Review

No Detection of BK Virus, JC Virus, KI, WU and Merkel Cell Polyomaviruses in Cerebrospinal Fluid of Patients with Neurological Complications after Hematopoetic Stem Cell Transplantation

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Abstract. *Neurological complications, often due to viral reactivation, after allogeneic hematopoetic stem cell transplantation (HSCT) are associated with increased mortality. Here, cerebrospinal fluid from 20 HSCT patients with neurological symptoms were analyzed and found to be negative by PCR for BK virus, JC virus, KI, WU and Merkel cell polyomavirus DNA.*

Neurological complications during the first months after hematopoietic stem cell transplantation (HSCT), where viral reactivation is an essential cause, are encountered in roughly 10% of patients and these patients have a higher mortality than those without neurological complications (1-3). The possibility to identify and treat viral infections in the central nervous system (CNS) would, thus, be beneficial. In fact, intensified strategies have been made to treat infections with *e.g.* adenoviruses, herpesviruses (cytomegalovirus, Epstein Barr virus), and also the two well-known human polyomaviruses (PyVs), BK virus (BK) and JC virus (JC). Primary infection with the latter two viruses results in no or mild symptoms and latent infection (4). However, in immunosuppressed patients, BK reactivation is associated with hemorrhagic cystitis (in HSCT patients) and polyomavirus associated nephropathy (in renal transplant patients), while reactivation of JC is associated with progressive multifocal leukoencephalopathy (PML) (4, 5). Other CNS diseases due to BK and JC reactivation have been reported (6, 7). In 2007-2008, three new human polyomaviruses were described: KI, WU found in nasopharyngeal aspirates, and Merkel cell polyomavirus (MC) in Merkel cell carcinoma (MCC) (8-10). Primary infection with no or mild symptoms with these viruses was suggested to be due to respiratory or fecal-oral transmission and they were more often detected in immunocompromized persons; while data regarding whether they infect the CNS are limited, one case report suggests that WU may be linked to PML (11-19). To extend the efforts to find causative agents for neurological symptoms in patients after HSCT, here we examined for the possible presence of KI, WU and MC in cerebrospinal fluid (CSF) of 20 patients with neurological complications after HSCT. Testing for BK and JC was included, since this is not always carried out in clinical practice.

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Key Words: Human polyomavirus, BK virus, JC virus, hemotopoetic stem cell transplantation, cerebrospinal fluid, KI, WU.

Patients and Methods

Patients. During 2000-2008, within the first year after HSCT, a lumbar puncture (LP) was performed on 46 out of the 598 HSCT patients transplanted at the Centre for Allogenic Stem Cell Transplantation, Karolinska University Hospital Huddinge, due to acute/subacute neurological complications and suspicion of viral infection. The CSF samples were analyzed depending on clinical suspicion and the remaining CSF stored at –20˚C. Sufficient amounts of CSF were available for further analysis for 20 out of 46 patients in this study, approved by the Regional Research Ethics Committee at Karolinska Institute. Data collection from original charts was carried out manually.

Table I. *Patients (n=20) with neurological complications within one year after HSCT, transplanted 2000-2008, where CSF was examined for the presence of polyomaviruses.*

Age, years	
Mean/Median	31/32 (range 1-60)
Gender	
Female/male	7/13
Diagnosis	
ALL	1
AML	3
MDS	5
KML	5
Lymphoma	3
Inborn errors of metabolism	1
Benign blood disease	2
Donor	
15 MUD	15
Cord blood	3
Sibling	5
Conditioning	
Busulphan/cyclophosphamide	8 (1 + etopside, 1+ melphalan)
TBI/cyklophosphamide	5 (1 + Flu)
Busulphan/fludarabine (reduced)	4
Fludarabine/cyclophosphamide (reduced)	2
Isophosphamide/carboplatin/etoposide	1
GVHD	
Acute non-severe, gr 1-2	8 (40%)
Acute severe	5 (25%)
Chronic grade 1	3 (15%)
Chronic grade 2	4 (20%)
Neurological complications	
Seizures	4 (2 suspected drug reactions,1 encephalitis 1 herniation, ukc)
Confusion/hallucinosis	4 (1 suspected, 1 documented drug side-effect, 1 ukc)
Nystagmus	1 (ukc)
Facial nerve palsy	1 (ukc)
Muscle weakness	1 (hypothyreosis)
Suspected CNS infection	2 (EBV, enterovirus)
Headache/headache and fever	7 (2 suspected encephalitis ukc, 1 leukemic relapse, 4 headache/meningitis ukc)
Liquor cell analysis	
Leucocyte cell count in liquor not available:	4
Leucocyte cell count zero	6
Leucocytosis within ref. values (poly <1, mono <0-5×10^6/ L)	6
Leucocytosis	4

HSCT: Hematopoietic stem cell transplantation, CSF: cerebrospinal fluid, ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, MDS: myelodysplastic syndrome; CML: chronic lymphoblastic leukemia, MUD: matched unrelated donor, TBI: total body irradiation, GVHD graft-versus-host disease, ukc: unknown cause.

PCR for detection of BK, JC, WU, KI and MC DNA. A PCR detecting BK and JC, with a sensitivity of 10 genomic copies of BK plasmid DNA and 5-10 copies JC plasmid DNA (20-22) was used. The CSF (10 μl) was heated at 94˚C for 9 min for denaturation before being added to the PCR mix as performed previously (20). A PCR for both KI and WU, detecting 10 copies of a KI VP1 gene containing plasmid was used (23) and the CSF (4 μl) was treated as above. A new PCR assay for detection of MC DNA was used with the primers, P137-MC573.F: GTCTCG CCAGCATTGTAGTCT and P138-MC739.R: GCAGTAAGCAG TAGTCAGTTTC, generating an amplicon of 177 bp from the early part of large T-antigen (LT). CSF (4 μl) samples were treated as above followed by 40 cycles of 30 s at 95˚C, 30 s at 53˚C and 45 s at 72˚C. MC DNA from a Merkel cell carcinoma was used as positive control and the assay had a detection limit of 10 MC gene copies.

Results and Discussion

The diagnosis, and details of the 20 HSCT patients included are summarized in Table I. The most frequent neurological symptoms were headache with or without fever, described in seven patients (35%), followed by seizures (20%) and

confusions/hallucinations (20%). At the end of the study period (January 2011), 9/20 (45%) patients with a mean follow up time of 3.4 years had survived. Neurological complications were related to the cause of death in two cases: one presented as a symptom of leukemic relapse, and the other as cranial herniation. BK, JC, KI, WU and MC DNA was however, not detected by PCR in any of the CSF samples of these patients.

The fact that KI, WU and MC were not found in the CSF of the patients, despite the patients' neurological symptoms, is in line with several recent studies (18, 19, 24). The lack of detection of JC and BK was expected, since the presence of BK or JC in the CSF of HSCT patients is uncommon and presence of JC would likely be associated with PML, which none of these patients presented.

One limitation of this study is the small number of samples and despite a large patient base (598 patients), where an LP for analysis of viral infectious agents was performed on 46 patients, only 20 CSF samples were available for further analysis. Nevertheless, these samples should be representative since they were not selected with any bias. In addition, in concordance with the fact that JC is frequently detected in the CSF of patients with PML (25), the PCR assays used, with detection limits of between 5-10 copies of the respective viruses, should be sensitive enough to detect viral DNA in the CSF if the corresponding viruses were responsible for the neurological disease.

Unfortunately, many episodes of neurological complications after HSCT are still given no definite diagnosis and this emphasizes the need for a broader search for causes, especially when new treatment options are becoming available. Drug toxicity was suspected in several cases, but often no clear diagnosis was given despite suspicion. Cyclosporin A causing CNS toxicity seizures and encephalopathy (26) may have contributed to the neurological symptoms in several cases, but was mentioned as a probable cause in only one.

In conclusion, in this pilot study, BK, JC, KI, WU, and MC DNA was not detected in available CSF samples from 20 allogeneic HSCT patients with neurological symptoms, and thus these viruses were not the major cause of these symptoms. However, due to the small number of cases, and since JC can infect the CNS in immunocompromised patients, larger studies are necessary before excluding the above viruses entirely as causative agents of neurological symptoms in HSCT patients.

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