCT-CI* comorbidity index, *MMD* HLA-mismatched from related or unrelated donors, *MRD* matched related, *MUD* matched unrelated, *PB* peripheral blood, *R* recipient, *UCB* umbilical cord transplantation.

[a]Cut-off based on the median age of patients.

the absence of CMV DNAemia. The adverse effect of high HCT-CI index, use of UCB as the source of hematopoietic stem cells, and the occurrence of grades III-IV aGvHD or CMV disease on survival reported in the former study [18] was confirmed herein. As previously reported, [18] here we noticed that use of PET for CMV DNAemia was associated with higher OM and NRM in univariate, but not multivariate models. The limited number of deaths among patients with pre-CMV DNAemia undergoing to PET unfortunately precluded meaningful separate analysis.

Table 4 Univariate and multivariate Cox-regression analysis of factors associated with risk of overall mortality and non-relapse mortality by day 365 after allogeneic hematopoietic stem cell transplantation.

Parameter	OM				NRM			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Sex (male vs. female)	0.93 (0.73–1.18)	0.54			0.89 (0.67–1.18)	0.42		
Age[a] (≥53 years vs. <53 years)	1.24 (0.98–1.57)	0.08	1.26 (0.98–1.62)	0.07	1.18 (0.89–1.56)	0.24		
Underlying disease								
Myeloid origin[b]	1 (ref)				1 (ref)			
Lymphoid origin[c]	0.81 (0.63–1.05)	0.11			0.84 (0.62–1.14)	0.26		
Other	1.08 (0.71–1.63)	0.73			1.30 (0.82–2.05)	0.27		
HCT-CI								
≥3	1.72 (1.27–2.33)	<0.001	1.66 (1.22–2.26)	0.001	1.43 (1.01–2.02)	0.05	1.39 (0.97–1.98)	0.07
1–2	1.18 (0.86–1.62)	0.32	1.16 (0.84–1.61)	0.37	0.99 (0.69–1.45)	0.99	0.98 (0.67–1.43)	0.91
0	1 (ref)		1 (ref)		1 (ref)		1 (ref)	
Stem cell source								
BM	1.09 (0.74–1.59)	0.67	1.02 (0.68–1.55)	0.91	1.17 (0.76–1.81)	0.48	1.07 (0.68–1.69)	0.77
UCB	1.34 (1.39–3.94)	0.001	2.43 (1.28–4.63)	0.007	2.11 (1.12–4)	0.02	2.24 (1.02–4.90)	0.04
PB	1 (ref)		1 (ref)		1 (ref)		1 (ref)	
Allograft type								
MMD	1.64 (1.16–2.33)	0.005	1.09 (0.71–1.69)	0.69	1.69 (1.11–2.57)	0.02	1.03 (0.60–1.75)	0.93
Haploidentical	1.35 (0.98–1.88)	0.07	1.33 (0.94–1.88)	0.11	1.54 (1.05–2.26)	0.03	1.41 (0.92–2.16)	0.12
MUD	1.31 (0.97–1.77)	0.08	1.20 (0.88–1.65)	0.26	1.45 (1.01–2.07)	0.04	1.27 (0.87–1.86)	0.21
MRD	1 (ref)		1 (ref)		1 (ref)		1 (ref)	
D/R paired CMV serostatus								
D + /R +	1.63 (0.96–2.77)	0.07			1.50 (0.83–2.72)	0.18		
D − /R +	1.70 (0.99–2.93)	0.06			1.42 (0.77–2.62)	0.27		
D + /R −	1 (ref)				1 (ref)			
R + vs R-[d]	1.66 (0.99–2.79)	0.06	1.58 (0.91–2.76)	0.10	1.47 (0.82–2.64)	0.19		
Conditioning regimen								
Myeloablative vs. reduced conditioning	1.11 (0.87–1.40)	0.40			1.17 (0.89–1.55)	0.27		
Containing ATG (yes vs. no)	1.37 (1.01–1.86)	0.04	1.37 (0.97–1.93)	0.07	1.53 (1.08–2.17)	0.02	1.60 (1.08–2.39)	0.02
aGvHD prophylaxis								
Based on mTOR inhibitors	0.67 (0.37–1.21)	0.19			0.92 (0.70–1.23)	0.58		
Based on Tacrolimus	0.95 (0.74–1.20)	0.65			0.67 (0.34–1.33)	0.25		
Based on Cyclosporine A	1 (ref)				1 (ref)			

Table 4 (continued)

Parameter	OM				NRM			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
aGvHD containing Cyclophosphamide	1.12 (0.86–1.45)	0.41			1.11 (0.81–1.51)	0.52		
aGvHD grades III-IV (Yes vs. no)	2.59 (1.94–3.44)	<0.001	2.80 (2.05–3.82)	<0.001	3.20 (2.33–4.40)	<0.001	3.34 (2.37–4.70)	<0.001
CMV DNAemia								
Before engraftment	1.23 (0.88–1.71)	0.23			1.05 (0.70–1.58)	0.81		
After engraftment	0.99 (0.76–1.29)	0.94			0.96 (0.71–1.31)	0.81		
No CMV DNAemia	1 (ref)		1 (ref)		1 (ref)			
Use of PET for CMV DNAemia (Yes vs. No)[e]	1.40 (1.11–1.77)	0.005	1.07 (0.82–1.40)	0.60	1.50 (1.13–1.99)	0.005	1.17 (0.86–1.60)	0.33
CMV disease	2.58 (1.64–4.07)	<0.001	1.71 (1.04–2.81)	**0.03**	2.62 (1.55–4.45)	<0.001	1.72 (0.97–3.06)	0.06
cGvHD	0.76 (0.37–1.54)	0.44			1.16 (0.57–2.39)	0.68		

aGvHD acute Graft versus Host disease, *ATG* Anti-Thymocyte globulin, *BM* bone marrow, *cGvHD* chronic Graft versus host diseae, *CMV* cytomegalovirus, *D* donor, *HCT-CI* hematopoietic cell transplantation-specific comorbidity index, *MMD* HLA-Mismatched from related or unrelated donors, *HR* hazard ratio, *MRD* matched related donor, *MUD* matched unrelated donor, *NRM* non-relapse mortality, *OM* overall mortality, *PB* peripheral blood, *PET* preemptive antiviral therapy, *R* receptor, *UCB* umbilical cord blood.

[a]Cut-off based on the median age of patients.

[b]Including Acute and chronic myeloid leukemia, Myelodisplastic syndrome, and myelofibrosis

[c]Including Lymphoma, acute and chronic lymphocytic leukemia and multiple myeloma.

[d]Included in the models instead of D or R CMV serostatus.

[e]The "No" subgroup includes both patients with self-resolving CMV DNAemia episodes and patients without documented CMV DNAemia throughout the study period.

The strength of the current study stems from its multicenter nature and the large cohort size. In addition to its retrospective design, the use of different PCR assays and blood matrices for CMV monitoring and the diversity of cut-offs for PET initiation and clinical practices across institutions could be construed as limitations of this study.

In summary, our data indicated that occurrence of pre-CMV DNAemia appeared not to have a detrimental impact on survival following allo-HSCT, despite predisposing to increased development of recurrent episodes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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