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ORIGINAL ARTICLE

Cytomegalovirus infection and disease after allogeneic hematopoietic stem cell transplantation: experience in a center with a high seroprevalence of both CMV and hepatitis B virus

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Abstract Cytomegalovirus (CMV) infection and disease are important concerns after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The similarity of hepatitis B virus (HBV) and CMV with regards to their chronic viral persistence and potential reactivation at the time of impaired cellular immunity has raised clinicians' interest in the occurrence and association between them among patients receiving allo-HSCT; however, only limited data have been obtained from a high seroprevalence region of both CMV and HBV. We monitored 117 adult allo-HSCT patients with both CMV polymerase chain reaction and pp65 antigenemia assay weekly until day 100. In 91.8% of our cases, donors and recipients were both CMV seropositive, and 13.7% of the patients were positive for HBV surface antigen. The incidences of CMV infection and disease were 45.3% and 6.8%, respectively. Grade II–IV acute graft-versus-host disease and anti-thymocyte globulin-containing conditioning regimen were associated with an increased risk of CMV infection in a multivariate analysis (hazard ratio 3.02, 95% CI 1.68–5.42, $p<0.001$ and hazard ratio 5.29, 95% CI 2.57–10.8, $p<0.001$). No survival disadvantage was found in patients who developed CMV infection ($p=0.699$) and CMV disease ($p=0.093$). No clinically significant HBV reactivation was found, and the underlying HBV infection in donors or recipients before allo-HSCT did not increase the risk of CMV infection and CMV disease and did not influence survival after allo-HSCT.

Keywords Cytomegalovirus · Hepatitis B virus · Preemptive therapy · Hematopoietic stem cell transplantation · Taiwan

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Introduction

Cytomegalovirus (CMV) infection/disease remains one of the important causes of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). It results from either reactivation of CMV in seropositive recipients or primary infection from seropositive donors to seronegative recipients [1]. In the past decade, there have been many advances in the development of antiviral agents, prophylaxis strategies, and diagnostic techniques for monitoring CMV infection status after allo-HSCT. Two strategies, prophylactic and preemptive therapy, are currently used. The preemptive therapy, defined as patients receiving antiviral therapy when

they have evidence of active but asymptomatic infection, is more widely used than prophylactic therapy and has decreased the incidence of CMV disease from 20–30% to 3–14% by day 100 [2–8]. The most widely used assays for the diagnosis and surveillance of CMV infection, which are the basis for preemptive therapy, are pp65 antigenemia assay and polymerase chain reaction (PCR) [3–10]. It is recommended that all allo-HSCT patients, regardless of whether they received CMV prophylaxis or not, be monitored for CMV infection weekly by either PCR or antigenemia assay [11].

Despite the significant decline in the incidence of CMV disease after implementation of preemptive strategy for allo-HSCT patients, overall survival is associated with the pretransplant CMV serostatus [2, 12–19]. The CMV-seropositive recipients and seronegative recipients of a seropositive graft appear to have a survival disadvantage. Transplants from unrelated or human leukocyte antigen (HLA)-mismatched donors and/or recipients of T cell-depleted allografts were predominantly affected by CMV [12–14, 18–20]. In Taiwan, the prevalence of CMV is much higher than western populations [21–25], with only 8.3% found to be CMV seronegative in a population-based survey [21]. This has led to a high rate of CMV donor seropositivity and recipient seropositivity (D+/R+) in our institute. That a high prevalence rate of CMV D+/R+ status might associate with unfavorable outcomes in our patient population is predicted, but deserves to be further investigated. Taiwan is also known as a high prevalence area of hepatitis B virus (HBV) and hepatitis C virus (HCV) [26–28], with both having similar characteristics to CMV with regards to their tendency towards chronic viral persistence and their potential reactivation at the time of impaired cellular immunity. However, little is known about the association between the underlying chronic hepatitis and the CMV reactivation after allo-HSCT. Here we analyzed CMV infection and CMV disease in adult patients undergoing allo-HSCT in a Taiwanese medical center, using both CMV PCR and CMV pp65 antigenemia assay weekly after engraftment until day 100. The clinical outcomes, risk factors for CMV infection and disease, the effects of preemptive therapy, and the association with underlying hepatitis were demonstrated.

Patients and methods

Patients and transplantation procedures

One hundred and seventeen adult patients undergoing allo-HSCT in Kaohsiung Medical University Hospital from January 1997 to December 2008 were reviewed. All recipients were monitored with CMV PCR weekly until posttransplantation day 100. The CMV pp65 antigenemia assay was examined simultaneously as of August 1999; therefore, 97 patients were monitored weekly for both CMV PCR and CMV pp65 antigenemia assay. These 117 patients formed the database for analysis after obtaining informed consent and approval from the Institutional Review Boards in Kaohsiung Medical University Hospital. The median follow-up was 41 months (range 1 to 144 months). All packed red blood cells and platelets were transfused using leukocyte-depleting filters. Only CMV-seronegative blood product was given to D−/R− recipients. Acyclovir was used as viral prophylaxis from day −7 to day −1 for all recipients except 13 (11.1%) seropositive recipients who received ganciclovir. No routine viral prophylactic agents were used in the posttransplant period. All patients received standard graft-versus-host disease (GVHD) prophylaxis with methotrexate and cyclosporine. Acute and chronic GVHD was graded according to standard criteria [29]. Steroid was used in patients with grade II–IV acute GVHD with varying durations. The clinical characteristics of these patients are listed in Table 1.

Serostatus of CMV, HBV, and HCV before allo-HSCT

The immunoglobin M (IgM) and IgG against CMV were recorded between donors and recipients before allo-HSCT. The complement fixation method was adopted for the CMV serostatus survey in 32 patients before May 2001 and was replaced by enzyme-linked fluorescent assay (VIDAS CMV IgM and IgG kit, Biomerieux, France) for more accurate sensitivity. Therefore, 85 recipients and donors were examined by enzyme-linked fluorescent assay and were used for the analysis of CMV serostatus before allo-HSCT. The hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (HBsAb) were determined by enzyme immunoassay between donors and recipients before allo-HSCT. The HCV antibody (anti-HCV) was detected simultaneously with the commercially available ELISA kit (Abbott, Chicago, IL, USA). Baseline serum alanine aminotransferase and abdominal echography were also recorded.

CMV pp65 antigenemia assay and CMV PCR

The CMV antigenemia assay was performed using the commercially available kit (CINAkit, Argene, France), which allowed the indirect immunofluorescence detection of lower matrix protein pp65 of CMV in peripheral blood leukocytes. A single stained cell seen per slide indicated a positive antigenemia, with a sensitivity estimated to be one positive cell per 1×10^5 cells. The peripheral blood leukocyte DNA was extracted by GFX genomic blood DNA purification kit (Amersham Biosciences), and CMV DNA was detected by PCR using primers and conditions as

Table 1 Basic characteristics of adult patients undergoing allo-HSCT (n=117)

Characteristics	Number of patients (%)
Age (years)	
Median	33
Range	13–61
Sex	
Male	63 (53.8)
Female	54 (46.2)
Underlying disease	
Acute myeloid leukemia	37 (31.6)
Acute lymphoblastic leukemia	29 (24.8)
Chronic myeloid leukemia	23 (19.7)
Myelodysplastic syndrome	4 (3.4)
Multiple myeloma	3 (2.6)
Non-Hodgkin's lymphoma	4 (3.4)
Severe aplastic anemia	14 (12.0)
Others[a]	3 (3.4)
Stem cell source	
Bone marrow	13 (11.1)
Peripheral blood	104 (88.9)
Donor	
HLA-identical sibling	76 (65.0)
Alternative	41 (35.0)
Ganciclovir prophylaxis before HSCT	
Yes	13 (11.1)
No	104 (88.9)
Conditioning	
Myeloablative	112 (95.7)
Reduced intensity regimen	5 (4.3)
TBI-containing regimen	109 (93.2)
Non-TBI regimen	8 (6.8)
ATG-containing regimen	15 (12.8)
Non-ATG-containing regimen	102 (87.2)
CD34+ cells ($\times 10^6$/kg)	
Median	7.62
Range	1.70–23.60
Acute GVHD	
Grades 0–I	76 (65.0)
Grades II–IV	41 (35.0)

[a] Others included one pure red cell aplasia, one large granulocytic leukemia, and one paroxysmal nocturnal hemoglobinuria

previously described [30]. The CMV PCR was qualitative rather than quantitative, with a sensitivity estimated to be 10 copies of the target sequence in a single PCR.

CMV infection, CMV disease, and preemptive therapy

The definition of CMV infection and CMV disease was based on the criteria reported previously [31]. Briefly, CMV infection was defined as isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen. Therefore, the presence of CMV pp65 antigenemia or two consecutive positive results of CMV PCR was defined as CMV infection and formed the basis of preemptive therapy [2, 6, 10]. CMV disease was defined by the presence of clinical symptoms or signs of end organ disease, combined with the evidence of CMV infection in a tissue biopsy specimen [31]. In cases of CMV pneumonia, the bronchoalveolar lavage fluid was obtained, and in cases of gastroenteritis or hepatitis, the biopsy specimens were obtained for extensive pathological and microbiological examination to establish a definite diagnosis of CMV disease.

Preemptive therapy with ganciclovir was initiated when CMV infection was first documented, proven by either positive CMV pp65 antigenemia or two consecutive positive results of CMV PCR. Ganciclovir was administered at a dose of 5 mg/kg twice daily for 2 weeks followed by 5 mg/kg once daily. The treatment was stopped when two consecutive negative results on both CMV PCR and CMV pp65 antigenemia were obtained. In cases of CMV disease, ganciclovir 5 mg/kg twice daily was initiated for 21 days, combined with CMV immunoglobin 500 mg/kg every other day for 20 days, followed by ganciclovir 5 mg/kg/day until two consecutive negative results of CMV PCR and CMV pp65 antigenemia were obtained.

Statistical methods

The following variables were analyzed to determine the risk factors for CMV infection and CMV disease, including age, gender, stem cell source, disease status at allo-HSCT, donor type, conditioning regimens (including total body irradiation-contained, anti-thymocyte globulin (ATG)-contained, or reduced intensity regimens), viral prophylaxis, CD34+ cell count, acute GVHD, and underlying chronic hepatitis B or C status. The categorical variables were analyzed by chi-square test and Fisher's exact test. Continuous variables were analyzed by independent t test for approximate normal distribution data and by nonparametric Wilcoxon rank-sum test for other distributions. The Kaplan–Meier analysis was performed to estimate survival, and the probabilities between subgroups were compared by log-rank test. Time to first positive evidence of CMV infection was also evaluated using the Kaplan–Meier method. Multivariate Cox proportional hazards regression analysis was performed to identify poor prognostic factors for CMV infection and disease. All statistics were calculated using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 was considered to indicate a statistically significant difference.

Results

CMV infection and CMV disease after allo-HSCT

For those receiving enzyme-linked fluorescent assay to determine CMV serostatus before allo-HSCT, CMV IgG was positive in 96.4% (82 of 85) recipients and 92.9% (79 of 85) donors. No CMV IgM was detected in any recipients or donors. In 85 pairs of donors/recipients, 78 (91.8%) were donor positive and recipient positive (D+/R+), 4 (4.7%) were donor negative and recipient positive (D−/R+), 1 (1.2%) was donor positive and recipient negative (D+/R–), and 2 (2.4%) were donor negative and recipient negative (D−/R−). Considering serostatus before allo-HSCT, CMV infection was seen in 52.6% (41 of 78) of D+/R+ patients, in 25% (1 of 4) D−/R+ transplantations, in 100% (1 of 1) D+/R− transplantation, and was not seen in 2 D−/R− transplantations.

All patients were engrafted successfully after allo-HSCT. The median time of WBC engraftment is 11 days (range 7–22 days). Neither positive CMV PCR nor positive CMV pp65 antigenemia was found during preengraftment period. The overall incidence of CMV infection after allo-HSCT was 45.3% (53 of 117) at a median of 34 days (range 7–167 days). In 53 patients with CMV infection, the overall survival rate is 56.7%. The most common causes of death in patients with CMV infection included disease relapse (34.8%), acute GVHD (21.7%), neutropenic sepsis (13.0%), chronic GVHD (13.0%), and CMV disease (8.7%). The CMV infection rate detected by CMV PCR was 41.0% (48 of 117) at a median of 36 days (range 7–89 days). The CMV pp65 antigenemia assay was examined simultaneously in 97 of 117 patients after August 1999, and a positive result was obtained in 21.6% patients (21 of 97) at a median of 32 days (range 7–167 days). There were 10 (8.54%) patients who have single positive CMV PCR. No evidence of CMV disease was found in these 10 patients at the time of positive PCR, and none of them with concurrent positive pp65 antigenemia. No preemptive therapy was performed in these patients except concurrent positive pp65 antigenemia or a second positive PCR. In 48 of 53 CMV-infected patients examined using the two methods, positive results of both PCR and pp65 antigenemia were found in 35.4% of patients (17 of 48). The pp65 antigenemia appeared 1 week earlier than CMV PCR in eight patients, at the same time in eight patients, and 1 week later in one patient. Twenty-five patients (52.1%) were found to have results of positive CMV PCR and negative antigenemia, and six patients (12.5%) were found to have positive antigenemia and negative CMV PCR. These data suggest that using a combination of both CMV PCR and CMV pp65 antigenemia assay as a monitoring tool could increase the detection rate of CMV infection for preemptive therapy.

CMV disease was diagnosed in eight patients (6.8%) at a median of 35 days (range 15–73 days), including six CMV pneumonia and two CMV enterocolitis. Tissue sampling for pathology review was performed in all patients with CMV disease, including BAL and bronchoscopic biopsy for CMV pneumonitis and endoscopic biopsy for CMV enterocolitis. Five patients died and two of them died which can be attributed to CMV disease (both had CMV pneumonia). Five patients (62.5%) responded to treatment with ganciclovir and CMV immunoglobulin. The attributable mortality rate of CMV disease in our cohort was 1.7% (two of 117). The causes of death in other three patients included one patient with chronic GVHD, one with neutropenic sepsis, and one with relapse.

Risk factors for CMV infection and CMV disease

The analysis of potential risk factors for CMV infection is shown in Table 2. In a univariate analysis, patients with grade II–IV acute GVHD ($p<0.001$), transplantation from alternative donors ($p=0.006$), and ATG-containing conditioning regimens ($p=0.001$) showed an increased risk in the development of CMV infection. The age, sex, disease status at allo-HSCT, stem cell source, TBI-containing regimen, reduced intensity regimen, ganciclovir prophylaxis, and CD34+ cell count showed no significant difference in association with CMV infection (Table 2). There were 41 recipients (35.0%) who developed grade II–IV acute GVHD. The cumulative incidence of CMV infection at day 100 was 73.2% (30 of 41) in patients with grade II–IV acute GVHD compared to 30.3% (23 of 76) in patients with grade 0–I acute GVHD (log-rank $p=0.0001$, Fig. 1a). In a multivariate analysis, grade II–IV acute GVHD (hazard ratio 3.02, 95% CI 1.68–5.42, $p<0.001$) and ATG-containing conditioning regimen (hazard ratio 5.29, 95% CI 2.57–10.8, $p<0.001$) were found to independently increase risk of CMV infection (Table 3).

The potential risks for CMV disease were also analyzed. Only grade II–IV acute GVHD was identified to show an increased risk in the development of CMV disease ($p=0.003$) from a univariate analysis. The age, sex, disease status at allo-HSCT, stem cell source, alternative donor, TBI-containing regimen, reduced intensity regimen, ATG-containing regimen, ganciclovir prophylaxis, and CD34+ cell count had no significant association with CMV disease. The cumulative incidence of CMV disease at day 100 was 17.1% (7 of 41) in patients with grade II–IV acute GVHD compared to 1.3% (1 of 76) in patients with grade 0–I acute GVHD (log-rank $p=0.0013$, Fig. 1b).

Table 2 Risk factors evaluated for the influence on probability of CMV infection

Factors	Number of patients	Number of CMV infection	*p* value[a]
Age	117	53	0.33
Sex			0.85
Male	63	28	
Female	54	25	
Underlying disease status at HSCT			0.58
Low risk[b]	59	25	
High risk	58	28	
Donor			0.006
Identical sibling donor	76	27	
Alternative donor[c]	41	26	
Conditioning regimens			0.66
Myeloablative	112	50	
Reduced intensity conditioning	5	3	
TBI-containing regimen			0.99
Yes	109	49	
No	8	4	
ATG-containing regimen			0.001
Yes	15	13	
No	102	40	
Stem cell source			0.25
Bone marrow	13	8	
Peripheral blood	104	45	
Viral prophylaxis before HSCT			0.77
Ganciclovir	13	5	
Acyclovir	104	48	
Acute GVHD			<0.001
Grades 0–I	76	23	
Grades II–IV	41	30	
CD34+ cell count	117	53	0.23

[a] Independent *t* test was used for continuous variables

[b] Acute leukemia in first remission, CML in the first chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblast, and severe aplastic anemia were defined as low-risk diseases

[c] Donors other than HLA-identical siblings were defined as alternative donors

Impact of CMV infection and CMV disease on survival

The probability of survival for all patients was 82.9% (97 of 117) at 100 days and 65.0% (76 of 117) at 1 year. Patients who developed CMV infection, either detected by CMV antigenemia assay or by consecutive CMV PCR, showed no statistically significant difference in 100-day survival (log-rank $p=0.727$) and overall survival (log-rank $p=0.699$, Fig. 2a). Patients who developed CMV disease showed a significant survival disadvantage before day 100 (log-rank $p=0.012$) and a borderline negative impact on overall survival compared to those who did not develop CMV disease (log-rank $p=0.093$, Fig. 2b).

Association between CMV infection and disease and underlying chronic hepatitis status

HBsAg was detected in 13.7% (16 of 117) of recipients and in 11.1% (13 of 117) of donors. All positive HBsAg patients showed negative HBsAb. No prophylactic lamivudine or entecavir was used after transplant within the analysis period. Anti-HCV was detected in 2.6% (3 of 117) of recipients and 1.7% (2 of 117) of donors. None of our recipients had clinically significant hepatitis B and hepatitis C disease. There was no significant difference in the development of CMV infection between HBsAg-positive and HBsAg-negative recipients ($p=0.79$) and between HBsAg-positive and HBsAg-negative donors ($p=0.77$). There was also no significant difference in the development of CMV infection between anti-HCV-positive and anti-HCV-negative recipients ($p=0.59$) and between anti-HCV-positive and anti-HCV-negative donors ($p=0.99$).

There was no significant difference in the occurrence of CMV disease between HBsAg-positive and HBsAg-negative recipients ($p=0.70$) and between HBsAg-positive and HBsAg-negative donors ($p=0.62$). Recipients with positive HBsAg showed no statistically significant difference in overall survival (log-rank $p=0.98$, Fig. 3a). Patients with both CMV infection and positive HBsAg showed no survival disadvantage at day 100 (log-rank $p=0.64$) and overall survival (log-rank $p=0.79$) when compared with other patients. Interestingly, in cases of CMV infection, the HBsAg-positive group shows a somewhat better survival compared to the HBsAg-negative group, but it is not statistically significant (log-rank $p=0.69$, Fig. 3b).

Discussion

The CMV seroprevalence rate varies among different study subjects. For example, the CMV seroprevalence in women of child-bearing age ranged from 30.4% to 89.7% in different countries and areas [22–25]. The D+/R+ rate was usually around 20% to 50% in studies investigating the impact of CMV serostatus on allo-HSCT [12, 14, 15, 32]. The prevalence of CMV-seropositive patients before allo-HSCT was 96.4% and 91.8% was D+/R+ transplantation in our cohort, confirming a high CMV seroprevalence in southern Taiwan and suggesting that there were more high-risk recipients undergoing allo-HSCT in this region. Our study offers a general survey from a high prevalent area of CMV infection and disease status after allo-HSCT.

In literature, the cumulative incidence of CMV infection after allo-HSCT varied from 24% to 84.3% in different

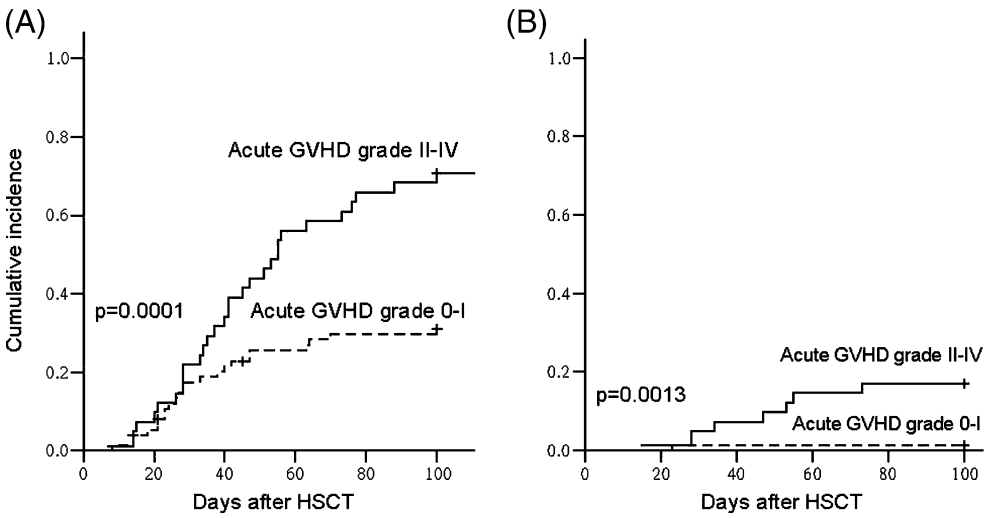

Fig. 1 The cumulative incidence of CMV infection (**a**) and CMV disease (**b**) at day 100 after allo-HSCT categorized by the severity of acute GVHD. Patients who developed grade II–IV acute GVHD (n=41) had a higher risk of CMV infection (p=0.0001) and CMV disease (p=0.0013) compared with grade 0–I acute GVHD (n=76)

populations [2–7, 10, 33–35]. In our observations, the incidence rate of CMV infection was 45.3%, which was similar to that reported, especially in East Asia [33–35]. The incidence of CMV disease was 6.8% and the mortality rate due to CMV disease was 1.7% in our recipients, which was also similar to that previously reported [3–7, 10, 33–37]. Although a high pretransplant seropositivity was noted in our cohort, the incidence of CMV infection and CMV disease did not differ much when compared with other studies. Further survival analysis also showed no survival disadvantage in patients who developed CMV infection and CMV disease in our cohort.

Risk factors for CMV infection and CMV disease after allo-HSCT include seropositive recipients, transplant from unrelated or HLA-mismatched donor, presence of acute GVHD, T cell-depleted transplant, use of total body irradiation in the conditioning regimen, and advanced age [1, 2, 11–20, 33–37]. In particular, GHVD, donor type, and pretransplant serostatus are considered to be strong indicators. In our analysis, grade II–IV acute GVHD and the use of ATG as part of conditioning regimens significantly increased the risk of CMV infection in a multivariate analysis. Grade II–IV acute GVHD is also a risk factor for CMV disease, consistent with other studies, and suggests that close CMV monitoring is important in patients who developed moderate to severe acute GVHD and ATG-containing conditioning regimens before allo-HSCT [1, 2, 11, 33–37]. Transplants from alternative donors, which were known as a risk factor for CMV infection in other studies, showed increase risk of CMV infection in a univariate analysis but not in a multivariate analysis. Since ATG was widely used for transplantations from alternative donors, close monitoring of CMV infection status is still important in these patients. Many factors have been suggested for the poor outcome among CMV-seropositive recipients. They included the direct effects of CMV, like breakthrough CMV disease or late CMV disease, and the indirect effects like increased risk of bacterial or fungal infection or ganciclovir-induced neutropenia [13, 31]. The impact of donor seropositivity remains controversial, especially for seropositive recipients [12–14, 32]. In our analysis, there is no difference in the rate of CMV infection and overall survival between the D+/R+ group and the D−/R+ group, suggesting that the donor's serostatus of CMV may not be important when the recipients are CMV seropositive.

CMV PCR and pp65 antigenemia were commonly used for monitoring CMV infection after allo-HSCT. In our patients with CMV infection, only 35.4% showed positive results from both examinations. There were 52.1% patients with positive CMV PCR but negative pp65 antigenemia, suggesting that utilizing only CMV pp65 antigenemia may not be sufficient to monitor CMV infection or disease. Since pp65 is an early antigen to be detected, and the result is correlated with the time of blood sampling, more

Table 3 Multivariate analysis comparing CMV infection risk among patients receiving allo-HSCT

Variable	Number of patients	Relative risk (95% CI)	p value
Donor			
Identical sibling donor	76	1.00	
Alternative donor	41	1.13 (0.60–2.16)	0.70
ATG-containing regimen			
No	102	1.00	
Yes	15	5.29 (2.57–10.8)	<0.001
Stem cell source			
Bone marrow	13	1.00	
Peripheral blood	104	1.27 (0.57–2.86)	0.56
Acute GVHD			
Grades 0–I	76	1.00	
Grades II–IV	41	3.02 (1.68–5.42)	<0.001

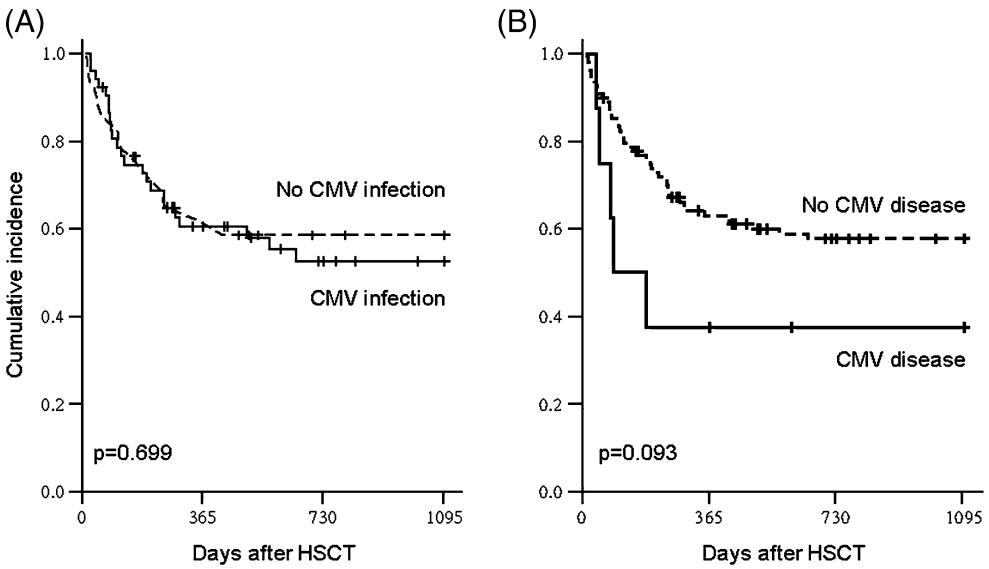

Fig. 2 The difference of overall survival in patients with the development of CMV infection (**a**) and CMV disease (**b**) after allo-HSCT. No significant survival difference was noted in patients who developed CMV infection (n=53) and CMV disease (n=8) compared with those who did not develop CMV infection (n=64) and CMV disease (n=109) after allo-HSCT (log-rank p=0.699 and 0.093, respectively)

frequent sampling may be considered to increase the detection rate. However, if weekly sampling will be performed, our experience suggests that a combination of both pp65 and PCR survey may increase the detection rate of CMV infection to allow earlier preemptive therapy.

Ganciclovir prophylaxis before and after allo-HSCT has been shown to reduce the incidence and severity in seropositive recipient [38], and there was no difference in risk of CMV disease and the survival rate between ganciclovir prophylaxis and preemptive therapy [4]. Since no apparent difference in CMV infection and disease was found in our patients using ganciclovir prophylaxis, the benefit of ganciclovir prophylaxis in our R+ patients is uncertain. Furthermore, using a monitoring tool with both CMV PCR and pp65 as the basis of preemptive therapy, the incidence of CMV infection and disease is not higher when compared with other studies. Based on these findings, we assume that in our high seroprevalence area, acyclovir may be sufficient as a virus prophylaxis before allo-HSCT, and well-monitored protocol should be performed to ensure preemptive therapy. Future studies and additional data are needed to access the benefit of ganciclovir prophylaxis in areas of high endemicity, especially for more high-risk patients like transplants from alternative donors.

In our analysis, the prevalence of HBsAg and anti-HCV in recipients and donors is similar to the general population in Taiwan [26–28]. Our results revealed that there was no significant association between the CMV reactivation and the serostatus of HBsAg and anti-HCV of donors and recipients. In these data, underlying HBV and HCV serostatus did not appear to influence the risk of CMV infection and CMV disease after allo-HSCT. No acute exacerbation of hepatitis B was noted, and there was no survival difference between the HBsAg-positive group and

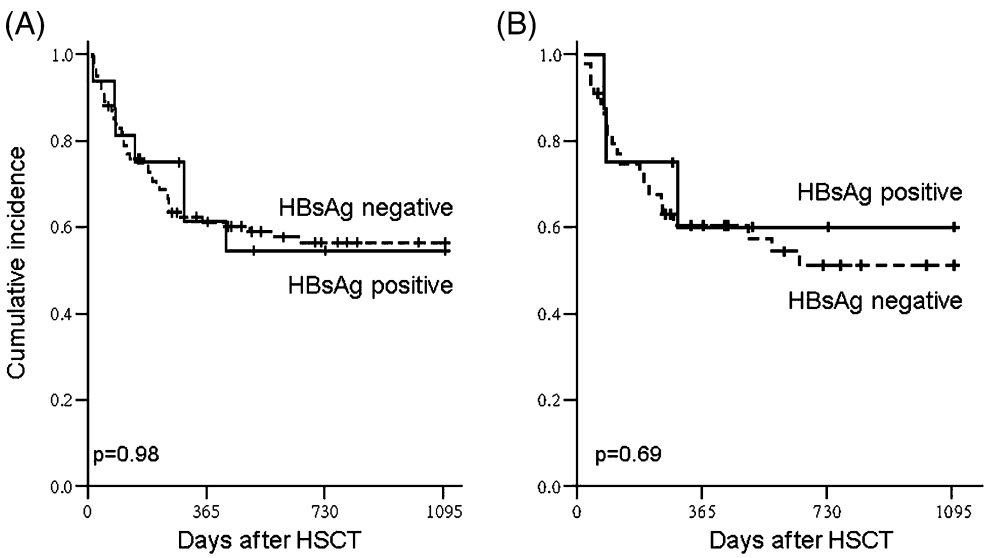

Fig. 3 The difference of overall survival in the HBsAg-positive (n=16) and HBsAg-negative (n=101) groups in all patients (**a**) and in patients with CMV infection (**b**) after allo-HSCT. No significant survival difference was noted in all patients and in patients with CMV infection with or without HBsAg (log-rank p=0.98 and 0.69, respectively)

HBsAg-negative group. In a high HBV prevalence area, HBsAg seropositivity did not appear to be a concern for CMV infection and disease in patients receiving allo-HSCT.

In conclusion, close monitoring of CMV infection and early preemptive antiviral therapy are important in a high seroendemic area, especially in patients who developed grade II to IV acute GVHD and ATG-containing conditioning regimens. A combination of both CMV pp65 antigenemia assay and CMV PCR could increase the detection rate and allow early preemptive therapy. No survival disadvantage in patients with CMV infection or CMV disease suggested the effectiveness of well-monitored protocol and preemptive therapy. HBV and HCV infection before allo-HSCT did not appear to alter the risk of CMV infection and CMV disease after allo-HSCT.

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