ORIGINAL ARTICLE VIROLOGY

Patients with refractory cytomegalovirus (CMV) infection following allogeneic haematopoietic stem cell transplantation are at high risk for CMV disease and non-relapse mortality

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Abstract

Pre-emptive therapy is an effective approach for cytomegalovirus (CMV) control; however, refractory CMV still occurs in a considerable group of recipients after allogeneic haematopoietic stem cell transplantation (allo-HSCT). Until now, hardly any data have been available about the clinical characteristics and risk factors of refractory CMV, or its potential harmful impact on the clinical outcome following allo-HSCT. We studied transplant factors affecting refractory CMV in the 100 days after allo-HSCT, and the impact of refractory CMV on the risk of CMV disease and non-relapse mortality (NRM). We retrospectively studied 488 consecutive patients with CMV infection after allo-HSCT. Patients with refractory CMV in the 100 days after allo-HSCT had a higher incidence of CMV disease and NRM than those without refractory CMV (11.9% vs. 0.8% and 17.1% vs. 8.3%, respectively). Multivariate analysis showed that refractory CMV infection in the 100 days after allo-HSCT was an independent risk factor for CMV disease (hazard ratio (HR) 10.539, 95% CI 2.467–45.015, p 0.001), and that refractory CMV infection within 60–100 days after allo-HSCT was an independent risk factor for NRM (HR 8.435, 95% CI 1.511–47.099, p 0.015). Clinical factors impacting on the risk of refractory CMV infection included receiving transplants from human leukocyte antigen-mismatched family donors (HR 2.012, 95% CI 1.603–2.546, p <0.001) and acute graft-versus-host disease (HR 1.905, 95% CI 1.352–2.686, p <0.001). We conclude that patients with refractory CMV infection during the early stage after allo-HSCT are at high risk for both CMV disease and NRM.

Keywords: Allogeneic haematopoietic stem cell transplantation, CMV disease, CMV infection, non-relapse mortality, refractory CMV
Original Submission: 11 February 2015; **Revised Submission:** 10 May 2015; **Accepted:** 8 June 2015
Editor: G. Antonelli
Article published online: 17 June 2015

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Introduction

Viral infections remain important causes of morbidity and mortality after allogeneic haematopoietic stem cell transplantation (allo-HSCT), especially cytomegalovirus (CMV) infection. A pre-emptive therapy approach enabled almost complete prevention of CMV disease; however, refractory CMV infection still developed in a subgroup of patients after allo-HSCT [1–6]. Refractory CMV infection, which was defined as CMV infection persisting for >2 weeks in spite of standard antivirus therapy, occurred in >40% of recipients after allo-HSCT, leading to a prolonged medication treatment time [1,5,7]. Until now, hardly any data have been available about the clinical characteristics and risk factors of refractory CMV infection, or regarding its potentially harmful impact on the clinical outcome after allo-HSCT. It has been well documented that clinical factors such as acute graft-versus-host disease

http://dx.doi.org/10.1016/j.cmi.2015.06.009

(GVHD), donor–recipient serostatus and receiving a graft from an unrelated donor are risk factors for CMV infection following allo-HSCT [2,8–11]. Whether these clinical factors have an impact on refractory CMV infection is unknown. Almyroudis *et al.* [6] demonstrated that persistent CMV reactivation occurred in 39% of T-cell-depleted haematopoietic stem cell transplants, despite treatment with currently available antivirals, and that the maximum CMV level was associated with persistent CMV reactivation. Ljungman *et al.* [8] demonstrated that the viral load kinetics after initiation of antiviral therapy were predictive of the risk of developing CMV disease, and that, in a group of 162 patients, the patients whose viral load decreased more slowly in the first week had a higher risk of CMV disease. Beyond that, hardly any data are available about the relationship between refractory CMV infection and CMV disease or mortality. Therefore, we performed a retrospective study to analyse the clinical characteristics and risk factors of refractory CMV infection, and the effects of refractory CMV infection on the risk of CMV disease and mortality, in a series of consecutive allo-HSCT recipients.

Materials and methods

Patient enrolment

Between January 2013 and July 2014, 488 patients who underwent allo-HSCT from human leukocyte antigen (HLA)-matched siblings or mismatched family donors with a myeloablative-conditioning regimen in the Peking University People's Hospital, Institute of Haematology, and who experienced CMV DNAemia during the first 100 days after allo-HSCT, were enrolled. Preliminary data showed that most of the CMV infections (50–70%) following allo-HSCT were identified in the 'early' period (<100 days) following the infusion of the graft, and late-onset CMV infections were strongly correlated with the early-onset cases [2,12]. Therefore, we focused on the patients who had CMV infections during the first 100 days after allo-HSCT. The institutional review board at the hospital approved the protocol, and all patients or their guardians signed consent forms approved by the institutional review board. The patients' characteristics are shown in Table 1.

Transplant protocol

The donor selection and the transplant protocol were carried out as previously reported [13–15]. Details are shown in Doc. S1. Donor lymphocyte infusion was performed for prophylactic or prevention purposes, according to previously reported criteria [16,17].

TABLE 1. Patient characteristics

Characteristic	All patients (n = 488)
Age of recipients (years), median (range)	29 (2–61)
Gender: no. of patients	
Male/female	280/208
Underlying disease, no. of patients	
Acute myeloid leukaemia	203
Acute lymphocyte leukaemia	158
Myelodysplastic syndrome	60
Chronic myeloid leukaemia	26
Severe aplastic anaemia	26
Lymphoma	9
Myeloma	4
Fanconi anaemia/myelofibrosis	1/1
Donor type, no. of patients	
Matched sibling	91
Mismatched family	397
Conditioning regimen, no. of patients	
BU/CY + ATG	360
TBI + CY + ATG	22
CY + ATG	8
BU + Flu + ATG	5
BU/CY	79
TBI + CY	6
TBI + Flu + CY	2
GVHD prophylaxis, no. of patients	
MMF + CsA + MTX	488
Infused nuclear cells (10^8/kg), median (range)	7.62 (1.63–20.07)
Infused $CD34^+$ cells (10^6/kg), median (range)	2.61 (0.33–84.05)
CMV-specific T-cell infusion, no. of patients	16
Donor lymphocyte infusion, no. of patients	65
Follow-up (days), median (range)	326 (34–916)

ATG, antithymocyte globulin; BU, busulfan; CMV, cytomegalovirus; CsA, cyclosporine A; CY, cyclophosphamide; Flu, fludarabine; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil; MTX, methotrexate; TBI, total body irradiation.

Sample preparation

Before transplantation, serum samples from recipients and donors were analysed by ELISA (Diesse, Sienna, Italy) for CMV-specific IgG antibodies. Heparin-treated peripheral blood (PB) was collected from the recipients weekly from day 15 to day 90, and at subsequent visits when CMV antigenaemia was detected.

Definition

CMV infection was defined as isolation of CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen. CMV DNAemia was defined as the detection of CMV DNA in samples of plasma, whole blood or isolated PB leukocytes. Refractory CMV infection was defined as CMV DNAemia lasting for >2 weeks in spite of administration of a full dose of antiviral drug therapy. Recurrent infection was defined as new detection of CMV infection in a patient who had previously documented infection and in whom the virus had not been detected for a period of at least 4 weeks during active surveillance [1]. CMV disease was diagnosed according to previously published criteria [1]. Other important definitions are shown in Doc. S1.

Monitoring for CMV infection and pre-emptive therapy

CMV infection was monitored by weekly plasma CMV DNA testing with real-time PCR (PG Biotech, Shenzhen, China). All

patients received prophylactic acyclovir from day 1 to day 30 and ganciclovir from day −10 to day −2. Pre-emptive therapy with either intravenous ganciclovir or intravenous foscarnet was given when the PCR tests were positive for >600 copies/mL CMV in two consecutive tests or >1000 copies/mL CMV in a single test on PB, as previous reported [18]. Treatment was given for 2 weeks at the full dose, and as maintenance for another 2 weeks until CMV DNA was cleared. For patients with available CMV-specific T-cell sources, adoptive transfer of CMV-specific T-cells was performed under the condition of refractory CMV infection or CMV disease. CMV-specific T-cells were generated and quality controlled in a central facility with good manufacturing practices, as described recently [5].

Statistical analyses

Differences in categorical variables between two groups were evaluated with the χ^2 test or Fisher's exact test. Continuous variables were compared by use of a non-parametric test. The association between clinical factors and refractory CMV infection, and the impact of refractory CMV infection on CMV disease and non-relapse mortality (NRM), were analysed with Kaplan–Meier analysis. For time-dependent variables, the proportional hazard assumption was examined. Then, a stratified Cox model was used to examine the effects of variables on the observation endpoints and for testing interaction terms with covariates. Statistical analyses were performed with IBM SPSS 19.0 statistical software (IBM SPSS Statistics, Armonk, New York, USA).

Results

Characteristics and clinical outcomes of recipients

Patient characteristics and clinical outcomes are shown in Tables 1 and 2, respectively. In total, 91 patients received stem cell grafts from matched sibling donors (MSDs), and 397 patients received grafts from HLA-mismatched donors. The overall survival of patients at the end of follow-up was 82.6%, the NRM rate was 12.3%, and the cumulative relapse rate was 7.99%.

CMV-specific T-cell transfusions were given to 16 patients. Of these, 11 patients were diagnosed with CMV disease and the other five with refractory CMV infection. T-cell transfusions were given approximately 4 weeks after the start of CMV infection, and the dose ranged from 1.9×10^4 cells/kg to 9.93×10^5 cells/kg. The therapy was effective in 12 patients, whose CMV titre decreased rapidly. The other four patients did not response to the cell therapy.

Refractory and recurrent CMV infection

CMV infection occurred in the recipients at a median time of 31 days (range: 6–100 days) after allo-HSCT, and the duration was 14 days (range: 1–125 days). In total, 247 (50.6%) recipients experienced refractory CMV infection in the 100 days after allo-HSCT. CMV was cleared at a median time of 27 days (range: 15–125 days) in these patients. Recurrent CMV infection was observed in 148 (30.3%) of the recipients at a median time of 90 days (range: 66–188 days) after allo-HSCT, and 110 of the patients were refractory during the first episodes (Table 3).

Regarding different donor type subgroups, in the patients with MSDs, the incidence rates of refractory CMV infection and recurrent CMV infection were 24.1% and 14.9%, whereas in the patients with HLA-mismatched donors, the rates were 54.9% and 31.5%, respectively.

CMV disease

CMV disease was diagnosed in 31 (6.4%) of the recipients at a median time of 57.5 days (range: 23–260 days) after allo-HSCT. Among these, there were 24 (77.4%) cases of pneumonia, six (19.4%) cases of gastroenteritis, and one (3.2%) case of encephalitis. CMV DNA was detected in both bronchoalveolar lavage fluid and intestinal mucosa of two patients. In one other

TABLE 2. Clinical outcomes

Outcome	All patients (n = 488)
Neutrophil engraftment (days), median (range)	13 (7–111)
Platelet engraftment (days), median (range)	14 (5–225)
Acute GVHD, no. of patients (%)	
None	149 (30.5)
Grade I	185 (37.9)
Grade II	122 (25)
Grade III	14 (2.9)
Grade IV	18 (3.7)
Relapse, no. of patients (%)	39 (7.99)
Rrelapse time (days), median (range)	180 (56–539)
Non-relapse mortality, no. of patients (%)	60 (12.3)
Non-relapse mortality time (days), median (range)	180 (56–580)
Overall survival, no. of patients (%)	403 (82.6)

GVHD, graft-versus-host disease.

TABLE 3. Cytomegalovirus (CMV) infection characteristics

CMV infection	All patients (n = 488)
CMV DNAemia time (days after allo-HSCT), median (range)	31 (6–100)
Duration of CMV infection (days), median (range)	14 (1–125)
Refractory CMV infection, no. (%)	247 (50.6)
Duration of refractory CMV infection (days), median (range)	27 (15–125)
Recurrent CMV infection, no. (%)	148 (30.3)
Duration of tecurrent CMV infection (days after allo-HSCT), median (range)	90 (66–188)
CMV disease, no. (%)	31 (6.4)
Pneumonia	24 (77.4)
Enteritis	6 (19.4)
Encephalitis	1 (3.2)
Duration of CMV disease (days), median (range)	57.5 (23–260)

allo-HSCT, allogeneic haematopoietic stem cell transplantation.

patient, CMV DNA was detected in BALF and cerebrospinal fluid (Table 3). Nine of the patients with CMV disease suffered NRM, and six patients died from CMV disease. The incidence rates of CMV disease in patients with MSDs and HLA-mismatched donors were 4.39% and 6.8%, respectively.

Clinical factors affecting refractory and recurrent CMV infection

We analysed clinical factors that may affect refractory and recurrent CMV infection, including patient age, sex, underlying disease, donor age, donor type, infused mononuclear cell number, infused $CD34^+$ cell number, white cell engraftment, platelet engraftment, acute GVHD, haemorrhagic cystitis, Epstein–Barr virus infection, and donor lymphocyte infusion, with Kaplan–Meier analysis. Factors with p-values of <0.1 were included in the subsequent Cox multivariate analysis (Table 4). The proportional hazard assumption was tested for time-dependent variables such as acute GVHD, Epstein–Barr virus infection, haemorrhagic cystitis, and hepatitis B virus infection. The results are shown in Table S1. Stratified Cox models were used to analyse the effects of variables on refractory CMV infection (Table S2) The results showed that patients receiving transplants from HLA-mismatched donors and with acute GVHD were at high risk for refractory CMV infection (hazard ratio (HR) 2.012, 95% CI 1.603–2.546, p <0.001; HR 1.905, 95% CI 1.352–2.686, p <0.001) (Table 5; Fig. 1a,b).

Next, we explored the impact of refractory CMV infection on CMV disease. In addition to the clinical factors mentioned above, refractory CMV infection was included (Table 4). Grade III–IV acute GVHD (HR 2.461, 95% CI 1.002–6.049, p 0.0017), and refractory CMV infection (HR 10.539, 95% CI 2.467–45.015, p 0.001) had significant influences on CMV disease in a stratified Cox model (Table 5; Table S2). Patients with refractory CMV infection had an increased incidence of CMV disease (11.9%) as compared with those without (0.8%) (p <0.001) (Fig. 2).

The impact of refractory CMV infection on NRM was explored in the same way. Refractory CMV infection was considered as a time-dependent variable, and the change time point was 60 days after allo-HSCT. This showed that refractory CMV infection within 60 days and 100 days after allo-HSCT was an independent risk factor for NRM (HR 8.435, 95% CI 1.511–47.099, p 0.015) (Table 5). Patients with refractory CMV infection had an increased incidence of NRM (17.1%) as compared with those without (8.3%) (p 0.012) (Fig. 3).

Although univariate analysis showed that both refractory CMV infection and CMV disease had impacts on overall survival, only CMV disease was an independent risk factor (data not shown).

Analysis in the subgroup with HLA-mismatched donors

We explored the risk factors for refractory CMV infection and the impact of refractory CMV infection on CMV disease and

TABLE 4. Univariate analysis of factors affecting refractory and recurrent cytomegalovirus (CMV) infection, CMV disease, and non-relapse mortality (NRM)

	p			
Variables	**Refractory CMV infection**	**Recurrent CMV infection**	**CMV disease**	**NRM**
Age of recipients (years): <30/≥30	0.002	0.902	0.435	0.006
Sex of recipients: male/female	0.432	0.561	0.600	0.243
Underlying disease: AML/ALL/MDS/ others	0.092	0.927	0.655	0.977
Status before allo-HSCT: CR1/CR2, CR3, NR, relapse	0.610	0.535	0.562	0.645
Infused nuclear cells: below/equal to or above the median (7.62×10^8/kg)	0.719	0.079	0.856	0.435
Infused $CD34^+$ cells: below/equal to or above the median (2.61×10^6/kg)	0.825	0.230	0.674	0.657
Donor type: matched sibling/ mismatched family	<0.001	<0.001	0.465	0.664
Acute GVHD				
None/grade I–IV	<0.001	0.024	0.062	0.088
None, grade I/grade II–IV	<0.001	0.036	<0.001	0.042
None, grade I–II/grade III–IV	0.206	0.535	<0.001	<0.001
EBV infection: without/with	<0.001	0.333	0.870	0.075
Haemorrhagic cystitis: without/ with	0.035	0.045	0.073	0.069
Refractory CMV infection: without/ with		<0.001	<0.001	0.012
Recurrent CMV infection: without/ with			0.01	0.17
CMV disease: without/with				<0.001

ALL, acute lymphocytic leukaemia; allo-HSCT, allogeneic haematopoietic stem cell transplantation; AML, acute myelocytic leukaemia; CR, complete remission; EBV, Epstein–Barr virus; GVHD, graft-versus-host disease; MDS, myeloid dysplastic syndrome; NR, no remission.

TABLE 5. Multivariate analysis of factors affecting refractory cytomegalovirus (CMV) infection, recurrent CMV infection, CMV disease, and non-relapse mortality

Variables	**HR**	**95% CI**	**p**
Refractory CMV infection			
Donor type: matched sibling/mismatched family	2.012	1.597–2.536	<0.001
Acute GVHD: none/grade I–IV	1.717	1.102–2.675	0.017
T_COV_: $t = 45$	0.464	0.167–1.288	0.140
Recurrent CMV infection			
Response to treatment: not refractory/refractory	3.691	2.424–5.619	<0.001
Donor type: matched sibling/mismatched family	2.457	1.585–3.807	<0.001
CMV disease			
Ranking of treatment: not refractory/refractory	10.539	2.467–45.015	0.001
Acute GVHD: none, grade I–II/grade III–IV	2.461	1.002–6.049	0.017
T_COV_: $t = 45$	3.246	0.175–60.177	0.429
Non-relapse mortality			
Ranking of treatment: not refractory/refractory	0.316	0.063–1.5759	0.160
Acute GVHD: none, grade I–II/grade III–IV	2.915	1.628–5.939	0.003
Age of recipients: below/equal to or above the median	1.973	1.126–3.458	0.018
T_COV_: $t = 60$	8.435	1.511–47.099	0.015

GVHD, graft-versus-host disease; HR, hazard ratio; T_COV_, Time-dependent covariate.

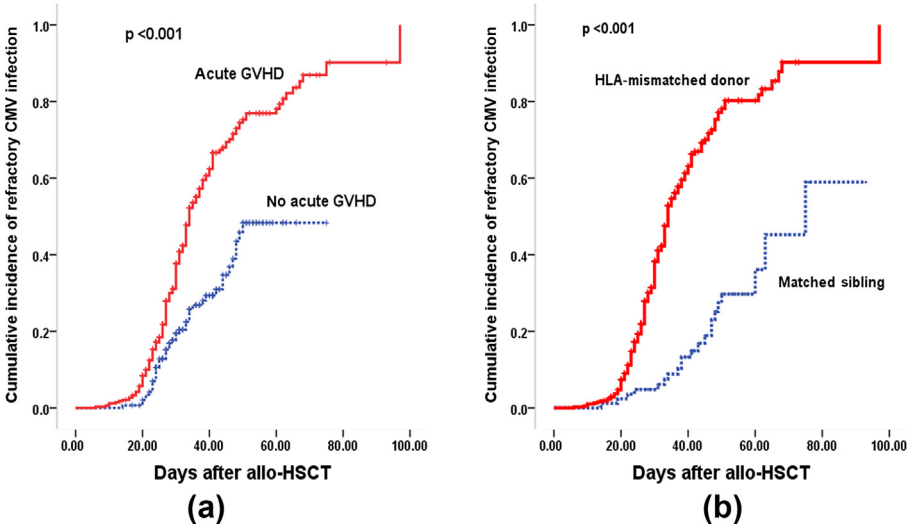

FIG. 1. Patients with acute graft-versus-host disease (GVHD) who (a) received transplants from mismatched family donors, and (b) were at high risk for refractory cytomegalovirus (CMV) infection (p 0.01 and p 0.003, respectively). allo-HSCT, allogeneic haematopoietic stem cell transplantation; HLA, human leukocyte antigen.

NRM only in the HLA-mismatched transplant subgroup. The outcomes were consistent with those of the whole cohort. The only significant risk factor for refractory CMV infection was grade I–IV acute GVHD (HR 1.793, 95% CI 1.246–2.58, p 0.002). Patient with refractory CMV infection were at high risk for CMV disease and NRM (HR 12.626, 95% CI 1.67–95.458, p 0.014; HR 2.066, 95% CI 1.067–4.002, p 0.031). Grade III–IV acute GVHD also had an impact on NRM (HR 2.912, 95% CI 1.299–6.529, p 0.009).

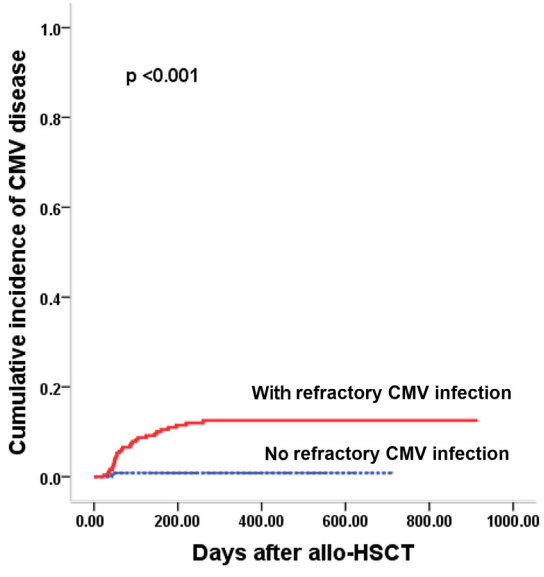

FIG. 2. Patients with refractory cytomegalovirus (CMV) infection had an increased incidence of CMV disease (11.8%) as compared with those without refractory CMV infection (0.8%) (p <0.001). allo-HSCT, allogeneic haematopoietic stem cell transplantation.

Discussion

Although refractory CMV infection occurs in a considerable group of recipients following allo-HSCT, limited data have been available about its clinical characteristics or risk factors, or its relationship with the development of CMV disease and NRM. We analysed a large cohort of patients with CMV infection after allo-HSCT, and, for the first time, demonstrated that refractory CMV infection during the 100 days after allo-HSCT was an independent risk factor for CMV disease and NRM. We also determined that patients receiving transplants from HLA-mismatched donors, and who showed acute GVHD, were more susceptible to refractory CMV infection.

CMV viraemia was once a well-recognized risk factor for CMV disease after allo-HSCT; nevertheless, pre-emptive antiviral therapy significantly decreased the incidence of CMV disease. However, approximately 50% of patients with CMV infection are refractory to the antiviral therapy [6,7,19,20]. Whether the refractory CMV infection after allo-HSCT was correlated with the development of CMV disease and NRM was not well explored in conjunction with pre-emptive antiviral therapy. In this study, we found that patients with refractory CMV infection during the first 100 days after allo-HSCT had a higher risk of CMV disease than those without, and that refractory CMV infection was an independent risk factor for CMV disease. In support of our results, Ljungman *et al.* [8] demonstrated that the rate of response of patients to antiviral therapy during the first week influenced the development of CMV disease, and that patients who responded more slowly had a higher risk. Among T-cell-depleted allo-HSCT patients, Almyroudis *et al.* [6] showed that 22% of patients with persistent CMV infection developed CMV disease. We also found that

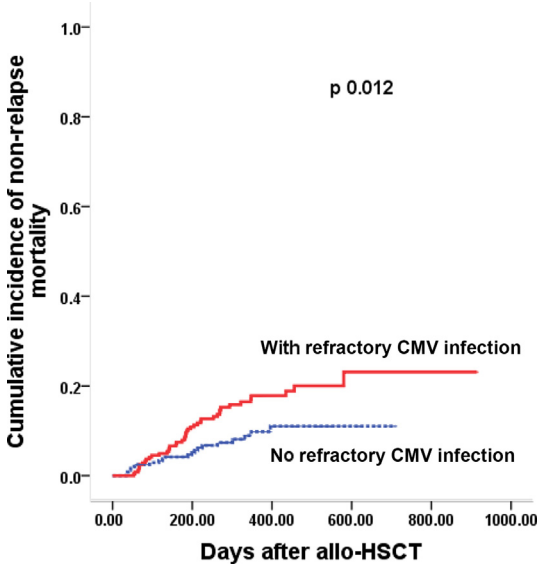

FIG. 3. Patients with refractory cytomegalovirus (CMV) infection had an increased incidence of non-relapse mortality (17.1%) as compared with those without refractory CMV infection (8.3%) (p 0.012). allo-HSCT, allogeneic haematopoietic stem cell transplantation.

refractory CMV infection within 60–100 days after allo-HSCT was an independent risk factor for NRM. This might be due to the susceptibility to CMV disease, or to toxicity caused by prolonged antiviral medication. These results indicated that refractory CMV infection is a severe complication in the early period after allo-HSCT. CMV-specific T-cell transfer was performed in 16 patients with refractory CMV infection, and the clinical outcome was promising. Several studies have stated that the infusion of low numbers of CMV-specific T-cells is safe and effective as a treatment for refractory CMV infection and CMV disease after allo-HSCT [5,21]. Controlled clinical trials are needed to investigate the optimal conditions for successful reconstitution of T-cell immunity after adoptive T-cell transfer. In addition, studies focused on the biomarkers of refractory CMV infection would enable early detection and make prophylaxis possible.

The analysis of clinical factors that impact on refractory CMV infection might help to identify patients at high risk. It is well understood that patients receiving transplants from HLA-mismatched or unrelated donors are more likely to have CMV infection after allo-HSCT [8,22,23]. Our study demonstrated that patients receiving transplants from HLA-mismatched donors were also more likely to suffer from refractory CMV infection than those receiving grafts from MSDs. This might be due to the high dose and prolonged use of immunosuppressive agents. We also demonstrated that donor type was not a risk factor for CMV disease, in either univariate or multivariate analysis. Nevertheless, as the endpoint of observation, CMV disease occurred in only a quite small proportion of this cohort, and the number of HLA-mismatched transplants was much larger than that of matched sibling transplants, which might have caused experimental bias and increased the statistical variability. Enlarging the cohort or using case pair analysis might have decreased the bias and confirmed the results. Also, other factors, such as more active antiviral therapy, were associated with HLA-mismatched transplants, which might have had a positive effect in preventing CMV disease. This was supported by another study [8]; however, all of these studies were retrospective, and further research exploring the viral kinetics and T-cell immunity reconstitution among patients with different donor types might shed light on this issue. The correlation between CMV infection and acute GVHD has been known for a long time [24]. Our data showed that acute GVHD was an independent risk factor for refractory CMV infection, even in the pre-emptive era.

Drug-resistant CMV infections caused by the human CMV phosphotransferase gene (UL97) and/or the polymerase gene (UL54) mutation were observed in 2–4% of patients with CMV reactivation after allo-HSCT, and always with severe outcomes [25–28]. Performing analyses of UL97 and UL54 in refractory CMV infection might identify further drug-resistant CMV infections. Monitoring these genes dynamically in refractory patients might help to demonstrate the beneficial impact of their monitoring on clinical outcome.

In conclusion, refractory CMV infection was a severe complication following allo-HSCT, and correlated with increased risks of CMV disease and NRM. Patients receiving transplants from HLA-mismatched donors and who developed acute GVHD were susceptible to refractory CMV infection. This group of patients was refractory to traditional antiviral drug treatment, and might need other antiviral strategies, such as adoptive CMV-specific T-cell transfer.

Author contributions

Huang Xiao-Jun: conception and design, securing research funding, and administrative, technical and logistic support. Huang Xiao-Jun and Liu-Jing: analysis and interpretation of the data, and drafting of the article. Liu-Jing: collection and assembly of data. All authors: final approval of the article, and provision of study materials or patients.

Transparency declaration

The authors state that they have no conflicts of interest.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (Grant No. 81370666) and The Key Programme of the National Natural Science Foundation of China (Grant No. 81230013).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.06.009.

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