

The association of conditioning regimen with cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation

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Received: May 2020, Accepted: November 2020

ABSTRACT

Background and Objectives: Infections is yet one of the life-threatening complications of the hematopoietic stem cell transplantation (HSCT). The myeloablative and immunosuppressive conditioning regimens, which are administered before HSCT, dampen the defense capacity of the recipients' immune systems. In this condition, opportunistic infections, especially viral infections such as cytomegalovirus (CMV) can be reactivated and cause morbidity and mortality in HSCT patients. Here, we aimed to find out any possible relationship between types of conditioning regimen and CMV reactivation in allogeneic HSCT patients.

Materials and Methods: We retrospectively analyzed the data of 145 CMV-seropositive cases out of total 201 allo-HSCT patients, including age, gender, underlying disease, conditioning regimen, prophylaxis regimen and occurrence of acute graft-versus-host disease (aGVHD) to evaluate their roles in CMV reactivation.

Results: Our result showed that conditioning regimen containing Busulfan and Fludarabine ($P=0.003$) or Cyclophosphamide ($P=0.02$) significantly decrease the early CMV reactivation. Patients who developed aGVHD ($P=0.003$) and those who received anti-thymocyte globulin (ATG) as prophylaxis regimen ($P=0.002$), had 1.84 and 2.63 times higher risks of CMV reactivation, respectively.

Conclusion: Our findings suggest the conditioning regimen, aGVHD and ATG as influencing factors for early CMV reactivation post-HSCT which should be considered in the future studies.

Keywords: Cytomegalovirus; Hematopoietic stem cell transplantation; Conditioning regimen

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is one of the most propitious treatment options for malignant and even some other diseases (1, 2). Conditioning regimen before HSCT, serves two main goals; decreasing the tumor burden, in the case of malignancy, and weaken the recipient immune system, so as to let the successful engraftment of the transplanted stem cells (3). One of the fatal complications after HSCT is cytomegalovirus (CMV) infection (4). CMV is a beta-herpesvirus, which infects a majority of the global population (5). The rate of

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CMV seroprevalence in populations varies between 40-95% with a global mean of 83%, based on the age, race, socioeconomic status and other factors (6, 7). CMV remains in a lifelong latent form after an asymptomatic primary infection in immunocompetent individuals. In this phase, anti-CMV antibodies present in the circulation making serological examinations positive. In any case of immune suppression such as solid-organ or hematopoietic stem cell transplantation, acquired immune-deficiency syndrome (AIDS), as well as harsh immunosuppressive drugs it can reactive and cause fatal consequences (8). CMV reactivation is a critical post-HSCT viral infection, which is estimated to happen after 37% and 12% of allogeneic and autologous HSCT, respectively, and is associated with an increased risk of mortality (9-12). Conditioning regimen is an immunosuppressive regimen influence the outcome of HSCT and might indirectly raise the possibility of CMV reactivation (13). Besides the conditioning regimen, donor and recipient CMV serological status, acute graft versus host disease (aGVHD), T cell depletion and anti-thymocyte globulin (ATG) can be risk factors for CMV infection (14). Direct effects of CMV reactivation include organ involvement in gut, liver, lung, and brain, whereas immunosuppression, graft failure and secondary infections are indirect effects of CMV reactivation (15). Although anti-viral therapy reduced CMV complications, drug toxicities and resistance are main concerns (16). Previous studies have investigated the effect of conditioning regimen on CMV reactivation. Some studies indicated that there is no significant differences in CMV reactivation between patients received different regimens (14, 17-19), while there are several evidence about effect of conditioning regimen on infections complications, including CMV (20, 21). Here we categorized our patients into four groups based on the conditioning regimen and evaluated their CMV status post-HSCT to find any possible relationship.

MATERIALS AND METHODS

Patients. The clinical records of 201 patients who underwent hematopoietic stem cell transplantation at HSCT center of Taleghani hospital, Tehran, Iran (a general hospital with special divisions of oncology and HSCT) from April 2016 until March 2019 were checked for CMV sero-status and finally 145

CMV-seropositive patients enrolled in this retrospective study. Before admission, the status of all patients was checked by cardiology, pulmonology, otorhinolaryngology, psychology and dental specialists. For confirmation of not having any active infections, including Toxoplasmosis, infection with Epstein-Barr virus (EBV), Varicella-Zoster virus (VZV), hepatitis B (HBV) and C virus (HCV), and CMV infection, real-time based molecular tests (all from Takapuzist, Iran) were also performed, based on the manufacturer protocol, on the peripheral blood samples of all patients and donors before admission. Patients' Karnofsky performance score was at least 70% which means that they could care themselves, however not strong and healthy enough to work (22). GVHD prophylaxis, HSCs mobilization and harvesting, graft manipulation, and aGVHD diagnosis were performed based our previous reports (23, 24). The availability of clinical data as well as positivity for CMV-specific IgG (seropositive) were inclusion criteria in this study. Patients with seronegative CMV status or those with a history of previous transplantation, were excluded. Ethical approval was waived by the local Ethics Committee of University (IR.SBMU.REC.1398.147). Regarding that the current study is retrospective, all the procedures were performed as part of the routine care.

Conditioning regimen. Seventy-seven patients received busulfan (BU) 0.8 mg/kg, four times a day for four days followed by cyclophosphamide (CY) 60 mg/kg/day for two days (myeloablative conditioning-1, MAC-1). Thirty-two patients received MAC-2 in which the cyclophosphamide of MAC-1 regimen is substituted with fludarabine (Flu) 30 mg/m² of body surface area once a day for five days. MAC-3 group includes 14 patients who received BU and FLU (as mentioned above) plus ATG 1.5 mg/kg/day for three days. All mentioned medications administered intravenously (IV). Seventeen patients received reduced-intensity conditioning (RIC) including fludarabine 30 mg/m²/day IV for five days, CCNU 100 mg/m²/day P.O. for two days and one dose of melfalan 40 mg/m². Five patients with aplastic or Fanconi anemia administrated by cyclophosphamide 50 mg/kg/day IV for four days and 1.5 mg/kg/day IV ATG for four days. All prescribed conditioning regimens were followed European Society for Blood and Marrow Transplantation (EBMT) criteria and also the HSCT committee decision based on the patients' disease,

age, comorbidity and clinical conditions (25).

CMV evaluation and anti-viral prophylaxis.

From admission until discharge, blood samples of patients were collected biweekly for CMV status evaluation. Nucleic acid was extracted from plasma by DynaBio Viral Nucleic Acid Extraction Mini Kit (Takapuzist, Iran). Detection of CMV copy number in the samples were performed using TaqMan-based DynaBio CMV Quantitative Real-Time PCR Kit (Takapuzist, Iran) and a Rotor-Gene 6000 real-time analyzer (Qiagen, Germantown, MD). Analytical sensitivity and linear range were 0.1 IU/ μ L and 0.5 IU/ μ L to 1×10^7 IU/ μ L, respectively. All patients received ciprofloxacin, fluconazole, and acyclovir for anti-bacterial, anti-fungal, and anti-viral prophylaxis, respectively.

Statistical analysis. The patients' characteristics were extracted from their clinical records and presented as frequency (%) or mean \pm standard deviation (SD). The demographic and clinical data of recipients were all included in the analysis as risk factors for predicting the occurrence of CMV disease. The Logistic regression model was utilized for the univariate analysis with CMV disease as the outcome of interest. Due to the small frequency of the post-transplant CMV positive patients, the dataset was unbalanced, therefore we applied the weighted Logistic regression to adjust the balance. The frequency of risk factors by CMV disease was also performed through a cross tabulation. All of the analyses were carried out using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) and R programming language version 3.5.2. The significance level was set at 0.05 for the Logistic regression models.

RESULTS

One-hundred and forty-five patients out of total 201 cases were CMV seropositive (72.1%) which their descriptive statistics are illustrated in Table 1. The analysis was performed only on these CMV-seropositive patients as well as their donors (145 patients and 145 attributed healthy donors). Less than half of the recipients (N=67, 46.2%) and more than half of the donors (82, 56.6%) were males. The most diagnosed disease was AML comprising 51.7% of all patients. GVHD occurred in 85 (42.3%) and CMV disease turned to be reactivated in 12 (5.9%) of the patients during their hospitalization period.

Association of risk factors with CMV disease.

In order to evaluate the CMV reactivation, only CMV-seropositive patients were included in the analysis. As the results illustrated in Table 2, the recipient and donor age was significantly increase odds of CMV disease by 3% (95% CI: (1.01-1.05); p-value=0.001 and 0.002, respectively). Donor gender had also a significant impact on the incidence of CMV disease in a way that males decreased the odds of

Table 1. The descriptive statistics of the CMV-seropositive patients under study

Variables	Subgroup	Frequency (%) /mean \pm SD
Recipient age	-	32.45 \pm 10.82
	Missing	3.0 (2.1)
Donor Age	-	31.69 \pm 11.16
	Missing	39 (26.9)
Recipient gender	Male	67 (46.2)
	Female	78 (53.8)
	Missing	0 (0.0)
Donor gender	Male	82 (56.6)
	Female	57 (39.3)
	Missing	6 (4.1)
Diagnosed disease	NHL	11 (7.6)
	HD	10 (6.9)
	AML	75 (51.7)
	ALL	35 (24.1)
	Aplastic Anemia	6 (4.1)
	Other	4 (2.8)
	Missing	4 (2.8)
GVHD	Yes	65 (44.8)
	No	75 (51.7)
	Missing	5 (3.4)
Conditioning regimen	MAC1	77 (53.1)
	MAC2	32 (22.1)
	MAC3	14 (9.7)
	RIC	17 (11.7)
	AA-AF	5 (3.4)
	Missing	0 (0.0)
	GVHD prophylaxis	CSA+MTX
	CSA+MTX+ATG	15 (10.3)
	Unclassified	41 (28.3)
CMV IgG	Positive	145 (72.1)
	Negative	21 (10.4)
	Missing	35 (17.4)
CMV reactivation	Positive	12 (8.3)
	Negative	133 (91.7)
	Missing	0 (0)

CMV disease by 35% compared to females (95% CI: (0.42-0.98); p-value=0.036). The patients who experienced the occurrence of aGVHD had 84% greater odds of CMV disease compared to the patients who did not (95% CI: (1.22-2.77); p-value=0.003). Among the conditioning regimens, receiving MAC1 and MAC2 significantly decreased the odds of CMV disease by 85% and 79%, respectively (95% CI: (0.03-0.48); p-value=0.003), (95% CI: (0.04-0.70); p-value=0.020). The patients who received ATG in their GVHD prophylaxis regimen, had 2.63 times greater odds of CMV disease occurrence compared to those who did not (95% CI: (1.44-5.00); p-value=0.002). None of the underlying diseases was found to have a significant effect on CMV reactivation (Table 2). All the significant variables in the univariate analysis

were entered the multiple model; however, none of them were significant. Table 3 illustrates the frequency of CMV disease in different categories of sex, age, diagnosis, conditioning regimen and GVHD prophylaxis. About 6% of both genders had CMV incidence. The patients aged more than 50 years old had the highest CMV incidence (25%). None of the Aplastic anemia patients and 15.4% of the NHL patients were CMV positive.

DISCUSSION

The main goal of our study was figuring out the relationship between conditioning regimen and CMV reactivation after allo-HSCT. Hence, only seropos-

Table 2. The univariate logistic regression model for CMV disease

Variables	Odds ratio	SE (95% CI)	P-value
Recipient age	1.03	0.009 (1.01-1.05)	0.001*
Donor age	1.03	0.01 (1.01-1.05)	0.002*
Patient gender			
Male	0.94	0.20 (0.62-1.39)	0.750
Female (RL ¹)	-	-	-
Donor gender			
Male	0.65	0.20 (0.42-0.98)	0.036*
Female (RL ¹)	-	-	-
Diagnosed disease			
NHL	0.70	0.44 (0.28-1.64)	0.424
AML	0.58	0.42 (0.25-1.30)	0.202
ALL	1.99	0.52 (0.71-5.64)	0.187
Aplastic Anemia	2.18	0.64 (0.64-8.33)	0.221
Other	5.37	550.08 (inf-146.15)	0.977
HD (RL ¹)	-	-	-
GVHD			
Yes	1.84	0.20 (1.22-2.77)	0.003*
No (RL ¹)	-	-	-
Conditioning regimen			
MAC1	0.15	0.64 (0.03-0.48)	0.003*
MAC2	0.21	0.66 (0.04-0.70)	0.020*
MAC3	0.43	0.70 (0.08-1.53)	0.230
AA-AF	0.002	535.41 (inf-93.73)	0.977
RIC (RL ¹)	-	-	-
GVHD prophylaxis			
CSA+MTX+ATG	2.63	0.31 (1.44-5.00)	0.002*
CSA+MTX (RL ¹)	-	-	-

1. Reference Level

* Significant at 0.05

Table 3. Demographic, diagnosis and treatment protocols by CMV disease

Variable	Subgroup	CMV (%)	
		Negative	Positive
Gender	Male	95.8	4.2
	Female	94.6	5.4
Age	<10	100	0.0
	10-25	94.9	5.1
	25-35	95.7	4.3
	35-50	95.8	4.2
	>50	80.0	20.0
Diagnosis	NHL	87.5	12.5
	HD	100	0.0
	AML	96.2	3.8
	ALL	92.5	7.5
	Aplastic Anemia	100	0.0
	Other	100	0.0
Conditioning regimen	MAC1	95.8	4.2
	MAC2	91.4	8.6
	MAC3	92.3	7.7
	RIC	100	0.0
	AA-AF	100	0.0
GVHD prophylaxis	CSA+MTX	93.3	6.7
	CSA+MTX+ATG	92.3	7.7

itive patients (145 patients out of 201 patients) who previously encountered with CMV were included in the descriptive table (Table 1) and analysis to found which conditioning regimen might affect the possibility of CMV reactivation. Significant decreases in CMV reactivation were observed in patients received busulfan + cyclophosphamide and busulfan + fludarabine, compared to RIC regimen. Although several studies have investigated the effect of conditioning regimen and CMV, none of them is exactly comparable with each other and that's because of different categorization for conditioning regimen based on the patients' condition, age and type of HSCT. Most of the investigations focused on non-myeloablative (NMA) conditioning and its impact on infection complications. Results from some of studies indicated that conditioning regimen has no effect on CMV infections. Venton and Goldsmith suggested that different kind of conditioning, includes myeloablative (MA) and reduced toxicity conditioning (RIC) had no effect on CMV reactivation (26, 27). Consistent with two latter studies, another report demonstrated that the rate of CMV incidence and antigenemia have

no significant differences between MA and RIC regimen (28). Also, same evidence in pediatric setting has been provided by Bartelink et al. study in which, comparison between two kinds of regimen, fludarabine plus busulfan and busulfan plus cyclophosphamide as conditioning, showed no impact on CMV incidence (19). Also, it has been indicated that dosage alternation in conditioning regimen as well as ATG reduction might have neutral effects on CMV incidence (29). In addition, Xuan et al. (14) demonstrated a comparable risk of CMV infections between intensified and standard conditioning. However, there are also accumulating documents about efficacy of conditioning on post-transplant CMV incidence in which beneficial aspects of RIC regimen have been suggested. Green et al. stated non-MA or MA without high dose TBI reduced the risk of CMV disease compared with patients received MA plus high dose TBI (30). In a study by Martino et al. CMV infection was significantly lower in patients conditioned by RIC regimen (31). Contrarily, several studies confirm our findings and showed disadvantages of RIC on CMV reactivation (20, 32). Lamba and colleagues found high rate of early CMV reactivation in recipients received alemtuzumab+RIC regimen (32). Manjappa et al. reported CMV reactivation in 63% of patients received RIC rather than 42% CMV reactivation in those who were administered with MA regimen (20). Moreover, in an investigation of umbilical cord blood transplantation with RIC regimen, rapid CMV reactivation has been observed (33). Regarding the fact that we evaluated early CMV reactivation in our investigation (during the hospitalization which was approximately 30 days), these studies are in accordance with our finding. Although some of these studies explained early reactivation (32, 33), delayed CMV disease after RIC-transplantation has been also mentioned in several studies (21, 34, 35). For instance, the rate of late CMV reactivation after HSCT is reported approximately 42-43% in patients received MA conditioning regimen following a 100-300 days monitoring (20, 31). Even 3.8 years of follow up showed 42% CMV reactivation in Iran (36). Due to short monitoring period of patients in our study (during hospitalization) the lower rate of CMV reactivation is expected and accounted as rapid CMV reactivation rate. Since infection complications rely on immune reconstitution status, different source of stem cells may cause discordant results by effecting immune recovery. Umbilical cord blood

contains more immature cells, responsible for delayed and poor immune cells engraftment (37). Another reason for this contrary may stem from addition of alemtuzumab, a T cell depletion agent used in Lamba's study.

Strong association between aGVHD and CMV has been found by our data and other reports (21, 28). Valadkhani et al. evaluated risk factors of CMV reactivation through 100 days follow up. Thirty-six percent of patients experienced CMV reactivation which is higher than ours and could be due to longer follow up period. Parallel to our study, they reported GVHD and donor age as risk factors for CMV reactivation. Since 79% of patients received same a pre-transplant chemotherapy, the effect of conditioning regimen was not reported (38). Another study in Iran confirm our finding that the primary disease is not a risk factor for CMV reactivation (36).

It has been expressed that GVHD prophylaxis agents may alter the rate of CMV infection (39). Our data suggest that ATG as GVHD prophylaxis 2.63 times increased the risk of CMV reactivation. Equally, in an investigation of haploidentical HSCT, high incidence of CMV was documented in patients received ATG-based prophylaxis (40). Delayed immune reconstitution induced by T cell depletion agents such as ATG, may explain the increased risk of opportunistic infection. However, another study in patients transplanted with unrelated donor, ATG as GVHD prophylaxis did not result significant differences in CMV reactivation.

There are some limitations in the current study. First, we monitored the CMV reactivation in a short period of time (during hospitalization \approx 30 days). Longer follow up could contribute valuable data about CMV status and illustrate more precise relationship between transplant risk factors with CMV reactivation. The reciprocal role of CMV reactivation in the outcome of HSCT is another interesting era of investigation which is not evaluated in the present study due to the timely process of HSCT outcome evaluation. Patients are currently being followed to evaluate the effect of CMV reactivation on chronic GVHD incidence, relapse, and overall survival. However, it has been shown that the CMV reactivation increases the rate of chronic and acute GVHD as well as non-relapse mortality (36). Also, paucity of data on sero-status of donors, preclude drawing further conclusion about the role of donor on this scenario.

Overall, we found that conditioning regimen can

influence the rate of early CMV reactivation in patients who exposed previously with CMV. Our study shows the benefits of MA conditioning regimen to reduce the early CMV reactivation. Besides, GVHD and ATG-containing regimens could be considered as post-HSCT risk factors for early CMV-reactivation. However, due to the heterogeneity of previous reports in source of HSCT and different MA or non-MA regimen, making a firm conclusion is difficult. Further studies should evaluate the effect of conditioning regimen on CMV reactivation, considering immune reconstitution and relapse of disease.

ACKNOWLEDGEMENTS

The authors would like to thank of Hematopoietic Stem Cell Research Center and Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran for providing the possibility of doing the study and helpful assistance.

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