Journal of Clinical Virology 95 (2017) 20–25

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Full length article

Risk factors for herpes simplex virus-1/2 viremia and clinical outcomes following unmanipulated haploidentical haematopoietic stem cell transplantation

Fei-Fei Tang[a], Xiao-Su Zhao[a], Lan-Ping Xu[a], Xiao-Hui Zhang[a], Yu-Hong Chen[a], Xiao-Dong Mo[a], Yu-Qian Sun[a], Kai-Yan Liu[a], Xiao-Jun Huang[a,b,*]

[a] Peking University People's Hospital, Peking University Institute of Hematology, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, China
[b] Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China

ARTICLE INFO

Keywords:
Herpes simplex virus (HSV)
Viremia
Haploidentical
Haematopoietic stem cell transplantation

ABSTRACT

Background: Herpes simplex virus (HSV)-1/2 can still be reactivated after allogeneic haematopoietic stem cell transplantation (allo-HSCT) even when the prophylactic acyclovir is used. However, the risk factors for HSV-1/2 viremia and the clinical outcomes following unmanipulated haploidentical HSCT remain unknown.
Objectives and study design: Nineteen patients with HSV-1/2 viremia and fifty-seven patients without HSV-1/2 viremia which were selected using the case-pair method after undergoing haploidentical HSCT were enrolled. We analysed the risk factors for HSV-1/2 viremia and compared the clinical outcomes between the two groups.
Results: The risk factors for HSV-1/2 viremia included HLA disparity ≥2 loci ($p = 0.049$) and cytomegalovirus (CMV) reactivation ($p = 0.028$). The incidences of platelet engraftment, oral mucositis and severe haemorrhagic cystitis (HC) in patients with and without HSV-1/2 viremia were 77% and 94% ($p = 0.003$), 78% and 13% ($p = 0.000$), and 25% and 6% ($p = 0.04$), respectively. Moreover, the median time to platelet engraftment in patients with and without HSV-1/2 viremia was +25 days (range, +11–+80) and +17 days (range, +8–+67) ($p = 0.004$), respectively. According to the multivariate analyses, HSV-1/2 viremia was associated with delayed platelet engraftment ($p = 0.038$), a higher incidence of oral mucositis ($p = 0.000$) and severe HC ($p = 0.038$). However, HSV-1/2 viremia was not associated with non-relapse mortality (34.0% vs. 31.5%, $p = 0.26$), leukaemia-free survival (60.9% vs. 57.9%, $p = 0.46$) and overall survival (61.2% vs. 60.7%, $p = 0.37$).
Conclusions: Based on our study results, we recommend that HSV-1/2 PCR should be performed upon clinical suspicion of HSV-1/2 infection.

1. Background

Since the advent of antiviral prophylaxis, the incidence of herpes simplex virus-1/2 (HSV-1/2) infections after allogenic haematopoietic stem cell transplantation (HSCT) has diminished [1]. However, HSV-1/2 reactivation remains a serious complication after allogenic HSCT(allo-HSCT). HSV-1 has a greater likelihood of causing recurrent oral infections, whereas HSV-2 is more likely to cause recurrent genital infections [2]. HSV-2 can also affect the oral mucosa, although such cases are thought to be much less common and do not tend to become recurrent [3]. In addition, certain HSV-1/2-associated diseases are severe and can occasionally be lethal. These include pneumonitis, enteritis, encephalitis, etc.

Previous reports of HSV-1/2 reactivation following transplantation have mainly focused on its relationship with oral ulcers. These reports have demonstrated that HSV-1/2 reactivation increases the incidence of oral ulcers [4–9]. However, few studies have investigated HSV-1/2 viremia. The manifestations of viremia and early infections are non-specific or even asymptomatic. Therefore, the monitoring, early diagnosis and pre-emptive treatments of HSV-1/2 diseases have been very important [10]. Polymerase chain reaction (PCR) is a useful and wide-ranging tool for monitoring and alternative diagnosis. Most previous reports regarding the use of HSV-1/2 PCR in post-transplantation monitoring have used oral mucosal specimens [7], and there were few reports that have used HSV-1/2 PCR with blood samples [8,9].

Duing to therapeutic interventions are well established for HSV, data regarding the prevalence of HSV in the blood after allo-HSCT are limited. Previous studies regarding HSV-PCR after allo-HSCT either almost included Human Leucocyte Antigen (HLA)-matched donors, unrelated donors, and cord blood [11] or reported the results of PCR of

* Corresponding author at: Peking University People's Hospital, Peking University Institute of Hematology, No. 11 Xizhimen South Street, Xicheng District, Beijing 100044, China.
E-mail addresses: huangxiaojun@bjmu.edu.cn, xiaojunhuang1@126.com, pk200608@126.com (X.-J. Huang).

http://dx.doi.org/10.1016/j.jcv.2017.07.018
Received 22 March 2017; Received in revised form 5 June 2017; Accepted 28 July 2017

oral mucosal specimens [7]. To our knowledge, no previous studies have evaluated the risk factors for HSV viremia and the clinical outcomes following unmanipulated haploidentical HSCT (haplo-HSCT). Therefore, the aim of this study was to explore the risk factors for HSV-1/2 viremia and evaluate the clinical outcomes following haplo-HSCT.

2. Objectives and study design

2.1. Patients

This is a retrospective analysis of qualitative PCR for HSV-1/2 in plasma. We began using PCR to detect HSV in plasma in April 2015. We applied real-time PCR (RT-PCR) in patients with a variety of clinical manifestations, who underwent haplo-HSCT. When a patient was suspected of having herpesvirus reactivation or the disease after haplo-HSCT, a peripheral blood (PB) sample was collected. Ultimately, three hundred and eighty-five patients, who were suspected of having a herpesvirus reactivation or the disease after haplo-HSCT at the Peking University Institute of Haematology, underwent HSV-PCR detection using plasma samples between April 2015 and September 2016. Nineteen patients developed HSV-1/2 viremia, while 366 patients were HSV-PCR negative. For each HSV-PCR positive case, a set of control cases were selected from the HSV-PCR negative cases. This matching method allowed for tight control of the confounding effects of time during the analysis. We randomly selected control subjects at a 3:1 ratio who were matched for (1) age (± 5 years), (2) year of transplantation (± 2 years), (3) type of disease (i.e., acute leukaemia, myelodysplastic syndrome (MDS), or chronic myelogenous leukaemia (CML)), and (4) status (i.e., standard risk or high risk) of the underlying disease. Finally, 57 patients with HSV-PCR negative were selected as the matched control group. The final study cohort comprised 76 consecutive children and adolescents, who underwent haplo-HSCT at the Peking University Institute of Haematology between April 2014 and September 2016. Informed consent was obtained from all patients. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committee of Peking University People's Hospital.

2.2. Conditioning regimens and graft-versus-host disease (GVHD) prophylaxis

All patients received a myeloablative conditioning regimen (MAC) without *in vitro* T-cell depletion. The conditioning therapy for haplo-HSCT was modified BUCY2 plus ATG (thymoglobulin) consisting of cytarabine (4 g/m2/day) intravenously on days −10 to −9; busulfan (3.2 mg/kg/day) intravenously on days −8 to −6; cyclophosphamide (1.8 g/m2/day) intravenously on days −5 to −4; Me-CCNU (250 mg/m2) orally once on day −3; and ATG (thymoglobulin,2.5 mg/kg/day; Sang Stat, Lyon, France) intravenously on days −5 to −2 [12,13]. All patients received fresh granulocyte colony-stimulating factor (G-CSF)-mobilized and unmanipulated G-CSF-primed bone marrow cells in addition to G-CSF-primed peripheral blood stem cells. All patients also received cyclosporine, mycophenolate mofetil, and short-term methotrexate for GVHD prophylaxis [12,13].

2.3. Post-alloHCT HSV infection prophylaxis and treatment

The HSV prophylaxis for patients who received HSCT consisted of orally acyclovir (800 mg/day) from day +1 until the discontinuation of all immunosuppressive agents [12,13]. Intravenous drip of acyclovir were carried out when patients with mucositis may have difficulty in taking oral antivirals. Patients with mucositis that did not affect oral antivirals were taking their acyclovir under the supervision of the nurses and their families. Therapeutic drug monitoring was not performed. The HSV-1/2 treatment for patients suspected of having herpesvirus reactivation or the disease consisted of intravenous acyclovir (5–10 mg/kg/dose) every 8 h for 7–10 days or until the symptoms and signs of the disease disappeared.

2.4. Viral PCR monitoring

Total DNA was extracted from 100 μL of EDTA-anticoagulated whole blood and viral DNA was quantitated by real-time qPCR using HSV1/2 Kit (DAAN Gene Co., Ltd. Of Sun Yat-sen University, Guangzhou, China). Blood DNA(5 μL) was added to the PCR reaction mixture in a total volume of 25 uL.The reaction was performed for 2 min at 50 °C, 15 min at 95 °C, and 40 cycles of 94 °C for 15 s and 55 °C for 45 s using qPCR (Applied Biosystems 7500, Foster City, CA). At the same time, positive and negative control were also used to confirm the results. The sensitivity was 100 copies/ml. CMV and EBV infection was monitored by weekly plasma DNA testing using real-time PCR (RT-PCR) (kits from PG Biotech Co., Ltd., Shenzhen, China with a detection of limit of 500 copies/ml).

2.5. Definitions of clinical endpoints

Viremia: HSV-1/2 viremia was defined as a positive PCR of a plasma sample. CMV viremia and EBV viremia were defined as a plasma PCR result showing > 1000 viral copies/mL.

Viral disease: Viral disease (e.g. pneumonia, hepatitis, and encephalitis) refer to CMV, EBV, and HSV-1/2 disease.

2.6. Engraftment

Neutrophil engraftment was defined as an absolute neutrophil count of 0.5×10^9/L or more for 3 consecutive days. Platelet engraftment was defined as a platelet count of 20×10^9/L or more for 7 consecutive days without transfusion.

Oral mucositis: The severity of oral mucositis was classified according to the toxicity criteria established by the National Cancer Institute [14].

Haemorrhagic cystitis (HC): HC grade was determined according to the suggestions of Bedi et al. [15]. Grade 0 indicated no HC, grade I indicated microscopic haematuria, grade II indicated macroscopic haematuria, grade III indicated the presence of visible clots, and grade IV indicated clots that had led to an obstruction. Grade III and IV were defined as severe HC.

Others: Overall survival (OS) times were measured as the date of HSCT until death from any cause. Leukaemia-free survival (LFS) was defined as the time from transplantation to relapse or death from any cause. Surviving patients were censored on the date of their last follow-up. Non-relapse mortality (NRM) was defined as death after allo-HSCT without disease progression or relapse. Relapse was defined by the appearance of morphological evidence of the disease obtained from testing samples from the peripheral blood, bone marrow, or extramedullary sites or else by the recurrence and sustained presence of pretransplantation chromosomal abnormalities. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined according to previously published criteria [16,17].

2.7. Statistical analysis

The data were up-to-date as of December 31, 2016, and represented a minimum of 100 days of follow-up. The data were censored at the time of relapse, NRM, or last follow-up. Demographic and clinical characteristics were compared between patients with HSV-positive and HSV-negative results using the $\chi^2$and Fisher's exact tests for dichotomous variables and the Mann–Whitney *U* test for continuous variables.

The probabilities for OS and LFS were estimated using the Kaplan–Meier method. Univariate and multivariate time-dependent Cox proportional hazard models were used to examine the risk factors associated with each of the following end-points of interest: HSV