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JC polyomavirus DNA detection in clinical practice

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ABSTRACT

Background: There are limited data about the use and clinical value of JC polyomavirus (JCPyV) DNA detection in various clinical indications.
Methods: We reviewed the clinical records of 410 patients from whom cerebrospinal fluid (CSF), plasma, urine, or tissue samples had been collected for JCPyV DNA polymerase chain reaction (PCR) between 2012 and 2018.
Results: JCPyV DNA was analyzed in 224 plasma, 190 CSF-, 32 urine and 10 tissue samples. 240 patients had a history of hematopoietic stem cell or solid organ transplantation, 159 had nephrological disease, 90 had hematologic malignancies, 58 had neurological disease, 37 had infectious disease and 23 had AIDS/HIV as underlying disease. Six patients had no underlying disease. The main reasons to take CSF or plasma samples were neurological symptoms of unknown etiology. Most urine samples were taken to monitor kidney transplantation patients. JCPyV DNA PCR contributed to the diagnosis of progressive multifocal leukoencephalopathy in eight patients (2.0%), of which seven had hematologic malignancy as an underlying disease.
Conclusions: JCPyV PCR is most informative among immunosuppressed patients with neurologic symptoms. CSF and brain biopsy are useful when there is clinical suspicion of PML, whereas plasma samples are not useful. The value of plasma samples is a matter of dispute in the screening of JCPyV-associated nephropathy, as BK polyomavirus is the causative agent in most polyomavirus-associated nephropathy cases. JCPyV detection is valuable in case the patient has past, current or planned treatment with immunosuppressive drugs.

List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
BKPyV	BK Polyomavirus
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
GCN	Granular Cell Neuronopathy
HC	Hemorrhagic Cystitis
HIV	Human Immunodeficiency Virus
HSCT	Hematopoietic Stem Cell Transplantation
JCPyV	JC Polyomavirus
JCPyVAN	JC Polyomavirus-associated Nephropathy
KT	Kidney Transplantation
NCCR	Non-Coding Control Region
PCR	Polymerase Chain Reaction
PML	Progressive Multifocal Leukoencephalopathy
PyVAN	Polyomavirus-Associated Nephropathy
VP1	Viral Protein 1

1. Background

JC polyomavirus (JCPyV) is a member of the Polyomaviridae family and has a 5 kb double-stranded circular DNA genome. It is a highly ubiquitous and usually harmless virus, as demonstrated by detectable antibodies in the majority of the healthy population [1, 2]. However, JCPyV has been implicated in a number of diseases when reactivated, or upon primary infection in an immunocompromised host. The virus is best known for its association with progressive multifocal leukoencephalopathy (PML).

Recent Finnish and Swedish population-based studies report PML-incidences of 0.072–0.12/100,000 person-years [3, 4, 5]. The

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prerequisite for PML is profound suppression of cell-mediated immunity, whether associated with diseases, such as lymphoproliferative malignancies or HIV, or treatment with immunosuppressive or immunomodulatory therapies as exemplified by natalizumab, a monoclonal antibody inhibiting lymphocytes migration to the brain. The virus can infect the granular cell layer of the cerebellum causing JCPyV neuronopathy (GCN) [6], characterized by a lytic infection by a JCPyV variant harboring a mutation in the VP1 coding region. Because both PML and GCN occur in the CNS, comorbidity is probable [7]. In rare cases JCPyV may be the causative agent of aseptic meningitis. JCPyV encephalopathy is caused by the infection of cortical pyramidal neurons and astrocytes [8]. JCPyV has occasionally been found in the brain of healthy individuals as well [9].

Polyomaviruses persisting in the body can reactivate after kidney transplantation (KT), causing renal dysfunction and graft loss. Although the major cause of polyomavirus-associated nephropathy (PyVAN) and hemorrhagic cystitis (HC) is the human BK polyomavirus (BKPyV) [10], in rare cases JCPyV reactivation may also cause problems among KT recipients. JCPyV-associated nephropathy (JCPyVAN) is a severe but very rare complication in KT recipients, with the incidence 0.9% in a cohort of 100 KT recipients [11]. Except for JCPyV replication in the kidneys and strong immunosuppression as a significant risk factor [12], other predisposing factors are not known. Asymptomatic JCPyV viruria has been reported in 16.8% – 27.2% and viremia in 14.2% of KT recipients [11], [13]. The presence of JCPyV in urine rarely signifies the development of nephropathy, and also the role of JCPyV viremia in evaluating the risk of PyVAN may be lower as compared to BKPyV [11]. JCPyV can also cause HC in hematopoietic stem cell transplantation (HSCT) recipients [14].

Because PML was rare among non-HIV patients until mid-2000′s [15], there are limited data about epidemiology, overall incidence trends and clinical characteristics of these patients. Even less is known about other patient groups where JCPyV infection is suspected and samples are taken for laboratory diagnosis. We conducted this study to assess the background conditions and clinical characteristics of these patients, as well as to establish which patient groups would benefit most of JCPyV PCR testing.

With the real-world use of new and effective immunosuppressive and immunomodulatory treatments, clinicians need to be aware of the possibility of JCPyV caused diseases, especially among immunocompromised patients with emerging progressive cognitive or other neurological symptoms. Because there is no effective and safe antiviral treatment, prevention of JCPyV reactivation and lytic infection is imperative.

2. Methods

In this retrospective study we assessed the clinical indications and the impact of JCPyV DNA testing in clinical practice in 2012–2018 in the Helsinki University Central Hospital, which is a tertiary care center serving a population of 1.5 million in southern Finland and a solid-organ transplant center serving the whole country of Finland. Altogether 770 CSF, urine, plasma or tissue samples from 410 patients were taken. In this study we included plasma samples of 224 patients, CSF samples of 190 patients, urine samples of 32 patients and tissue samples of 10 patients. From some patients repeated samples or different sample types were taken. Quantitative PCR testing for JCPyV DNA from plasma, CSF or urine and qualitative PCR for tissue samples was performed at the Department of Virology and Immunology, Diagnostic Center of the Helsinki University Hospital. Only patients with available medical records were included in the study. The data were obtained from the Helsinki University Hospital records.

The requests for JCPyV PCR examinations came from individual clinicians on clinical grounds only, without knowledge of this study. Underlying disease, stem-cell transplantations, and solid organ transplantations were recorded.

The study protocol was approved by the review board of the Inflammation Center at the Helsinki University Hospital.

2.1. JCPyV DNA PCR

Quantitative real-time PCR was performed essentially as described previously for CSF [16].

Briefly, nucleic acids were extracted from 200 µl CSF using the EasyMag extraction platform (bioMérieux, Marcy-l'Étoile, France) and eluted into 25 µl, and 10 µl were used for the PCR reaction. Nucleic acid extraction from 200 µl plasma or 100 µl urine was performed in the Magna Pure instrument (Roche Life Science, Rotkreuz, Switzerland). Nucleic acids extracted from plasma were eluted into 50 µl and those extracted from urine into 100 µl, and 10 µl of the eluates were used for the PCR reaction. Nucleic acids from tissue samples were extracted by protenase K treatment followed by phenol-chloroform extraction.

The target region amplified by the PCR primers is within the large T antigen gene of JCPyV [17].

The level of quantification is 125 copies/ml for CSF samples, 400 copies/ml for plasma, and 3000 copies/ml for urine. The method can detect even lower levels but without reliable quantification. Quantitative result in viral copies/ml is given for CSF, plasma and urine samples, whereas a qualitative positive or negative result is given for tissue samples. Here, however, the quantitative assay was interpreted and used qualitatively, and all samples giving positive signals from both of the duplicate PCR reactions at any level were considered positive.

2.2. Statistical analysis

Statistical analysis and graphs were produced using RStudio v.3.6.2. For the mean and median calculations for age and percentage calculations, missing values were omitted, and patients from whom multiple samples had been taken were only included once in each testing episode. When tallying the number of patients that tested positive for JCPyV, patients who had at least one positive result were interpreted as positive.

3. Results

Altogether 770 CSF, urine, plasma or tissue samples from 410

Table 1
Clinical characteristics of study patients and samples.

	Total	Tissue samples	CSF	Plasma	Urine
Number of total samples	770	11	223	486	50
Number of JCPyV positive samples, n (%)	72 (9.4)	1 (9.1)	9 (4.1)	15 (3.1)	47 (94.0)
Number of patients	410*	10	190	224	32
Number of JCPyV positive patients, n (%)	42 (10.2)	1 (10.0)	7 (3.7)	5 (2.2)	29 (90.6)
Sex, M/F	254/156	5/5	111/79	141/83	22/10
Age, y (mean, median)	38.0/41.0	37.9/37.5	53.2/58.0	25.8/16.0	33.9/30.5
Number of children (<16 y), n (%)	126**(30.7)	4 (40.0)	9 (4.7)	116 (51.8)	12 (37.5)
Patients with transplant or HSCT, n (%)	240 (58.5)	7 (70.0%)	47 (24.7%)	184 (82.1%)	29 (90.6%)

* There were 410 unique patients. Multiple and/or different samples were collected from some patients.

** There were 126 unique patients below 16 years of age. Multiple and/or different samples were collected from some patients.

patients were taken (Table 1), and from some patients both plasma and urine, plasma and CSF, or CSF and tissue samples were taken. The demographic and clinical information of participants is presented in Table 1.

The most frequent clinical reason for taking CSF samples was suspicion of PML. Common symptoms raising suspicion of JCPyV infection were various neurological manifestations such as cognitive symptoms, balance and gait problems, fever, epileptic seizures and speech problems (n = 21, 96.4%). The only specific symptom for which urine samples were taken was dysuria (n = 2, 4.0%). Most plasma (n = 445, 91.6%) and urine samples (n = 48, 96.0%) were taken from asymptomatic transplant patients at monitoring visits. Plasma samples were also occasionally taken when patients had nonspecific signs of infection: fever, dysuria, fluctuating cognitive symptoms or diarrhea (n = 41, 8.4%) (Table 2).

The majority of CSF samples were taken within the specialties of neurology (n = 112, 58.9%) and hematology (n = 24, 12.6%). Most plasma samples were sent by pediatrician (n = 134, 59.8%) or transplant surgeons (n = 28, 12.5%). Out of 50 urine samples, 37 (74%) were collected at KT screening visits (Fig. 1).

Next, we explored the underlying diseases among the patients. We listed the three most frequent underlying diseases per sample type. A complete overview of underlying diseases is presented in Table 3. HIV/AIDS patients were separated from other infectious diseases due to their enhanced risk of PML. The most common underlying diseases among patients from whom CSF was collected for JCPyV DNA PCR were hematologic malignancy (n = 71), neurologic diseases (n = 47) or infectious diseases (n = 28). Patients providing tissue samples (five kidney, four brain and one conjunctival biopsy) had nephrological diseases (n = 5), hematologic malignancies (n = 3) or infectious diseases (n = 2). Plasma samples were taken from patients with nephrological diseases (n = 142), hematologic malignancies (n = 24) or neurologic diseases (n = 15). Urine samples were collected from patients with nephrological diseases (n = 20), hematologic malignancies (n = 5) or diabetes mellitus (n = 3).

As JCPyV reactivation with putatively serious consequences is particularly common among transplant recipients, we assessed the rate of transplantation events behind JCPyV sampling (Table 1). Of all 410 patients altogether 240 (58.5%) were transplant recipients. The majority of CSF samples were taken from patients with no transplant (n = 143 /190, 75. 3%). Most plasma samples (n = 161/224, 71.9%), urine samples (n = 24/32, 75.0%) and tissues samples (n = 5/10, 50.0%) were taken from patients with KT (Fig. 2).

We assessed the aptness of JCPyV DNA testing among the different patient groups. The proportion of positive samples was 10% (1/10) for tissues samples, 3.7% (7/190) for CSF, 2.2% (5/224) for plasma, and 90.6% (29/32) for urine (Table 1). All seven patients with a positive JCPyV finding from the CSF were subsequently diagnosed with PML. Two of them had magnetic resonance imaging (MRI) changes only in the cerebellar hemisphere, suggesting that GCN is likely the correct diagnosis. Both of these patients had ataxia as a clinical feature and one of them had also some cognitive symptoms. Both patients have survived. Among all seven patients diagnosed with PML, six had hematologic malignancies as a predisposing disease and one had AIDS. The patient with positive JCPyV finding from brain biopsy had been diagnosed with PML, and had hematologic malignancy as a predisposing factor. The remaining three brain tissue samples, as well as all kidney tissue samples (n = 5) and the one conjunctival biopsy sample from KT patient, were JCPyV negative.

Out of all 486 plasma samples, 15 samples (3.1%) from altogether five patients were found to contain JCPyV DNA. None of these patients had JCPyV associated diseases. One of the positive plasma samples was taken because the patient with hematological malignancies had both cognitive and gait problems. All other plasma samples were taken from KT patients at their post-transplant monitoring visits and four of them turned out JCPyV positive.

A high proportion, 47 out of 50 (94%) urine samples were JCPyV positive. Majority of the patients were KT patients, and the samples were taken during monitoring visits. Only one sample was taken because of dysuria.

4. Discussion

PML, although a rare disease, has the best-established causative association with JCPyV [18], followed by GCN [6]. Other JCPyV-associated diseases such as JCPyV encephalopathy, meningitis [8, 19], JCPyVAN, and HC are extremely rare [11], and none of them was represented in our cohort. According to our results, patients with hematologic malignancies have the highest risk to develop PML. This is due to the fact that both the disease and the treatment together suppress the immune system. Seven out of 90 patients (7.8%) with hematological malignancies and one out of 23 patients (4.4%) with HIV/AIDS had JCPyV PCR-confirmed PML in this study. There was one biopsy-confirmed PML patient whose JCPyV PCR from CSF remained negative at the time of diagnosis. Two months later, immunohistochemistry from A brain biopsy showed staining for JCPyV. The underlying disease of this patient was chronic lymphocytic leukemia. We do not have an obvious explanation for the negative CSF result, although our assay may not have been sufficiently sensitive to detect very low copies of JCPyV DNA in CSF. Other possible causes for negative CSF results include early stage of PML lesions with no virus leakage to CSF or location of the PML lesions remote from CSF spaces. In some cases, efficient reconstitution of CNS immune surveillance can also cause negative JCPyV PCR results [20]. For the other seven PML patients JCPyV DNA PCR from CSF was an informative tool in the PML diagnostic process. Our findings confirm the diagnostic utility of CSF PCR and of brain biopsy when there is a strong clinical suspicion of PML or GCN among immunosuppressed patients.

In the field of neurology, in order to stratify the risk of PML among multiple sclerosis (MS) patients on ongoing or planned natalizumab

Table 2
Symptoms leading to sampling and proportions of JCPyV positive findings.

Symptom	Total	Tissue positive/negative, n (%)	CSF positive/negative, n (%)	Plasma positive/negative, n (%)	Urine positive/negative, n (%)
Cognitive symptoms	82	1 (1.2)/1 (1.2)	4 (4.9)/69 (84.1)	1 (1.2)/6 (7.3)	0/0
Balance and gait problems	39	1 (2.6)/0	2 (5.1)/31 (79.5)	1(2.6)/4 (10.3)	0/0
Fever	29	0/0	1 (3.4)/19 (65.5)	0/9 (31.0)	0/0
Epileptic seizures	24	0/2 (8.3)	0/21 (91.3)	0/1 (4.2)	0/0
Speech problems	21	0/0	4 (19.0)/13 (61.9)	0/4 (19.0)	0/0
Headache	19	0/0	1 (5.3)/15 (78.9)	0/3 (15.8)	0/0
Visual problems	15	1 (6.7)/0	0/11 (73.3)	0/3 (20.0)	0/0
Dysuria	12	0/0	0/5 (41.7)	0/5 (41.7)	2 (16.7)/0
Sensory abnormalities	11	0/0	0/11 (100.0)	0/0	0/0
Hemiparesis	4	0/0	0/4 (100.0)	0/0	0/0
Diarrhea	5	0/0	0/2 (40.0)	0/3 (60.0)	0/0
Graft rejection	3	0/1 (33.3)	0/1 (33.3)	0/1 (33.3)	0/0
Tremor	1	0/0	0/1 (100.0)	0/0	0/0

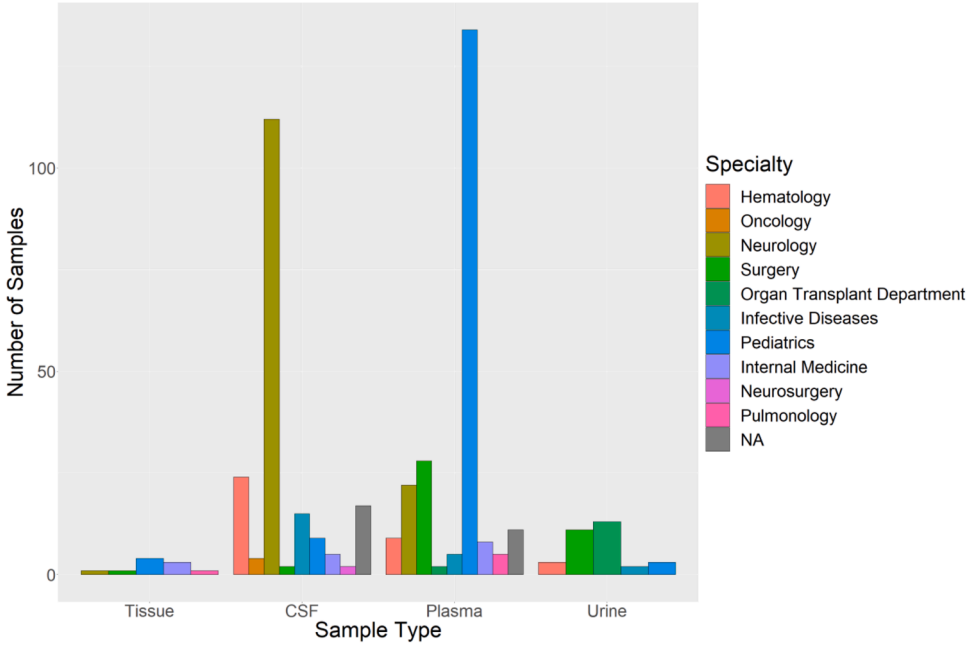

Fig. 1. Number of samples by specialty of treating physician.

Table 3
Underlying diseases per sample type.

Underlying disease	Total number of samples *	Tissue samples n (%)	CSF samples n (%)	Plasma samples n (%)	Urine samples n (%)
Nephrological diseases	159	5 (3.1)	10 (6.3)	142 (89.3)	20 (12.6)
Hematologic malignancies	90	3 (3.3)	73 (81.1)	24 (26.7)	5 (5.6)
Neurological diseases	58	0	47 (81.0)	15 (25.9)	0
Infectious diseases (other than HIV)	37	2 (5.4)	28 (75.7)	13 (35.1)	0
Other diseases	27	0	6 (22.2)	20 (74.1)	2 (7.4)
HIV/AIDS	23	0	22 (95.7)	2 (8.7)	1 (4.3)
Rheumatological diseases	17	0	8 (47.1)	10 (58.8)	1 (5.9)
Diabetes mellitus	17	1 (5.9)	6 (35.3)	9 (52.9)	3 (17.6)
Malignancy (other than hematological)	14	0	8 (57.1)	6 (42.9)	0
No predisposing diseases or treatments	6	0	5 (83.3)	2 (33.3)	0
Primary immunodeficient diseases	3	0	1 (33.3)	1 (33.3)	1 (33.3)

* Multiple/different samples were collected from some patients and some patients had more than one underlying disease.

regimen, there is a well-practiced use of JCPyV antibody testing to reveal past JCPyV exposure. Although the specificity of this method is limited due to the high prevalence of JCPyV antibodies also in the healthy population, the absence of antibodies practically excludes PML risk in 20–60% of the population at the time of testing. Still, additional tools would be needed to narrow down the true PML risk population.

Although JCPyV viruria is common among healthy blood donors as well as among KT recipients, only a small number of PyVAN cases have been attributed to JCPyV [21]. Our data suggest that JCPyV excretion to urine, particularly among KT recipients, is common, and positive JCPyV finding from urine is often unspecific. Urine may thus not be the appropriate sample for diagnosis or monitoring of JCPyV associated disease. The majority of plasma samples in our study were taken from KT patients as part of the polyomavirus screening protocol and none of the patients had JCPyV associated diseases. JCPyV findings from plasma samples were not associated with the symptoms of the patients. Given the extremely low incidence of JCPyVAN, current guidelines do not recommend routine monitoring of JCPyV after KT [22].

The study was limited in being single-centered and using observational retrospective method. For some patients medical records were not available and they were thus excluded from the study.

5. Conclusion

JCPyV is a ubiquitous virus which persists in our body but rarely causes disease even among immunosuppressed individuals. Because of the severity of JCPyV associated diseases, the possibility of JCPyV detection should be exploited to improve management among selected patient populations. It is recommended for all medical specialties to employ JCPyV-associated disease risk stratification before and during the use of immunosuppressive or immunomodulatory treatments. Although in other patient populations JCPyV detection may be of little added value, it is important among patients with past, current or planned treatment with immunosuppressive or immunomodulatory drugs.

Ethics approval

The study protocol was approved by the Ethical Board of the Inflammation Center at the Helsinki University Hospital.

Consent for publication

Not applicable.

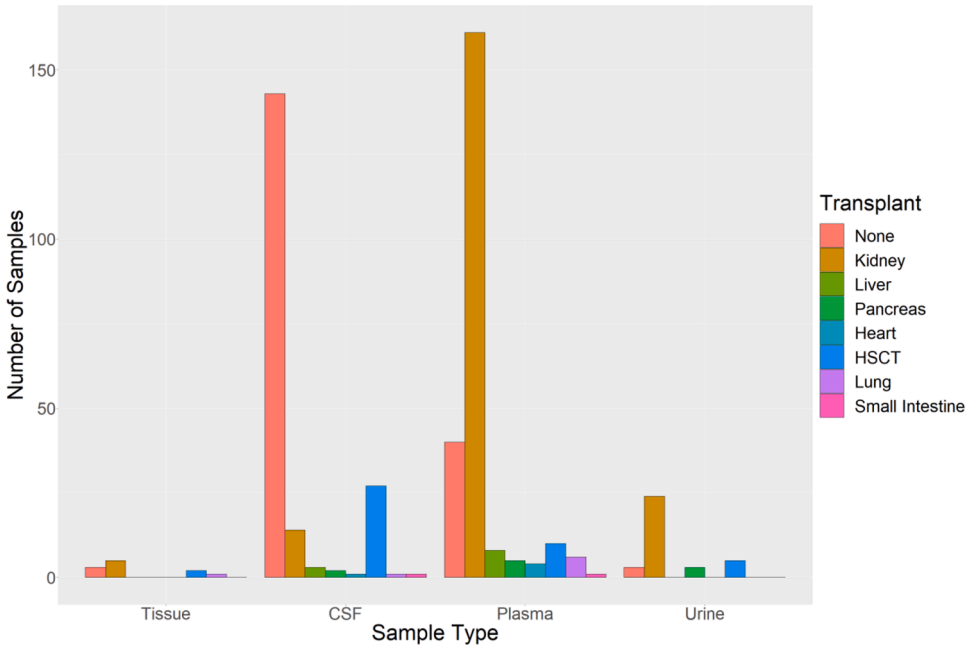

Fig. 2. Type of transplantation leading to sampling.

Availability of data and material

The data were collected from the clinical and laboratory records at the Helsinki University Central Hospital. Some data can be made available on reasonable request.

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Patient consent statement

This study does not include factors necessitating patient consent.

CRediT authorship contribution statement

Marge Kartau: Writing – original draft, Formal analysis. **Eeva Auvinen:** Data curation, Writing – original draft. **Auli Verkkoniemi-Ahola:** Data curation, Writing – review & editing. **Laura Mannonen:** Data curation, Writing – review & editing. **Ilkka Helanterä:** Data curation, Writing – review & editing. **Veli-Jukka Anttila:** Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Not applicable.

Bibliography

[1] U. Moens, M. Van Ghelue, X. Song, B. Ehlers, Serological cross-reactivity between human polyomaviruses, Rev. Med. Virol. 23 (2013) 250–264, https://doi.org/10.1002/rmv.1747.
[2] S. Hanaei, M.A. Sahraian, M. Mohammadifar, S.V. Ramagopalan, M. Ghajarzadeh, M. Ghajarzadeh, Prevalence of anti-JC virus antibody seropositivity in patients with multiple sclerosis: a systematic review and meta-analysis, Intervirology 62 (2019) 169–173, https://doi.org/10.1159/000507367.
[3] M. Kartau, A. Verkkoniemi-Ahola, A. Paetau, M. Palomäki, R. Janes, M. Ristola, et al., The incidence and predisposing factors of john cunningham virus-induced progressive multifocal leukoencephalopathy in Southern Finland: a population-based study, Open Forum Infect. Dis. 6 (2019) ofz024, https://doi.org/10.1093/ofid/ofz024.
[4] J.O.T. Sipilä, M. Soilu-Hänninen, P. Rautava, V. Kytö, Progressive multifocal leukoencephalopathy in Finland: a cross-sectional registry study, J. Neurol. 266 (2019) 515–521, https://doi.org/10.1007/s00415-018-09167-y.
[5] E. Iacobaeus, S. Burkill, S. Bahmanyar, R. Hakim, C. Byström, M. Fored, et al., The national incidence of PML in Sweden, 1988-2013, Neurology 90 (2018) e498–e506, https://doi.org/10.1212/WNL.0000000000004926.
[6] C.S. Tan, I.J. Koralnik, Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis, Lancet Neurol. 9 (2010) 425–437, https://doi.org/10.1016/S1474-4422(10)70040-5.
[7] M.T. Wijburg, D. Siepman, J.J.J. van Eijk, J. Killestein, M.P. Wattjes, Concomitant granule cell neuronopathy in patients with natalizumab-associated PML, J. Neurol. 263 (2016) 649–656, https://doi.org/10.1007/s00415-015-8001-3.
[8] M.N. Khoury, D.C. Alsop, S.P. Agnihotri, R. Pfannl, C. Wuthrich, M.-.L. Ho, et al., Hyperintense cortical signal on magnetic resonance imaging reflects focal leukocortical encephalitis and seizure risk in progressive multifocal leukoencephalopathy, Ann. Neurol. 75 (2014) 659–669, https://doi.org/10.1002/ana.24144.
[9] F.A. White, M. Ishaq, G.L. Stoner, R.J. Frisque, JC virus DNA is present in many human brain samples from patients without progressive multifocal leukoencephalopathy, J. Virol. 66 (1992) 5726–5734, https://doi.org/10.1128/JVI.66.10.5726-5734.1992.
[10] H.H. Hirsch, P.S. Randhawa, AST infectious diseases community of practice. BK polyomavirus in solid organ transplantation-guidelines from the American society of transplantation infectious diseases community of practice, Clin. Transplant 33 (2019) e13528, https://doi.org/10.1111/ctr.13528.
[11] C.B. Drachenberg, H.H. Hirsch, J.C. Papadimitriou, R. Gosert, R.K. Wali, R. Munivenkatappa, et al., Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: a prospective evaluation, Transplantation 84 (2007) 323–330, https://doi.org/10.1097/01.tp.0000269706.59977.a5.
[12] B. Höcker, J. Tabatabai, L. Schneble, J. Oh, F. Thiel, L. Pape, et al., JC polyomavirus replication and associated disease in pediatric renal transplantation: an international certain registry study, Pediatr. Nephrol. Berl Ger. 33 (2018) 2343–2352, https://doi.org/10.1007/s00467-018-4029-9.
[13] I. Helanterä, H.H. Hirsch, E. Auvinen, L. Mannonen, M. Nummi, M. Wernli, et al., High-level JCPyV viruria after kidney transplantation-clinical and histopathological findings, J. Clin. Virol. Off. Publ. Pan. Am. Soc. Clin. Virol. 85 (2016) 75–79, https://doi.org/10.1016/j.jcv.2016.10.018.
[14] P. Manna, K. Grantham, S. Reddy, R. Nath, JC virus reactivation in hemorrhagic cystitis complicating bone marrow transplantation patients, Biol. Blood Marrow Transplant 11 (2005) 73, https://doi.org/10.1016/j.bbmt.2004.12.216.
[15] K.L. Amend, B. Turnbull, N. Foskett, P. Napalkov, T. Kurth, J. Seeger, Incidence of progressive multifocal leukoencephalopathy in patients without HIV, Neurology 75 (2010) 1326–1332, https://doi.org/10.1212/WNL.0b013e3181f73600.
[16] Seppälä H., Virtanen E., Saarela M., Laine P., Paulín L., Mannonen L., et al. Single-molecule sequencing revealing the presence of distinct JC polyomavirus populations in patients with progressive multifocal leukoencephalopathy. J. Infect. Dis. 2016:jiw399. 10.1093/infdis/jiw399.

[17] A. Dumoulin, H.H. Hirsch, Reevaluating and optimizing polyomavirus BK and JC real-time PCR assays to detect rare sequence polymorphisms, J. Clin. Microbiol. 49 (2011) 1382–1388, https://doi.org/10.1128/JCM.02008-10.
[18] A.L. Atkinson, W.J. Atwood, Fifty years of JC polyomavirus: a brief overview and remaining questions, Viruses 12 (2020) E969, https://doi.org/10.3390/v12090969.
[19] A. Behzad-Behbahani, P.E. Klapper, P.J. Vallely, G.M. Cleator, A. Bonington, BKV-DNA and JCV-DNA in CSF of patients with suspected meningitis or encephalitis, Infection 31 (2003) 374–378, https://doi.org/10.1007/s15010-003-3078-5.
[20] J. Kuhle, R. Gosert, R. Bühler, T. Derfuss, R. Sutter, O. Yaldizli, et al., Management and outcome of CSF-JC virus PCR-negative PML in a natalizumab-treated patient with MS, Neurology 77 (2011) 2010–2016, https://doi.org/10.1212/WNL.0b013e31823b9b27.
[21] I. Lautenschlager, T. Jahnukainen, P. Kardas, J. Lohi, E. Auvinen, L. Mannonen, et al., A case of primary JC polyomavirus infection-associated nephropathy, Am. J. Transplant Off. J. Am. Soc. Transplant Am. Soc. Transpl. Surg. 14 (2014) 2887–2892, https://doi.org/10.1111/ajt.12945.
[22] H.H. Hirsch, N. Babel, P. Comoli, V. Friman, F. Ginevri, A. Jardine, et al., European perspective on human polyomavirus infection, replication and disease in solid organ transplantation, Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 20 (Suppl 7) (2014) 74–88, https://doi.org/10.1111/1469-0691.12538.