Published in final edited form as:

Transplantation. 2009 August 15; 88(3): 360–366. doi:10.1097/TP.0b013e3181ae5ff9.

Polyomavirus Infection and Its Impact on Renal Function and Long-Term Outcomes after Lung Transplantation

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Abstract

Background—Polyomavirus infection causes nephropathy after kidney transplantation but has not been thoroughly investigated in nonrenal organ transplantation.

Methods—Ninety lung transplant recipients were enrolled and provided urine samples over 4.5 years. Samples were analyzed for BK virus (BKV), JC virus (JCV), and simian virus 40 (SV40) by conventional and quantitative real time polymerase chain reaction (PCR).

Results—Fifty-nine (66%) patients had polyomavirus detected at least once, including 38 (42%) for BKV, 25 (28%) for JCV, and 6 (7%) for SV40. Frequency of virus shedding in serial urine samples by patients positive at least once varied significantly among viruses: JCV, 64%; BKV, 48%; SV40, 14%. Urinary viral loads for BKV ($10^{5.4}$ copies/ml) and JCV ($10^{6.0}$ copies/ml) were higher than for SV40 ($10^{2.5}$ copies/ml; $p=0.001$ and $p=0.0003$, respectively). Polyomavirus infection was associated with a pre-transplant diagnosis of chronic obstructive pulmonary disease [odds ratio (OR) = 6.0; $p=0.016$], but was less common in patients with a history of acute rejection [OR=0.28; $p=0.016$]. SV40 infection was associated with sirolimus-based immunosuppression ($p=0.037$). Reduced survival was noted for patients with BKV infection ($p=0.03$). Patients with polyomavirus infection did not have worse renal function than those without infection, but in patients with BKV infection, creatinine clearances were lower at times when viral shedding was detected ($p=0.038$).

Conclusion—BKV and JCV were commonly detected in the urine of lung transplant recipients; SV40 was found at low frequency. No definite impact of polyomavirus infection on renal function was documented. BKV infection was associated with poorer survival.

Keywords

Polyomavirus; lung transplantation; kidney disease

Introduction

Renal dysfunction is a common problem after solid organ transplantation, even among recipients of nonrenal organs [1–7]. The incidence of end-stage renal disease after lung or

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heart–lung transplantation varies from 4 to 16% [3,5–7]. The etiology of renal dysfunction after transplantation is multifactorial, but the use of calcineurin inhibitors in these patients is most frequently implicated as the primary underlying cause [6,8–9]. Common comorbid conditions related to transplantation, such as hypertension and diabetes mellitus, have also been found to contribute to the progression of renal dysfunction [1–2,7].

Another possible source of renal injury after transplantation is nephritis due to viral infection. BK virus (BKV), JC virus (JCV), and simian virus 40 (SV40) are nonenveloped, double-stranded DNA polyomaviruses. More than 80% of adults are serologically positive for BKV and JCV. These viral infections are usually acquired in childhood possibly via a respiratory or oral-fecal route [10–11]. SV40 is believed to have been introduced into the human population through contaminated poliovirus vaccines administered between 1955 and 1962. Seroprevalence of SV40 in adults has been estimated to be between 2% and 20%; in some instances, SV40 neutralizing antibodies were detected in individuals with no history of exposure to contaminated vaccines [12–15]. Nephropathy associated with polyomaviruses, predominantly BKV, has been reported in 1–10% of renal transplant recipients and often leads to loss of the allograft [16]. Polyomavirus nephropathy in other solid organ recipients and nephropathy related to JCV and SV40 have only been described in a few cases [17–21]. BKV infection is also associated with hemorrhagic cystitis in bone marrow transplant recipients [22].

Few studies have examined the impact of polyomavirus infection on nonrenal transplant patients [23–28]. Most of these studies have failed to show an association between BKV or JCV viral detection and renal dysfunction [24,26,28], whereas one found an association between BKV reactivation and impaired renal function in heart recipients, but only upon univariate analysis [23]. Another study examined BKV and JCV in blood samples collected from patients participating in a large randomized trial of cytomegalovirus prophylaxis and found that detection of polyomaviruses was more common in kidney recipients than in other transplant recipients [25]. In a previous report [27], we documented that urinary polyomavirus infections are frequent in lung transplant recipients. In this extended prospective study, we further examined the dynamics of polyomavirus infection after lung transplantation and evaluated its impact on renal function and other long-term outcomes.

Methods

Study population

Adult lung and heart–lung transplant recipients receiving continuing medical care at the Vanderbilt Transplant Center were enrolled prospectively from April 2002 through April 2006 and followed until January 2007. Patients were eligible for the study if they had received a lung transplant or a heart–lung transplant and were ambulatory. Pediatric patients (<18 years of age) and patients who had a renal transplant or were on hemodialysis were excluded. All patients signed informed consent forms according to a protocol approved by the Institutional Review Board from each institution for this study.

Standard demographic and historical data were collected on each patient, as well as donor information. At each visit the following clinical information was collected: serum creatinine and current immunosuppressive regimen. A creatinine clearance (CrCl) was calculated with a standard Cockcroft-Gault formula using an ideal body weight formula [29]. Patients' medical histories were examined for the diagnosis of acute rejection and chronic graft dysfunction. Acute rejection was defined if there was either grade 1 or higher-grade rejection as diagnosed by transbronchial biopsy or if there was a strong clinical suspicion of acute rejection that was treated with augmented immunosuppression. Chronic graft dysfunction was diagnosed using two separate definitions: (1) evidence of bronchiolitis obliterans from a transbronchial or open

lung biopsy, or (2) evidence of bronchiolitis obliterans syndrome, defined as a sustained decrease of >20% of FEV1 on pulmonary function testing. Antiviral prophylaxis in our lung transplant recipients consisted of oral acyclovir with intermittent intravenous ganciclovir or oral valganciclovir therapy given preemptively within the first 3–6 months of transplantation as determined by virological monitoring using cytomegalovirus blood antigenemia. Additionally, our patients received lifelong prophylaxis against *Pneumocystis jiroveci* with sulfamethoxazole/trimethoprim three times weekly.

Sample collection and virological analysis

Urine samples were collected as described previously [27] from patients at approximately 3 to 6 month intervals when they came for routine clinic visits. Frozen samples were shipped to Baylor College of Medicine for blinded laboratory analysis. Sample processing and DNA extractions were performed in a laminar flow hood within a BSL3 facility free from viruses and plasmids following procedures to avoid contamination [27].

DNA was extracted from 500 µl urine/sample using the Gentra Puregene Tissue Kit (Qiagen, Valencia, CA) and following the manufacturer's instructions. DNA samples were tested by conventional polymerase chain reaction (PCR) using assay conditions and universal polyomavirus primers able to detect BKV, JCV, and SV40 sequences [17,30]. Those samples that were positive by conventional PCR were assayed by real-time quantitative PCR (RQ-PCR) to identify the specific virus present (BKV, JCV, or SV40) and to determine viral loads. Specific primers and probes directed against the N-termini of the large T-antigen genes of SV40, BKV, and JCV and reaction conditions have been described [31]. This approach for virus identification was used on specimens collected after 2003. A PCR reaction was considered virus-positive if ≥ 25 copies/reaction were detected. If a second virus appeared to be present in a sample, it was considered positive if the viral load was within 2 logs of the other virus. Viral loads were calculated and expressed as genome copies/ml of urine.

Data Analysis

Each polyomavirus was analyzed independently. For example, patients with BKV infection were compared to those without BKV infection, even if they were infected with a different polyomavirus. Also, patients with any polyomavirus infection were compared to patients without polyomavirus infection. Differences in categorical and continuous variables by exposure groups were assessed using Fisher's exact test and the Mann-Whitney test, respectively. For the analysis of quantitative viral load levels in the urine, a mean viral load (log10 transformed) was calculated for each patient who had at least one positive urine specimen for a polyomavirus. Differences in quantitative viral loads by exposure groups were assessed using the Mann-Whitney test.

Risk factors for polyomavirus infection

The following risk factors were analyzed for an association with polyomavirus infection: age, sex, underlying lung disease, donor age and sex, time since transplantation at enrollment, immunosuppressive medications, history of acute rejection at any time after transplantation, and postoperative renal failure (defined as more than a 50% drop in pre-transplant CrCl during the initial transplant hospitalization). If a patient received more than one immunosuppressive regimen during the follow-up period, the regimen was documented that the patient was taking when he/she first started shedding any polyomavirus (viral shedding and infection are synonymous). For patients who did not shed any virus, the predominant regimen during the period of follow-up was used in the data analysis. For univariate analyses, continuous variables were compared using the Mann-Whitney test and categorical variables were compared using the Fisher's exact test. Logistic regression was used to adjust for potential confounding.

Effect of polyomavirus infection on renal function

CrCl was analyzed employing two approaches. The first involved calculating a mean CrCl for each patient over the study period. Also, the percent drop from the patient's pretransplant CrCl to the CrCl at the time of enrollment was calculated. Patients infected with polyomavirus had their mean CrCl and percent drop from pretransplant CrCl univariately compared to those without infection using the Mann-Whitney test. Linear regression was used to adjust for potential confounding.

A second analysis used the individual, repeated CrCl measurements per patient. We assessed marginal differences in CrCl depending on presence of urinary virus using linear regression accounting for correlation induced by the repeated measurements clustered by patient. A separate analysis with this statistical method using a quantitative viral load in the urine was also performed. The viral loads (log10 transformed) of each virus were analyzed using the individual repeated measurements and related to renal function using regression models described above. This analysis was performed using all urine samples, but a separate analysis was performed for each virus where only the urine samples from patients who had at least one urine positive for that specific polyomavirus were used.

To account for potential confounding, the above regression models were also adjusted for the following variables: sex, age, time since transplantation, postoperative renal failure (as defined above), type of calcineurin inhibitor (cyclosporine vs. tacrolimus), and presence of hypertension and diabetes mellitus.

Chronic graft dysfunction and death

To evaluate a relationship between polyomavirus infection and chronic graft dysfunction and death, a time-to-event analysis was used, with follow-up beginning the day of transplantation. Survival curves stratified by viral group were constructed using the Kaplan-Meier method and compared using log-rank statistics.

All information was entered into a computer database and statistics were performed using Stata Software Version 9 (Statacorp, College Station, Texas). A two-sided p-value ≤ 0.05 was considered significant. Means were used when data were evenly distributed, and medians when data were skewed.

Results

Ninety lung transplant recipients were enrolled from April 2002 to April 2006 and followed till January 2007. The demographic characteristics of these patients are listed in Table 1. A total of 524 urine samples were collected over a total of 249 patient-years of follow-up. Average duration of follow-up per patient was 2.8 years (range 51 days to 4.7 years). A mean of 5.8 (1–13) urine samples were collected per patient.

Dynamics of urinary viral shedding

A total of 182 (35%) of 524 urine specimens tested positive for at least one polyomavirus. For every patient who was positive for a polyomavirus at least once, an average of 3.1 (range 1–8) samples tested positive. Patients with polyomavirus infection did not have more urine samples collected than those not infected with polyomavirus (5.5 samples vs. 6.3 samples, respectively; $p=0.22$). Ninety-five urine specimens tested positive for BKV (18%), 94 tested positive for JCV (18%), and 7 tested positive for SV40 (1%).

Fifty-nine (66%) of the 90 patients were positive for a polyomavirus in at least one urine specimen. This included 38 patients (42%) with BKV, 25 (28%) with JCV, and 6 (7%) with

SV40. Ten patients were positive in the urine for two viruses. These combinations included BKV and JCV (n=7), JCV and SV40 (n=1), and BKV and SV40 (n=2). The frequency of shedding varied according to the virus detected. Sixty-four percent of the urine samples were positive for JCV in patients who had JCV detected at least once. The corresponding percentages for BKV and SV40 were 48% and 14%, respectively. The frequency of shedding was significantly lower for SV40 than for BKV ($p<0.001$) and JCV ($p<0.001$), and the frequency of BKV shedding was significantly lower than that of JCV ($p=0.003$). Patients infected with SV40 did have more urine samples collected per patient (8.3; range 4–11) compared to patients infected with JCV ($p=0.03$) and BKV ($p=0.01$).

Quantitative analyses for viral load were done on 95 urine samples from 38 patients for BKV, 94 samples from 25 patients for JCV, and 7 samples from 6 patients for SV40. The viral loads for the individual viruses are demonstrated in a box plot (Figure 1). Mean viral loads for BKV ($10^{5.4}$ copies/ml) did not differ from JCV ($10^{6.0}$ copies/ml, $p=0.21$), but viral loads for SV40 ($10^{2.5}$ copies/ml) were lower than for BKV ($p=0.001$) and for JCV ($p=0.0003$).

Risk factors for polyomavirus infection

On univariate analysis, patients with JCV infection were more likely to be male (80% of patients infected with JCV were male, compared to 43% of patients without JCV infection, $p=0.002$). Urinary infection with SV40 was associated with a sirolimus-based immunosuppression regimen (33% of patients with SV40 infection on sirolimus vs. 5% of patients without SV40 infection, $p=0.050$). While there was no significant difference in the age of patients infected with SV40 compared to those not infected with SV40, there was a trend for infection with SV40 to be associated with year of birth when examined categorically (Table 2). All patients with SV40 were born before 1962, and thus, could have potentially received contaminated vaccines.

On multivariate analysis, having chronic obstructive pulmonary disease (COPD) as the underlying diagnosis was significantly associated with having infection with any polyomavirus [odds ratio (OR) (95% CI) 6.0 (1.4–26.0); $p=0.016$, Table 3]. Additionally, a history of acute rejection was negatively associated with polyomavirus infection [OR 0.28 (0.09–0.89); $p=0.032$], and there was a trend for patients receiving cyclosporine to have more polyomavirus infection. When infection with individual polyomaviruses was examined, acute rejection remained negatively associated with BKV infection on multivariate analysis [OR 0.33 (0.11–1.01); $p=0.054$]. Male sex was associated with JCV infection and SV40 infection was significantly associated with sirolimus-based immunosuppression.

Polyomavirus infection and renal function

The patients' mean CrCl during the study period and their percent drop from pretransplant CrCl when they enrolled into the study were evaluated for an association with polyomavirus infection. On univariate analysis, only infection with JCV showed any significant association with renal function, but mean CrCl was actually better (65.1 ml/min) in patients with JCV infection than in patients without JCV infection (56.1 ml/min; $p=0.035$). This difference in mean CrCl could possibly be attributed to gender, since men had significantly higher CrCl than women. Patients with SV40 infection had a larger drop from pretransplant CrCl (58%) at the time of study enrollment than patients without SV40 infection (34%; $p=0.0007$). It was also noted that patients on sirolimus had greater reductions in renal function from their pretransplant values, which may potentially explain the association between SV40 infection and sirolimus therapy. When these analyses of renal function were adjusted for other variables (age, sex, pretransplant CrCl, time after transplantation that patient was enrolled, type of calcineurin inhibitor, and the presence of hypertension and diabetes mellitus), none of the polyomaviruses were significantly associated with mean CrCl or percent drop from pretransplant CrCl.

Longitudinal analysis (taking into account repeated measurements) of renal function revealed no association between presence of any of the polyomaviruses or the viral load of any polyomavirus and renal function. However, we performed a separate analysis to evaluate whether presence of virus or viral load affected renal function exclusively in patients who had at least one urine positive for that particular virus. Among patients who had at least one urine positive for BKV, the presence of urinary BKV infection and increasing BK viral load were significantly associated with worsening renal function. The presence of BKV in a urine specimen was associated with a 4.2 ml/min decrease in CrCl compared to when no virus was detected in the urine ($p=0.015$). Additionally, a ten-fold increase in viral load of BKV was associated with a 0.8 ml/min decrease in CrCl ($p=0.038$). When adjusted for other variables, these associations remained significant for urinary infection with BKV (3.6 ml/min drop in CrCl; $p=0.038$), but only trended to significance for BKV viral load (0.5 ml/min drop in CrCl; $p=0.080$). Analysis for patients infected with JCV and SV40 found no association with renal function.

Two (2%) of the 90 patients enrolled in our study developed renal failure requiring hemodialysis. One of the patients had no polyomavirus detected in 4 collected urine samples, while the other patient had 2 of 3 urine specimens positive for BKV.

Chronic graft dysfunction and survival

Of the 90 patients enrolled in the study, 44 patients (49%) had a diagnosis of chronic graft dysfunction by the end of the study period. No association could be detected between the diagnosis of chronic graft dysfunction and infection with any of the polyomaviruses. Thirty patients (33%) died during the study period. The causes of death included chronic graft dysfunction (50%), infection (20%), cardiac disease (10%), neoplasm (7%), and other causes (13%). Presence of BKV infection was associated with an increased risk of death ($p=0.03$; Figure 2). When we examined individual causes of death, there was an association between BKV infection and death from chronic graft dysfunction (26% of patients infected with BKV died of chronic graft dysfunction, compared to 10% of patients without BKV infection; $p=0.047$).

Discussion

Human diseases caused by polyomavirus infection are increasingly recognized in recipients of solid organs with a spectrum of findings ranging from asymptomatic urinary viral shedding to severe nephropathy [10,16,24]. Our study revealed that polyomavirus infection is common after lung transplantation, with 66% of patients having at least one urine specimen positive for a polyomavirus. Our current study also confirms the results of our previous pilot study that both the frequency of polyomavirus urinary shedding and the urinary viral loads are significantly higher for JCV and BKV than for SV40 [27].

One interesting discovery in our study was that patients infected with polyomavirus, particularly BKV, were less likely to have had a history of acute rejection. It may be that the presence of polyomavirus infection after lung transplantation indicates a high level of immunosuppression, which would lead to less allograft rejection. In kidney transplant recipients, however, a history of treatment for acute rejection has been identified as a risk factor for reactivation of BKV [16,32–33]. It has been postulated that the use of high-dose steroid pulses or antilymphocyte preparations to treat kidney rejection allows for viral replication and invasiveness [32]. An alternative explanation might be that the injury to the allograft kidney caused by acute rejection creates a tissue environment advantageous for viral replication and that the immunosuppressive treatments used to treat rejection play a less important role. The BKV-specific immune response has not been well-defined previously, but examining the dynamics and immune response to this viral infection in nonrenal transplant recipients may

aid in clarifying the mechanism that this virus uses in causing more frequent invasive disease in kidney transplant recipients.

While we could not detect a significant difference in renal function between patients infected with polyomavirus and those not infected, we did find that patients infected with BKV experience worsening renal function during the times they were shedding BKV in the urine. Additionally, increasing viral loads of BKV were associated with reduced renal function in these patients. This association could be related to direct virological effects on the kidneys or may merely be due to more intense pharmacological immunosuppression. A Spanish study examining BKV infection in kidney, heart, and liver transplant recipients found that detecting BKV in the urine or plasma of heart transplant recipients was associated with increased serum creatinine [23]. Confirming these findings would likely require a large study of kidney injury in nonrenal organ recipients with careful control of calcineurin inhibitor exposure.

We also found an increased risk of death in association with BKV infection that appeared to be driven by patients infected with BKV dying more frequently of chronic graft dysfunction. Yet patients with BKV infection did not develop more chronic graft dysfunction and were actually less likely than those patients without BKV infection to develop acute rejection. Thus, the reasons for the association of BKV with increased mortality from chronic graft dysfunction are difficult to explain. One possibility is that BKV infection is not a cause of chronic graft dysfunction, but that the chronic viral infection in some way amplifies the poorly characterized immunological processes involved in chronic graft dysfunction leading to more severe manifestations. Such a process has also been proposed but not proved for cytomegalovirus infection [34].

Twenty-eight percent of our patients had evidence of JCV infection. This is similar to the prevalence of urinary JCV infection in immunocompetent individuals [24]. We did not discover a relationship between JCV infection and renal impairment. However, except for a few case reports of JCV nephropathy, there is little evidence to suggest that JCV infection is a significant cause of renal injury [21,35]. The most important human disease caused by JCV infection is progressive multifocal leukoencephalopathy (PML). No cases of PML were identified in our cohort, but this disease is rare after solid organ transplantation [36]. Male sex was significantly associated with JCV infection in our study, which has been documented in other studies of immunocompetent patients [37]. Surprisingly, COPD as the indication for lung transplantation was also associated with polyomavirus infection. The reason for this is unknown. There have been few studies examining the relationship between polyomavirus infection and lung disease, such as idiopathic pulmonary fibrosis and cancer. None of these investigations have shown a relationship between BKV or JCV infection and lung disease, but COPD was not specifically studied [38–39]. It is possible that polyomavirus infection is related to a factor associated with COPD that was not incorporated into our multivariate model.

We observed a trend for infection with SV40 to be associated with year of birth. Specifically, all infected patients were born before 1962 and could have potentially received contaminated vaccinations. Studies examining SV40 seroprevalence have demonstrated the presence of SV40-specific antibodies in individuals born after 1962 [12–15]. Additionally, SV40 has been detected in urine, feces, and tissues of children, providing evidence that this virus is actively circulating in the community [11,13,30,40–41]. We discovered that patients on sirolimus were more likely to have detectable SV40 infection. Patients are often placed on a sirolimus-based immunosuppression regimen when they develop renal dysfunction ascribed to calcineurin inhibitors. This clinical fact may explain the association we found between SV40 infection and sirolimus, since both groups of patients (those infected with SV40 and those on sirolimus) had greater declines from their pretransplant CrCl at the time of study enrollment.

Our study has certain limitations. Polyomavirus-associated nephropathy is a diagnosis made by tissue biopsy. We did not perform kidney biopsies to determine if any of the patients with polyomavirus infection had invasive polyomavirus-associated disease. Additionally, we did not do virological testing of blood samples on our patients. In renal transplant recipients, polyomavirus viremia is more predictive of invasive polyomavirus nephropathy than polyomavirus viruria [32,42]. In a previous report, we had tested 148 blood samples from lung transplant recipients for polyomaviruses, and none of them yielded positive results. Similarly, BKV viremia was not detected among 60 nonrenal solid organ transplant recipients followed for 9 months posttransplantation [28]. Therefore, testing blood specimens for polyomavirus in this population may not be an effective way of examining the relationship between polyomavirus infection and renal dysfunction. If polyomaviruses do cause injury to the kidney in lung recipients, it may more likely cause a subacute or chronic process and not the classic acute nephropathy seen in kidney recipients. Our patients were enrolled at variable intervals of time since transplantation, and we did incorporate data on timing of enrollment into our multivariate analysis. It would be ideal, however, to enroll patients at the time of transplantation and follow them prospectively to obtain a more accurate picture of the dynamics of polyomavirus shedding after lung transplantation. We enrolled patients after transplantation and some patients had already lost considerable renal function compared to their pretransplant values. Indeed, we documented that patients with SV40 infection had a larger drop in CrCl from pretransplant values than patients without SV40 infection, but could not directly associate it with the viral infection as we did not have viral monitoring available before enrollment.

In conclusion, these data show that shedding of all three polyomaviruses (BKV, JCV, SV40) occurs among adult lung transplant recipients. Infection with polyomavirus was associated with a negative history of acute rejection and could potentially be a marker for a more immunosuppressed host. BKV infection was associated with an increased risk of death. Studying polyomavirus infection in nonrenal transplant recipients eliminates the variable of graft rejection and may aid in defining the pathogenesis of polyomavirus-associated nephropathy in kidney transplant recipients. A larger prospective study of polyomavirus infection in a lung transplant population beginning before or at the time of transplantation is warranted.

Abbreviations

BKV, BK virus
 JCV, JC virus
 SV40, Simian virus 40
 PCR, Polymerase chain reaction
 OR, Odds ratio
 CrCl, Creatinine clearance
 FEV1, Forced expiratory volume in one second
 BSL3, Biosafety level 3
 RQ-PCR, Real-time quantitative polymerase chain reaction
 PML, Progressive multifocal leukoencephalopathy
 COPD, Chronic obstructive pulmonary disease
 IPF, Idiopathic pulmonary fibrosis
 CF, Cystic fibrosis
 PPH, Primary pulmonary hypertension
 AAD, Alpha 1 antitrypsin deficiency

Acknowledgments

The project described was supported by grant number CA104818 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or of the National Institutes of Health.

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Figure 1. Quantitative viral load in urine by virus

The mean viral load for each patient is used in generating the box plots. Patients who shed two viruses are included in both boxes. The numbers of patients in each box are: BKV (n=38), JCV (n=25), and SV40 (n=6). BKV vs. JCV $p=0.21$ (Mann-Whitney); BKV vs. SV40 $p=0.001$; JCV vs. SV40 $p=0.0003$. In each box and whisker plot, the lower and upper edges of the box indicate the 25th and 75th percentiles; the horizontal line within the box represents the median; the whiskers indicate either the minimum and maximum values or a distance of $1.5 \times$ the interquartile range from the edge of the box (whichever distance is smaller).

Figure 2. Actuarial curves representing overall survival in patients grouped by BKV infection. Log-rank statistics; $p=0.03$.

Table 1

Patient characteristics of cohort

Characteristic	n=90
Sex (male:female, n)	48:42
Age (years) ^a	50.8 ± 12.5
Underlying lung disease ^b , n (%)	
COPD	28 (31%)
IPF	27 (30%)
CF	12 (13%)
PPH	7 (8%)
AAD	5 (5%)
Other	11 (12%)
Type of transplant, n (%)	
Single lung	48 (53%)
Double lung	39 (43%)
Heart–lung	3 (3%)
Donor age (years) ^a	28.2 ± 13.4
Donor sex (male:female, n)	54:36
Time since transplant patient enrolled ^c	915 days (18 days–12.1 years)
Immunosuppression at enrollment, n (%)	
Cyclosporine	68 (76%)
Tacrolimus	21 (23%)
Sirolimus	4 (4%)
Everolimus	2 (2%)
Mycophenolate mofetil	49 (54%)
Azathioprine	39 (43%)
Prednisone	90 (100%)
CrCl at enrollment (ml/min) ^a	59.4 ± 22.7
Hypertension, n (%)	73 (81%)
Diabetes mellitus, n (%)	26 (29%)

^aValue given as mean ± standard deviation.

^bCOPD=chronic obstructive pulmonary disease; IPF=idiopathic pulmonary fibrosis; CF=cystic fibrosis; PPH=primary pulmonary hypertension; AAD=alpha 1 antitrypsin deficiency; Other=Eisenmenger's syndrome (n=3), sarcoid (n=3), silicosis (n=3), giant cell pneumonitis (n=1), restrictive cardiomyopathy/pulmonary hypertension (n=1).

^cValue given as median (range).

Table 2

SV40 infection by year of birth^a

	Year of birth, n (%)		Total, n	P-value
	<1941	1941–1962		
SV40 infection	3 (50%)	3 (50%)	6	0.082
No SV40 infection	14 (17%)	50 (59%)	84	

^aYear of birth categories determined based on risk of receiving vaccinations contaminated with SV40 [12].

Table 3

Risk factors for polyomavirus infection: Multivariate analysis

	Adjusted OR ^a (95% CI)	P-value
Any polyomavirus		
COPD ^b	6.0 (1.4–26.0)	0.016
Acute rejection	0.28 (0.09–0.89)	0.032
Cyclosporine-based immunosuppression	2.7 (0.9–8.4)	0.089
BKV		
Acute rejection	0.33 (0.11–1.01)	0.054
JCV		
Male sex	12.1 (2.5–58.9)	0.002
COPD	4.36 (0.9–21.2)	0.068
Postoperative renal failure	0.28 (0.07–1.1)	0.061
SV40		
Sirolimus-based immunosuppression	2307 (1.57–3.4 million)	0.037

^a All odds ratios (OR) are adjusted for patient's age, sex, underlying lung disease, duration of time since transplantation that patient was enrolled, donor age and sex, history of postoperative renal failure (defined as $\geq 50\%$ drop in pretransplant CrCl during transplant hospitalization), immunosuppression, and history of acute rejection. Only variables that had a P-value < 0.1 are listed.

^b COPD= Chronic obstructive pulmonary disease.