JC VIRUS DNA IN THE PERIPHERAL BLOOD OF RENAL TRANSPLANT PATIENTS: A ONE-YEAR PROSPECTIVE FOLLOW-UP IN FRANCE

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Fig.1: Follow up of patients with JCV-DNA positive in whole blood.
Horizontal bars represent months of follow-up.
Red horizontal bars are those patients treated with rituximab
Virus loads are expressed as $\log_{10}$ copies/mL.
Red stars represent detection of Decoy cells.

**JC VIRUS DNA IN THE PERIPHERAL BLOOD OF RENAL TRANSPLANT
PATIENTS: A ONE-YEAR PROSPECTIVE FOLLOW-UP IN FRANCE**

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Shortened title: JCV infection in renal transplantation

Abstract

There is little information on JC virus (JCV) infection in renal transplant patients. A long-term prospective follow-up study was conducted to assess the incidence of JCV DNA in the blood of 103 adult renal transplant patients enrolled prospectively between 1 January and 31 December, 2006. Patients were monitored until April, 2008. JCV DNA was quantified by a real-time polymerase chain reaction in whole blood samples collected regularly for at least one year post-transplant.

JCV was detected in seven patients (6.8 %) (31/1487 whole blood samples) at a median time of 139 days post-transplant. The median JC virus load of the first positive DNA blood sample was $3.4 \log_{10}$ copies/mL ($1.9 - 5.7 \log_{10}$ copies/mL). Induction therapy were either anti-CD25 monoclonal antibodies ($n=5$) or antithymocyte globulins ($n=2$). Post-transplant immunosuppressive treatment included steroids with tacrolimus/mycophenolate mofetil (MMF) ($n=2$), or ciclosporin/MMF ($n=1$), or belatacept/MMF ($n=4$). Two patients were also treated with rituximab. All seven patients infected with JCV had other viral infections(s): BK virus (3), Epstein-Barr virus (2), Cytomegalovirus (1) or both BK virus and Epstein-Barr virus (1). Three patients had BKV-associated nephropathy and decoy cells shedding. JCV infection was not associated with acute rejection episodes or nephropathy, regardless of the virus load. No patient developed progressive multifocal leukoencephalopathy during follow-up.

Thus the incidence of JCV infection in renal transplant patients was low and not associated with any specific clinical manifestations. JCV replication must still be diagnosed and differentiated from BK virus infection because of its non-aggressive course.

Key words: Polyomavirus, nephropathy, progressive multifocal leukoencephalopathy, solid-organ transplantation, immunosuppressive treatment

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Introduction

JC virus (JCV) is a member of the *Polyomaviridae*, which include other human viruses such as BK virus, KI [Allander et al., 2007], WU [Gaynor et al., 2007] and SV40. JC and BK virus infections are frequent and occur early in childhood. Seropositivity increases with age; BKV reaches a seroprevalence of 91% in children aged 5-9 years, while seroprevalence of JCV reaches only 50% by the age of 60-69 years [Knowles et al., 2003]. The transmission routes are not yet clear but are thought to be via the respiratory tract, although oral-fecal transmission is also possible.

Both viruses remain latent in the kidney, in B lymphocytes [Lafon et al., 1998; Monaco et al., 1996] and in the central nervous system after primary infection, but they may be reactivated at any time. JCV excretion in the urine can reach 46 % in immunocompetent patients, and the virus usually remains asymptomatic [Pagani et al., 2003; Rossi et al., 2007]. However, reactivation of either virus in immunosuppressed patients can cause several clinical syndromes. JCV can become reactivated in patients infected with HIV, transplant recipients and patients with cancer, and may produce progressive multifocal leukoencephalopathy, a fatal neurological demyelinating disease [Hou and Major, 2000]. Polyomavirus-associated nephropathy in renal transplant patients is mostly due to BKV, but recent studies have shown that JCV infection may also be involved [Drachenberg et al., 2007].

Little is known at present on the incidence of JCV infection and its clinical consequences in these patients.

This prospective long-term follow-up study assessed the incidence of JCV shedding in whole blood samples from renal transplant recipients. The results were correlated with immunosuppressive treatment and the virological parameters of the patients who excreted JC virus.

Materials and methods

Patients

Adult renal transplant recipients under the care of Toulouse University Hospital were enrolled prospectively from 1 January to 31 December, 2006 and were monitored until April, 2008.

Any induction therapy was based on antithymocyte globulins or anti-CD25 monoclonal antibodies. All patients were treated with steroids for post-transplant immunosuppression. They were also given tacrolimus/mycophenolate mofetil (MMF), or cyclosporin A/MMF, belatacept/MMF, or cyclosporin/everolimus. Renal biopsies were taken when acute rejection was suspected. The patients were given three injections of methylprednisolone if acute rejection was confirmed. Antithymocyte globulins were given to cases of steroid-resistant acute rejection. Finally, patients with humoral acute rejection were treated by plasmapheresis and rituximab.

Recipients were given prophylaxis for human cytomegalovirus (HCMV). Seropositive recipients who had received a transplant from a seropositive or a seronegative donor, and high-risk patients (a seronegative recipient with a seropositive donor) were given valganciclovir prophylaxis (900 mg/d if their creatinine clearance was > 60 ml/min, and 450 mg/d if their creatinine clearance was < 60 ml/min) during the first 3 to 4 months post-transplantation.

Virological monitoring

All renal transplant recipients were tested prospectively for JCV excretion. Whole blood samples were collected into potassium EDTA tubes from the time of transplantation to at least one year post-transplant.

Methods

Measurement of blood JCV DNA

JC virus DNA was extracted automatically from whole blood and detected by quantitative real-time PCR. The DNA was extracted from 200 µL samples using a MagNA Pure™ instrument (Roche Diagnostics, Meylan, France) and the MagNA Pure LC DNA Isolation Kit I® according

100 to the manufacturer's instructions. JCV DNA was detected using a Light Cycler™ system
101 (Roche).

102 The primers were located in the region coding for the agnoprotein: HMJC: 5'-ATA CAg TgC
103 TTT gCC TgA ACC-3' and HMJC6: 5'-CAA CTg AgC AAT AgC ACT ACC-3'. The fluorogenic
104 probe was HSMSJC3: 6FAM-5'ACT ggA gCT CCg ggg gCT gTA 3'TAMRA. Real-time PCR was
105 carried out using Fast Start DNA Master hybridization probes (Roche Diagnostics, Meylan,
106 France). The use of specific primers and probes insures no cross reactivity with BKV. Extracted
107 DNA (5 µL) was added to the PCR mixture containing 2 mmol/L MgCl₂, 0.500 µmol/L of each
108 primer, and 0.100 µmol/L of probe. The PCR conditions were initial denaturation for one cycle
109 of 2 min at 50°C followed by 2 min at 95°C, followed by 45 cycles of 20 s at 95°C and 60 s at 58
110 °C. Cooling was realised 30 s at 40 °C. The reaction, data acquisition, and analyses were all
111 performed on a Light Cycler instrument. The PCR was checked for contamination using a
112 negative sample. JCV DNA load in whole blood is expressed as log₁₀ genome copy number per
113 mL. Coupled with the MagNA Pure™ extraction, the detection limit was 500 copies genome per
114 mL (i.e. 2.7 log₁₀ copies/mL).

115 Patients were also tested for other opportunistic viral infections: cytomegalovirus (CMV),
116 Epstein-Barr virus (EBV), and BK virus (BKV) were quantified in whole blood and urine
117 samples (BKV).

118 HCMV DNA, EBV DNA, and BKV DNA were extracted automatically from whole blood with
119 the MagNA Pure® instrument. HCMV DNA was quantified on a Light Cycler 1.0™ (Roche), as
120 described previously [Mengelle et al., 2003]. EBV DNA was quantified using a Light Cycler EBV
121 Quantification Kit® (Roche), according to the manufacturer's instructions [Gulley et al., 2006;
122 Hill et al., 2006; Kozic et al., 2006]. BKV DNA was quantified on a Light Cycler 2.0™ (Roche),
123 as described previously [Basse et al., 2007].

124 *Serological markers*

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3 125 Hepatitis B antigens were tested using the Ag HBs Architect (Abbott, Abbott Park, Illinois,
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5 126 USA), and anti-HCV antibodies were tested using the anti-HCV Architect (Abbott). HBV DNA
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8 127 and HCV RNA were quantified using the COBAS Ampliprep-COBAS Taqman96™ (CAP-
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10 128 CTM) system (Roche).

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12 129 Anti-HCMV IgG and IgM were assayed using the Liaison CMV IgG and Liaison CMV IgM
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14 130 assays according to the manufacturer's instructions (DiaSorin, Saluggie, Italy) Anti-VCA IgG and
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16 131 anti-EBNA antibodies were tested using the Liaison VCA IgG and Liaison EBNA IgG. Anti-
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18 132 VCA IgM antibodies were detected with the Liaison EBV IgM assays according to the
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20 133 manufacturer's instructions (DiaSorin).

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22 134 *Statistical analyses*

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26 135 StatView 5.0 was used for all statistical analyses. The results are shown as means or medians.
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Results

A total of 103 renal transplantations (68 men) were performed at the Toulouse University Hospital between 1 January and 31 December, 2006. The mean age of the patients was 49.8 years (range: 24-77 years). Patients were enrolled for at least one year post-transplant. A total of 1487 whole blood samples were collected, and the median frequency of sampling was 12 (2-32) per patient.

JCV was detected in 31 whole blood samples from seven of the 103 transplant patients (6.8%), two women and five men. Their mean age was 44 (23-70) years. Six patients had undergone a first transplantation and one patient had undergone a second transplantation. The underlying diagnoses were polycystic kidney disease (n=2), thrombotic microangiopathy (n=1), focal segmental glomerulosclerosis (n=1), interstitial nephropathy (n=1), IgA nephropathy (n=1), and an undetermined cause.

Induction therapies were anti-CD25 monoclonal antibodies (n=5) or antithymocyte globulins (n=2). Their post transplant immunosuppressive therapy included steroids with tacrolimus/MMF (n=2), or ciclosporin/MMF (n=1), or belatacept/MMF (n=4). Two patients were given rituximab; one for a posttransplant focal segmental glomerulosclerosis relapse (375 mg/m² twice), and the other for an acute humoral rejection (375 mg/m²/week for 4 weeks).

No patient had hepatitis B surface antigens or anti-HCV antibodies. All patients were negative for serum HBV DNA and HCV RNA. Five patients were anti-HCMV seropositive, and all were anti-EBV positive at the time of transplantation.

The first positive JCV DNA sample was detected at a median time of 139 days post-transplant (43-250). The median JC virus load of the first positive DNA blood sample was 3.4 log₁₀ copies/mL (1.9 – 5.7 log₁₀ copies/mL). JCV DNA was detected in only one blood sample taken from three patients: at 43, 180, 225 days post-transplant and the virus loads were 4.5, 5.7 and 1.9 log₁₀copies/mL. The patient whose virus load was 5.7 log₁₀ copies/mL was treated with rituximab.

JCV DNAemia was persistent in the other four (median 66 days, range 21-447). JCV DNA was detected five times over 49 days in the one patient in this group who was treated with rituximab, and the virus loads were very low (3.1, 2.5, 2.5, 0.6 and 2.5 log₁₀ copies/mL).

All patients had other viral infection(s): BKV (3), EBV (2), CMV (1) and BK+EBV (1). EBV infection occurred soon after transplantation: 27, 29, and 43 days, whereas the other infections occurred later: BK at 51, 115, 127, and 223 days post-transplant, and CMV at 194 days post-transplant (in a HCMV seropositive patient). Fig. 1 summarizes the virological data for those patients in whom JCV was detected.

The urine of three patients contained decoy cells. The blood samples of these patients also contained BKV DNA: the first quantified BKV virus loads were 2.8, 4 and 4 log₁₀ copies/mL, and the urine BKV virus loads were 6.94, 10.83, and 10.89 log₁₀ copies/mL. First BKV DNA was detected at a median time of 121 days post-transplant (51-223), and was prior to or at the same time as JCV DNAemia. All three patients had a biopsy-confirmed polyomavirus-associated nephropathy and had experienced acute rejection before BKV replication. The acute rejection episodes had been treated with steroid injections alone (1), steroid injections and polyclonal antibodies (1), and steroid injections, muromonab CD3, and rituximab (1). Pathological analysis of a kidney biopsy taken from the fourth patient with BKV replication showed no features of polyomavirus-associated nephropathy.

The median CD3 count was 733/mm³ (178– 1717) and the median CD4 count was 470/mm³ (78-1184) at the time of first JCV DNAemia, and the median creatinine was 1.39 mg/dL (1.12-2.93). The median microalbuminuria was 94 mg/mL (25-244), the median hematuria was 9 cells/mm³ (0-28), and the median leukocyturia was 3 cells/mm³ (0-80).

Discussion

Although BKV is known to be associated with polyomavirus-associated nephropathy in renal transplant recipients, little is known on the consequence of a JCV infection. In this study, the incidence of JCV infection was determined in a long-term follow-up of renal transplant patients. The incidence was similar to that found by Razonable et al., [2005], who detected JCV DNA in the blood of 7.6% of renal transplant patients during the first year post-transplant. The incidence of JCV is similar to that of BKV DNAemia that was reported previously for the renal transplant patients at Toulouse University Hospital [Basse et al., 2007]. Some authors have not detected JCV DNA in the blood of renal transplant and liver transplant patients [Randhawa et al., 2005], whereas others have reported rare, transient and low viremia [Drachenberg et al., 2007]. In this study, whole blood samples rather than plasma or leukocyte specimens were used to quantify JCV DNA. Whole blood samples might increase the sensitivity of JCV detection, as has been described for other viruses [Basse et al., 2007; Drachenberg et al., 2007; Fafi-Kremer et al., 2004; Garrigue et al., 2006; Gouarin et al., 2004], and could explain why current results differ from those of other studies.

Urine samples were not tested regularly for JCV. BKV viruria is rare in immunocompetent patient and is clearly associated with disruptions of cellular immunity, as demonstrated in bone marrow transplant recipients [Doerries, 2006] and in patients infected with HIV [Hirsch and Steiger, 2003]. In contrast, JCV is found commonly in the urine of immunocompetent patients: JCV remains latent in the renal parenchyma after primary infection, and may be excreted in non-immunosuppressed individuals. It is found frequently in the urine of elderly persons in particular [Chang et al., 2002; Kitamura et al., 1997]. This was why urine samples were not tested regularly; positive results would have had to be interpreted cautiously.

The association of JCV and polyomavirus-associated nephropathy has been documented in isolated cases [Kazory et al., 2003; Randhawa et al., 2005; Wen et al., 2004]. Only the blood of patients with polyomavirus-associated nephropathy contained JCV DNA, even at low

concentrations [Drachenberg et al., 2007]. Such an association was not observed in the current study, none of the patients whose blood contained JCV DNA suffered from any polyomavirus-associated nephropathy whatever the virus load. Only three patients had decoy cells at the time of JCV excretion: they were all infected with BKV with a very high virus loads in whole blood and urine, indicating BKV-associated nephropathy, as shown by others [Randhawa et al., 2005; Viscount et al., 2007].

None of the patients had any neurological symptoms and no cases of multifocal leukoencephalopathy were diagnosed during follow-up. Several cases of multifocal leukoencephalopathy have been reported recently in haematological [Pelosini et al., 2008] and bone marrow transplant patients [Goldberg et al., 2002] treated with rituximab. Kamar et al. [2009] reported that the incidence of JCV DNAemia in 73 rituximab-treated solid-organ transplant patients was 5.5%, but no neurological disorder was found during long-term follow-up. In the present study, only two of the seven JCV-infected patients were treated with rituximab, these results are very similar to those of the previous study. Overall, the JC virus loads were not correlated with any clinical manifestations.

Frequent mixed infections with other opportunistic virus were detected in whole blood sample. In particular, mixed BKV and JCV infections were observed in whole blood samples of four patients. Three patients had biopsy-confirmed BKV nephropathy, but no significant relationship could be established between mixed infection and clinical manifestations since these opportunistic infections were detected prior to or at the same time as JCV. This high incidence of mixed infections, which reflects heavy immunosuppression, has not been described previously [Drachenberg et al., 2007; Randhawa et al., 2005; Watcharananan et al.], although immunosuppressive therapy was as potent as in this study: i.e., kidney transplant patients induced with anti-CD52 antibodies (alemtuzumab) followed by tacrolimus monotherapy.

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235 In conclusion, the present monocentric study shows that JCV infections are relatively uncommon
236 in renal transplant recipients. They were not associated with acute renal rejection, or with the
237 presence of decoy cells in the JCV DNAemia positive patients, suggesting that the course of JCV
238 infection is lower and less aggressive than of BKV. No association between JCV infection and
239 neurological manifestations was also detected. Overall, these results are comparable to those of
240 Razonable et al., [2005], who found episodes of subclinical JCV DNA in the blood of solid-organ
241 transplant patients.
242 However, JCV infection should be surveyed regularly in transplant recipients as the presence of
243 JCV DNA in the blood may differentiate between JCV and BKV infections. Therefore, detection
244 of JCV indicates over-immunosuppressed patients who may benefit from reduction of
245 immunosuppressive treatment.

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