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Herpesvirus infections in hematopoietic stem cell transplant recipients seropositive for human cytomegalovirus before transplantation

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SUMMARY

Background: Viral infections are a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). The effect of herpesvirus infections in human cytomegalovirus (HCMV)-seropositive (IgG-positive/IgM-negative) HSCT recipients remains poorly understood. The risk factors associated with Epstein–Barr virus (EBV), HCMV, and human herpes virus type 6 (HHV-6) infections after HSCT, both alone and in combination, were investigated in this study.

Methods: Peripheral blood specimens were collected from 44 HSCT recipients and examined for viral DNA using quantitative fluorescence PCR assays. Risk factors for EBV, HCMV, and HHV-6 infections were analyzed by binary logistic regression, and relationships between these viruses were analyzed using the Chi-square test.

Results: EBV, HCMV, and HHV-6 were detected in 50%, 45.45%, and 25% of HCMV-seropositive (IgG-positive/IgM-negative) HSCT recipients, respectively. Male sex ($p = 0.007$) and conditioning regimens including anti-thymocyte globulin (ATG) ($p = 0.034$) were strongly associated with an increased risk of EBV infection. Graft-versus-host disease (GVHD) prophylaxis with corticosteroids was a risk factor for both EBV ($p = 0.013$) and HCMV ($p = 0.040$) infections, while EBV infection ($p = 0.029$) was found to be an independent risk factor for HHV-6 infection. Pre-existing HHV-6 infection was associated with lower rates of HCMV infection ($p = 0.002$); similarly, pre-existing HCMV infection was protective against HHV-6 infection ($p = 0.036$).

Conclusions: HCMV-seropositive (IgG-positive/IgM-negative) HSCT recipients exhibited a high rate of herpesvirus infections, particularly EBV. ATG and male sex were strongly associated with an increased risk of EBV infection. GVHD prophylaxis with prednisone was found to affect both EBV and HCMV infections. Prior infection with EBV was shown to promote HHV-6 infection. Taken together, these data highlight the need for active monitoring of herpesvirus infections in patients undergoing HSCT.

1. Introduction

Hematopoietic stem cell transplantation (HSCT) has proven to be an effective measure in the treatment of hematological malignancies. However, this procedure is not without significant risks, particularly that of viral infections, which remain one of the major causes of morbidity and mortality after HSCT.[1,2] Transplantation is often accompanied by the use of potent immunosuppressive drugs to both prevent and treat graft-versus-host disease (GVHD). The use of these drugs results in a severely compromised immune system, making HSCT patients more vulnerable to primary viral infections and reactivation.

Herpesviruses are among the most common opportunistic viral infections in HSCT recipients. Of these infections, human cytomegalovirus (HCMV) pneumonia and enteritis are the most serious and often fatal complications, with a mortality rate exceeding 50% after HSCT.[3] Primary Epstein–Barr virus (EBV) infection and lymphoproliferative disorders can occur after T-cell-depleted HSCT,[4] while human herpesvirus 6 (HHV-6),[5] a member of the β-herpesvirus family along with HCMV, can achieve

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lifelong latency in the host. Reactivation of these latent infections after HSCT has been associated with a variety of symptoms including skin rash, fever, interstitial pneumonitis, bone marrow suppression, encephalitis, and GVHD.[6–9]

The vast majority of research into herpesvirus infections in HSCT recipients has focused on HCMV, with very little known regarding either EBV or HHV-6. Moreover, few studies have examined the effects of these viruses in combination, particularly in the context of HCMV-seropositive (IgG-positive/IgM-negative) HSCT recipients. In previous studies examining the relationships between the herpesviruses, β-herpesviruses were found to transactivate each other, while HCMV infection appeared to trigger HHV-6 and/or HHV-7 co-infection and vice versa.[10] However, the relationship between EBV and β-herpesvirus infections remains poorly understood. In this study, HSCT recipients who were seropositive for HCMV (IgG-positive/IgM-negative) before transplantation were examined to assess the relationships between HCMV, EBV, and HHV-6 infections after HSCT and to identify potential risk factors for viral infection.

2. Methods

2.1. Human subjects and samples

HSCT recipients treated at the study hospital between January 2012 and June 2012 were tested for seropositivity (IgG and IgM) to HCMV prior to transplantation; almost all of the patients were infected with HCMV before transplantation. Following the exclusion of the few HCMV IgG-negative patients, 44 patients with IgG-positive/IgM-negative HCMV were enrolled in this study. Detailed demographic and clinical data for these patients are shown in Table 1. Plasma samples were collected once weekly in the first month, twice in the second and third months after transplantation, and then every 1–2 months until December 2012 (range 3 months to 1 year). In total, 392 peripheral blood specimens (range 5–17 samples per patient) were collected, from which peripheral blood leukocytes (PBLs) were isolated and stored at −70 °C until DNA extraction. For some patients, the number of follow-up samples was limited due to early death or loss to follow-up.

Table 1
Characteristics of the study patients

Characteristics of patients	Value
Patients, *n*	44
Age, years, median (range)	26 (16–55)
Sex, *n* (%)	
Male	23 (52.30%)
Female	21 (47.70%)
Underlying disease	
Acute myelogenous leukemia	19 (43.20%)
Acute lymphoblastic leukemia	18 (40.90%)
Non-Hodgkin lymphoma	1 (2.27%)
Myelodysplastic syndrome	5 (11.36%)
Lymphosarcoma cell leukemia	1 (2.27%)
Conditioning regimen	
Ara-c + BUCY + MeCCNU + ATG	21 (47.73%)
Ara-C + BUCY + MeCCNU	4 (9.09%)
BUCY + ATG + MeCCNU	5 (11.36%)
Ara-C + BUCY + ATG	1 (2.27%)
BUCY + MeCCNU	11 (25.00%)
BUCY + ATG	1 (2.27%)
BUCY	1 (2.27%)
Type of donor	
HLA-identical sibling	16 (36.36%)
Mismatched related donor	14 (31.82%)
Matched unrelated donor	11 (25.0%)
Mismatched unrelated donor	3 (6.82%)
Stem cell source	
Peripheral blood	42 (95.45%)
Peripheral blood and bone marrow	2 (4.55%)
GVHD prophylaxis	
Mycophenolate mofetil + cyclosporine	10 (22.73%)
Mycophenolate mofetil + cyclosporine + prednisone	34 (77.27%)
aGVHD	
Grade 0–I	36 (81.82%)
Grade II–IV	8 (13.64%)
Death[a]	5 (11.36%)
Pulmonary fungal infection	2 (4.55%)
Pulmonary hemorrhage	1 (2.27%)
Hemorrhage of digestive tract	1 (2.27%)
Pulmonary fungal infection and hemorrhage of digestive tract	1 (2.27%)

Ara-C, cytosine arabinoside; BU, busulfan; CY, cyclophosphamide; MeCCNU, methylcyclohexylnitrosamine; ATG, anti-thymocyte globulin; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; aGVHD, acute graft-versus-host disease.
[a] Death: these deaths had no direct correlation with the viral infections.

2.2. Conditioning regimen and post-transplant treatment

HSCT recipients were treated with or without anti-thymocyte globulin (ATG) before transplantation. Patients were treated with mycophenolate mofetil plus cyclosporine in combination with short-term methotrexate and intravenous ganciclovir (5 mg/kg per day) for HCMV, for 7 days prior to transplantation. This was followed by long-term mycophenolate mofetil plus cyclosporine and prednisone, or mycophenolate mofetil plus cyclosporine for GVHD prophylaxis, sulfamethoxazole for *Pneumocystis carinii* pneumonia (4 tablets, twice daily), and intravenous ganciclovir for HCMV (5 mg/kg per day for the first 2 weeks) after transplantation. Acute GVHD and chronic GVHD were diagnosed and graded according to standard criteria.[11] Corticosteroids were used in patients with grade II–IV acute GVHD, with varying durations.

2.3. DNA detection of herpesviruses

2.3.1. Primers and probes

Herpesvirus DNA was extracted using a commercial DNA extraction kit (Promega Biological Technology Co. Ltd, Beijing, China) in accordance with the manufacturer's instructions. The primers and probes used to detect EBV, HCMV, and HHV-6 have been described previously.[4,12,13] Briefly, PCR primers and probes for EBV were selected from BALF5,[4] those for HCMV from the immediate early (IE) gene,[13] and those for HHV-6 from the U31 gene.[12] All primers and probes were synthesized by ZeHeng Technology (Shanghai, China).

2.3.2. Quantitative fluorescence PCR assay

Quantitative fluorescence PCR was performed using a TaqMan PCR Kit (Takara, Dalian, China) and run on an ABI 7500 Real-Time PCR System (USA), as described previously.[3] Standard strains were used as positive controls in each amplification (B95-8 for EBV, AD169 for CMV, and GS for HHV-6A). In addition to a blank control, distilled water was used as a negative control. Real-time fluorescence was measured, and cycle threshold (Ct) values were calculated for each sample .

Specificity was confirmed using viral DNA from standard strains. Sensitivity was confirmed by TaqMan Qualitative fluorescence PCR using serial dilutions of standard strains, with a minimum detectable Ct value of 48 relative to undiluted samples, which produced Ct values of 16. No peaks were detected in the negative control.

2.4. Statistical analyses

All statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Qualitative variables, such as the clinical characteristics of the HSCT recipients, were recorded as the percentage of the positive results, and differences in these variables were evaluated using the Chi-square test. Quantitative variables, such as age, were recorded as the median and range. Risk