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Multiplex analysis of Human Polyomavirus diversity in kidney transplant recipients with BK virus replication

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ABSTRACT

Background: While the pathogenicity of the two initially identified Human Polyomaviruses (HPyVs), BK Virus (BKPyV) and JC Virus (JCPyV) has been intensely studied, there is only limited data, on whether the occurrence of the recently discovered HPyVs correlates with high level BKPyV replication and progression towards Polyomavirus associated nephropathy (PVAN).

Methods: Therefore, we performed a comprehensive longitudinal genoprevalence analysis of 13 HPyVs using a novel multiplex assay including 400 serum and 388 urine samples obtained from 99 kidney transplant recipients (KTRs), grouped by quantitative BKPyV DNA loads and evidence of manifest BKPyV associated disease (histologically verified PVAN, high urinary decoy cell levels and concurrent decrease of renal function).

Results: In total, 3 different non-BKPyV/JCPyV HPyVs, Human Polyomavirus 9, Merkel Cell Polyomavirus (MCPyV) and Trichodysplasia Spinulosa associated Polyomavirus were detected in 11 blood and 21 urine samples from 21 patients. Although DNAemia of these viruses occurred more frequently during high level BKPyV DNAemia and PVAN, the increase of the detection frequency due to progression of BKPyV replication did not reach statistical significance for blood samples. The positive detection rate of MCPyV in urine, however, was significantly higher during BKPyV DNAemia in 19 KTRs of our cohort who suffered from histologically verified PVAN ($p = 0.005$). In one individual with PVAN, continuous long-term shedding of MCPyV in urine was observed.

Conclusion: In our cohort the recently discovered HPyVs HPyV9, TSPyV and MCPyV emerged in blood from KTRs with variable kinetics, while detection of MCPyV DNAuria occurred more frequently during BKPyV DNAemia in patients with PVAN.

1. Introduction

BK Virus (BKPyV) and JC virus (JCPyV) were isolated as first Human Polyomaviruses (HPyVs) from urine of a nephropathic kidney transplant recipient (KTR) and from brain tissue of a patient with progressive multifocal leukencephalopathy and named by the initials of the respective patients [1,2].

To date, the following additional HPyVs are known to exist: Karolinska Institute and Washington University Polyomaviruses, Merkel cell Polyomavirus (MCPyV), Trichodysplasia spinulosa associated Polyomavirus (TSPyV), Human Polyomaviruses 6, 7, 9, 12, Malawi Polyomavirus, Saint Louis Polyomavirus, New Jersey Polyomavirus and Lyon IARC polyomavirus, which has not yet been assigned to a polyomavirus species [3–7].

Prolonged and asymptomatic shedding of these viruses occurs in healthy individuals, and in immunocompromised individuals they may

Abbreviations: BKPyV, BK Polyomavirus; DNA, deoxyribonucleic acid; D, donor; eGFR, estimated glomerular filtration rate; HPyV, Human Polyomavirus; HPyV9, Human Polyomavirus 9; KTRs, kidney transplant recipients; JCPyV, JC Polyomavirus; KTRs, kidney transplant recipients; LOD, limit of detection; MCPyV, Merkel Cell Polyomavirus; ml, milliliter; PCR, polymerase chain reaction; PVAN, Polyomavirus associated nephropathy; qPCR, quantitative PCR; R, recipient; TSVPyV, Trichodysplasia spinulosa associated polyomavirus

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cause severe disease [3,8]. MCPyV was associated with development of Merkel cell carcinoma [9], while TSPyV can cause a rare follicular skin disease [10]. For the recently discovered HPyVs no definitive association with any major clinical manifestation has been verified.

BKPyV and JCPyV, in contrast, have been functionally linked with clinical manifestations in KTRs [11–13]. Indeed, Polyomavirus associated nephropathy (PVAN) is a severe disease, that may affect up to 10% of all KTRs and cause allograft loss in 10%–100% of affected patients [11–15]. While BKPyV is the primary causative agent of PVAN, JCPyV can also trigger PVAN in rare cases [12,15,16].

Thus, the question opens up whether the newly discovered HPyVs occur more frequently in KTRs with BKPyV-induced PVAN. So we performed a comprehensive genoprevalence analysis of 13 HPyVs in blood and urine samples collected pairwise from KTRs with BKPyV DNAemia with and without evidence for progression towards PVAN.

2. Materials and methods

2.1. Patients and samples

This retrospective study included 400 plasma and 388 urine samples from 99 KTRs (35 female, 64 male; mean age: 57 years, range: 19–79), who received a kidney transplant between March 2008 and September 2014. Detailed clinical information is given in Supplemental Material and Methods and Supplemental Table 1. From each patient, plasma and urine samples were collected pairwise at the same day respectively due to virological routine post-transplant surveillance (386 sample pairs, 14 plasma and 2 urine samples were acquired without corresponding samples). The median number of samples per patient was 3 (plasma and urine) respectively. The median interval between sample acquisition post-transplant was 79 days for plasma and 84 days for urine (range: 11–561). Samples were acquired during the following periods post-transplant (plasma/urine): 0 (at transplantation): n = 5/4, post-transplant day 1-90: n = 66/62, 91-180: n = 107/102, 181-270: n = 72/72, 271-360: n = 46/47, 361-450: n = 24/22, 451-540: n = 19/19, 541-630: n = 13/13, 631-720: n = 15/15, > 720: n = 33/32.

The study protocol was approved by the ethics committee of the Medical University of Vienna (EK2064/2016). Since the samples had been acquired for virological diagnosis in the past, the ethics committee concluded that no written informed consent from patients was required (EK1035/2016).

2.2. Quantitative BKPyV and JCPyV PCR

In each urine and plasma sample BKPyV and JCPyV DNA was quantified due to routine surveillance post-transplant using a testing schedule and protocol included in Supplementary Material and Methods.

2.3. DNA isolation for HPyV multiplex assay

For HPyV multiplex assays DNA was isolated from EDTA-plasma by QIAmp Blood-Mini kit, and from urine by QIAamp Viral RNA kit (both Qiagen, Germany), according to the manufacturer's instructions. Isolated DNA from 200 µL EDTA-plasma was finally eluted into 100 µL AE buffer and DNA from 140 µL urine was eluted into 60 µL of AVE buffer (both Qiagen, Germany).

2.4. HPyV multiplex PCR

Viral DNA of each sample was measured using a novel bead-based multiplex PCR for 13 HPyVs, as described previously [17,18]. More detailed information is given in Supplementary Material and Methods.

2.5. Bead-based suspension assay

To semi-quantify all 13 HPyVs simultaneously, we applied bead-based suspension assay (Luminex) as described previously [17]. Detailed information on the protocol, the confirmation method and assessment of sensitivity and specificity is given in Supplemental Material and Methods [10,19,20].

2.6. Statistical methods

Agreement between BKPyV/JCPyV qPCR and multiplex PCR was analyzed using a two-way contingency table and Altman scheme (almost perfect: kappa 1.00, very good: kappa 0.81-0.99, good: kappa 0.61–0.80, fair: kappa 0.21–0.40 and poor: kappa < 0.20). The association between the detection rate non-BKPyV/JCPyV HPyVs and evidence for PVAN progression was analyzed using Fisher´s exact test. For all statistical tests, a two-sided p-value of < 0.05 was considered statistically significant and GraphPad Prism version 5.0 software was used.

3. Results

3.1. Detection of BKPyV and JCPyV by qPCR

Out of 400 plasma and 388 urine samples, BKPyV DNA was detected by quantitative PCR (qPCR) in 260 plasma and 305 urine samples and JCPyV in 67 plasma and 170 urine samples. Eighty-three of the 99 KTRs (84%) particularly displayed one or more episodes of BKPyV DNAemia post-transplant (median BKPyV DNA load: 3.6×10^4 copies/ml, range: 1.0×10^2-1.5×10^9). Of these 83, 50 additionally displayed JCPyV DNAemia at any time during follow-up (median viral load: 3.2×10^3 copies/ml, range: 1.0×10^2-8.0×10^5). Two patients (2%) displayed JCPyV DNAemia alone. Fourteen out of the 99 KTRs (14%) did not display BKPyV or JCPyV detection, neither in urine nor in blood. Patient baseline characteristics did not significantly differ among these groups (Supplemental Table 1).

3.2. Comparison of single and multiplex PCRs for BKPyV and JCPyV detection

All samples quantified for BKPyV and JCPyV DNA by qPCR were retested with multiplex PCR. The comparative results are shown in Table 1. To evaluate the performance of multiplex PCR Cohen's kappa values were calculated based on qPCRs positivity. Agreement between the two assays was good to very good (kappa for BKPyV in plasma = 0.89, for BKPyV in urine = 0.91, for JCPyV in plasma = 0.76 and for JCPyV in urine = 0.75). Discrepant test results mainly occurred at DNA loads < 1×10^3 copies/mL.

3.3. Detection of non-BKPyV/JCPyV HPyVs

Newly discovered HPyVs (other than BKPyV and JCPyV) could be detected in a total of 21 of all 99 KTRs (21.2%). In total, 3 different non-BKPyV/JCPyV HPyVs were detected. As shown in Fig. 1, Human Polyomavirus 9 (HPyV9) was detected in a blood sample from one patient and MCPyV was detected in 9 blood and 21 urine samples from a total 20 KTRs (Fig. 2). In one of those 20 patients, TSPyV was detected in a subsequently acquired blood sample.

The detection rates of MCPyV, HPyV9 and TSPyV in plasma samples were 8.1% (8/99), 1.1% (1/99) and 1.1% (1/99) respectively. Viral DNA concentrations in plasma were low and were only found in patients who displayed BKPyV and/or JCPyV DNAemia during the follow-up.

3.4. MCPyV detection

MCPyV was detected in 9 blood samples from 8 KTRs, in 2 patients

Table 1
Comparison of BKPyV/JCPyV qPCR and multiplex PCR.

BKPyV in EDTA-plasma Kappa = 0.88				
		qPCR		
Multiplex PCR		+	–	Total
	+	237	0	237
	–	23	140	163
	Total	260	140	400
BKPyV in Urine Kappa = 0.91				
		qPCR		
Multiplex PCR		+	–	Total
	+	302	8	310
	–	3	75	78
	Total	305	83	388
JCPyV in EDTA-plasma Kappa = 0.77				
		qPCR		
Multiplex PCR		+	–	Total
	+	45	0	44
	–	23	333	356
	Total	67	333	400
JCPyV in Urine Kappa = 0.76				
		qPCR		
Multiplex PCR		+	–	Total
	+	125	0	125
	–	45	218	263
	Total	170	218	388

exactly at the day of transplantation, in the remaining individuals between the 32nd and the 239th day post-transplant (Fig. 2). The median MCPyV DNA load was 5.9×10^2 copies/mL (range: 1.2×10^2-1.4×10^3 copies/mL). MCPyV detection in plasma coincided with BKPyV DNAemia in 6 KTRs and with PVAN in one patient respectively. In one of these individuals MCPyV was detectable in blood 2 months prior to BKPyV DNAemia (patient #73, Fig. 2).

In urine, MCPyV DNA was detected in 16 KTRs (16.2%) with a median viral load of 6.3×10^2 copies /mL (range: 1.2×10^2-4.2×10^4 copies/mL). In contrast, to MCPyV detection in blood, MCPyV DNAuria occurred during the entire post-transplant follow-up, ranging from the transplantation day to the 796th day post-transplant. Out of the 21 episodes of MCPyV DNAuria, 9 occurred simultaneously with BKPyV DNAemia and 5 emerged when PVAN was diagnosed (Fig. 2).

In 6 cases MCPyV was detected in multiple blood and/or urine samples from the same patient respectively. As shown in Fig. 2, one of these patients (patient #74) displayed persistent MCPyV DNA shedding in urine (with 4 positive samples over a period of 686 days) which started when PVAN developed and continued during subsequent and prolonged high-level BKPyV DNAemia.

3.5. HPyV 9 and TSPyV detection

HPyV9 DNA (3.4×10^3 copies/mL) was detected in a blood sample from a 27-year-old male at the same time point when BKPyV DNA loads in urine and in blood peaked and PVAN was verified histologically (126th day post-transplant). In two consecutive samples (obtained after 2 and 12 months), as well as in all corresponding urine samples, HPyV 9 was undetectable.

Furthermore, TSPyV DNA was found in one blood sample from a 59-year-old male (patient #78, Fig. 2) at the 32nd month post-transplant, who did not display BKPyV DNAemia at the same time, but had experienced a combined episode of MCPyV DNAuria and BKPyV DNAemia 111 days earlier. The TSPyV DNA load, however, was too low to be quantified.

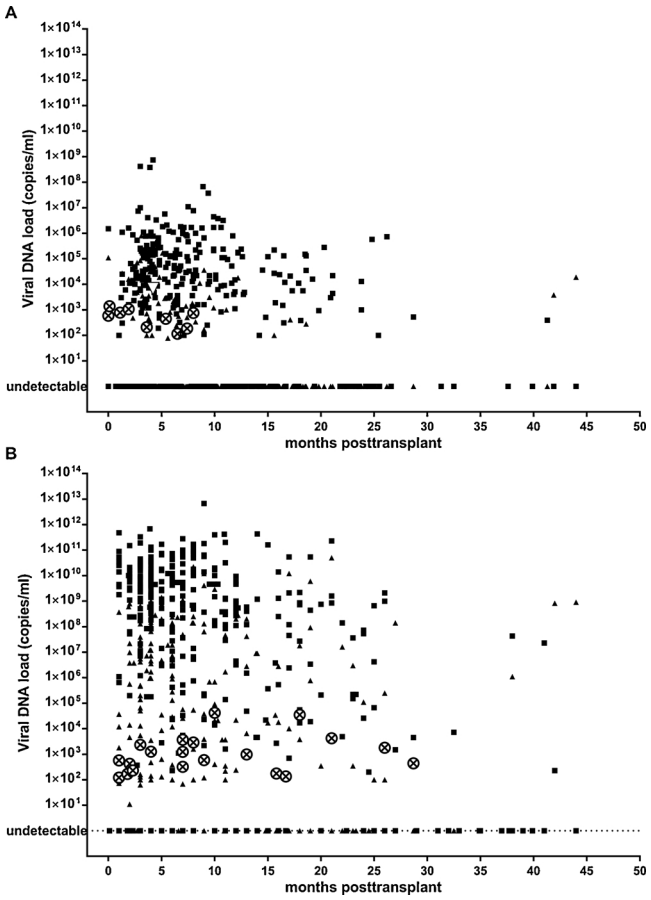

Fig. 1. HPyV DNA in blood and urine in KTRs over time. The insert shows the logarithm of the MCPyV DNA copy number per mL (y) plotted against the post transplantation time (x). A: HPyVs in blood. The square box indicates BKPyV DNA, upwards triangle shows JCPyV DNA, ringed cross indicates MCPyV DNA, and downwards triangle is HPyV9 DNA. In one patient TSPyV was detected by multiplex analysis, the DNA load, however, was too low to be quantified by qPCR and is therefore not shown. B: HPyVs in urine. The square box indicates BKPyV DNA, upwards triangle shows JCPyV DNA, and ringed cross indicates MCPyV DNA.

3.6. Detection rate of non-BKPyV/JCPyV HPyVs in relation to severity of BKPyV DNAemia

Finally, we analyzed whether HPyV detection (other than BKPyV and JCPyV) differed among patients with BKPyV DNAemia in relation to the clinical severity of this DNAemia. Therefore, we grouped the 83 BKPyV DNAemic KTRs based on quantitative BKPyV DNA loads and PVAN evidence (Table 2). Nine-teen KTRs of our cohort only displayed peak DNA loads lower than 10^4 copes/ml during any BKPyV DNAemia episode. Decoy cell levels and incidence rates of a concurrent decrease of renal function were significantly lower in these KTRs than in patients with BKPyV DNA levels > 10^4 copies/ml. BKPyV DNA loads exceeded 10^4 copies/ml in 45 KTRs, in whom significantly higher decoy cell levels and higher incidence rates of decreased renal function occurred, but no biopsy was performed during BKPyV DNAemia (Table 2). In 19 KTRs, presence of PVAN was additionally confirmed in a biopsy (also see Supplemental Material and Methods), and these individuals displayed the highest BKPyV DNA loads, decoy cell levels and highest rates

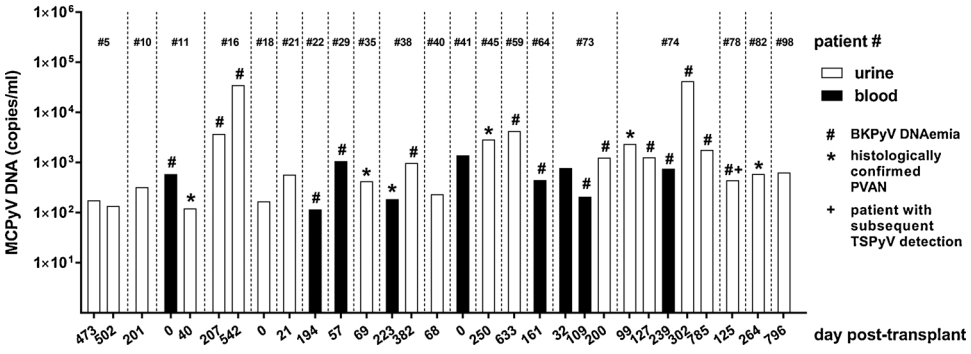

Fig. 2. Patients with episodes of MCPyV DNA detection during the follow-up post-transplantation. The logarithm of the MCPyV DNA copy number per mL is shown on the Y-axis, and day after the transplantation shown on the x-axis. Dashed lines separate individual patients. White and black bars indicate positive MCPyV findings in urine and in blood, respectively. Hash indicates a positive MCPyV DNA detection episode which coincided with BKPyV DNAemia. Stars indicate MCPyV DNA detection episodes which coincided with histological verification of PVAN.

of a concurrent decrease of renal function (Table 2).

As shown in Table 2, the detection rate of MCPyV in urine, but not in blood, was significantly higher during BKPyV DNAemia episodes in these 19 KTRs with verified PVAN than in the other patients with BKPyV DNAemia ($p = 0.005$). For the detection of non-BKPyV/JCPyV HPyVs in blood, there was no statistical evidence for such an association between progression of BKPyV replication and increased detection rates, and this applied to detection during or in absence of BKPyV DNAemia (Table 2). Of note, additional JCPyV DNAemia occurred more frequently in KTRs with high level BKPyV DNAemia (Table 2).

4. Discussion

In order to identify whether the newly discovered HPyVs occur more frequently in KTRs with clinically progressed BKPyV replication we performed a retrospective, comprehensive genoprevalence analysis of 13 HPyVs in a cohort of KTRs with and without high level BKPyV DNAemia, grouped by viral loads and histological evidence of PVAN. Although HPyV9 and MCPyV DNAemia frequently occurred during BKPyV DNAemia episodes and PVAN, we found no statistical evidence for an increased occurrence of the newly identified HPyVs in blood due to clinically manifest BKPyV replication. A higher detection rate of MCPyV DNAuria however, was observed in our cohort when patients developed PVAN.

Indeed, to our knowledge, only one publication has described all 13 first-detected HPyVs in longitudinal kidney transplantation cohort [21]. In this previous study low detection frequencies of non-BKPyV/JCPyV HPyVs were reported. Recently, an analysis on the impact of HPyV9 and TSPyV coinfection on BKPyV DNAemia demonstrated a positive association between HPyV9 seropositivity and subsequent PVAN development, while TSPyV DNAemia was only detected in a small number of KTRs [22].

With the aim of extending these existing findings, we focused on investigating HPyVs in a specifically selected KTR cohort with high-

Table 2
Non BKPyV/JCPyV HPyV detection in relation to clinical severity of most severe BKPyV DNAemia episode during follow-up.[a]

		no BKPyV and JCPyV DNAemia	BKPyV DNA load in blood			Difference among the groups
			$< 10^4$ copies/ml	$> 10^4$ copies/ml	$> 10^4$ copies/ml histologically verified PVAN	
number of patients		14	19	45	19	
peak BKPyV DNAemia	**DNA load** (median, range; copies/ml)	–	1.60×10^3, 1.00×10^2-5.50×10^3	3.90×10^4, 1.00×10^2-1.00×10^7	7.80×10^5, 1.10×10^4- 1.50×10^9	$p < 0.0001$*
decoy cells	**%** (median, range)	–	1, 0-80	70, 0-95	90, 60-99	$p < 0.0001$*
decrease of renal function [b]	**number of patients, %**	–	1/19, 5%	14/45, 31%	17/19, 89%	$p < 0.0001$**
additional JCPyV DNAemia	**number of patients, %**	–	1/19, 5%	19/45, 42%	7/19, 37%	$p = 0.001$**
	DNA load (median. range; copies/ml)	–	1.00×10^2	1.80×10^3, 1.00×10^2-8.00×10^5	3.60×10^4, 5.00×10^2-5.00×10^5	$p = 0.018$***
detection of non BKPyV/JCPyV HPyVs (in number of patients)						
during BKPyV/JCPyV DNAemia	in urine	–	none	3 (MCPyV)	6 (all MCPyV)	$p = 0.005$**
	in blood	–	1 (MCPyV)	3 (2xMCPyV. 1xHPyV9)	3 (all MCPyV)	$p = 0.560$**
in absence of BKPyV/ JCPyV DNAemia	in urine	1 (MCPyV)	3 (all MCPyV)	3 (all MCPyV)	none	$p = 0.291$**
	in blood	none	1 (TSPyV)	2 (all MCPyV)	none	$p = 0.999$**

Abbreviations: BKPyV: BK Polyomavirus, JCPyV: JC polyomavirus, HPyV: Human Polyomavirus, eGFR: estimated glomerular filtration rate. PVAN: Polyomavirus associated nephropathy.

[a] with respect to:(1) highest BKPyV DNA load in blood, (2) highest decoy cell level, (3) eventual decrease of eGFR and (4) eventual verification of PVAN by histology.

[b] ≥15% decrease of eGFR. as compared to mean of the 3 preceding measurements.

* non-parametric Kruskall-Wallis t-test.

** Fisher´s exact test.

*** Mann-Whitney t-test.

level BKPyV DNAemia and PVAN and analyzed pairs of blood and urine samples. In agreement with previous data, we detected two newly discovered HPyVs, HPyV 9 and TSPyV in blood samples, respectively, confirming generally low detection frequencies of these viruses [20,21]. We detected MCPyV at higher frequencies in blood, but even more in urine samples.

Of note, in our study cohort the detection rates of MCPyV in urine, but not in blood, were higher in patients with histologically verified PVAN than in patients with mere high level BKPyV DNAemia, and persistent MCPyV shedding with multiple positive urine samples was specifically observed in an individual with PVAN. Similar to our findings, previous studies reported that MCPyV viruria and occasionally prolonged shedding were detected in adult and pediatric KTRs [21,23]. Furthermore, Husseiny et al. found MCPy viruria in 30% of recipients, and that low-level shedding of MCPyV in urine occurred in immunosuppressed and immunocompetent subjects [24]. Together with our data this indicates that MCPyV might persist in renal tubular or bladder epithelial cells and could reactivate similarly to BKPyV, although the primary latency site is not entirely elucidated [21].

Another accordance with previous studies was that we detected non-BKPy/-JCPyV HPyVs in blood at low DNA levels, with most of the viral DNAemia episodes occurring at a single time point mostly during the first 4 months post-transplant [21,23–26]. This observation indicates that the early phase of high dose immunosuppression after transplantation could not only trigger BKPyV but also MCPyV reactivation [13,15,21].

Indeed, we here initially applied a new multiplex method in a large cohort of KTRs, able to simultaneously assess 13 HPyVs with a single assay. Since qPCR was used for clinical routine surveillance of BKPyV and JCPyV replication, we were able to determine the inter-assay variability and found good agreement between the multiplex assay and qPCR, which further validates the newly developed method. The detection limits for BKPyV and JCPyV were lower using qPCR, proposing that the multiplex assay is more suitable for screening studies for the full spectrum of HPyVs rather than clinical follow-up of BKPyV and JCPyV.

Another interesting aspect of the current data, was that HPyV9 DNAemia occurred in one sample with a high BKPyV DNA load, obtained from a patient during an episode of histologically verified PVAN. A previous study first described the presence of HPyV9 in blood samples from KTRs; however, the prevalence in our study, was significantly lower than the originally reported one [20]. As noted, our multiplex assay displayed a lower sensitivity for BKPyV than qPCR; and divergent detection rates could be due to differences in primer sets and sample materials [21,25]. Notably, HPyV9 DNA was absent from the patient's corresponding urine sample, as well as from all urine samples from the KTRs, suggesting that HPyV9 may not be excreted through the urinary tract at detectable levels.

In summary, we systematically and comprehensively studied the genoprevalence of 13 HPyVs with a new assay in a cohort of KTRs with high-level BKPyV DNAemia and demonstrated that DNAemia of MCPyV and to a lesser extent of HPyV9 and TSPyV emerged with variable kinetics during post-transplant follow-up. MCPyV DNAuria, however, was detected in our cohort with a higher frequency in patients who in addition to BKPyV DNAemia also displayed histological evidence of PVAN.

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Ethical approval

The study protocol was approved by the ethics committee of the Medical University of Vienna (EK2064/2016). Since the samples had been acquired for virological diagnosis in the past, the ethics committee concluded that no written informed consent from patients was required (EK1035/2016).

Authorship statement

Yilin Wang contributed new analytic tools and participated in performance of the research, writing of the paper and data analysis, Robert Strassl and Ilkka Helanterä participated in conceptualization of research design, Gregor Bond participated in research design and writing of the paper, Stephan Aberle contributed analytic tools. Klaus Hedman contributed new analytic tools and participated in research design and writing of the paper, Lukas Weseslindtner participated in research design, performance of the research, data analysis and writing of the paper.

Declaration of Competing Interest

None declared

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.08.012.

References

[1] S.D. Gardner, A.M. Field, D.V. Coleman, B. Hulme, New human papovavirus (B.K.) isolated from urine after renal transplantation, Lancet 1 (7712) (1971) 1253.
[2] B.L. Padgett, D.L. Walker, G.M. ZuRhein, R.J. Eckroade, B.H. Dessel, Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy, Lancet 1 (7712) (1971) 1257.
[3] U. Moens, A. Krumbholz, B. Ehlers, et al., Biology, evolution, and medical importance of polyomaviruses: an update, Infect. Genet. Evol. 54 (2017) 18.
[4] T. Gheit, S. Dutta, J. Oliver, et al., Isolation and characterization of a novel putative human polyomavirus, Virology 506 (2017) 45.
[5] M.C. Feltkamp, S. Kazem, E. van der Meijden, C. Lauber, A.E. Gorbalenya, From Stockholm to Malawi: recent developments in studying human polyomaviruses, J. Gen. Virol. 94 (Pt 3) (2013) 482.
[6] E.S. Lim, A. Reyes, M. Antonio, et al., Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing, Virology 436 (2) (2013) 295.
[7] N. Scuda, J. Hofmann, S. Calvignac-Spencer, et al., A novel human polyomavirus closely related to the african green monkey-derived lymphotropic polyomavirus, J. Virol. 85 (9) (2011) 4586.
[8] T. Dalianis, H.H. Hirsch, Human polyomaviruses in disease and cancer, Virology 437 (2) (2013) 63.
[9] H. Feng, M. Shuda, Y. Chang, P.S. Moore, Clonal integration of a polyomavirus in human Merkel cell carcinoma, Science 319 (5866) (2008) 1096.
[10] E. van der Meijden, R.W. Janssens, C. Lauber, J.N. Bouwes Bavinck, A.E. Gorbalenya, M.C. Feltkamp, Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient, PLoS Pathog. 6 (7) (2010) e1001024.
[11] H.H. Hirsch, W. Knowles, M. Dickenmann, et al., Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients, New Engl. J. Med. 347 (7) (2002) 488.
[12] C.B. Drachenberg, H.H. Hirsch, J.C. Papadimitriou, et al., Polyomavirus BK versus JC replication and nephropathy in renal transplant recipients: a prospective evaluation, Transplantation 84 (3) (2007) 323.
[13] H.H. Hirsch, N. Babel, P. Comoli, et al., European perspective on human polyomavirus infection, replication and disease in solid organ transplantation, Clin. Microbiol. Inf. 20 (Suppl 7) (2014) 74.
[14] D. Sawinski, S. Goral, BK virus infection: an update on diagnosis and treatment, Nephrol. Dial. Transplant. 30 (2) (2015) 209.
[15] J. Trofe-Clark, D.B.K. Sawinski, Other, Polyomaviruses in kidney transplantation, Semin. Nephrol. 36 (5) (2016) 372.
[16] S. Delbue, M. Ferraresso, L. Ghio, et al., A review on JC virus infection in kidney transplant recipients, Clin. Dev. Immunol. 2013 (2013) 926391.
[17] M. Sadeghi, Y. Wang, T. Ramqvist, et al., Multiplex detection in tonsillar tissue of all known human polyomaviruses, BMC Infect. Dis. 17 (1) (2017) 409.
[18] Y. Wang, A. Keinonen, S. Koskenmies, et al., Occurrence of newly discovered human polyomaviruses in skin of liver transplant recipients and their relation with

squamous cell carcinoma in situ and actinic keratosis – a single-center cohort study, Transplant. Int. 32 (5) (2019) 516.
[19] S. Goh, C. Lindau, A. Tiveljung-Lindell, T. Allander, Merkel cell polyomavirus in respiratory tract secretions, Emerg. Infect. Dis. 15 (3) (2009) 489.
[20] E. van der Meijden, H.F. Wunderink, C.S. van der Blij-de Brouwer, et al., Human polyomavirus 9 infection in kidney transplant patients, Emerg. Infect. Dis. 20 (6) (2014) 991.
[21] S. Bialasiewicz, R.J. Rockett, K.A. Barraclough, et al., Detection of recently discovered human polyomaviruses in a longitudinal kidney transplant cohort, Am. J. Transplant. 16 (9) (2016) 2734.
[22] A.L. van Rijn, H.F. Wunderink, C.S. de Brouwer, E. van der Meijden, J.I. Rotmans, M.C.W. Feltkamp, Impact of HPyV9 and TSPyV coinfection on the development of BK polyomavirus viremia and associated nephropathy after kidney transplantation, J. Med. Virol. 91 (6) (2019) 1142.
[23] L. Signorini, M. Belingheri, F. Ambrogi, et al., High frequency of Merkel cell polyomavirus DNA in the urine of kidney transplant recipients and healthy controls, J. Clin. Virol. 61 (4) (2014) 565.
[24] M.I. Husseiny, B. Anastasi, J. Singer, S.F. Lacey, A comparative study of Merkel cell, BK and JC polyomavirus infections in renal transplant recipients and healthy subjects, J. Clin. Virol. 49 (2) (2010) 137.
[25] M. Fajfr, L. Pliskova, R. Kutova, et al., Human polyomavirus 9 in immunocompromised patients in the University Hospital in Hradec Kralove, Czech Republic, J. Med. Virol. 89 (12) (2017) 2230.
[26] E. van der Meijden, H.F. Wunderink, C.S. van der Blij-de Brouwer, et al., Human polyomavirus 9 infection in kidney transplant patients, Emerg. Infect. Dis. 20 (6) (2014) 991.