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Incidence, risk factors and the effect of polyomavirus infection in hematopoietic stem cell transplant recipients

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Abstract

Objective: The effect of polyomavirus infection in HSCT recipients is poorly understood.

Methods: We evaluated 38 HSCT recipients. Polyomavirus was detected by nested qualitative polymerase chain reaction (PCR) assays of urine. The risk factors for BK virus and JC virus were analysed. The kidney and liver functions of infected and uninfected patients were compared.

Results: BK virus, JC virus, and simian virus 40 were detected in 21%, 42%, and 0% of HSCT recipients respectively. HCMV infection was found to be an independent risk factor for JC virus infection (odds ratio (OR): 8.528), while transplants with mismatched HLA are more susceptible to BK virus infection (OR: 12.000). Liver function of JC virus-infected subjects was worse than that of uninfected subjects.

Conclusion: We must be vigilant for opportunistic polyomavirus infections in HSCT recipients, especially those with HCMV co-infection or a mismatched HLA transplant. When unexplained liver function deterioration is observed, JC virus infection should be considered.

Keywords

Haematopoietic stem cell transplantation, polyomavirus, BK virus, JC virus, risk factors, haemorrhagic cystitis

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Introduction

With the development of transplantation technology and the emergence of new potent immunosuppressive regimens, haematopoietic stem cell transplant (HSCT) is now the most efficient way to treat very high risk haematological malignancies. Because the recipients are consequently immunocompromised, they are more vulnerable to opportunistic infections by viruses such as human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and polyomaviruses.[1] These viral infections are among the most common causes of morbidity and mortality after HSCT.[2] Polyomaviruses, members of the Polyomaviridae family, include BK virus, JC virus and simian virus 40 (SV40). Following initial infections in childhood with no or mild symptoms,[3] polyomaviruses may be latent in the kidney epithelium, genitourinary tract epithelium and lymphocytes.[4] When BK virus or JC virus reactivates in hosts that are immunosuppressed (such as HSCT or solid organ transplant recipients) or immunocompromised (such as AIDS patients), it can lead to severe clinical diseases. For example, BK virus reactivation can lead to polyomavirus-associated nephropathy in kidney transplant patients,[1,3,5] while JC virus is more likely to be associated with progressive multifocal leukoencephalopathy (PML).[1,3] Some studies revealed that polyomavirus, mainly BK virus,[3,6,7] might associate with haemorrhagic cystitis (HC) after HSCT,[3,6,7] whereas other studies showed no association.[8,9] However, it was unclear if JC virus also induces HC in HSCT recipients. Additionally, BK virus reactivation may result in renal failure and allograft failure in kidney transplant patients,[10] but it was unknown if reactivation of this virus or of JC virus might similarly affect the kidney function in HSCT.

In this study, we examined the occurrence of BK virus, JC virus and SV40 after HSCT in China, analysed the risk factors for these viral infections, investigated if viral infections of JC virus or BK virus are associated with HC in HSCT recipients, and explored the effect of these viruses on kidney and liver functions.

Patients and methods

Patients, samples and follow-up

Allogeneic HSCT recipients treated at the First Affiliated Hospital, College of Medicine, Zhejiang University, China, in 2010 were enrolled in this study. Patients who died or were lost to follow-up within one year after transplantation were excluded. Overall, 38 subjects with a median of age of 23 years were enrolled; they were transplanted for a variety of haematologic malignancies, as described in Table 1.

Allogeneic HSCT recipients were examined in our outpatient clinic at regular intervals after transplantation. Follow-up was conducted biweekly in the first month, then once per month until one year post-transplant. As urine specimens are easier to acquire than blood samples, which are invasive and painful to obtain, urine samples were collected to test for JC virus and BK virus DNA at 6 and 12 months post-transplant and stored at $-70^{\circ}C$. At each follow-up visit, all clinical data were collected, including HC-related symptoms. This study was approved by the ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. All patients gave their informed consent to participate in the study.

Conditioning regimen, immunosuppressive and anti-graft-versus-host disease program

HSCT recipients received myeloablative or non-myeloablative conditioning before transplantation (Table 1). They also received

Table 1. Patient characteristics.

	Value
Patients	38
Age (Median and Range, years)	23(12–46)
Sex (M/F)	17/21(45%/55%)
Underlying disease	
Acute myelogenous leukaemia	17(45%)
Acute lymphoblastic leukaemia	9(24%)
Chronic myeloid leukaemia	8(21%)
Non-Hodgkin lymphoma	1(3%)
Myelodysplastic syndrome	2(5%)
Paroxysmal nocturnal haemoglobinuria	1(3%)
Donor cell source	
Related donor	15(39%)
Unrelated donor	23(61%)
HLA (Donor and Recipient)	
Complete match	29(76%)
Incomplete match	9(24%)
Conditioning regimen	
BUCY	22(58%)
BUCY + MeCCNU	2(5%)
BUCY + ATG	3(8%)
BUCY + Ara-C	3(8%)
BUCY + MeCCNU + ATG	1(3%)
BUCY + MeCCNU + ATG + Ara-C	1(3%)
Fludarabine + Myleran + ATG	6(16%)
Immunosuppressive drugs[#]	
Mycophenolate Mofetil + Cyclosporin	24(63%)
Mycophenolate Mofetil + Cyclosporin + Other drugs*	14(37%)
GVHD	
Yes	18(47%)
No	20(53%)
HCMV infected	
Yes	7(18%)
No	31(82%)
HC	
Yes	7(18%)
No	31(82%)

HLA, human leukocyte antigen; BU, busulfan; CY, cyclophosphamide; MeCCNU, methylcyclohexylnitrosamine; ATG, anti-thymocyte globulin; Ara-C, cytosine arabinoside; GVHD, graft-versus-host disease; HCMV, human cytomegalovirus; HC, Haemorrhagic cystitis.
[#]GVHD prophylaxis therapies
*Prednisone and/or tacrolimus and/or azathioprine

cyclosporine, mycophenolate mofetil and a short-term methotrexate course from day −7 to day −1, followed by a long-term course of cyclosporine and mycophenolate mofetil with or without prednisone, tacrolimus or azathioprine as prophylaxis for graft-versus-host disease (GVHD). Intravenous ganciclovir (5 mg/kg per day) was given from day −7 to day 14 for HCMV prophylaxis, and sulfamethoxazole was administered for pneumocystis carinii pneumonia (PCP) prophylaxis (four tablets per day, twice daily) after transplantation. For cases in which GVHD occurred, the immunosuppressive program was intensified. Prednisone was commonly the first line therapy, and other medications, such as tacrolimus or high doses of azathioprine, etanercept, and intravenous immunoglobulin, were used with varying durations according to the patient's response.

Polyomavirus detection

Polyomavirus DNA was extracted from urine samples using AxyPrep Body Fluid Viral DNA/RNA minikits (AiSiJin Technology, Hangzhou, China) according to the manufacturer's instructions. BK virus, JC virus and SV40 DNA were detected by nested polymerase chain reaction (PCR). The primers amplified a conserved region of the T-antigen nucleotide sequences for BK virus, JC virus and SV40.[11] All of the primers were synthesized by Invitrogen Biotechnology Co. Ltd. (Shanghai, China).

The reaction system and amplification conditions were the same as those in our previous report.[12] For each amplification, positive PCR products (BK virus, 353 bp; JC virus, 189 bp; and SV40, 135 bp, all sequenced by ZeHeng Technology Co. Ltd., Shanghai, China) served as positive controls and distilled water served as a negative control.

The PCR products stained with ethidium bromide were analysed by electrophoresis

on 1.5% agarose in 0.5 × TBE gels and visualized by ultraviolet light. Positive products were sequenced by ZeHeng Technology Co. Ltd. and analysed by BLAST software.

The sensitivity of the complete procedure was checked by amplifying serial dilutions of plasmids in 25 µl of distilled water before testing the urine samples. The extraction and amplification conditions were the same as those used for the clinical samples, and all dilutions were assayed in triplicate.

Renal function and liver function detection

The glomerular filtration rate (GFR) is the optimal indicator for renal function.[13] Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), and direct bilirubin (DB) were the most common indicators for measuring liver function in our enrolled patients. At each visit in this study, serum creatinine (Cr), ALT and AST were measured by the biochemical laboratory of the First Affiliated Hospital and GFR was converted according to the modified equations suitable for Chinese individuals.[13]

Statistical analyses

Statistical analyses were performed using SPSS software (version 16.0, SSPS Inc., Chicago, IL, USA). Patients' ages are expressed as the median, and other quantitative variables are expressed as the mean values ± standard deviation (SD) for normally distributed data. These data were compared using a *t*-test. Qualitative variables are expressed as a percentage of positive results, and the differences between these variables were evaluated by using the chi-square or the Fisher's exact test. Virus risk factors were analysed by a binary logistic regression. All *p*-values were based on a two-tailed test of significance ($p < 0.05$).

Results

Polyomavirus infection

Two samples were collected for every patient; urine samples were collected in the sixth month and the twelfth month after HSCT and stored at −70°C. The patient was considered to be virus-infected if at least one sample was positive. Of the 38 total patients, eight patients were infected with BK virus (21%) and 16 patients were infected with JC virus (42%), including four patients who infected with both viruses (11%). No patients were infected with SV40. JC virus was detected in both urine samples for only one of the 38 patients; this virus was detected in the sixth month and twelfth month post-transplantation samples of seven and eight patients, respectively. BK virus was not detected in both urine samples for any of the patients; this virus was detected in the sixth month and twelfth month post-transplantation samples of five and three patients, respectively. Both viruses were detected from the same sample for all four of the patients infected with BK virus and JC virus. The rate of JC virus coinfection in BK virus-infected patients was significantly higher than the rate of JC virus infection in those without BK virus infection ($p = 0.001$). Additionally, the rate of BK virus coinfection in JC virus-infected patients was also significantly higher than the rate of BK virus infection in those without JC virus infection ($p = 0.025$).

Risk factors for polyomavirus infection

We attempted to analyse the potential risk factors,[12,14–17] including recipient's age, gender, sex difference between donor and recipient, donor being related to recipient, ABO blood type, mismatched ABO blood type between donor and recipient, mismatched human leukocyte antigen (HLA) between donor and recipient, myeloablative conditioning, conditioning regimen

Table 2. Binary logistic analysis for BK virus and JC virus risk factors.

	Factors	Coefficient(B)	OR(95%CI)	*P*-value
BK virus	HLA-mismatch	2.485	12.000(1.053–136.794)	0.045
JC virus	HCMV Co-infection	2.143	8.528(1.092–66.578)	0.041

OR, odds ratio; CI, confidence intervals; HLA, human leukocyte antigen; HCMV, human cytomegalovirus.

(including anti-thymocyte globulin (ATG)), GVHD, immunosuppressive program (including prednisone corticosteroids, tacrolimus, and high dose of azathioprine) and HCMV infection. Our analyses found that HCMV infection is a risk factor for JC virus infection (odds ratio (OR): 8.528, $p = 0.041$). Additionally, a mismatched HLA transplant is 12 times more susceptible to BK virus infection than a matched HLA transplant (OR: 12.000, $p = 0.045$) (Table 2).

Haemorrhagic cystitis

Of the 38 total patients, seven patients (18%) had HC, including six patients within 3 months after transplantation, and one patient in the fifth month post-transplantation. The median time of occurrence was 48 days. Four of the seven patients were infected with at least one polyomavirus; one patient was infected with both BK virus and JC virus, one patient was infected with only BK virus and two patients were infected with only JC virus. Our limited data on polyomavirus infection (20 cases) and HC (7 cases) did not reveal any clear association between polyomavirus and HC.

The influence of renal and liver function

The Cr, GFR, TB and DB differences between BK virus-infected patients and uninfected patients were not statistically significant, nor were the differences in these metrics between JC virus-infected and uninfected patients (Table 3). In contrast, although the ALT and AST were not significantly different between BK virus-infected patients and uninfected patients, the ALT and AST in JC virus-infected patients were both significantly higher than those in patients who were not infected with JC virus (ALT, $p = 0.041$; AST, $p = 0.025$). Given that HCMV infection may affect renal function and liver function, both the renal and liver functions were compared between HCMV-infected patients and HCMV-uninfected patients; however, no significant differences were found.

Discussion

The infection rates of polyomavirus, BK virus, and JC virus in this study were 63%, 21% and 42%, respectively, which are similar to published results.[18,19] Our previous research demonstrated that immunosuppressed patients are more susceptible to JC virus than to BK virus.[12] Here, in the four patients co-infected with BK virus and JC virus, prior infection with BK virus or JC virus was shown to hasten additional infection by other polyomaviruses (JC virus or BK virus), but the exact mechanism for this remains unknown. It is possible that the virus-specific IgG raised against one polyomavirus may not confer cross-protective immunity against other polyomaviruses. Alternatively, polyomaviruses may affect immune regulation, facilitating the replication and further infection of other polyomaviruses. Further research is needed to validate these hypotheses.

Many previous publications have reported an association between HCMV and polyomavirus infection.[20–23] This association may be related to the immunosuppression required to prevent GVHD. Although most of those studies revealed a

Table 3. Liver function and kidney function between polyomavirus-infected and -uninfected patients.

	BK virus-infected (n = 8)	Uninfected (n = 30)	*P*-value	JC virus-infected (n = 16)	Uninfected (n = 22)	*P*-value
ALT (u/L)	82.125 ± 52.865	59.167 ± 63.518	0.355	87.750 ± 51.450	46.727 ± 63.470	0.041
AST (u/L)	54.375 ± 24.213	35.500 ± 25.974	0.073	50.625 ± 31.580	31.364 ± 18.927	0.025
TB (μmol/L)	14.375 ± 7.539	14.700 ± 9.278	0.928	15.312 ± 7.255	14.136 ± 9.982	0.691
DB (μmol/L)	4.750 ± 3.370	5.100 ± 2.917	0.772	4.812 ± 2.613	5.182 ± 3.261	0.711
Cr (μmol/L)	60.875 ± 29.304	47.833 ± 11.498	0.055	51.438 ± 10.776	49.955 ± 20.882	0.797
GFR (ml/min)	193.005 ± 99.703	223.760 ± 60.618	0.277	193.826 ± 51.409	234.347 ± 77.849	0.078

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DB, direct bilirubin; Cr, creatinine; GFR, glomerular filtration rate.

correlation between BK virus and HCMV,[22,23] we found here that there were also some associations between HCMV and JC virus infections. We identified HCMV as a risk factor for JC virus infection, in agreement with previous studies[12,20,21] that showed that cytomegalovirus replication enhances JC virus replication and infection. The possible mechanism of action may be as follows. The immunoregulatory ability of cytomegaloviruses might increase the risk of opportunistic JC virus infections by modulating molecules involved in immune recognition and inflammation.[12,24] Additionally, expression of the cytomegalovirus immediate-early 2 gene activates early promoters of JC virus (which induce expression of T-antigen), and various late JC virus genes are then replicated and expressed[12,21]

Here, we found that mismatched HLA between the donor and recipient is an independent risk factor for BK virus infection; however, this topic is controversial.[15,17,25] We hypothesize that the effect of HLA mismatch is due to the donor immune response. During BK virus infection, CD8-positive cytotoxic T cells can only attack the virus-bearing target cells that share the same class I HLA antigens.[26] Notably, when HLA is mismatched, the CD8-positive cytotoxic T cells might lose this ability. Thus, an impaired immunosurveillance of BK virus by virus-specific T-cell responses and the major histocompatibility complex restriction of the T-cell response might allow the BK virus to escape virus-specific immunity in a situation with mismatched HLA.[17]

HC is a frequent complication after allogeneic HSCT. Here, the HC rate was 18%, and various studies have reported HC rates between 10% and 70%.[7] Some reports[5–7] found that HC is associated mainly with BK virus and also maybe JC virus.[7] Seven patients in this study had HC, but, in contrast to previous reports[27], their HC was not related to polyomavirus and none of the polyomavirus-infected patients developed polyomavirus-associated HC. One previous report[6] revealed that only 10–25% of BK viruria develops into clinically significant cystitis and that BK viremia and peak BK viremia (≥10,000 copies/mL), but not viruria, were independent risk factors for HC.[6] If the polyomavirus replication was not at these peak levels in our samples, it may explain the lack of association between HC and polyomavirus infection that we observed. Furthermore, there are many other potential risk factors for HC besides polyomavirus, such as the type of conditioning chemotherapy (ex: BU-based conditioning regimens),[28] HLA mismatch, GVHD, adenovirus, and HCMV[7,28].

BK virus and/or JC virus are important causes of polyomavirus nephropathy in recipients of renal transplants,[1,3] but their significance in HSCT was poorly described. Polyomavirus nephropathy begins as a localized viral presence in the tubular epithelial cells of the kidney and progresses to diffuse and destructive T cell-mediated interstitial nephritis.[16] This interstitial infiltration can cause scarring of the kidney parenchyma, loss of function, and kidney failure.[16] However, during follow-up, no neurological complications were found among the JC virus-positive patients in this study.

Here, we did not observe an increase in the Cr level, regardless of whether or not BK virus or JC virus infection occurred. Ramos' theory of the three stages of polyomavirus infection[29] reveals that polyomavirus only causes tissue destruction and classical polyomavirus-associated nephropathy, which occur in 5–10% of transplant patients, during the third stage. Therefore, it is not surprising that we did not find renal dysfunction in any of the polyomavirus-infected patients because their polyomavirus infections would still be in the first or second stage, during which there is no clinical abnormality and renal function is still normal.

To the best of our knowledge, almost no studies have focused on the effect of polyomavirus on the liver function of transplant recipients. Only Demir-Onder K et al.[30] reported that BK virus is a factor that should be considered when unexplained renal or liver function deterioration is observed in liver transplant recipients. To our surprise, we found that JC virus infection could cause liver function damage. The exact pathophysiologic basis underlying this situation remains unknown. It is possible that JC virus reaches the liver via the blood flow through a mechanism that allows it to escape virus-specific immune surveillance, subsequently inducing liver cell damage by direct virus attack as well as by indirect immune attack. Further research is needed to validate this hypothesis.

As with all studies, our work has some limitations. First, we only collected urine samples for qualitative PCR detection; we did not collect blood samples and conduct quantitative PCR detection. Furthermore, for consistency, samples were collected at 6 and 12 months post-transplantation regardless of when symptoms occurred, so the results do not necessary match up with the timing of symptoms. These shortcomings will have some impact on the accuracy of the results of this study. Lastly, this study only investigated BK virus, JC virus and SV40. Further studies are needed to investigate other related pathogens, such as KI polyomavirus (KIPyV), wupolyomavirus (WUPyV) and MC polyomavirus (MCPyV).

In summary, the data presented in this study reveal the frequent occurrence of polyomavirus in HSCT recipients, with JC virus being the most common pathogen. HCMV infection is a risk factor for JC virus infection, and mismatched HLA may induce BK virus infection. Polyomavirus-related HC, polyomavirus-induced nephropathy and renal dysfunction were not found. Nevertheless, JC virus-related liver function damage was present. Therefore, we should be alert to potential opportunistic polyomavirus infections in HSCT recipients, especially those with the risk factors mentioned above. Furthermore, when unexplained liver function deterioration is observed, polyomavirus JC virus infections should be considered as a possible cause.

Authors' contributions

Jianhua Hu, Jun Fan and Lanjuan Li designed the study; Siying Li and Yaping Huang performed the experiments; Meifang Yang, Xuan Zhang and Hong Zhao acquired the patients; Lichen Xu and Huihui Dong collected the data; Jianhua

Hu, Meifang Yang and Lichen Xu analysed the data; and Jianhua Hu and Siying Li prepared the manuscript.

Declaration of conflicting interests

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