Pediatric Hematology and Oncology, 31:743–753, 2014

ISSN: 0888-0018 print / 1521-0669 online
DOI: 10.3109/08880018.2013.859188

informa
healthcare

ORIGINAL ARTICLE
Stem Cell Transplant

Cytomegalovirus Infection in Pediatric Allogenic Hematopoietic Stem Cell Transplantation. A Single Center Experience

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We report a retrospective analysis of Cytomegalovirus (CMV) infection: incidence, recurrence, resistance, and subsequent disease of 81 children who underwent allogenic hematopoietic stem cell transplantation (HSCT). The recipient and/or donor's CMV serology was positive prior to transplant [recipient (R+) and/or donor (D+)]. CMV was monitored by RT-PCR starting from the first week post transplant. Forty patients showed CMV infection (49, 5%). Of them 10 manifested CMV disease leading to four deaths. In univariate analysis, factors associated with CMV infection were CMV R+ $P < .01$, CMV R+/D+ pair $P < .01$, nonbone marrow (BM) stem cell source $P < .05$, nonirradiation conditioning regimen $P < .05$, Antithymocyte globulin (ATG) $P < .01$. Factors associated with CMV resistance were: >1 HLA allele mismatch $P < .05$, CMV R +/D– pair $P < .01$, CMV D– $P < .01$, non-BM $P < .05$, nongenoidentical transplant $P < .01$. CMV disease was influenced by >1 HLA allele mismatch ($P < .001$), non-BM ($P < .01$). On multivariate analysis, CMV R+/D− ($P < .05$), corticosteroids ≥2 mg/kg $P < .01$, ATG $P < .01$ and non-BM ($P < .05$) were independent factors for CMV infection. CMV R+ transplant is associated with more CMV infection and resistance to preemptive treatment. Prolonged immune suppression (IS) worsens outcome of CMV infection and should be shortened whenever possible.

Keywords CMV, HSCT, infection, preemptive, serology

INTRODUCTION

Cytomegalovirus (CMV) infection is one of the most frequent causes of early postallogenic hematopoietic stem cell transplantation (HSCT) related morbidity and mortality. Prophylactic acyclovir, RT-PCR for CMV monitoring and preemptive ganciclovir (GNC), foscavir (FSC), and cidofovir (CIDO) has tremendously decreased the incidence of CMV infection and subsequent disease [1–6]. Many factors predict the

Received 14 September 2013; accepted 22 October 2013.
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outcome for CMV infection and disease [7, 8]. They include the recipient (R) / donor (D) pair CMV pre-transplant sero-status. However, contradictory results exist concerning the severity of infection and disease when CMV sero positive patients are receiving their graft from either a CMV negative [9–11] or positive donor [12, 13]. Other factors include the source of hematopoietic stem cells [bone marrow (BM), peripheral blood stem cells (PBSC), or cord blood (CB)], the type of transplant [geno-identical (genoid), matched unrelated, mismatched], prolonged immune suppression (IS), graft versus host disease (GVHD), heavy conditioning regimen, and initial high risk disease [7, 14, 15]. The aim of this study is to analyze our experience in CMV infection in patients with a special emphasis on various prognostic factors regarding morbidity and mortality.

PATIENTS AND METHODS

A retrospective analysis conducted in the Pediatric Hematology oncology department, Université Claude Bernard Lyon, France. Children from neonatal period to 18 years old were included in the study if they were transplanted at least once with otherwise consecutive transplants from related or unrelated donor, either from BM, peripheral blood hematopoietic stem cells, or CB. Indications of the transplant were hematological, immune or metabolic disorders. This study included 81 patients where at least the recipient or donor was CMV serologically positive prior to transplantation. Studied patients were transplanted in the period between February 2000 and December 2006. It was conducted after the approval of the ethic committee at the hospital. Patient's characteristics are shown in (Table 1).

Conditioning regimen was given according to initial pathology and might include total body irradiation and antithymocyte globulin (ATG). Acute *GVHD* was graded from I to IV.

GVHD prophylaxis or treatment: IS included cyclosporine A and or low dose <2 or high dose ≥ 2 mg/kg/day methyl prednisolone.

HLA Antigen Detection: In BM or PBSC transplant, 10 antigens were tested for HLA matching class I A, B, C and class II DR, DQ. In CB, 6 antigens were tested for HLA matching of class I A and B and Class II DR. In order to homogenize histo-compatibility

TABLE 1 Patients Characteristics ($n = 81$)

Variables	*n*	Percentage
Recipient age:		
Median (Range)	7.3 years (1,2 months – 17.88 years)	
Recipient gender		
Male	45	56%
Female	36	44%
CMV serology recipient/donor pairs		
−/+	28	35%
+/−	21	26%
+/+	32	39%
Herpes viridae prophylaxis		
Acyclovir	49	60%
Valaciclovir	29	36%
Others	3	4%
Initial diagnoses		
Hematological malignancies	44	54%
Nonmalignant diseases	37	46%
Benign hematology	*19*	
Immunodeficiency	*10*	
Metabolic disorders	*8*	

TABLE 2 Transplant Characteristics ($n = 81$)

	n	Percent
Season		
Winter	20	25%
Spring	18	22%
Summer	24	30%
Autumn	19	23%
Stem cell source		
Marrow	59	73%
Blood	22 (PBSC, $n = 10$; CB, $n = 12$)	27%
Transplant type		
Genoidentical	33	41%
Matched unrelated donor	48	59%
Graft matching		
0 mismatch	49	60%
1 mismatch	26	32%
2 mismatch	5	6%
3 mismatch	1	2%
Conditioning regimen		
TBI	23	28%
No TBI	58	72%
ATG	56	69%
No ATG	25	31%
Immune suppression		
Acute GVHD	47	58%
II or less	57	70%
III/IV	24	30%
GVHD Treatment		
No and CTC $<$ 2 mg/kg	47	58%
CTC $>$ 2 mg/kg and others	34	42%

ATG: antithymocyte globulin, CTC: Corticosteroids F: Female, GVHD: graft versus host disease, M: Male, PBSC: Peripheral blood stem cell, TBI: Total body irradiation.

data between CB and other stem cell sources (BM or PBSC) 3/6 matching, 4/6 or 5/6 matching and 6/6 matching in CB were considered 8/10, 9/10, and 10/10 matching respectively in other stem cell sources.

CMV prophylaxis: All patients received prophylactic acyclovir for children at the dose of 10–15 mg/kg/day or valaciclovir for adolescents at the dose of 500 mg $\times$ 2 /day starting from day –10 of transplant. Prophylactic treatment was either discontinued on immune reconstitution or substituted for another antiviral whenever CMV infection was confirmed. Transplant characteristics shown in (Table 2).

CMV monitoring: Viral load was assessed in serum by real time quantitative PCR on weekly basis starting during the first week and until day +100 post-transplant. Serum samples were recorded as copy number /mL. Identified viral DNA copies <500 was evaluated as qualitatively positive.

Quantitative CMV PCR Procedure

CMV DNA procedure was done using the QIAamp Blood Kit (Qiagen®, Germany). The plasmid was constructed by cloning a CMV PCR product yielded by the qualitative PCR in the pGemTeasy plasmid using the Original TA Cloning® Kit from Invitrogen (San Diego, USA). *Escherichia coli* strains were subsequently transformed with this plasmid. After amplification in *E. coli*, this plasmid was extracted using a Qiagen Midi Kit and its concentration determined by spectrophotometry. Several plasmid dilutions were tested varying from 5 $\times$ 10 E 2 to 2 $\times$ 10 E 8 copies/mL.

Real-Time Quantitative CMV PCR Procedure

The PCR primers used for this assay were derived from the qualitative PCR procedure described previously by Levy et al. [16]. The primers were selected in the HXFL4 gene encoding region. The upstream and downstream primer sequences were 5′ ACC AAC ATA AGG ACT TTT CAC ACT TTT 3′ and 5′ GAA TAC AGA CAC TTA GAG CTC GGG GT 3′, respectively. The fluorogenic probe was 5′ CTG GCC AGC ACG TAT CCC AAC AGC A 3′. The probe was labeled at the 5′ end with FAM and at the 3′ end with TAMRA. The PCR primers and probe were synthesized by Eurogentec (Liège, Belgium). PCR reactions were carried out using the Taqman^{TM} PCR reagent Kit (AB Applied Biosystems).

CMV Infection: It defined as early if present before D +100 post-transplant or late if afterwards. Infection was determined by the first appearance of CMV load either qualitatively (positive $<$ 500 copies/mL), or quantitatively $\geq$ 500 cDNA. The fate of both could be either spontaneous resolution or progression to more than $\geq$ cDNA. *Preemptive Anti-CMV Therapy:* Generally, It was started preemptively if documented $\geq$ 500 cDNA /mL in peripheral blood at 2 consecutive times 1 week apart. The duration to negative DNA load was recorded. Preemptive therapy consisted of GNC loading dose 10 mg/kg/day intra venous (IV) (5 mg/kg $\times$ 2 /day). Pharmacokinetics was done to adjust the dose. This dose was maintained for 2 weeks, followed by maintenance dose of 5 mg/kg/day till negativity. In CB transplantation, in order to avoid the toxic effect of GNC to hemopoietic stem cells, FSC 180 mg/kg/day (90 mg/kg $\times$ 2 /day) IV was used as a preemptive treatment. The primary outcome of preemptive therapy was either resolution of cDNA (with subsequent definitive cure or recurrence) or resistance. Resistance was an indication to substitute for another treatment; CIDO 5 mg/kg/once a week. The final outcome was either definitive cure or refractoriness. *CMV Recurrence:* Any confirmed reactivation of CMV after resolution. *CMV Resistance:* Any resistance to initial preemptive treatment to achieve a state of resolution. The time point of nonresolution to be considered resistant infection was mostly the end of 2 week antiviral induction therapy. *CMV refractoriness:* Definitive failure of therapy to eradicate a resistant CMV infection after 2nd or more lines of treatment. The time point for refractoriness was the appearance of CMV disease manifestation or mortality. *The kinetics of cDNA copies:* were assessed in relation to presence or absence of preemptive treatment with an end point progression, or resolution of cDNA. *CMV Disease:* positive detection of CMV viral DNA and associated end organ involvement including: interstitial pneumonitis, retinitis, encephalitis, gastroenteritis, hepatitis, and cystitis. *Prognostic factors:* several factors might influence CMV infection, recurrence and resistance disease. Tested variables included: recipient age, gender, recipient and donor pair serology, herpes prophylaxis, season of transplant, stem cell source, transplant type, HLA match, TBI, ATG containing regimen, acute GVHD grading, and steroids.

Statistical Methods

All calculations were performed using SPSS 16.0 Inc, Chicago IL, USA,

Comparison of nominal characteristics was done by Chi-square or Fischer's exact test and continuous by Mann–Whitney. P value $<$ .05% was statistically significant, while more than .05 and less than .1 denoted tendencies to be statistically significant and both were included into multivariate analysis. A backward stepwise logistic regression test was used to determine factors that independently affected them with confidence interval 95% from the standard of errors. Kaplan Meier curve was used to calculate survival estimates of the parameter.

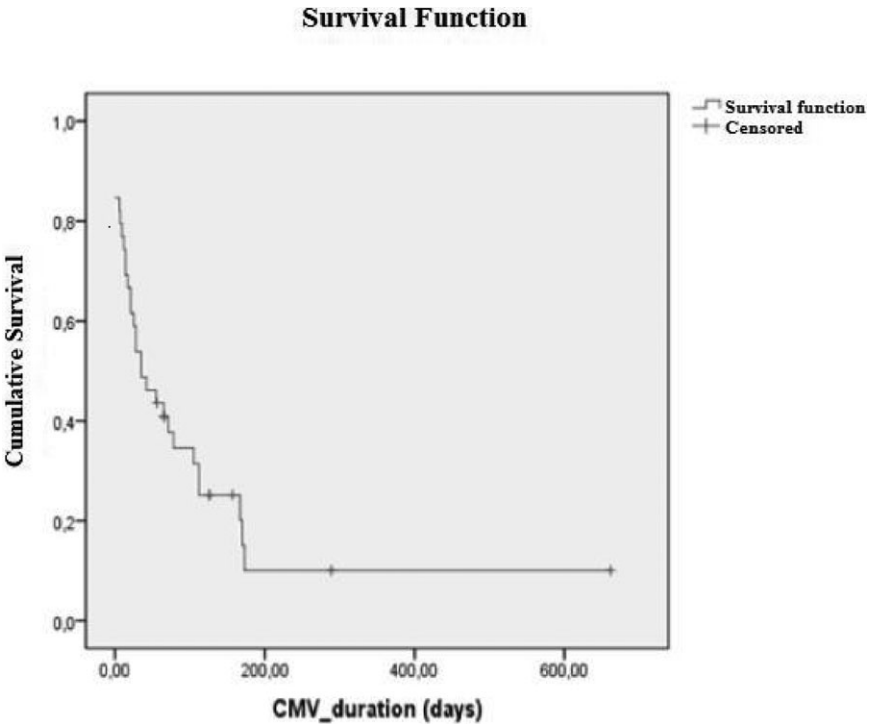

FIGURE 1 Survival of CMV infection viremia.

RESULTS

Outcome of transplant: A total of 62/81 patients (76.5%) were alive at least 6 months post-transplant and up to 78 months. Four patients died from CMV disease: interstitial pneumonitis ($n = 3$) at 60, 62, and 114 days respectively and CMV gastroenteritis ($n = 1$) at 175 days post-transplant.

Kinetics of CMV Infection: Forty patients (49.3%) had positive CMV PCR. All positive DNA copies were early infection (during the first 100 days post-transplant), of them, 11 patients (13.5%) continued to have positive CMV copies afterwards. The median CMV onset of infection was 11 days post-transplant (range 2–72 days). The median DNA copies magnitude was 4113 (range 500–1.580.000). The median estimation of duration of CMV infection viremia was 35 days, standard error 15.6 CI 95% (4.4–65.6) and its probability to progress is 10% of all CMV infection (Figure 1).

Fate of CMV Infection and Viral Load and Preemptive Treatment: Out of 40 patients with documented CMV infection, 14 patients (35%) showed spontaneous resolution. Preemptive therapy was given in 26 patients (65%). The details of preemptive treatment and response are shown in (Figure 2). Exceptions could happen according to physicians opinion depending on patient's condition. Two nongenoidentical transplant patients received preemptive therapy despite qualitative CMV < 500 DNA copies and resolved. While three genoidentical transplant patients showed spontaneous resolution of quantitative > 500 DNA copies without preemptive treatment (Figure 3). Of the 40 patients who had initial CMV infection [either qualitatively <500 cDNA $n = 26$, or > 500 cDNA $n = 14$], three groups could be distinguished:

- Group I ($n = 13$) (32.5%) with qualitative CMV < 500 cDNA. Of them $n = 11$ showed spontaneous resolution without preemptive and $n = 2$ received earlier preemptive therapy.

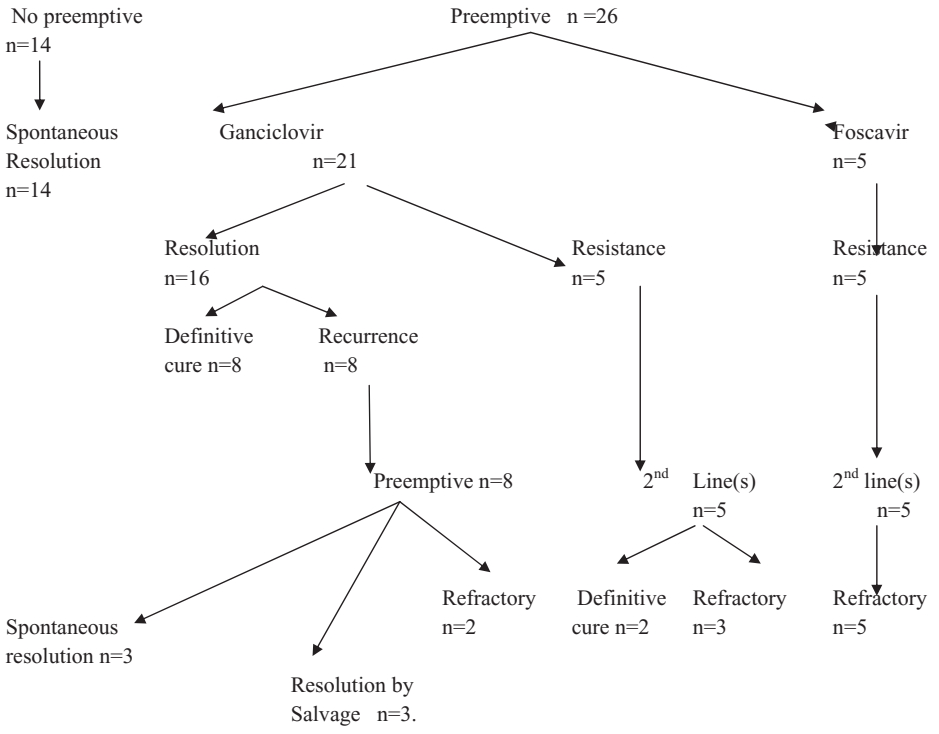

FIGURE 2 Fate of CMV infection and preemptive treatment.

- Group II qualitative < 500 cDNA did not receive preemptive and progressed to quantitative > 500 cDNA $n = 13$ (32.5%). At that time, $n = 10$ resolved with treatment and three patients resolved spontaneously without treatment.
- Group III quantitative > 500 cDNA $n = 14$ (35%) all received treatment with various fates.

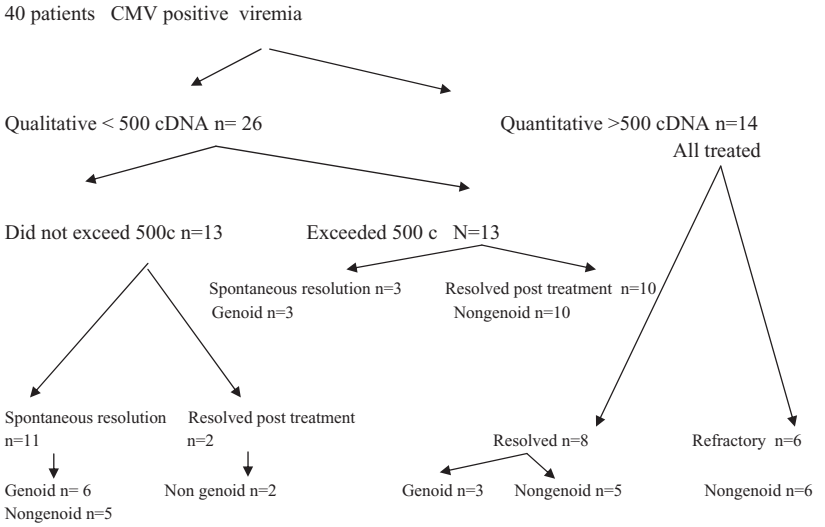

FIGURE 3 Fate of CMV Infection according to cDNA load and type of transplant.

The fate of CMV infection and viral load is shown in (Figure 3).

CMV Disease: Ten patients, (25% of CMV infection), presented with CMV disease as follows; interstitial pneumonitis $n = 5$ (clinical and radiological), gastrointestinal tract CMV (virology of diarrhea) $n = 4$, and laryngitis (clinical and secretions virology) $n = 1$. The median estimation of duration of CMV disease (end organ involvement and viremia) was 30 days SE 26,818 CI 95% (0.00–83).

Prognostic Factors: In univariate analysis (Table 3):

Statistically significant factors to CMV infection were: CMV positive recipient ($P = .002$), CMV serology R+/D+ pair ($P < .01$), non-BM stem cell source ($P < .05$), non-TBI Conditioning regimen ($P < .05$), and ATG containing regimen ($P < .01$) (Table 3a). Otherwise there were border line statistical significant factors to CMV recurrence (Table 3b). Factors influencing CMV resistance included: > 1 HLA allele mismatch ($P < .05$), negative CMV Donor ($P < .01$), CMV R+/D– pair ($P < .01$), non-BM stem cell source ($P < .05$), nongenoidentical transplant type ($P < .01$) (Table 3c). Finally, factors statistically significant toward CMV disease included: increased mismatch II-III ($P < .001$), non-BM stem cell source ($P < .01$), (Table 3d).

In multivariate analysis (Table 4), CMV R+/D– pair ($P < .05$), non-BM stem cell source ($P < .05$), ATG containing regimen ($P < .01$), and high dose steroids ($P < .01$) continued to be independent factors for CMV infection. In logistic regression, CMV infection odds ratio was 6.2 in CMVR+/D– than R–/D+, CI95%: 1.39–27.7, was 5.916 if non-BM stem cell than BM stem cells CI95%: (1.33–26.42), was 9.603 if ATG than non-ATG CI95%: (2.25–41) and 6.696 in high dose corticosteroids than low dose CI95% (1.72–26.06). However CMV infection odds ratio was 3.696 in CMV R+/D+ than R+/D– but statistically nonsignificant $P = .12$.

DISCUSSION

CMV is a major cause of morbidity and early mortality in the setting of pediatric HSCT [17]. We conducted a study on the outcome of CMV in a population of children with various hematological, immune deficiency and metabolic diseases undergoing HSCT over a period of 7 years. In our series, CMV disease occurred in 25% of patients with CMV infection. Contrarily, George et al. showed a lesser incidence; in 315 adult patients undergoing allo HSCT CMV infection was 13% [18]. Lower results were reported by Patel who showed an incidence of 5%, and Yoon as 5.9% in a homogeneous population of ALL patients [19, 20]. In our study, CMV disease was responsible for 10% mortality of CMV infection population in the 200 days post-transplant. These figures denote CMV high morbidity and mortality impact. Nearly half of the patients who had CMV disease died mainly from interstitial pneumonitis. This goes in the same direction with other experiences where mortality from CMV disease is between 30% and 100% [18, 20–23]. In order to prevent the disease, real-time PCR assay is a reliable marker for CMV monitoring. It allows an earlier diagnosis starting from Day 0 post-transplant giving the chance to start preemptive treatment [4, 19–20]. In our population, high incidence of CMV infection was documented. Almost half of our transplanted children showed DNA positive copies when tested by RT PCR technique. This is concordant with an adult study showing an incidence of CMV infection reaching 39% [18]. Contrarily, lower incidence of infection of 24% and 12.8% were reported, but with a variability in CMV cutoff definition [24, 25]. In order to prevent abuse of antiviral treatment in already self-limited disease, Najioullah suggested a cut-off of 500 c-DNA to start preemptive treatment [4]. In a study of 42 SCT recipients, a cut-off of 288 CMV DNA copies/mL levels between two consecutive PCR positive samples was an optimal value to start preemptive therapy and the analysis of the kinetics of DNA copies levels at a median of 7 days post treatment allowed the prediction of the

TABLE 3 Univariate Analysis in (a) CMV Infection, (b) Recurrence, (c) Resistance, and (d) Disease

Variable	Number	Event	Incidence%	Significance
CMV infection:				
Age mean CMV+ infection 6.77 years		40	49.38	
CMV-infection 7.97 years		41	50.61	.3
Gender				
Male	45	20	44.4	
Female	36	20	55.5	.37
Recipient CMV serology				
Positive	53	33	62.3	
Negative	28	7	25	<.01
Donor CMV serology				
Positive	60	27	45	
Negative	21	13	61.9	.212
CMV R/D serology pair				
R/D negative/positive	28	7	25	
R//D positive/negative	21	13	61.9	
R/D positive/positive	32	20	62.5	<.01
Herpes prophylaxis				
Acyclovir	49	29	59.2	
Valacyclovir	29	9	31	.08
Season winter/autumn	39	24	61.5	
Season spring/summer	42	16	38	.06
Bone marrow (BM)	59	25	42.4	
Non-BM	22	15	68.2	<.05
Genoidentical	33	12	36.4	
Non geno-identical transplant type	48	28	58.3	.07
HLA 0/I mismatch	75	35	46	
HLA II-III mismatch	6	5	83.3	.08
TBI containing regimen	23	7	30.4	
Non TBI containing regimen	58	33	56.9	<.05
ATG	56	34	60.75	
Non ATG	25	6	24	<.01
Acute graft vs. host disease	47	22	52.9	
No graft vs. host disease	34	18	46.8	.66
High Dose CTC and others	34	21	61.8	
No HDCTC	47	19	40.4	.06
CMV recurrence:				
0–1 mismatch	35	7	20	
2–3 mismatch	5	1	0	.06
Genoidentical	12	0	0	
No genoidentical	28	8	28.6	.08
High-risk disease	9	8	27.6	
Low-risk disease	11	0	0	.08
CMV resistance:				
0–1 mismatch	35	9	25.7	
2–3 mismatch	5	3	60	*<.05*
Positive CMV recipient	33	12	36.4	
Negative CMV recipient	7	0	0	.08
Positive CMV donor	27	4	14.8	
Negative CMV donor	13	8	61.5	*<.01*
CMV pair recipient +/donor –	13	8	61.5	
CMV pair recipient –/donor +	7	0	0	
CMV pair recipient +/donor +	20	4	20	*<.01*
BM	25	4	16	
Non BM stem cell source	15	8	53.3	*<0.05*
Genoidentical transplant	12	0	0	
Non geno-identical transplant type	28	12	42.9	*<.01*

TABLE 3 Univariate Analysis in (a) CMV Infection, (b) Recurrence, (c) Resistance, and (d) Disease (Continued)

Variable	Number	Event	Incidence%	Significance
CMV disease:				
0–1 mismatch	75	6	8	
2–3 mismatch	6	4	66.6	*<.001*
BM	59	3	5.1	
Non-BM stem cell source	22	7	31.8	*<.01*

response to CMV therapy [26]. In a study discussing the clinical utility of CMV real-time PCR in HSCT recipients, the cut-off value for preemptive therapy was determined to be approximately between 2×10^4 copies/mL (sensitivity, 80.0%; specificity, 50.0%) and 3×10^4 copies/mL (sensitivity, 90.0%; specificity, 70.0%) [27]. However, the optimal cut-off value for the initiation of preemptive therapy remains to be determined [28]. In our study, the onset of CMV infection was always before D+100, emphasizing its role in early post-transplant morbidity. Interestingly, the incidence of infection was as early as 2 days post-transplant, and 1/3 of infections were documented during the first week of transplant. In CMV infection, seropositive recipient is a major pejorative prognostic factor whatever the status of the donor was. This indicates a higher probability of reactivation in seropositive recipient patients receiving transplant from seronegative donor rather than CMV primary infection transmitted from a positive donor to a negative recipient. However the most pejorative combination occurred when CMV R+/D– followed by both recipient and donor were seropositive (CMV R+/D+), which was associated with increased CMV resistance. Similarly, George et al. and Patel et al. reported the same observation expressing the role of reactivation and primary infection [18–19]. In contrast, stem cells transplant from a seropositive CMV donor could be preferred for seropositive recipient requiring active transferred immunity [29]. In our study, ATG containing regimen and non-BM stem cell source were associated with increased CMV infection. This can be partially explained by increased IS. However, there is a debate about CMV infection in patients with unrelated transplants and those undergoing T cell depletion [2, 18–20, 23, 30]. Conditioning regimen not including TBI was associated with higher risk of CMV infection. The lack of allogenic lympho-hematopoietic cells reconstitution, and subsequent deficient donor antihost cytotoxic T lymphocyte (CTL)-mediated against CMV is the probable explanation as suggested by Welniak et al. [31]. Interestingly, we have noticed a tendency toward increased CMV in autumn and winter, this could direct our interest toward a seasonal variation in need to be explored thoroughly on larger scale of patients and in comparison to R–/D– pair allotransplant. Vulnerable status prior

TABLE 4 Multivariate Analysis in CMV Infection

Variable	Odds ratio	CI 95%	*P*
CMV R/D pair (+/−)	1 (reference)		
CMV R/D pair (−/+)	0.161	0.036–0.719	<.05
CMV R/D pair (+/+)	3.696	0.77–17. 68	.12
BM stem cell source	1 (reference)	1.33–26.42	<.05
No BM stem cell source	5.916		
No ATG	1 (reference)		
ATG	9.603	2.25–41	<.01
Low dose steroids < 2 mg/kg	1 (reference)		
High dose steroids ≥ 2 mg/kg	6.696	1.72–26.06	<.01

to transplant or prolonged IS was associated with increased incidence of recurrence and resistance to clearance [32, 33]. This was confirmed in our study as more CMV recurrence and/or refractoriness were seen whenever IS, non-BM stem cell source, increased mismatch in the setting of transplant were detected. This goes with our results of spontaneous resolution of viremia in patients with allo genoidentical transplant characterized by less IS, even if viral count exceeded 500 cDNA. CMV disease is influenced by R+/D–, T-cell-depletion, unrelated donor graft, and exposure to GVHD treatment [19]. In multivariate analysis, CMV infection was associated with R+/D– CMV pair, high dose corticosteroids, ATG, and non-BM stem cell source. Similar results were shown in a study where analysis of risk factors for CMV infection in 117 children who underwent allo HSCT showed higher CMV antigenemia in CMV seropositive recipients, nongenoidentical transplants, T-cell depleted grafts, ATG-containing regimens, or steroid for acute GvHD [20]. Our results were also in the line with a previous study assessing the impact of patient/donor CMV sero-status pair on outcome of 125 allo transplanted children using CMV PCR. CMV D– was the only factor associated with a significantly higher incidence of disease, suggesting that in CMV R+/D+ is associated with a reduced incidence of CMV disease and a favorable outcome following preemptive treatment [24].

Conclusion and Recommendation

CMV R+/D– pair transplant is associated with more CMV infection and resistance to preemptive treatment and needs reinforced anti CMV prophylaxis. Prolonged IS favors CMV infection and thus disease and mortality. The shorter and the lesser the IS period to control acute GvHD is, the better the results are.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Bueno J, Ramil C, Green M. Current management strategies for the prevention and treatment of cytomegalovirus infection in pediatric transplant recipients. *Paediatr Drugs*. 2002;4:279–290.

[2] Meijer E, Boland GJ, Verdonck LF. Prevention of cytomegalovirus disease in recipients of allogeneic stem cell transplants. *Clin Microbiol Rev*. 2003;16:647–657.

[3] Nakamura R, Cortez K, Solomon S, et al. High-dose acyclovir and preemptive ganciclovir to prevent cytomegalovirus disease in myeloablative and non-myeloablative allogeneic stem cell transplantation. *BMT*. 2002;30:235–242.

[4] Najioullah F, Thouvenot D, Lina B. Development of real-time PCR procedure including an internal control for the measurement of HCMV viral load. *J Virol Methods*. 2001;92:55–64.

[5] Nichols WG, Boeckh M. Recent advances in the therapy and prevention of CMV infections. *J Clin Virol*. 2000;16:25–40.

[6] Hazar V, Kansoy S, Kupesiz A, et al. High-dose acyclovir and pre-emptive ganciclovir in prevention of cytomegalovirus disease in pediatric patients following peripheral blood stem cell transplantation. *BMT*. 2004;33:931–935.

[7] Meyers JD, Flournoy N. Thomas ED Risk factors for cytomegalovirus infection after human marrow transplantation. *J Infect Dis*. 1986;153:478–488.

[8] Van Prooyen HC, Visser JJ, van Oostendorp WR, et al. Prevention of primary transfusion-associated cytomegalovirus infection in bone marrow transplant recipients by the removal of white cells from blood components with high-affinity filters. *Br J Haematol*. 1994;87:144–147.

[9] Howden BP, Michaelides A, Spelman DW, et al. Cytomegalovirus viral load monitoring after allogeneic bone marrow transplantation in patients receiving antiviral prophylaxis. *BMT*. 2003;32:795–800.

[10] Gor D, Sabin C, Prentice HG, et al. Longitudinal fluctuations in cytomegalovirus load in bone marrow transplant patients: relationship between peak virus load, donor/recipient serostatus, acute GVHD, CMV disease. *BMT*. 1998;21:597–605.

[11] Grob JP, Grundy JE, Prentice HG, et al. Immune donors can protect marrow transplant recipients from severe cytomegalovirus infections. *Lancet*. 1987;1:774–776.
[12] Enright H, Haake R, Weisdorf D, et al. Cytomegalovirus pneumonia after bone marrow transplantation. Risk factors and response to therapy. *Transplantation*. 1993;55:1339–1346.
[13] Qamruddin AO, Oppenheim BA, Guivier M, et al. Screening for cytomegalovirus (CMV) infection in allogeneic bone marrow transplantation, using quantitative whole blood polymerase chain reaction (PCR) method: analysis for potential risk factors for CMV infection. *BMT*. 2001;27:301–306.
[14] Boers AE, van der Hold R, van Esser JWJ, et al. Increased transplant related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after T-cell depleted stem cell transplantation. *Blood*. 2000;95:2040–2045.
[15] Nichols WG, Corey L, Gooley T, et al. Rising pp65 antigenemia during pre-emptive anticytomegalovirus therapy after allogeneic hematopoietic stem cell transplantation: risk factors, correlation with DNA load, and outcomes. *Blood*. 2001;97:867–874.
[16] Levy R, Najioullah F, Thouvenot D, et al. Evaluation and comparison of PCR and hybridization methods for rapid detection of cytomegalovirus in clinical samples. *J Virol Methods*. 1996;6:103–111.
[17] Ljungman P. CMV infections after hematopoietic stem cell transplantation. *BMT*. 2008;42: 70–72.
[18] George B, Pati N, Gilroy N, et al. Pre-Trans[plant cytomegalovirus (CMV) serostatus remains the most important determinant of CMV reactivation after allogeneic hematopoietic stem cell transplantation in the era of surveillance and preemptive therapy. *Transpl Infect Dis*. 2010;12:322–329.
[19] Patel SR, Ridwan RU, Ortín M. Cytomegalovirus reactivation in pediatric hemopoietic progenitors transplant: a retrospective study on the risk factors and the efficacy of treatment. *Pediatr Hematol Oncol*. 2005;27:411–415.
[20] Yoon HS, Lee JH, Choi ES, et al. Cytomegalovirus infection in children who underwent hematopoietic stem cell transplantation at a single center: a retrospective study of the risk factors. *Pediatr Transplant*. 2009;13:898–905.
[21] Castagnola E, Cappelli B, Erba D, et al. Cytomegalovirus infection after bone marrow transplantation in children. *Hum Immunol*. 2004;65:416–422.
[22] Chong AL, Clinton F, Breatnach F, et al. Cytomegalovirus infection in paediatric haemopoietic stem cell transplantation. *Ir Med J*. 2008;101:17–21.
[23] Paris C, Kopp K, King A, et al. Cytomegalovirus infection in children undergoing hematopoietic stem cell transplantation in Chile. *Pediatr Blood Cancer*. 2009;53:453–458.
[24] Matthes-Martin S, Lion T, Aberle SW, et al. Pre-emptive treatment of CMV DNAemia in paediatric stem cell transplantation: the impact of recipient and donor CMV serostatus on the incidence of CMV disease and CMV-related mortality. *BMT*. 2003;31:803–808.
[25] Bordon V, Bravo S, Van Renterghem L, et al. Surveillance of cytomegalovirus (CMV) DNAemia in pediatric allogeneic stem cell transplantation: incidence and outcome of CMV infection and disease. *Transpl Infect Dis*. 2008;10:19–23.
[26] Gimeno C, Solano C, Latorre JC, et al. Quantification of DNA in plasma by an automated real-time PCR assay (cytomegalovirus PCR kit) for surveillance of active cytomegalovirus infection and guidance of preemptive therapy for allogeneic hematopoietic stem cell transplant recipients. *J Clin Microbiol*. 2008;46:3311–3318.
[27] Choi SM, Lee DG, Lim J, et al. Comparison of quantitative cytomegalovirus real-time PCR in whole blood and pp65 antigenemia assay: clinical utility of CMV real-time PCR in hematopoietic stem cell transplant recipients. *Korean Med Sci*. 2009;24:571–578.
[28] Peres RM, Costa CR, Andrade PD, et al. Surveillance of active human cytomegalovirus infection in hematopoietic stem cell transplantation (HLA sibling identical donor): search for optimal cutoff value by real-time PCR. *BMC Infect Dis*. 2010;10:147.doi: 10.1186/1471-2334-10-147.
[29] Nichols WG, Boeckh M, Carter RA, et al. Transferred herpes simplex virus immunity after stem-cell transplantation: clinical implications. *J Infect Dis*. 2003;187:801–808.
[30] Walker CM, van Burik JA, De For TE, Weisdorf DJ. Cytomegalovirus infection after allogeneic transplantation: comparison of cord blood with peripheral blood and marrow graft sources. *Biol Blood and Marrow Transplant*. 2007;13:1106–1115.
[31] Welniak LA, Blazar BR, Anver MR, et al. Opposing roles of interferon-gamma on CD4+ T cell-mediated graft-versus-host disease: effects of conditioning. *Biol Blood Marrow Transplant*. 2000;6:604–612.
[32] Meijer E, Dekker AW, Verdonck LF. Influence of antithymocyte globulin dose on outcome in cytomegalovirus-seropositive recipients of partially T celldepleted stem cell grafts from matched-unrelated donors. *Br J Haematol*. 2003;12:473–476.
[33] Almyroudis NG, Jakubowski A, Jaffe D, et al. Predictors for persistent cytomegalovirus reactivation after T-cell-depleted allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis*. 2007;9:286–294.