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A Difficult Decision: Atypical JC Polyomavirus Encephalopathy in a Kidney Transplant Recipient

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Abstract

Background—A number of cerebral manifestations are associated with JC Polyomavirus (JCPyV) which are diagnosed by detection of JCPyV in cerebrospinal fluid (CSF), often with the support of cerebral imaging. Here we present an unusual case of a kidney transplant patient presenting with progressive neurological deterioration attributed to JCPyV encephalopathy.

Methods—Quantitative JCPyV PCR (qPCR) was used prospectively and retrospectively to track the viral load within the patient blood, urine, CSF, and kidney sections. A JCPyV VP1 ELISA was used to measure patient and donor antibody titers. Immunohistochemical (IHC) staining was used to identify active JCPyV infection within the kidney allograft.

Results—JCPyV was detected in the CSF at the time of presentation. JCPyV was not detected in pretransplant serum, however viral loads increased with time, peaking during the height of the

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neurological symptoms (1.5×10^9 copies/mL). No parenchymal brain lesions were evident on imaging, but transient cerebral venous sinus thrombosis was present. Progressive decline in neurological function necessitated immunotherapy cessation and allograft removal, which led to decreasing serum viral loads and resolution of neurological symptoms. JCPyV was detected within the graft's collecting duct cells using qPCR and IHC. The patient was JCPyV naïve pretransplant, but showed high antibody titers during the neurological symptoms, with the IgM decrease paralleling the viral load after graft removal.

Conclusions—We report a case of atypical JCPyV encephalopathy associated with cerebral venous sinus thrombosis and disseminated primary JCPyV infection originating from the kidney allograft. Clinical improvement followed removal of the allograft and cessation of immunosuppression.

Introduction

A 27 year-old man presented to hospital with confusion and headache 9 months after receiving a kidney transplant. Past medical history included diarrhea-associated haemolytic uremic syndrome as an infant, with subsequent chronic kidney disease and progression to end-stage kidney disease. He commenced peritoneal dialysis and 4 years later received a 3/6 HLA mismatch, deceased donor transplant. The donor was Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) IgG positive and the patient CMV and EBV IgG negative. Immunosuppression comprised basiliximab induction (20mg on day 0 and day 4), mycophenolate sodium (720 mg BD), tacrolimus (2 mg BD) and prednisolone (8mg daily). The posttransplantation period was uncomplicated, achieving a baseline creatinine of 1.13mg/dL without episodes of acute rejection or treatment with T or B cell depleting antibodies. Standard transplant center protocol for monitoring BKPyV was followed posttransplant (plasma was monitored at 1,2,3,6,9,12,18, and 24 months).

The patient had a 10-day history of headache, vertigo, confusion and intermittent right sided weakness. On examination, he was afebrile, and disoriented to time and place. The blood pressure was 112/78 mm Hg and pulse 110 beats per minute. He had a bilateral upper limb resting tremor, reduced sensation in the right arm and leg and an ataxic gait. There were no papilledema, meningismus, rash, hepato-splenomegaly or lymphadenopathy. Creatinine was 1.37mg/dL, haemoglobin 14.1 g/dL, white blood cell count (WBC) $6 \times 10^3/\mu\text{L}$ and platelets $193 \times 10^3/\mu\text{L}$. Trough tacrolimus level was 4.8 ng/mL. Coagulation studies were significant for a 20210G>A heterozygous prothrombin gene mutation. However prothrombin, activated partial thromboplastin time, lupus anticoagulant, activated protein C resistance, antithrombin 3, protein C and S studies were normal.

A brain X-ray computed tomogram (CT) was normal, but MRI (including venogram) showed an acute left transverse venous sinus thrombosis (Figure 1a) without brain parenchymal lesions (not shown). The patient was anticoagulated with heparin and then warfarin.

An electroencephalogram (EEG) showed frequent bursts and runs of higher voltage symmetrical delta activity with no epileptiform discharges, suggesting either raised intra cranial pressure or a diffuse encephalopathy. BK polyomavirus (BKPyV), CMV, EBV and

Herpes Simplex (HSV) DNA were not detectable by polymerase chain reaction (PCR) in peripheral blood. During the following week, the delirium worsened with increasing confusion and disorientation. A CT scan of the chest, abdomen and pelvis showed no evidence of posttransplant lymphoproliferative disease. Two weeks after the initial presentation cognition improved. Repeat MRI showed resolution of the venous cerebral thrombosis (not shown) with no parenchymal lesions or meningeal inflammation (Figure 1b). However 3 weeks after admission, the delirium worsened and repeat EEG reported a frequent generalized delta activity consistent with severe diffuse encephalopathy. An alternative diagnosis was sought as this deterioration was considered inconsistent with venous sinus thrombosis. A lumbar puncture was performed and analyses of the cerebrospinal fluid (CSF) showed an increased protein concentration of 92mg/dL, low glucose (48.6mg/dL), WBC of $1.3 \times 10^1/\mu\text{L}$ (mononuclear cells 99%, polymorphs 1%) and red blood cell count of $9 \times 10^0/\mu\text{L}$. Flow cytometry, India ink, cryptococcal antigen and Ziehl-Neelsen studies were normal; and culture for bacterial and fungal pathogens was negative.

Due to progressing symptoms, a repeat CSF exam was performed 5 days later showing the presence of JC Polyomavirus (JCPyV) DNA by PCR. The CSF was PCR negative for CMV, EBV, HSV 1 and 2, varicella-zoster virus, adenovirus, rabies and lyssavirus. The CSF cell count could not be ascertained due to blood contamination.

The diagnosis of JCPyV-associated encephalopathy was established and the implications discussed with the patient and family. Over a period of 17 days, the mycophenolate dose was initially halved and then ceased; and the tacrolimus dose was reduced aiming for a level of 4ng/mL, however there was no improvement in the delirium. Retrospective quantitative PCR (qPCR) showed increasing JCPyV loads in blood starting 5 months prior to onset of neurological symptoms, but negative in the pretransplant serum.

Sequential collections of blood remained positive for JCPyV by qPCR (Figure 2a). Due to a lack of improvement in neurological symptoms and concerns of permanent cognitive impairment or death, he underwent transplant nephrectomy and cessation of immunosuppression 7 weeks after his initial presentation. Histopathology of the explanted kidney demonstrated mild tubulointerstitial scarring, moderate vascular sclerosis and microcystic change, but no evidence of rejection. Tubules and collecting ducts within the medulla occasionally showed mild nuclear abnormalities including enlargement and nuclear staining with SV40 antibody that cross-reacts with JCPyV (Figure 3). No SV40 staining was evident in the renal cortex.

Once immunosuppressive therapy was ceased, the patient's delirium resolved over 2 weeks. Nine months after discharge from hospital the patient, was stable on hemodialysis, with regained baseline cognition and complaints of only occasional left arm paresthesia and mild vertigo. A CSF exam performed 9 months posttransplant nephrectomy showed normalization of protein levels to 23mg/dL, with a WBC count of $1.2 \times 10^1/\mu\text{L}$ (mononuclear 97%, polymorphs 3%) and RBC count of $3 \times 10^0/\mu\text{L}$.

In contrast, no JCPyV was detected in the blood of the patient who received the deceased donor's contralateral kidney. However this patient did subsequently develop BK viraemia without polyomavirus-associated nephritis.

Materials and Methods

Real-time PCR and Genomic Analyses

DNA from blood, serum, urine, flocced nasal swabs (Copan Diagnostics, USA) and CSF (200µL of each sample) was extracted using Roche High Pure Viral Nucleic Acid kits (Roche Diagnostics, Australia), while Qiagen DNeasy Blood & Tissue kits (Qiagen, Australia) were used for kidney tissue (approximately 25mg) DNA extraction, as per manufacturers' instructions. Nasal swabs were suspended in 1mL of PBS prior to extraction. Fresh scalpel blades and plasticware were used to collect the different sections of kidney tissue.

Endogenous retrovirus 3 (ERV3)¹ was used for cellular quantification. Real-time PCRs specific for BKPyV (assay V3A)² and JCPyV (assay JL1)³ were used for both initial diagnostic testing and subsequent quantification. Primer targets did not share homology with any of the newly discovered human polyomaviruses. Quantified A549 tissue culture and a 3373bp pMA-RQ(ampR) synthetic plasmid containing the JL1 assay target (Life Technologies, Germany) were used to generate standard curves for quantification of ERV3 and JCPyV, respectively.

JCPyV genomes were generated using PCR with 10 overlapping primer pairs (Supplementary Table 1) and standard Sanger sequencing. Genome assembly and analyses were performed using CLC Main Workbench 6.6.1 (CLC Bio, Denmark).

Immunohistochemistry

Following nephrectomy, one-third of the graft was fixed for further histopathological investigations. Immunohistochemical detection of JCPyV was performed using a monoclonal mouse antibody against the large T antigen (LTA_g) of SV40, which also cross-reacts with JCPyV's LTA_g (Calbiochem, catalogue no DP02, at a 1:200 dilution). Sections were cut onto coated slides and automated immunoperoxidase staining was performed using the Ventana Benchmark Ultra (Ventana Medical Systems, USA). This method included heat retrieval at pH 8 and the Optiview detection system (Roche Diagnostics, USA).

JCPyV and BKPyV Serology

JCPyV and BKPyV serology was conducted as previously described⁴, apart from JCPyV proteins being substituted for BKPyV. Briefly, JCPyV and BKPyV VP1 capsid proteins were generated using a baculovirus expression system, with the resulting self-assembled virus-like particles being used as antigens in microtitre plate-based ELISAs. Serum was used at a 1:100 dilution.

Results

Virus Detection and Quantification

Blood viral loads gradually increased, and were extremely high in CSF, serum and urine during the peak of the patient's neurological symptoms, in addition to JCPyV detection in the upper respiratory tract. (Figure 2a,b)

JCPyV loads were highest in the medulla of the kidney explant (23.6 copies/cell), followed by the minor calyx (2.71 copies/cell), renal pelvis (1.64 copies/cell), and cortex (0.57 copies/cell). BKPyV was not detected in any sample.

Subsequent to cessation of immunosuppression, viral loads in serum gradually decreased by 5 logs, although 9 months later, JCPyV continued to be detected at low levels in serum and CSF. (Figure 2a,b)

Genetic Analyses

Six full length JCPyV genomes were sequenced from a range of samples collected throughout the study period (Figure 2a), as well as from the medulla section of the explant. All 7 genomes were identical. The virus' noncoding control region (NCCR) was identical to that of healthy archetype JCPyV isolate CY (AB038249), and did not contain any mutations in the Agnoprotein or Viral Protein 1 (VP1) genes previously associated with progressive multifocal leukoencephalopathy (PML) or other neurological diseases⁵⁻¹⁰. Examination of the relevant sequencing chromatograms did not reveal evidence of minor JCPyV populations harbouring genomic mutations. Attempts to sequence the final CSF were not successful. The sequenced genome was deposited as isolate QLD-01 (Accession number HG764413).

Serology

The patient was JCPyV seronegative pretransplant up until the commencement of neurological symptoms, when serum viral loads peaked. IgG titres thereafter rapidly rose, correlating with decreasing viral loads and IgM titres (Figure 2b). Likewise, the contralateral KTx recipient was also JCPyV naïve pretransplantation, in contrast to the donor's seropositivity for JCPyV (Supplementary Table 2). Both recipient and the donor sera were seropositive for BKV, however the case patient's pretransplantation seroreactivity was substantially lower (Supplementary Table 2).

Discussion

After primary infection, JCPyV latency is established within the kidney, although other sites have also been described^{5,11,12}. JCPyV reactivates under immunosuppressive conditions causing varied neurological diseases including PML¹⁰, granule cell neuronopathy⁷, meningitis¹³ and JCPyV encephalopathy¹⁴. In kidney transplant recipients (KTRs), JCPyV reactivation is not uncommon, however JCPyV associated neurological events are rare¹⁵.

JCPyV involvement in our patient was not considered at the time of initial presentation due to the MRI finding of cerebral venous sinus thrombosis, but was diagnosed after progression of the patient's delirium. The increasing JCPyV viral loads in blood, high JCPyV viral load

in CSF, and progressive neurological deterioration suggested JCPyV cerebral involvement. A previous review of PML outcomes in 9 KTRs reported 7 deaths and residual neurological damage in 2 survivors¹⁶. Given the high risk of permanent neurological sequelae or death in other JCPyV-associated neurological diseases¹⁶, immunosuppression was ceased and the patient underwent a transplant nephrectomy with the aim of immune reconstitution and control of the viral infection. Although nephrectomy resulted in the patient's return to dialysis; there were significant reductions in blood and CSF viral loads and a resolution of delirium and neurological symptoms occurred. Interestingly, our patient continued to shed JCPyV in his CSF without the presence of significant neurological symptoms which has not been previously described. Two years posttransplant nephrectomy the patient remains stable on hemodialysis with only mild neurological symptoms consisting of occasional arm paresthesia and vertigo.

The JCPyV NCCR and the major viral capsid protein VP1 are key determinants of the virus' tissue tropism^{8,9,12}. Rearrangements in JCPyV's NCCR arise from unchecked replication following immunosuppression, and along with mutations in VP1 and the JCPyV agnoprotein, have been associated with the development of various neurological diseases^{6,7,10,13}. In this case however, the virus remained genetically stable over 8 months, although it was not possible to address the presence of mutant JCPyV species in localized cerebral areas, as described previously⁶. While the presence of JCPyV variant sub-populations in the CSF cannot be completely excluded, the lack of minor peak signals in the sequencing data suggests that the dominant strain within the CSF is indeed archetype, rather than being a manifestation of blood contamination. JCPyV NCCR alterations occur frequently during immunosuppression within both the CNS and blood,¹⁷ however CSF-originating archetype JCPyV within the setting of neurological disease is not an unprecedented, albeit rare, occurrence.¹⁸ Thus, these findings suggest that archetype JCPyV is capable of infecting the CNS, a proposition which is further reinforced by Sock et al¹⁹ report of archetype NCCR's capacity for activity within glial cells, although at a lower level to that of rearranged NCCR variants.

Neurological symptoms were not consistent with dural sinus thrombosis which in conjunction with the worsening symptoms despite the radiological resolution of the thrombus, point to an alternate etiology. The development of posterior reversible encephalopathy syndrome (PRES) can be linked to acute increases in blood pressure and use of immunosuppressive agents, and describes a clinical syndrome of headache, visual disturbance, confusion, decreased level of consciousness, and seizures which are associated with characteristic radiological findings of posterior cerebral white matter oedema.²⁰ Whilst PRES is an important differential diagnosis and was considered, it was thought unlikely because both the patient's blood pressure and tacrolimus dosing were stable, along with the absence of any neuroimaging abnormalities within the brain parenchyma on any of the 3 MRI scans performed (Sagittal T1, coronal T2, axial FLAIR, DWI, ADC, T1, SWI sequences of the brain, MRV and 3D Time Of Flight MRA Axial, Coronal and Sagittal T1 MPRAGE post gadolinium sequences). The use of mycophenolate has been widely associated with JCPyV replication, which we found to peak concurrently with neurological symptoms. Cessation of mycophenolate restored the humoral response and led to a reduction in viral replication and symptoms. The multiple lines of evidence and the timing of events

therefore suggests JCPyV as the primary etiological agent of pathogenesis in this case. While the neurological presentation was similar to that of previously described JCPyV encephalopathy¹⁴; the presence of archetype JCPyV genome, cerebral venous sinus thrombosis and a lack of typical radiological lesions led us to consider this a novel case of atypical JCPyV-associated encephalitis.

The high viral loads found in the blood and urine represents a poorly regulated systemic JCPyV infection, highlighted by JCPyV's unexpected detection within the nasal cavity. The respiratory tract has long been suspected of being a potential route of JCPyV transmission, however presence of the virus in airways has not been previously described. The JCPyV detection in the respiratory swab may represent a rare occurrence of JCPyV replication within the respiratory tract, or alternatively, may purely be due to a spillover event from high viral loads circulating within the blood. Of note, the initial CSF was contaminated with blood during collection, which indicates that the actual CSF JCPyV load may have been lower than calculated; however we consider the virus presence as genuine, as 9 months later, JCPyV continued to be detected within the blood-free CSF. This observation also reinforces the need to take sample quality into consideration when assessing diagnostic CSF results. Viral loads were dramatically higher in the medulla of the kidney compared to other explant sections, which, together with strong LTag protein staining within the collecting duct cells of the medulla points to an active localized infection within the kidney graft. The infrequent nuclear staining patterns observed in this case are a consistent feature found in previous reports of JCPyV nephropathy^{21–23}, although the lack of graft dysfunction or evidence of acute rejection has been recorded less frequently.²¹

The observed seronegativity pretransplant, a robust rise and fall of the IgM response, and the seroconversion and rise in the IgG titer, concurrent with the decreasing serum viral loads demonstrate all the hallmarks of a primary infection. The serological data from the case report patient and donor, in concert with the archetype JCPyV replication in the graft and identical circulating genomes throughout the body point to an unchecked primary infection seeded by a JCPyV positive graft.

An interfering BKPyV effect has been proposed by Cheng and colleagues²⁴, whereby a high humoral response to BKPyV was observed to have a protective effect against JCPyV viruria in the KTR population. The observed protective effect may be due to cross-reactive antibodies, or more likely, from a parallel cross-reactive cellular immunity response as proposed by Rossi et al.²⁵. The role of the humoral response in regulating polyomavirus infection is still not well understood, however it appears to increase with rising polyomavirus replication levels.²⁶ In contrast, the T cell response has been shown to not only regulate the polyomavirus infections, but to also be capable of cross-protection between BKPyV and JCPyV.²⁷ In the absence of a sufficient T cell response, high titres of antibodies may be sufficient to clear the virus from the blood.²⁷

Despite both KTRs sharing a JCPyV positive donor and both seropositive for BKPyV pretransplant, only the case report patient went onto develop JCPyV infection. Unlike the contralateral patient however, the case report patient had much lower seroreactivity to BKPyV; thus, the combination of JCPyV naivety and a low BKPyV humoral response may

have been sufficient to lead to a donor-derived uncontrolled JCPyV infection. In contrast, the higher BKPyV seroresponse in the contralateral KTR may have been sufficient enough to offer cross-protection to either prevent or regulate the JCPyV infection to sub-clinical levels.

This case, along with a recent report of JCPyV-associated nephritis²³ highlight the potential of JCPyV to cause severe disease in naïve patients after kidney transplantation, and argues for the value of including JCPyV screening in patients with graft dysfunction or neurological disease.

JCPyV is capable of binding and aggregating type-O erythrocytes, and this mechanism is the basis of proposed increased PML risk amongst patients with type-O blood²⁸. Indeed, the current case's blood type was type-O, which suggests that high virion loads in conjunction with the prothrombin gene mutation²⁹ created the environment for virus-mediated hemagglutination, and consequently, venous sinus thrombus development. Additionally, elevated CSF WBC and protein counts suggested the presence of meningeal inflammation, which could also have contributed to the development of the thrombus, as the 2 are known to be associated³⁰. The thrombus may have then facilitated the release of JCPyV into CSF by reverse flow through arachnoid villae and possible infection of the choroid plexus, resulting in the observed persistent JCPyV presence in the CSF and lack of cerebral lesions.

This is a unique case of atypical JCPyV-associated encephalopathy presenting with disseminated primary JCPyV infection, cerebral venous sinus thrombosis and a lack of typical brain lesions on MRI scans. The demonstration of JCPyV in blood, CSF, the LTA_g staining in the renal collecting duct cells, the robust serological response, coupled with the neurological presentation, and improvement of the patient following graft nephrectomy and cessation of immunosuppression suggests JCPyV to be the causative agent in this case. It is also notable that the viral genome isolated from the blood and CSF was not of the usual neuroinvasive type. These findings emphasize the importance of comprehensive CSF examination in immunosuppressed patients with delirium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ADC	Apparent Diffusion Coefficient
CMV	Cytomegalovirus
CSF	Cerebrospinal Fluid
CT	X-Ray Computed Tomogram
DWI	Diffusion Weighted Imaging
EBV	Epstein-Barr Virus
EEG	Electroencephalogram
ERV3	Endogenous Retrovirus 3
FLAIR	Fluid Attenuated Inversion Recovery
HSV	Herpes Simplex Virus
JCPYV	JC Polyomavirus
KTR	Kidney Transplant Recipient
LTA_g	Large T Antigen
MPRAGE	Magnetization-Prepared Rapid Gradient Echo
MRI	Magnetic Resonance Imaging
MRV	Magnetic Resonance Venography
MRA	Magnetic Resonance Angiography

NCCR	NonCoding Control Region
PCR	Polymerase Chain Reaction
PML	Progressive Multifocal Leukoencephalopathy
PRES	Posterior Reversible Encephalopathy Syndrome
qPCR	Quantitative Polymerase Chain Reaction
SWI	Susceptibility Weighted Imaging
VP1	Viral Protein 1
WBC	White Blood Cell Count

Figure 1.

a) MR Venogram showing left transverse sinus thrombosis in axial oblique (left) and coronal oblique (right) views. **b)** MRI axial FLAIR (left) and T1 post gadolinium contrast injection (right), taken 2 weeks after presentation to hospital showing no evidence of meningeal enhancement or parenchymal lesions.

Figure 2.

a) Timeline showing clinical progression and associated JCPyV loads. All viral loads are shown as JCPyV copies/mL of respective fluid. Asterisks denote which samples had their full JCPyV genomes sequenced. **b)** Progression of the patient's antibody responses, as determined by a VLP-based ELISA plotted against viral load in serum. Open circles in the viral load plots indicate viral loads extrapolated from whole blood, which equate to a 1 log

decrease in serum, based on parallel sample evaluations (data not shown). Significant clinical history is denoted below the x-axis. ND = Not detected

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Figure 3.
Immunohistochemical SV40 LTag staining within the explanted kidney medulla at 400× magnification showing staining within collecting duct cell nuclei.