A Longitudinal Molecular Surveillance Study of Human Polyomavirus Viremia in Heart, Kidney, Liver, and Pancreas Transplant Patients

Raymund R. Razonable,1 Robert A. Brown,3 Atul Humar,2 Emma Covington,2 Emma Alecock,2 Carlos V. Paya,1 and the PV16000 Study Group*

1Division of Infectious Diseases and Internal Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota; 2University of Toronto, Toronto, Canada; 3Roche Products, Welwyn Garden City, Herts, United Kingdom

In this study of 263 heart, kidney, liver, and pancreas transplant patients, BK virus (BKV) and JC virus (JCV) DNAemia were observed most commonly in kidney and/or pancreas transplant patients (26%), although they were also observed, to a lesser extent, in heart (7%) and liver (4%) transplant patients. The majority of episodes of polyomavirus DNAemia were subclinical, although, in some cases, BKV DNAemia was associated with kidney rejection, and JCV DNAemia was accompanied by nonspecific symptoms. Hence, BKV and JCV DNAemia are not uncommon during the first year after kidney, heart, liver, and pancreas transplantation, and they could be associated with certain clinical syndromes in transplant patients.

Infections with human polyomaviruses BK virus (BKV) and JC virus (JCV) are widespread, with seroprevalence in 90% of adults [1]. After primary infection, these viruses persist in the kidneys, blood, and brain—sites that serve as reservoirs for reactivation and as vehicles for transmission to susceptible hosts.

Several syndromes are attributed to polyomavirus in transplant patients [2]: BKV causes tubulointerstitial nephritis, ureteral stenosis, and graft dysfunction in kidney transplant patients and hemorrhagic cystitis in hematopoietic stem cell transplant (HSCT) patients, whereas JCV causes progressive multifocal leukoencephalopathy (PML). JCV has also been associated with nephropathy in kidney transplant patients [3]. Beyond these clinical syndromes, the effect of BKV and JCV viremia on transplant outcomes is less clear. Specifically, the incidence and clinical manifestations of BKV viremia in non-kidney solid organ transplant (SOT) patients are undefined. Likewise, the incidence and clinical manifestations of JCV viremia after SOT and its effect on transplant outcomes remain to be investigated.

Studies describe an in vitro interaction between polyomaviruses and cytomegalovirus (CMV) [4–6]: CMV enhances polyomavirus replication [4, 6, 7], and, conversely, polyomaviruses have transactivating properties that enhance CMV replication [5]. However, clinical data that support these interactions in vivo are limited. Organ transplantation, which allows reactivation of viruses, offers a unique environment to study these viral interactions. Hence, we conducted the present study to investigate the epidemiology and clinical relevance of BKV and JCV in a cohort of SOT patients at high risk of CMV disease.

Patients and methods. Two hundred sixty-three (72%) of the 364 CMV-seronegative recipients of a heart, kidney, liver, or pancreas from a CMV-seropositive donor (CMV D+/R+) who participated in the PV16000 trial that compared valganciclovir (n = 168) and oral ganciclovir (n = 95) for prevention of CMV disease were studied [8]. All patients received oral ganciclovir or valganciclovir prophylaxis for 100 days. Peripheral blood samples were collected from all patients within 10 days after transplantation (before prophylaxis); on days 14, 42, 70, and 100 (during prophylaxis); and at months 4, 4.5, 6, 8, and 12 after transplantation. The blood samples were stored at −70°C until use in the present study.

There were 2232 blood samples collected from the 263 patients (mean, 8.5 samples/patient), all of which were analyzed for BKV and JCV DNAemia at the Mayo Clinic research laboratory (Rochester, MN). Viral DNA was extracted from 200 μL of whole blood (Isoquick; ORCA Research) and was eluted in 25 μL of DNAse-free and RNAse-free water. Five microliters of eluted DNA was added to 15 μL of Mastermix Solution (Roche Molecular Biochemicals) containing primers and probes that amplify the viral capsid protein VP2, as described elsewhere [9]. BKV and JCV DNA were quantified by use of a LightCycler (Roche Molecular Biochemicals). The primer-probe combi-
nation detected both viruses, which were differentiated by their peaks in the melting curve analysis [9].

During the PV16000 trial, clinical events during the first year after transplantation were prospectively recorded in a database that was maintained by the sponsor. In the present study, these events were correlated with the presence and degree of polyomavirus replication. The association between polyomavirus DNAemia and CMV disease, serum creatinine and creatinine clearance, allograft rejection, and other clinical symptoms was evaluated.

The overall and organ-specific incidences of BKV and JCV DNAemia were calculated on the basis of the total number of patients and for each organ type. Prevalence was calculated on the basis of the total number of patients for each specific time point. Data are presented as proportions, means, and medians. Statistical analysis was performed by use of the χ² test or Fisher’s exact test, as appropriate, and by use of the Wilcoxon 2-sample test. The level of statistical significance was P < .05.

Results. Thirty-two (12.2%) of 263 CMV D+/R− patients developed BKV DNAemia (range, 2–22,680 copies/mL) at the median time to onset of DNAemia, which was day 100 after transplantation. The prevalence of BKV DNAemia was highest (6.2%) at 4.5 months (figure 1A). Half of the BKV DNAemia episodes were transient (detected at 1 time point). The incidence of BKV DNAemia was similar during and after antiviral prophylaxis, although it was observed more commonly in patients receiving oral ganciclovir prophylaxis (17.9%) than in patients receiving valganciclovir prophylaxis (8.9%) (P = .03).

Twenty-four (75%) of the 32 BKV DNAemic patients were kidney transplant patients. Hence, in 92 kidney transplant patients, the first-year incidence of BKV DNAemia was 26% (figure 1B). The majority of BKV DNAemic episodes in kidney transplant patients were subclinical. In 6 patients (25%), incidence of BKV DNAemia coexisted with clinical or biopsy-proven acute graft rejection; 2 of these patients lost their allograft because of persistent poor graft function or recurrent acute rejection. A higher peak BKV load was observed in patients who developed graft rejection (4108 vs. 652 copies/mL) than in those who lost...
Figure 2. A, Prevalence of JC virus (JCV) DNAemia in 263 solid organ transplant patients receiving ganciclovir or valganciclovir during the first year after transplantation. B, First-year posttransplant incidence of JCV DNAemia in heart, liver, kidney, and kidney-pancreas transplant patients. The dotted line represents end of prophylaxis.

Thirteen (4.9%) of 263 SOT patients developed JCV DNAemia during the first year after transplantation (range, 6–220 copies/mL). The median time to onset of JCV DNAemia was 100 days after transplantation. The prevalence of JCV DNAemia was highest (1.8%) at month 8 (figure 2A). JCV DNAemia was observed in 7 (7.6%) of 92 kidney transplant patients, 1 (20%) of 5 kidney-pancreas transplant patients, 3 (6.7%) of 45 heart transplant patients, and 2 (1.7%) of 121 liver transplant patients (figure 2B). In most episodes (9/13 [69%]), JCV DNAemia was transient, and it occurred in a similar proportion of patients receiving valganciclovir (6.0%) and oral ganciclovir (3.2%) prophylaxis (P = NS).

The majority (8/13 [61.5%]) of JCV DNAemic episodes were subclinical. Only 5 patients (38.5%) had clinical symptoms at the time of JCV DNAemia. One patient had biopsy-proven acute heart rejection, whereas the other 4 patients had fatigue, lethargy, dyspnea, or tremors. No patient developed manifestations of PML.

Seventeen (53%) of 32 BKV DNAemic patients developed their graft (11,623 vs. 607 copies/mL), although the difference did not reach statistical significance. In 2 other patients (8.3%), hematuria with or without dysuria was temporally related to BKV DNAemia. In another patient, renal artery stenosis was diagnosed 1 month after transient BKV viremia.

Eight (25%) of 32 BKV DNAemic patients were recipients of heart (3/45 [6.7%]) or liver (5/121 [4.1%]) allografts (figure 1B); none of these patients received a simultaneous kidney transplant. Notably, all 3 heart allograft recipients (and 1 liver allograft recipient) developed BKV DNAemia after treatment for acute graft rejection. The biopsy specimens from the patients were not specifically examined for BKV, but a detailed review of the clinical records did not indicate evidence of allograft dysfunction at the time of BKV DNAemia. Within a month before or after BKV DNAemia, none of the BKV DNAemic nonkidney SOT patients had serum creatinine or creatinine clearance that would indicate renal dysfunction. No BKV DNAemic nonkidney SOT patient developed renal failure at 1 year after transplantation. One heart transplant patient had dysuria at the time of BKV DNAemia.
CMV viremia. Ten of these patients (32%) developed CMV disease (including 5 with simultaneous BKV DNAemia and CMV disease), compared with 16% of patients without BKV DNAemia ($P = .03$). However, the peak CMV load in BKV DNAemic patients was similar to that in patients without BKV DNAemia (17,193 vs. 10,498 copies/mL; $P = \text{NS}$). Likewise, the mean peak BKV load was not significantly different between CMV viremic and nonviremic patients (807 vs. 1766 copies; $P = \text{NS}$).

Four (31%) of 13 JCV DNAemic patients developed CMV viremia; 1 patient developed CMV disease 2 months after transient JCV DNAemia. Coexistence of CMV and JCV replication was not observed. The peak CMV load was similar between JCV DNAemic patients and those without JCV DNAemia (8232 vs. 11,473 copies/mL; $P = \text{NS}$). Likewise, the mean peak JCV load was not significantly different between CMV viremic and nonviremic patients (68 vs. 104 copies; $P = \text{NS}$).

Discussion. During the first year after SOT, BKV and JCV viremia is not uncommon in CMV D+/R SOT patients. BKV DNAemia was observed even in nonkidney SOT patients. The majority of episodes of polyomavirus DNAemia were transient and subclinical, although an association between BKV and graft dysfunction in kidney transplant patients was observed, and an association between JCV and nonspecific symptoms was suggested.

The majority of episodes of BKV DNAemia in kidney transplant patients were subclinical. In a minority of episodes, BKV DNAemia was associated with graft dysfunction and urologic manifestations. Notably, the incidence of BKV DNAemia and its median onset in our cohort was higher and earlier than in recent studies, which reported incidences between 5%–13% and a median onset at 23 weeks [10]. This disparity likely reflects differences in study design—the present study serially collected up to 10 blood samples/patient, thereby increasing the likelihood of detecting viremia. Indeed, the rate that we have reported may underestimate the true incidence of BKV infection, since the present study did not account for nonviremic or transiently viremic BKV infections that occurred in between the scheduled collections of blood samples. In addition, this disparity could reflect differences in patient population—the present study included patients at highest risk of CMV disease, who have been described to have a higher predisposition to BKV [1], possibly as a result of the cointeraction between BKV and CMV [5, 7]. Interestingly, many BKV DNAemic patients had concomitant CMV disease—an observation consistent with reports of BKV and CMV coinfections in kidney transplant [10] and HSCT [11] patients.

Anecdotal reports indicate that BKV infection occurs in recipients of organs other than the kidneys [12]. The present study has demonstrated that BKV DNAemia occurs in heart and liver transplant patients at rates that are 4- and 6-fold lower, respectively, than those in kidney transplant patients. The mechanism behind the higher incidence of BKV infection in kidney transplant patients than in liver and heart transplant patients is unclear, but it could imply the transmission of a new BKV strain (through a kidney allograft that harbors BKV) into a susceptible patient [13]; BKV is not known to persist in liver and cardiac cells and is likely not transmitted by these organs. Indeed, a previous study demonstrated higher rates of BKV infection in BKV-seropositive patients who received kidney allografts from BKV-seropositive donors than in those who received kidney allografts from BKV-seronegative donors [14]. Local factors, such as inflammation, could also permit BKV reactivation [13], as implied by the higher incidence of BKV infection in recipients of kidney transplants from deceased donors (who have prolonged cold ischemia time) than in recipients of kidney transplants from living donors [13]. That immunosuppressive regimens play a role has also been suggested [13]. Pharmacological immunosuppression was not specifically controlled for in the PV16000 trial, thereby limiting our ability to analyze the effect of immunosuppressive drugs on BKV. Nonetheless, immunosuppression may not completely explain the higher incidence of BKV in kidney transplant patients, since these patients are not generally more immunosuppressed than are liver and heart transplant patients.

The clinical relevance of BKV DNAemia in recipients of organs other than kidneys remains to be established, although a recent report suggested that BKV may cause chronic renal dysfunction in heart and stem cell transplant patients [12]. In the present study, the majority of episodes of BKV DNAemia in nonkidney SOT patients were subclinical. No association with increasing creatinine was established, although 1 episode of dysuria was observed. The short, 1-year follow-up period in the present study may have limited our ability to assess the long-term effect of BKV DNAemia on renal function. A longer period of follow-up is suggested for future studies on the relevance of BKV in nonkidney SOT patients.

JCV infection is a rare complication after SOT. In the present study, the overall JCV DNAemia rate was 5%. JCV DNAemia was more common in kidney and/or pancreas transplant patients, possibly reflecting reactivation of donor-derived JCV, which also persists in the kidneys. Although a majority of episodes were subclinical, in 5 patients, JCV DNAemia occurred concurrently with acute heart rejection, tremors, fatigue, lethargy, or dyspnea. Although these associations are not necessarily causal, the role that JCV plays in graft rejection deserves further study, since JCV has been demonstrated in specimens of patients with nephropathy [3]. In the present study, a correlation between JCV and renal dysfunction in kidney and non-kidney SOT patients was not observed.

In conclusion, polyomavirus DNAemia is not uncommon...
during the first year after SOT. JCV DNAemia appears to be more common than is indicated by historical data, whereas BKV DNAemia occurs not only in kidney transplant patients but also in heart and liver transplant patients. Studies assessing the long-term clinical relevance of BKV in heart and liver transplant patients are encouraged.

**PV16000 Study Group.** A complete list of the members of the Valganciclovir Solid Organ Transplant Study Group follows, in alphabetical order, by country: Australia—Josie Eris (Royal Prince Alfred Hospital, Camperdown), Anne Keogh (St. Vincent’s Hospital, Darlinghurst), Tim Mathew (Queen Elizabeth Hospital, Woodville), Geoff McLaughan (Royal Prince Alfred Hospital, Camperdown), Kathy Nicholls (Royal Melbourne Hospital, Parkville), and Simone Strasser (Royal Prince Alfred Hospital, Camperdown); Canada—Atul Humar (Toronto General Hospital, Toronto), Richard Lalonde (Montreal Chest Institute, Montreal), Paul Marotta (London Health Sciences Center University Campus, London), Jutta Preiksaitis (University of Alberta Hospital, Edmonton), and Eric Yoshida (Vancouver Hospital and Health Science Centers, Vancouver); France—Iradj Gandjbakch (Pitie-Salpetriere Hospital, Paris), Yvon Lebranchu (Bretonneau Hospital, Tours), Christophe Legendre (Saint-Louis Hospital, Paris), and Faouzi Saliba (Hopital Paul Brousse, Villejuif); Ireland—Oscar Traynor (St. Vincents University Public Hospital, Dublin); Italy—Paolo Angeli (Azienda Ospedaliera Di Padova, Padova) and Francesco Menichetti (Ospedale Cisanello, Pisa); New Zealand—Ed Gane (Auckland Hospital, Auckland); United Kingdom—Ali Bakran (Royal Liverpool University Hospital, Liverpool), John Forseythe (Edinburgh Royal Infirmary, Edinburgh), Nigel Heaton (Kings College Hospital, London), Peter Lodge (St. James Hospital, Leeds), Derek Manas (Freeman Hospital, Newcastle Upon Tyne), Peter Morris (Churchill Hospital, Oxford), Jayan Parameshwar (Papworth Hospital, Papworth Everard), and Nizar Yonan (Wythenshawe Hospital, Manchester); and United States—Barbara Alexander (Duke University Medical Center, Durham, NC), Emily Blumberg (Hospital of the University of Pennsylvania, Philadelphia, PA), Daniel C. Brennan (Barnes Jewish Hospital, St. Louis, MO), Robert Brown (Columbia Presbyterian Medical Center, New York, NY), Ronald W. Busuttil (UCI School of Medicine, Los Angeles, CA), Ken Chavin (Medical University South Carolina, Charleston, SC), David Conti (Albany Medical Center, Albany, NY), Angelo DeMatteos (Oregen Health Sciences University, Portland, OR), Ed Dominguez (University of Nebraska Medical Center, Omaha, NE), Howard J. Eisen (Temple University School of Medicine, Philadelphia, PA), Dan Fishbein (University of Washington, Seattle, WA), Thomas Fishbein (Mt. Sinai Medical Center, New York, NY), Robert Fisher (Medical College of Virginia Hospital, Richmond, VA), Richard Freise (University of California, San Francisco, San Francisco, CA), Marquis Hart (UC San Diego Medical Center, San Diego, CA), Thomas Heffron (Emory University, Atlanta, GA), Ray E. Hersherberger (Oregon Health Sciences University, Portland, OR), Richard J. Howard (University of Florida, Gainesville, FL), Sandra A. Kemmerly (Alton Ochsner Medical Institution, New Orleans, LA), Richard Knight (Mt. Sinai Medical Center, New York, NY), Bernard Kubak (UCI School of Medicine, Los Angeles, CA), Shimon Kusne (University of Pittsburgh, Pittsburgh, PA), Steven Mawhorter (Cleveland Clinical Foundation, Cleveland, OH), Martin Mullen (Loyola University Medical Center, Maywood, IL), Carlos Paya (Mayo Clinic, Rochester, MN), Mark Pescevitz (Indiana University Medical Center, Indianapolis, IN), John Pirsch (University of Wisconsin Medical School, Madison, WI), Timothy L. Pruett (University of Virginia Health Systems, Charlottesville, VA), Jeffrey Punch (University of Michigan Medical Center, Ann Arbor, MI), John Rabkin (Oregon Health Sciences University, Portland, OR), Robert Rubin (Massachusetts General Hospital, Boston, MA), John Scandling (Stanford University Medical Center, Palo Alto, CA), Michael Shapiro (Hackensack University Medical Center, Hackensack, NJ), Randi Slibovsky (Albert Einstein Medical Center, Philadelphia, PA), Kenneth Washburn (University of Texas Health Service Center, San Antonio, TX), and Sam Weinstein (LifeLink Transplant Institute, Tampa, FL).

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**References**