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Original Article

CD34+ cell dose and CMV viremia after haploidentical hematopoietic stem cell transplantation: a retrospective study

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Abstract: Background: Patients with hematologic malignancies who undergo Haplo-HSCT (Haploidentical hematopoietic stem cell transplantation) are at a significantly higher risk for CMV (Cytomegalovirus) infection than individuals with normal immune function. This increased susceptibility to CMV is a major contributor to the morbidity and mortality seen in patients following hematopoietic stem cell transplantation. Methods: This study involved a retrospective analysis of 113 patients who underwent allogeneic hematopoietic stem cell transplantation for malignant hematological diseases. Relevant variables were assessed through univariate analysis, and those identified in the previous literature as potential influencers of the occurrence of CMV viremia were also included in the multivariate regression analysis. Results: Among the 113 patients with malignant hematologic diseases undergoing Haplo-HSCT, 56 cases (49.56%) were identified with CMV viremia. Initial univariate analysis highlighted patient age, graft source, and CD34+ cell dose as risk factors for CMV viremia post-transplantation. However, subsequent logistic regression analysis revealed that CD34+ cell dose stood out as an independent protective factor against CMV viremia (OR = 0.797, 95% CI: 0.644-0.987, P = 0.037). Conclusion: Our study reveals that a higher CD34 cell dosage serves as an independent protective factor against the development of CMV viremia after Haplo-HSCT in patients with hematologic malignancies.

Keywords: CMV viremia, haplo-HSCT, malignant hematological disease

Introduction

Malignant hematological diseases are a group of clonal malignancies that originate in the hematopoietic system. This category includes various conditions such as leukemia, myelodysplastic syndrome (MDS), lymphoma, and multiple myeloma, among others. Cytomegalovirus is a member of the herpesvirus family, and like other herpesviruses, it can remain dormant in host tissue and persist for years. CMV infection is highly prevalent in the population, often asymptomatic, with approximately 60-80% of healthy adults testing positive for CMV antibodies [1]. When immune function is compromised, the CMV virus can transition from a latent infection to an active and dominant infection in the body. Due to their weakened immune system, patients undergoing Haplo-HSCT are at a much higher risk of developing severe CMV infection, which is among the primary causes of morbidity and mortality in this patient population [2]. The majority of CMV infections in these patients occur between 50-90 days following the transplantation [3].

Cytomegalovirus can lead to various adverse events such as CMV disease, acute/chronic graft-versus-host disease (GVHD), opportunistic infections, and bone marrow suppression, severely affecting the prognosis of transplant patients [4-6]. The incidence of CMV disease ranges from 10% to 40%, with the most predominant type being CMV pneumonia, which carries a mortality rate as high as 70% [7]. The presence of plasma CMV-DNA is associated with an increased incidence of CMV disease; factors such as age, gender, immune status, pre-transplant use of anti-thymocyte globulin (ATG), and Haploidentical hematopoietic stem cell transplantation [4, 8-10] are all risk factors for the occurrence of CMV viremia. However, we

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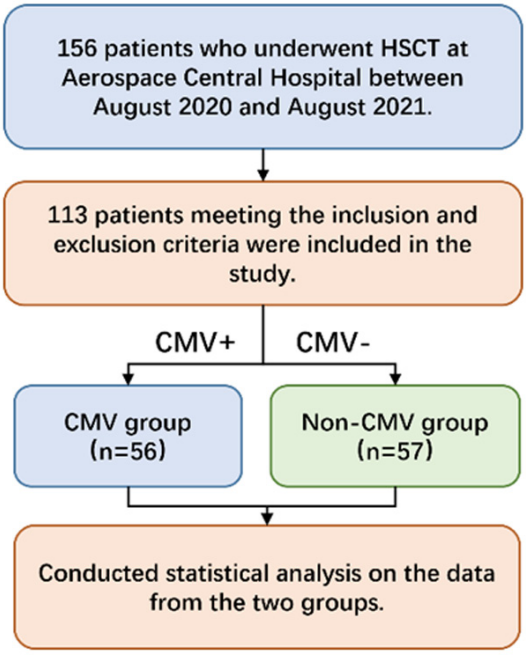

Figure 1. Flow chart of this study.

still hoped to identify other modifiable factors that may influence the occurrence of CMV viremia.

We meticulously designed a retrospective analysis involving 113 patients who underwent allogeneic hematopoietic stem cell transplantation for malignant hematologic diseases. Our methodology not only encompassed traditional univariate analysis but also included multivariate regression analysis, considering variables identified in previous literature as well as exploring novel factors such as MNC and CD34+ cell dose. This comprehensive approach enabled us to include areas not previously explored in existing research, aiming to uncover overlooked determinants of CMV viremia in transplant patients.

Methods

Participants

This study included a total of 113 patients with hematologic malignancies who underwent Haplo-HSCT at the Aerospace Central Hospital from August 2020 to August 2021. The inclusion criteria for this study were as follows: (I) Patients diagnosed with hematologic malignancies who were scheduled to undergo hematopoietic stem cell transplantation. (II) Both the donor and patient's peripheral blood CMV-DNA copy number had to be less than 1×10^3/L prior to the transplantation procedure. The exclusion criteria for this study were as follows: HLA (Human Leukocyte Antigen)-matched HSCT, absence of detectable plasma CMV-DNA load, incomplete important clinical data, and loss to follow-up of patients.

Data collection

The study collected medical record data from the Aerospace Center Hospital from August 2020 to August 2021. Experienced researchers conducted data extraction to ensure the accuracy and completeness of the data. The data included patient demographics (age, gender), disease characteristics (stage of underlying disease at transplantation, HCT-CI (Hematopoietic Cell Transplantation-Specific Comorbidity Index) score, donor-related factors (type, ABO mismatch, age, gender), pre-transplant conditioning regimen, stem cell source, cell dose (mononuclear cells and CD34+ cells), and based on the presence of ≥ 500 copies/mL [11-14] of plasma CMV-DNA within 100 days after undergoing haplo-HSCT, which is defined as CMV viremia, the study subjects were divided into two groups: the CMV group and the Non-CMV group (**Figure 1**).

Data analysis and statistics

Statistical analysis employed single-factor statistical techniques, including chi-square test and independent sample t-test, to evaluate the impact of CMV viremia. Logistic regression analysis was used to identify possible risk factors associated with CMV viremia. All statistical analyses were conducted using SPSS software.

Results

General conditions and clinical features of patients

The study conducted at the Aerospace Central Hospital between August 2020 and August 2021 was a retrospective case-control study focusing on patients treated with HSCT. A total of 156 patients who underwent HSCT were initially enrolled in the study. After screening to ensure adherence to inclusion and exclusion criteria, 113 patients were ultimately included

Table 1. Patient and transplant characteristics

Characteristic	CMV group (56)	Non-CMV group (57)	*P* value
Sex of patient			0.644
Male	31 (55.36)	34 (59.65)	
Female	25 (44.64)	23 (40.35)	
Age of patients			0.024
Mean ± SD	30±268	23±274	
Min - Max	2-59	1-62	
Median (IQR)	31	27	
Underlying disease			
Acute myeloid leukemia	40 (71.43)	42 (73.68)	0.447
Acute lymphoid leukemia	8 (14.28)	6 (10.52)	
Myelodysplastic syndromes	2 (3.57)	4 (7.02)	
Chronic myeloid leukemia	1 (1.79)	3 (5.26)	
Lymphoma	4 (7.14)	1 (1.75)	
Plasma cellular leukemia	1 (1.79)	0 (0)	
Acute mixed leukemia	0 (0)	1 (1.75)	
Disease status			0.459
CR	35 (62.50)	30 (55.56)	
NR	21 (37.50)	24 (44.44)	
HCT-CI			0.932
Mean ± SD	0.84±0.621	0.82±1.058	
Min - Max	0-3	0-5	
Median (IQR)	1.00	1.00	
Donor type			0.689
HLA mismatched, related	51 (91.07)	54 (94.74)	
HLA mismatched, unrelated	4 (7.14)	2 (3.51)	
Unrelated cord blood	1 (1.79)	1 (1.75)	
Graft source			< 0.001
Peripheral blood	39 (69.64)	55 (96.49)	
Bone marrow + Peripheral blood	16 (28.57)	1 (1.75)	
Umbilical cord blood	1 (1.79)	1 (1.75)	
Conditioning			0.792
Reduced intensity	9 (16.07)	8 (14.03)	
Myeloablative	47 (83.93)	49 (85.96)	
ABO disparity			0.487
None	30 (55.56)	30 (52.63)	
Minor	5 (9.26)	11 (19.30)	
Major	14 (25.93)	12 (21.05)	
Bidirectional	5 (9.26)	4 (7.02)	
Sex of donor		56	0.825
Male	39 (73.58)	43 (76.79)	
Female	14 (26.42)	13 (23.21)	
Age of donor			0.431
Mean ± SD	32.92±162.38	34.98±199.94	
Min - Max	9-63	7-59	
Median (IQR)	33.00	38.50	
MNC, $\times 10^8$/kg			0.769
Mean ± SD	9.80±9.40	10.00±15.67	
Min - Max	4.84-19.32	1.91-23.5	
Median (IQR)	9.54	8.98	
CD34+ cell, $\times 10^6$/kg			< 0.001
Mean ± SD	5.15±5.58	6.88±7.35	
Min - Max	1.05-9.12	1.2-16.92	
Median (IQR)	5.00	6.70	

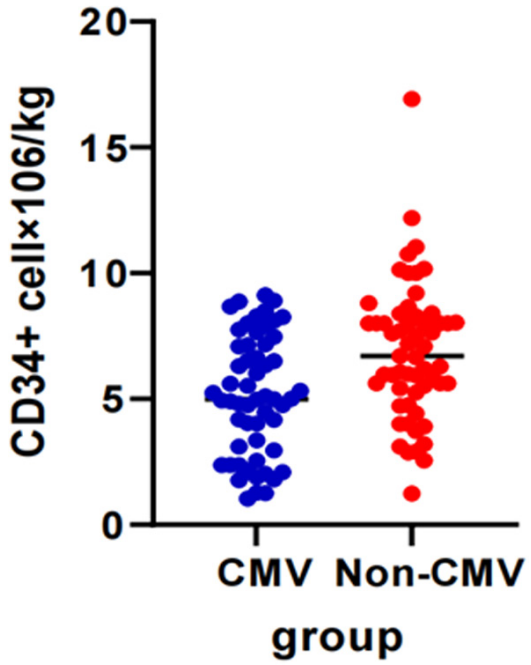

Figure 2. Scatter plot of CD34+ cell doses between Non-CMV and CMV groups. Red indicates Non-CMV, blue indicates CMV.

in the analysis for further investigation. After Haplo-HSCT, the cumulative incidence of CMV viremia at 100 days was found to be 49.56%. Among the patients in the CMV group, there were 56 individuals with various malignant hematologic diseases, including 40 cases of acute myeloid leukemia (AML), 8 cases of acute lymphoblastic leukemia (ALL), 4 cases of lymphoma, 2 cases of myelodysplastic syndrome (MDS), 1 case of chronic myeloid leukemia (CML), and 1 case of plasmacytic leukemia. The non-CMV group comprised 57 patients with hematologic malignancies, consisting of 42 cases of AML, 6 cases of ALL, 1 case of acute mixed cell leukemia (MAL), 3 cases of CML, 4 cases of MDS, and 1 case of lymphoma. Detailed patient and transplant demographics, including age, gender, donor type, conditioning regimen, and other relevant information, can be found in **Table 1**. There were no significant differences ($P > 0.05$) observed in patient gender, underlying disease, phase of underlying disease at transplant, HCT-CI score, donor type, ABO disparity, donor age, donor sex, pretransplant conditioning regimen, mononuclear cell dosage, or other factors (**Table 1**).

The median age of patients in the CMV group was 31 years, while in the non-CMV group it was 27 years ($P = 0.024$). In the CMV group, the graft sources were peripheral blood, peripheral blood combined with bone marrow, and cord blood, accounting for 39 cases (69.64%), 16 cases (28.57%), and 1 case (1.79%), respectively. In the non-CMV group, the sources of transplants were peripheral blood, peripheral blood combined with bone marrow, and cord blood, accounting for 55 cases (96.49%), 1 case (1.75%), and 1 case (1.75%), respectively. Statistical analysis revealed a significant difference between the two groups ($P < 0.001$). For CD34 stem cell dosage, the median in the CMV viremia group was 3.03×10^6/kg, whereas in the non-CMV group it was 6.70×10^6/kg ($P < 0.001$) (**Figure 2**). Again, we highlight that the observed differences in patient age, graft sources and CD34 stem cell dosage were statistically significant (**Table 1**).

Logistic regression analysis

In the logistic regression analysis, the variables with $P < 0.05$ in the univariate analysis were included, along with factors identified in previous literature as possible influencers of the occurrence of CMV viremia. These factors included the age and sex of patients, graft source, donor type, and CD34 cell dosage, HCT-CI score, and disease status.

The results revealed that the CD34 cell dose acted as an independent protective factor against CMV viremia. Specifically, for each unit increase in the number of CD34 cells, there was a corresponding decrease in the risk of CMV viremia by a factor of 0.797 (OR = 0.797, 95% CI: 0.644-0.987, $P = 0.037$). This finding emphasizes the importance of CD34 cell dose for predicting the risk of CMV viremia in haplo-HSCT patients and suggests that a higher CD34 cell dose may provide a protective effect against CMV viremia (**Table 2**).

The receiver operating characteristic (ROC) curve analysis results (**Figure 3**) showed that the area under the curve (AUC) for predicting CMV viremia in HSCT patients based on CD34+ cells was 0.673. The cut-off value was calculated as 5.244×10^6/kg using the Youden index method.

Discussion

HSCT is currently an important method for treating malignant hematological diseases,

Table 2. Multivariable logistic regression analysis of CMV viremia in HSCT patients

Influencing Factor	B	SE	Wald	DF	P	OR	95% CI
Sex of patients	0.654	0.513	1.623	1	0.203	1.923	0.703-5.259
Age of patients	0.008	0.014	0.305	1	0.581	1.008	0.980-1.037
Peripheral blood			5.312	2	0.070		
Bone marrow + Peripheral blood	0.115	1.144	0.010	1	0.920	1.122	0.119-10.558
Cord blood	2.613	1.500	3.037	1	0.081	13.647	0.722-257.999
Disease status	0.021	0.499	0.002	1	0.966	1.022	0.384-2.715
HCT-CI	0.276	0.309	0.795	1	0.373	1.317	0.719-2.414
CD34 cell	-0.227	0.109	4.345	1	0.037	0.797	0.644-0.987

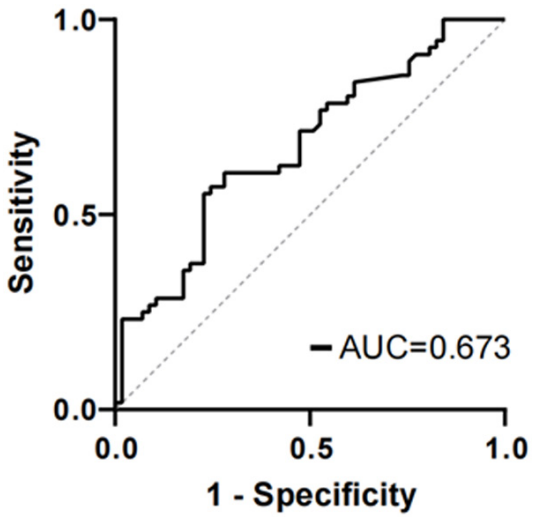

Figure 3. ROC curve analysis of CD34+ cell dose to predict CMV viremia in HSCT patients.

and cytomegalovirus is one of the main causes of infection and poor prognosis in patients undergoing allogeneic hematopoietic stem cell transplantation. Therefore, clinicians need to identify factors that may influence the occurrence of CMV viremia and are considered controllable.

Among the 113 patients with malignant hematological diseases included in this study, the incidence of CMV viremia after transplantation was 49.56%. We used scatter plot to compare the number of CD34 cells between the CMV and non-CMV groups. The stem and leaf images clearly demonstrated that the non-CMV group had a significantly higher number of CD34 cells compared to the CMV group. Furthermore, a single factor analysis was performed, revealing a significant difference in the CD34+ cell dose between the two groups. Multivariate analysis showed that CD34+ cell dose was an independent protective factor for CMV viremia in HSCT patients. This observation aligns with the results reported in several prior articles. According to Xu et al. [15], the transplantation of purified cord blood CD34+ stem cells into immunocompromised mice was found to boost hematopoietic and immune reconstitution by augmenting the population of CD34+ cells in the transplant. Zheng et al. [16] compared patients with CMV viremia in dermatomyositis to patients without CMV viremia. The study findings demonstrated significant reductions in the levels of total T cells, total B cells, NK cells, CD4+ T cells, and specific subsets of CD4+ T cells (Th1, Th2, Th17, and Treg cells) among patients with CMV viremia. Zhao et al. [17] identified a noteworthy correlation between an increased count of natural killer (NK) cells and a lower occurrence of CMV viremia subsequent to bone marrow transplantation. Moreover, they established a positive relationship between the quantity of NK cells and the number of CD34+ cells present in the graft (OR = 0.739, $P < 0.001$). These studies collectively suggest that a decline in the number of CD34 cells may impede immune reconstitution, consequently elevating the incidence of CMV viremia. Hence, it is important to prioritize the prevention of CMV viremia wheenever the dose of CD34 cells is low.

Currently, CD34+ cell dose detection is considered a reliable method to determine if peripheral blood stem cells are suitable for transplantation collection. The recommended range for the optimal number of stem cells collected is 4-6×10^6/kg CD34+ cell, with a minimum requirement of CD34+ cell ≥ 2×10^6/kg. The ROC curve analysis revealed an area under the curve of 0.673, indicating the predictive value of CD34+ cell dose for CMV viremia in HSCT

patients. Employing the Youden index method, a cut-off value of 5.244×10^6/kg was determined, suggesting that exceeding this threshold can reduce the incidence of CMV viremia. This assessment is crucial for ensuring the collection of a sufficient number of viable stem cells for successful transplantation. In a study by Gaballa et al. [18], it was demonstrated that administering higher doses of CD34+ cells (> 5.2×10^6/kg) led to accelerated platelet engraftment and faster immune recovery in patients undergoing Haplo-HSCT. Moreover, these higher doses were also associated with a reduced occurrence of GVHD [18]. These findings align with our own research.

In summary, an increase in CD34 cell count has been shown to effectively reduce the occurrence of CMV viremia, with levels above 5.244×10^6/kg associated with a decreased incidence of CMV viremia. These findings have potential for further application in clinical practice. However, it is important to acknowledge a limitation of this study, which stems from the data being sourced from a single institution with a limited sample size.

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Disclosure of conflict of interest

None.

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