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Journal of Clinical Virology 143 (2021) 104944

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

JC and Human polyomavirus 9 after kidney transplantation: An exploratory serological cohort study

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ARTICLE INFO

Keywords:
Polyomavirus
Serology
Transplantation
Blood donor

ABSTRACT

Introduction: Human polyomaviruses (HPyVs) cause disease in immunocompromised patients. BK polyomavirus (BKPyV) for instance persistently infects the kidneys. In kidney transplant recipients, (KTRs) BKPyV can cause allograft nephropathy. JCPyV, MCPyV, TSPyV and HPyV9 reside in the kidneys too, or have been detected in urine. In this study, we investigate exposure to JCPyV, MCPyV, TSPyV and HPyV9 after kidney transplantation by serological means.

Materials and methods: Serum samples from 310 KTR collected before and 6 months after transplantation (n = 620), from 279 corresponding kidney donors collected before transplantation, and from blood donor controls collected one year apart (n = 174) were assessed for HPyV species-specific IgG responses using a multiplex immunoassay. KTR HPyV IgG kinetics were compared to those of healthy blood donors by linear mixed modeling, and related to those of their donors by linear regression.

Results: In the KTR, increased IgG levels during follow-up were observed for JCPyV (14.8%), MCPyV (7.1%), TSPyV (10.6%), and for HPyV9 (8.1%), while blood donor antibody levels remained stable. Seroconversion was observed for JCPyV (6.5%), MCPyV (2.3%), TSPyV (1.3%), and for HPyV9 (6.5%). The linear mixed model analysis showed that antibody increase was significant for JCPyV ($p < 0.001$) and HPyV9 ($p < 0.001$). Post-transplant JCPyV and HPyV9 antibody responses were associated with donor antibody levels against these HPyVs, respectively.

Conclusions: KTR are exposed to JCPyV and HPyV9 after transplantation. Whether the allograft serves as the source, as indicated by the donor serostatus association, deserves further study.

1. Introduction

On average, each individual is persistently infected with nine different human polyomaviruses (HPyVs)[1]. In the immunocompetent host, HPyV infections are controlled by the immune system and not accompanied by symptoms and disease. When immunity is compromised, for instance in long-term immunosuppressed solid organ transplant (SOT) recipients[2], HPyVs can freely replicate, damage tissues and cause disease. The *Polyomaviridae* currently contain 13 HPyV species[3].

Most clinical complications are seen with the BK polyomavirus (BKPyV), which causes BKPyV-associated nephropathy (BKPyVAN) in kidney transplant recipients (KTR), and haemorrhagic cystitis in primarily hematopoietic stem cell transplantation patients. The phylogenetically closely related JC polyomavirus (JCPyV) can also cause nephropathy [4], but is particularly known for causing progressive multifocal leukoencephalopathy (PML) in AIDS patients and in multiple sclerosis patients treated with specific immunomodulatory drugs, such as natalizumab[5,6]. Both BKPyV and JCPyV can be detected in blood, CSF and urine of affected patients. The Merkel cell polyomavirus (MCPyV) causes approximately 80% of Merkel cell carcinoma (MCC) in the skin[7]. MCC is rare and found primarily in elderly and sometimes in long-term immunocompromised patients[8–10]. MCPyV has been detected in blood, albeit at very low amounts[11,12]. The

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https://doi.org/10.1016/j.jcv.2021.104944
Received 10 June 2021; Received in revised form 6 August 2021; Accepted 9 August 2021
Available online 16 August 2021

Trichodysplasia spinulosa polyomavirus (TSPyV) causes trichodysplasia spinulosa (TS), an extremely rare, dysplastic follicular skin disease seen in severely immunocompromised (often SOT) patients[13,14]. TSPyV DNA can be detected in blood, CSF and urine of SOT patients as well, with high viral loads during primary infection [14]. Human polyomavirus 9 (HPyV9) has been identified in blood and urine of KTR[15], but an association with disease in immunocompromised patients has not been established[16,17]. For HPyV6 and HPyV7 an association with a skin disorder in severely immunosuppressed has been described[18], but systemic infection (for instance accompanied by viremia) has not been reported for HPyV6. For HPyV7, viremia has been described, albeit at low loads[19]. Similar observations were made for KIPyV and WUPyV related to respiratory infections in severely immunocompromised hosts [20–22]. For NJPyV one convincing case of combined myositis, retinitis and vasculitis has been described, accompanied by detectable viral loads in blood[23]. For the other HPyVs (HPyV10, STLPyV, and LIPyV), disease associations are absent and detection of virus in blood and/or urine is extremely rare.

Not much is known about HPyV transmission and infection. Most natural infections occur (early) in childhood and are thought to result from oral ingestion or inhalation. For some HPyVs, for example BKPyV and JCPyV, and possibly TSPyV, KIPyV and WUPyV, it is believed that after primary infection of the oropharynx, they replicate in tonsils and possibly salivary glands followed by spread via the circulation[24,25]. In this way, they can reach the end organ that becomes persistently infected, for instance the kidneys in the case of BKPyV and JCPyV. Whether 'strict' cutaneous HPyVs (for example HPyV6) are transmitted through direct skin-skin contact is not known.

From studies in KTR, it is very likely that BKPyV can be transmitted via the allograft from donor to recipient[26,27]. In a small group of KTR, with the help of viral genome sequencing, it was recently suggested that JCPyV as well can be transmitted through kidney transplantation[28]. A serological study in a pediatric KTR kidney transplant population, suggested the same after observing JCPyV seroconversion in about half of the seronegative children[29]. Furthermore, SOT is a known risk factor for development of PML[30]. At the moment it is unknown whether donor-derived JCPyV, opposed to autologous reactivating JCPyV, is causing these post-transplantation PML cases. Transplantation-related transmission has not been suggested for HPyVs other than BKPyV and JCPyV.

In this study, by analysing a cohort of KTR, we aimed to provide additional, serological evidence for allograft-transmission of JCPyV and a number of other HPyVs with viremic potential. With the help of a multiplex immunoassay [31], we determined JCPyV, TSPyV, MCPyV and HPyV9-specific antibody responses and seroconversions before and after transplantation in KTR, while BKPyV was included in the analyses for comparison. Healthy blood donor (HBD) sera collected one year apart were included for comparison, as well as pretransplantation kidney donor serum samples when available.

2. Materials and methods

2.1. Population and samples

Two stored serum samples, one collected before transplantation and one approximately six months after transplantation, were analyzed from each of 310 adult KTR transplanted between 2014 and 2018 in the Leiden University Medical centre (LUMC), with a mean age of 49.9 (range 19.0 – 74.8) and 188 male (60.6%). The date of collection of samples taken before kidney transplantation ranged from 261 days to 1 day before transplant, with a median of 8 days before transplant. Samples taken after transplantation ranged from 33 to 299 days post-transplant, with a median of 177. For 279 kidney transplant patients, serum samples of their respective kidney donor were also available for analysis. The study adhered to the General Data Protection Regulation, the code of conduct for medical research and the code of conduct for responsible use of human tissue. The data management plan was approved by the data protection officer of the LUMC. The medical ethical committee of the LUMC determined this research was outside the scope of the medical research involving human subjects act (reference: B19.067/ML/1111).

Paired, anonymized HBD serum samples ($n = 174$) were acquired one year (median of 397 days) apart, as described in a previous study [16], adhering to the code of conduct for responsible use.

2.2. Polyomavirus multiplex immunoassay

A customized Luminex multiplex immunoassay was used to assess IgG antibody responses against the major capsid protein Viral Protein 1 (VP1) of JCPyV, MCPyV, TSPyV, HPyV9 and BKPyV. This assay was previously described in detail[31]. Briefly, VP1 fusion proteins were expressed in E. coli and coupled to uniquely colored, magnetic fluorescent beads (Bio-Rad Laboratories, Hercules, CA, USA). The serum samples were blocked in 1:100 dilution in blocking buffer to suppress non-specific binding. Biotinylated goat-α-human IgG ($H + L$) (1:1000) followed by streptavidin-R-phycoerythrin (SAPE) (1:1000) were used to detect IgG responses against the individual VP1 antigens. To control for intertest variability, a serially diluted pool of four serum samples with known IgG response was added to each plate. Antibody responses were measured in a Bio-Plex 200 analyzer (Bio-Rad Laboratories, Hercules, CA, USA) and analyzed using Bio-Plex Manager 6.1 software. Specific antibody responses were calculated by subtracting from each sample the median fluorescence intensity (MFI) values of a blank sample (no serum added) and of beads coupled to GST protein only as a background measurement. Cut-off values for seropositivity were determined as described previously using a seronegative population and a bin-width distribution analysis[31]. The thresholds for the KTR, expressed in MFI, were 846 for JCPyV, 550 for MCPyV, 126 for TSPyV, 1000 for HPyV9 and 1079 for BKPyV. The HBD were tested on a different Bio-Plex 200 analyzer and therefore cut-offs were determined separately. The thresholds for HBD were 666 for JCPyV, 747 for MCPyV, 638 for TSPyV, 274 for HPyV9 and 3085 for BKPyV.

2.3. Antibody response kinetics

For this study the serological status of an individual was determined by calculating the slope between the sampling time points before and after transplantation as follows: (MFI after transplantation – MFI before transplantation) / number of days between sampling. Samples were categorized as 'stable' if the slope remained within the range of plus or minus two standard deviations of the slope from zero. If the slope was outside of these ranges, individuals were called having either 'increased' or 'decreased' antibody levels. The same categorization was applied for the KTR and the HBD, although a correction factor (the ratio between the median follow-up of the populations) was applied to account for the difference in follow-up time between KTR and HBD cohorts. In case individual follow-up antibody responses passed the seropositivity cut-off threshold and showed either an increased or decreased response, these were categorized as seroconversion (- → +) or as seroreversion (+ → -), respectively.

2.4. Statistics

Statistical analysis was performed in RStudio 1.2.1335[32] and R 3.6.2[33] with packages Tidyverse[34], lme4[35], lattice[36], limma [37], and ggplot2[38]. To compare responses between populations (KTR and HBD) and account for the correlation between repeated measurements from the same individual, linear mixed models with random intercepts were applied. These models apply fixed effects (group (KTR and HBD), time (in MFI / day)) and random intercepts (i.e. unique for each subject) to model polyomavirus IgG responses over time. In addition, an interaction term between time and group was used since this improved

the model as assessed by a likelihood ratio test (ANOVA). Linear regression models were applied to study factors of influence on post-transplant IgG response.

3. Results

Serum samples from 310 kidney transplant recipients collected before and after transplantation, and from 87 healthy blood donors collected 12 months apart were assessed for IgG antibody responses to JCPyV, MCPyV, TSPyV, HPyV9 and BKPyV. Compared to the HBD, whose HPyV antibody responses remained largely stable, the KTR showed more dynamic HPyV serologic profiles (Fig. 1). Increased antibody levels were observed in 14.8% of KTR for JCPyV, 7.1% for MCPyV, 10.6% for TSPyV, 8.1% for HPyV9 and 11.9% for BKPyV (Table 1). In total, 101 KTR showed increased antibody levels for any HPyV. The majority, 63 (62.4%), increased for just one HPyV (21 solely for JCPyV, 18 for BKPyV, 12 for MCPyV, 9 for TSPyV and 3 for HPyV9), while 38 (37.6%) showed increased antibody levels for more than one HPyV (Fig. 2). The most prevalent combinations were JCPyV, HPyV9 and BKPyV; JCPyV and HPyV9; TSPyV and MCPyV, which occurred in five KTR. Most increases for HPyV9 (21/25) occurred in conjunction with increases for JCPyV (19/25) and BKPyV (11/25).

Seroconversion was observed in 6.5% of KTR for JCPyV, 2.3% for MCPyV, 1.3% for TSPyV, 6.5% for HPyV9 and 0.6% for BKPyV (Table 1). The percentage seroconverters among the baseline seronegatives was 14.7% (20/136) for JCPyV, 9.6% (7/73) for MCPyV, 6.6% (4/61) for TSPyV, 8.1% (20/247) for HPyV9 and 10% (2/20) for BKPyV. Decreasing HPyV antibody levels were less frequently observed, varying from 2.9 - 5.5%, with seroreversions between 0.3 and 1.3%.

In Fig. 3, the change in antibody levels over time is shown for the KTR. The antibody response against JCPyV and HPyV9 increased over time following the pattern of that against BKPyV. Antibody responses against MCPyV and HPyV9 remained more or less stable during follow-up. In order to compare trends in HPyV antibody responses between KTR and HBD, linear mixed models with random intercepts were used. Responses at baseline were comparable between KTR and HBD for JCPyV ($p = 0.220$), MCPyV ($p = 0.520$) and TSPyV ($p = 0.444$), but higher in HBD for BKPyV ($p < 0.001$) and HPyV9 ($p < 0.001$) (Table 2). As expected, the BKPyV response increased significantly over time after kidney transplantation, while BKPyV response declined slightly in the HBD (8.26, 95% Confidence Interval (CI): 4.87–11.65, $p < 0.001$) (Table 2). Interestingly, the JCPyV and HPyV9 antibody response also increased significantly in KTR compared to the HBD (6.61, 95% Confidence Interval (CI): 4.36–8.86, $p < 0.001$, and (4.74, 95% Confidence Interval (CI): 2.59–6.88, $p < 0.001$, respectively). No increase in responses after transplantation was observed for MCPyV and TSPyV.

Since the observed increase in JCPyV and HPyV9 posttransplantation antibody response resembled that of BKPyV, we analyzed a possible correlation with donor HPyV response level, as we have demonstrated previously for BKPyV[26]. As expected, the pretransplant antibody level was most influential on post-transplant response ($p<0.001$ for all analyzed polyomaviruses). Furthermore, a high baseline donor antibody level was associated with significant increase in post-transplant levels for JCPyV ($p = 0.037$), HPyV9 ($p = 0.005$) and BKPyV ($p = 0.014$) (Table 3). For HPyV9, the size of the effect depended on the recipient serological status before transplantation, as is evidenced by the interaction term ($p = 0.016$, Table 3), suggesting a high antibody level in the transplant recipient protects against a rise in HPyV9 antibody levels.

4. Discussion

In this study, we analyzed IgG responses against selected HPyVs with viremic potential in KTR before and after transplantation, in comparison with HPyV IgG responses in HBD over a comparable period of time. Increased IgG responses after kidney transplantation were observed for JCPyV and HPyV9, comparable to what has been shown for BKPyV,

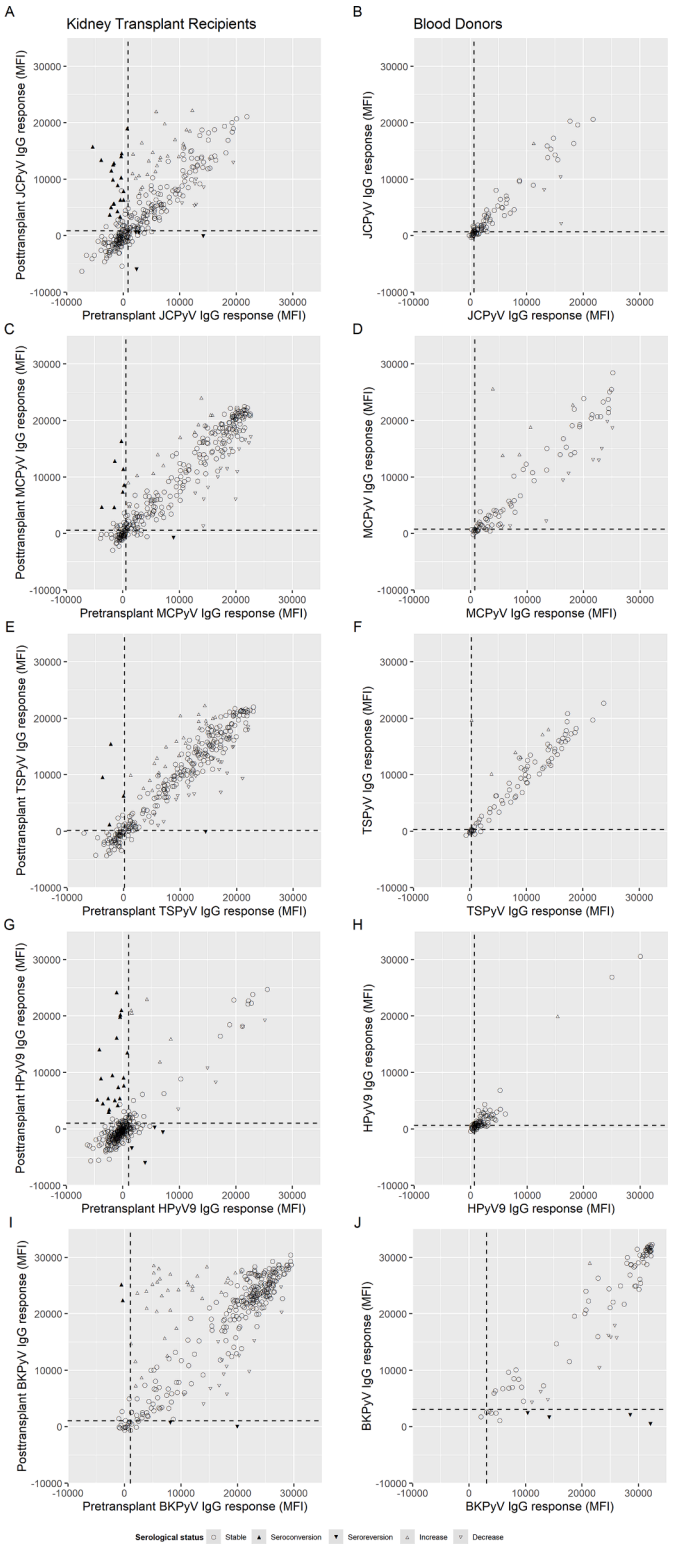

Fig. 1. Individual IgG responses of kidney transplant recipients and blood donors during follow-up. Shown are IgG responses for KTR (left panels) and blood donors (right panels) in median fluorescent intensity (MFI) against JCPyV (A, B); MCPyV (C, D); TSPyV (E, F); HPyV9 (G, H) and BKPyV (I, J). First measurement is shown on the x-axis, the second measurement on the y-axis.

Table 1
Human polyomavirus IgG antibody response kinetics among kidney transplant recipients and blood donors.

IgG kinetics	JCPyV N (%)	MCPyV N (%)	TSPyV N (%)	HPyV9 N (%)	BKPyV N (%)
Kidney transplant recipients *(N = 310)*					
Stable	252 (81.3)	272 (87.7)	252 (81.3)	276 (89.0)	256 (82.6)
Increased	46 (14.8)	22 (7.1)	33 (10.6)	25 (8.1)	37 (11.9)
- *seroconverted*	20 (6.5)	7 (2.3)	4 (1.3)	20 (6.5)	2 (0.6)
Decreased	12 (3.9)	16 (5.2)	25 (8.1)	9 (2.9)	17 (5.5)
- *seroreverted*	4 (1.3)	1 (0.3)	1 (0.3)	4 (1.3)	2 (0.6)
Blood donors *(N = 87)*					
Stable	83 (95.4)	72 (82.8)	81 (93.1)	86 (98.9)	74 (85.1)
Increased	1 (1.1)	5 (5.7)	1 (1.1)	1 (1.1)	1 (1.1)
- *seroconverted*	0	0	0	0	0
Decreased	3 (3.4)	10 (11.5)	0	0	12 (13.8)
- *seroreverted*	0	0	0	0	4 (4.6)

while MCPyV and TSPyV IgG responses remained stable in the post-transplantation period, just like all analyzed HPyV IgG responses in the HBD. The observed increase in JCPyV and HPyV9 IgG responses were associated with kidney donor IgG response against the relevant HPyV.

The lack of increased IgG responses against MCPyV and TSPyV after transplantation may not come as a surprise. These HPyVs are generally believed to primarily infect the skin [39], a superficial organ and possibly less sensitive to fluctuations in central antiviral host immunity. For MCPyV this indeed could be the case, although small amounts of MCPyV DNA are consistently detected in blood[11,12,40]. However, for TSPyV it has been shown to circulate at high loads (>10^6 genome equivalent copies/ml blood) in KTR for months, in the presymptomatic phase of TS[41]. Therefore, it is likely that TSPyV, next to skin, replicates as well in internal, not yet identified, organ or tissue. In such a context it is difficult to explain why IgG responses against TSPyV remain stable, while those against JCPyV and BKPyV clearly rise, unless the (transplanted) end organ, the kidney in the case of JCPyV and BKPyV, is of pivotal importance here. In this regard, the observed increasing serological trend for HPyV9 in KTR, comparable to JCPyV and BKPyV, might suggest that HPyV9 is nephrotropic. Cross-seroreactivity against HPyV9, BKPyV and JCPyV VP1 antigens to explain the concurrent rise in serum antibodies has been ruled out previously[1,25].

For BKPyV we have previously shown that post-transplantation increase in seroreactivity is related to duration and peak viral load of post-transplantation episodes of viremia[42]. Unfortunately we are unaware of JCPyV activity and load after kidney transplantation in our KTR cohort, but it might be expected that JCPyV viremia can be detected in (a proportion of) KTR with increased JCPyV IgG responses, since JCPyV and BKPyV are phylogenetically closely related and share the same end organ. Since the kidney donor IgG response seems to influence both the JCPyV IgG response and the HPyV9 IgG response after transplantation, it appears that JCPyV and HPyV9, similar to BKPyV, are transplanted together with the kidney transplant, in a subset of kidney transplant patients. For JCPyV, this has been suggested previously by molecular comparison of JCPyV genomes before and after transplantation[28]. Since JCPyV is a significant pathogen in the kidney transplant population, the influence of acquiring JCPyV through the kidney allograft should be subject of further study, especially for JCPyV seronegative recipients.

Assuming seroconversion results from primary infection, we compared the frequency of seroconversions in the KTR to the HBD controls, to find evidence for transplantation-related transmission of HPyVs. The highest seroconversion rates in KTR were observed for JCPyV and HPyV9, both 6.5%, which clearly differed from the HBD, where no seroconversions were noticed. For HPyV9, the majority of the

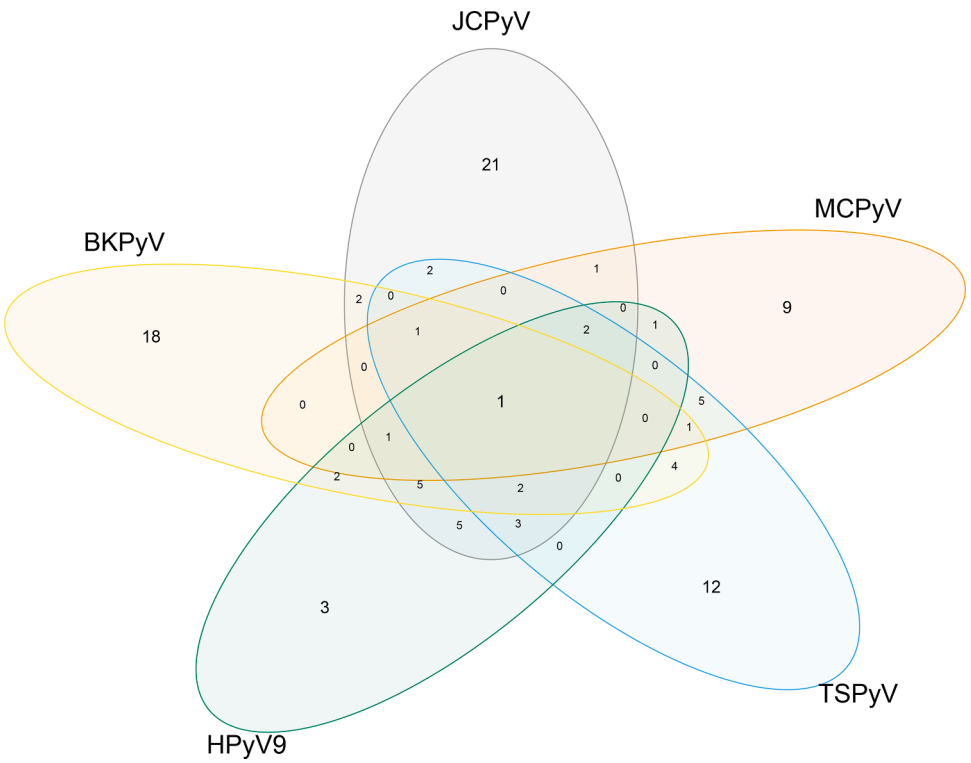

Fig. 2. Venn diagram of kidney transplant recipient with increased IgG responses (including seroconversions) against HPyVs. The numbers in the Figure indicate the number of KTRs that showed an increased IgG response to the corresponding polyomavirus(es).

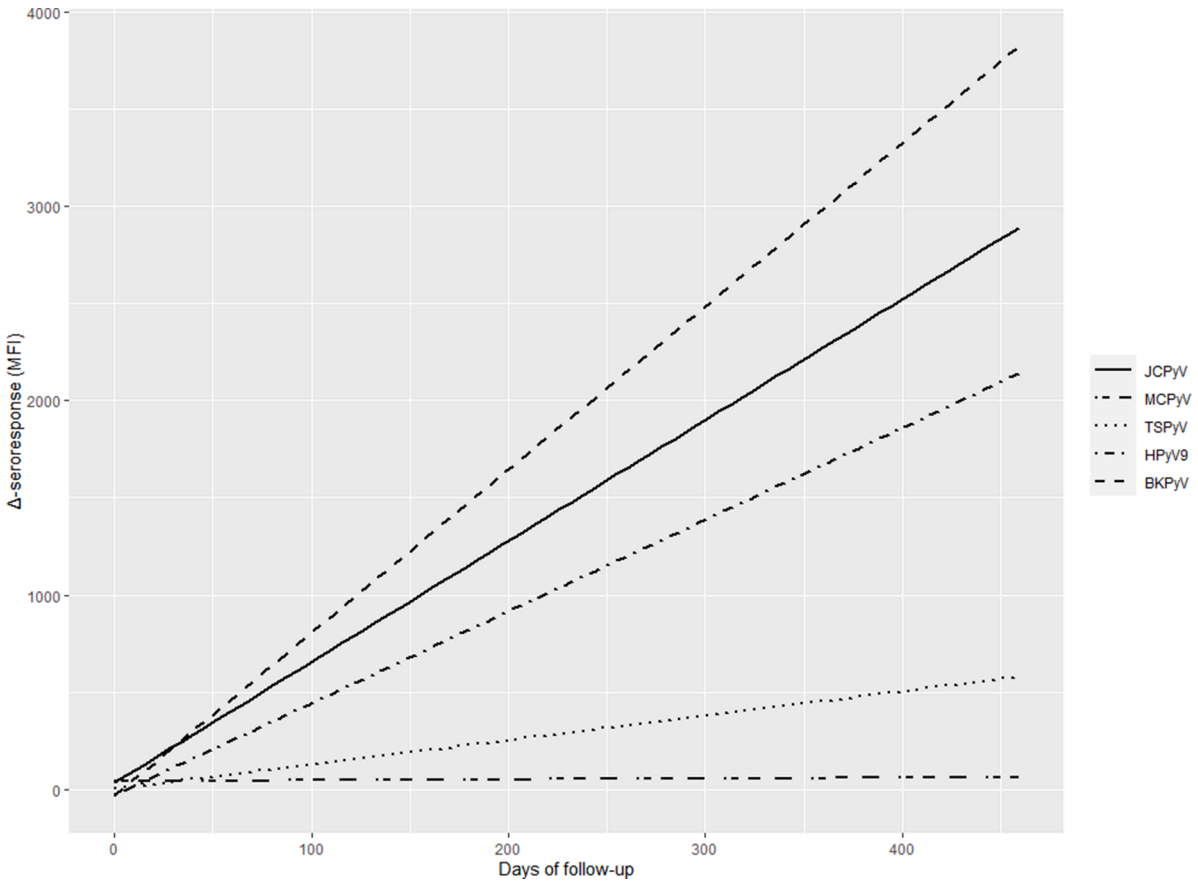

Fig. 3. Difference in HPyV IgG response during follow-up for the kidney transplant recipients ($N = 310$). Shown are linear regression lines for IgG responses against the different polyomaviruses with the first timepoint set at zero MFI and the difference in MFI calculated by subtraction (polyomavirus IgG level of the second timepoint minus the corresponding IgG level of the first timepoint).

Table 2
Linear mixed effects models of polyomavirus IgG levels during follow-up.

HPyV	Predictors*	Estimates	95% CI	p-value
JCPyV	Intercept	3770.70	3104.57 – 4436.83	**<0.001**
	Group	893.47	−532.20 – 2319.14	0.220
	Time	6.61	4.36 – 8.86	**<0.001**
	Group * Time	−7.39	−10.43 – −4.35	**<0.001**
MCPyV	Intercept	9428.54	8510.92 – 10,346.16	**<0.001**
	Group	644.79	−1316.83 – 2606.41	0.520
	Time	0.22	−1.76 – 2.20	0.827
	Group * Time	−2.01	−4.69 – 0.66	0.140
TSPyV	Intercept	9334.44	8504.31 – 10,164.56	**<0.001**
	Group	−692.83	−2467.29 – 1081.64	0.444
	Time	1.33	−0.39 – 3.05	0.131
	Group * Time	−0.23	−2.55 – 2.09	0.846
HPyV9	Intercept	483.37	−84.78 – 1051.53	0.096
	Group	2003.58	786.95 – 3220.21	**0.001**
	Time	4.74	2.59 – 6.88	**<0.001**
	Group * Time	−5.57	−8.47 – −2.68	**<0.001**
BKPyV	Intercept	17,122.68	16,083.76 – 18,161.59	**<0.001**
	Group	5767.08	3543.90 – 7990.27	**<0.001**
	Time	8.26	4.87 – 11.65	**<0.001**
	Group * Time	−14.38	−18.95 – −9.81	**<0.001**

* Linear mixed models with random intercepts and fixed effects 'Group' (with kidney transplant patients as the reference group, opposed to blood donors) and 'Time' (in MFI / day). 'Group * Time' is the interaction term, which improved the fit of the model (as tested by ANOVA).

sero-increasers (80%; 20 out of 25, Table 1) were actually sero-converters, whereas for JCPyV this constituted 43%. Since BKPyV seroprevalence in the general population is extremely high[1,43], there is no use in comparing the observed seroconversion numbers for this virus. Altogether, these data are suggestive of donor origin of at least a proportion of JCPyV and HPyV9 infections after transplantation in KTR. The actual size of kidney allograft-mediated HPyV transmission could be larger but is difficult to assess serologically, because transmission in seropositive recipients does not result in seroconversion. However, it could result in increased IgG responses, as we observed especially for JCPyV.

Further research is necessary to confirm our findings and to determine the clinical relevance of, for example, JCPyV allograft exposure for developing viremia, JCPyV-associated nephropathy, and perhaps even PML after kidney transplantation. A study in adult KTR has previously shown a correlation between kidney donor JCPyV IgG response and JCPyV viruria, suggesting the donor kidney to be the origin of JCPyV viruria in the recipient[44]. Recently, transmission of JCPyV through the kidney allograft was demonstrated for a small number of kidney transplantation donor and recipient pairs with the help of metagenomic sequencing [28], comparable to what has been shown for BKPyV[26, 45].

In conclusion, this study provides evidence that KTR are exposed to JCPyV and HPyV9 after transplantation (next to BKPyV). The origin of this exposure could lie in the transplanted kidney. Whether donor screening could provide insight in determining KTR risk of developing for instance JCPyV infection and related complications could be subject of further study.

Sources of support

Funded by Sanquin Blood Supply Foundation

Table 3
Linear regression models of age, sex, donor MFI (per 1000) and MFI before transplant (per 1000).

HPyV	Predictors*	Estimates	95% CI	p-value
JCPyV	Intercept	1478.88	−527.43 – 3485.20	0.148
	Age	−0.58	−37.15 – 36.00	0.975
	Sex	417.88	−611.43 – 1447.18	0.425
	Donor MFI	80.03	4.80 – 155.26	**0.037**
	MFI before Tx	786.81	700.19 – 873.42	**<0.001**
MCPyV	Intercept	−94.62	−1634.34 – 1445.10	0.904
	Age	22.08	−5.19 – 49.36	0.112
	Sex	−67.06	−840.27 – 706.16	0.865
	Donor MFI	23.66	−27.66 – 74.99	0.365
	MFI before Tx	897.82	851.19 – 944.45	**<0.001**
TSPyV	Intercept	2138.27	584.61 – 3691.94	**0.007**
	Age	−25.24	−51.46 – 0.99	0.059
	Sex	399.16	−339.14 – 1137.45	0.288
	Donor MFI	7.07	−41.70 – 55.85	0.775
	MFI before Tx	901.62	855.10 – 948.14	**<0.001**
HPyV9	Intercept	823.64	−1002.89 – 2650.16	0.375
	Age	−1.26	−35.37 – 32.85	0.942
	Sex	217.19	−746.68 – 1181.06	0.658
	Donor MFI	188.46	56.63 – 320.30	**0.005**
	MFI before Tx	864.39	759.39 – 969.39	**<0.001**
	Donor MFI* MFI before Tx	−25.92	−47.07 – −4.78	**0.016**
BKPyV	Intercept	3456.13	359.01 – 6553.26	**0.029**
	Age	−1.54	−48.89 – 45.81	0.949
	Sex	476.73	−863.24 – 1816.70	0.484
	Donor MFI	89.58	18.45 – 160.71	**0.014**
	MFI before Tx	783.28	709.80 – 856.77	**<0.001**

* Interaction terms (Donor MFI * MFI before Tx) were tested for all polyomaviruses, but are only shown here if these improved the fit of the model (as tested by ANOVA). Tx: transplantation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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