

## Lack of Significant Co-Activation of BKV and CMV in Renal Transplant Patients: An Institutional Experience

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**Abstract:** BK virus (BKV) is a significant contributor to renal allograft dysfunction due to its prevalence in immunocompromised transplant patients. Cytomegalovirus (CMV) infection is also common in the same group. Since both BKV and CMV are ubiquitous infections and reactivation of both viruses can occur in immunosuppressed states, such as renal transplantation, we performed a retrospective analysis of BKV and CMV co-activation in renal transplant patients. Between January 2013 and December 2014, 222 renal transplant patients were tested for BKV and CMV using real-time polymerase chain reaction (PCR) in the Molecular Diagnostics Laboratory at The University of Texas Medical Branch in Texas. Assessment of CMV viral load was performed using plasma samples, while BKV viral load was assessed in both plasma and urine samples. We found that 53 patients (26.6%) were positive for BKV, 10 patients (7.2%) were positive for CMV, 6 patients (2.7%) were positive for co-reactivation, and 153 patients were negative for both viruses (68.92%). There was no significant association between BKV and CMV co-activation in renal transplant patients.

**Key words:** BK virus, BKV, cytomegalovirus, CMV, renal transplant recipient

### INTRODUCTION

Polyoma BK virus (BKV) and cytomegalovirus (CMV) are opportunistic viral pathogens frequently encountered in renal transplant patients<sup>1,2</sup>. They can both contribute to allograft rejection because of the use of potent immunosuppressive treatments in transplant patients<sup>1,2</sup>. Typically, BKV infection is asymptomatic early in life. The virus persists in the kidney and the renal tubular epithelial cells where it remains latent until reactivation via immunosuppression in renal transplant patients<sup>1,2</sup>. CMV also stays latent in renal tubular epithelial cells<sup>3,4</sup>. CMV in renal transplant patients can arise as a result of primary infection, virus from the donor organ, or persistent latent infection in the recipient themselves<sup>3,4</sup>. It has been thought that CMV and BKV could have trophic effects on each other leading to coinfection in renal transplant recipients<sup>5</sup>. The studies by Toyoda *et al.* and Nada *et al.*<sup>6,7</sup> are two of the very few in the literature that have been able to show a weak association between concomitant BKV and CMV infection. Their study allowed the conclusion that BKV may be a permissive risk factor for CMV; a conclusion consistent with previous reports<sup>5</sup>. However, studies by Nasiri *et al.* and Kaul *et al.* found a lack of BKV and CMV coexistence in kidney transplant recipients<sup>8,9</sup>.

Both BKV and CMV are common opportunistic pathogens in renal transplant recipients. The incidence of BKV activation has been reported to range between 30% and 60%<sup>1</sup> and symptoms of CMV infection are observed in approximately two-thirds of kidney transplant patients<sup>4</sup>, therefore the probability of BK-CMV co-activation

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can be high in this patient population, which has not been reported in the literature<sup>8,9</sup>. We therefore wanted to analyze BKV-CMV co-activation in our renal transplant patients to determine whether the two viruses are possible risk factors for one another.

### **MATERIALS AND METHODS**

#### **Patients, Specimens, and BKV and CMV PCR Assays**

BKV and CMV viral DNA testing results were generated in 222 renal transplant patients referred to the Molecular Diagnostics Laboratory at The University of Texas Medical Branch (UTMB) in Texas between January 2013 and December 2014. Assessment of CMV viral load was performed using plasma samples, while BKV viral load was assessed in both plasma and urine samples. BK virus DNA was detected by real-time PCR using minor groove binding (MGB) Alert BK Virus Probe and Primers (Nanogen, San Diego, CA), with PCR enzyme mix LightCycler FastStart DNA Master Hyb Probe (Roche, Indianapolis, IN) on a Smart Cycler II real-time PCR machine (Cepheid, Sunnyvale, CA, USA), as described<sup>10</sup>. The dynamic range of the BKV assay was 390-3.9×10<sup>8</sup>copies/ml (2.6-8.6 log copies/ml). The lower limit of the quantification (LLOQ) value of 390 copies/ml served as the limit of detection for this study. CMV DNA was measured using Abbott CMV analytic specific reagent (ASR) on m2000 real-time PCR system (Abbott Molecular Inc., Des Plaines, IL, USA). Sample preparation was carried out on m2000sp using the magnetic bead m2000 System DNA extraction kit, and PCR amplification and detection of CMV were conducted on them m2000rt using RealTime CMV kits as described<sup>11,12</sup>. The dynamic range of the assay was 200-1×10<sup>8</sup> copies/mL (2.3-8 log copies/ml). The lower limit of the quantification (LLOQ) value of 200 copies/ml served as the limit of detection for this study.

Retrospective study of clinical data was generated from medical records via pre-approved access of electronic medical records (EPIC) of UTMB renal transplant patients. Patients who had at least one plasma and/or urine sample drawn for BKV and CMV monitoring between January 2013 and December 2014 were included in the study. The study was approved by the UTMB institutional review board (IRB).

#### **Statistical Analysis**

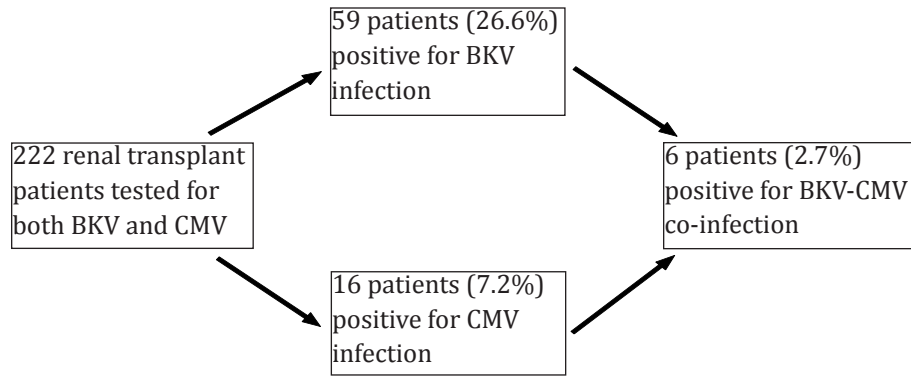
Data was analyzed using Statistical Analysis Software (SAS) version 9.4 (SAS Institute Inc. Cary, NC, USA). The significance of co-infection was determined statistically using a two-tailed Fisher's exact test, p< 0.05 was considered statistically significant.

### **RESULTS AND DISCUSSION**

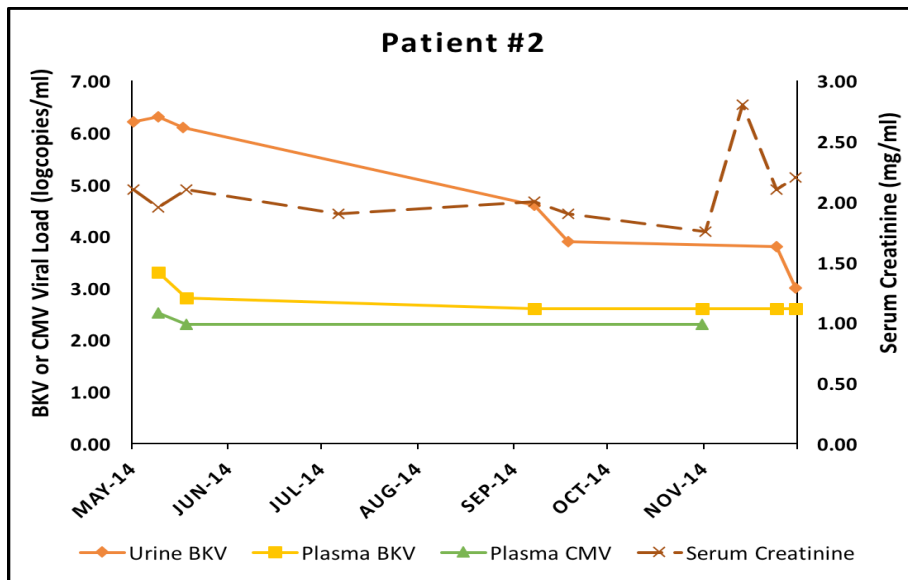
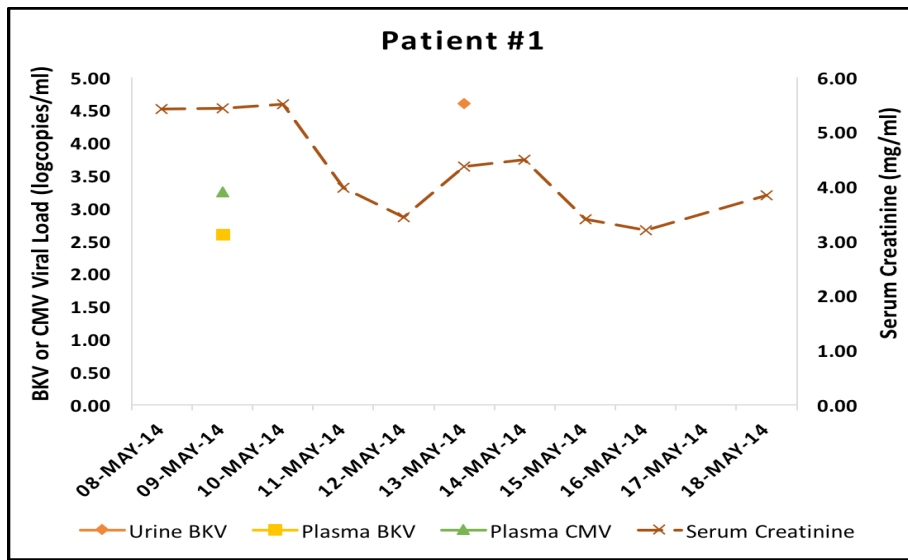
A total number of two hundred and twenty-two renal transplant patients tested for both BKV and CMV were included in the study. The average age of the patients is 50 years, 61.71% were males and 38.29% were females. Four hundred and sixty-five urine BKV, six hundred and fifteen plasma BKV, and four hundred and sixty-five plasma CMV samples were measured and included in the study. Some patients were tested multiple times, and BKV and CMV tests were requested together or separately. As summarized in Figure 1, the positive rate for BKV was 26.6% (59/222), the positive rate for CMV was 7.2% (16/222), and 2.7% (6/222) patients were positive for both viruses. Two-tailed Fisher's exact test showed that there was no significant association between BKV and CMV infections (p< .05). All 6 cases of active BKV-CMV infections demonstrated elevated trends in two common renal function markers, serum creatinine and BUN (Figure 2 and Table 1).

In the present study, although six patients measured for urine BKV, plasma BKV, and CMV viral loads indicted the presence of concomitant BKV and CMV infection, there was no significant link between the two viruses in renal transplant patients, leading us to conclude that these two viruses are therefore not co-activators for each other. However, renal dysfunction might result from a synergistic relationship between BKV-CMV active co-infection as suggested by the elevated serum creatinine levels in all 6 patients. Further studies are needed to validate and establish possible cause-and-effect relationship between BKV and CMV co-activation and renal function.

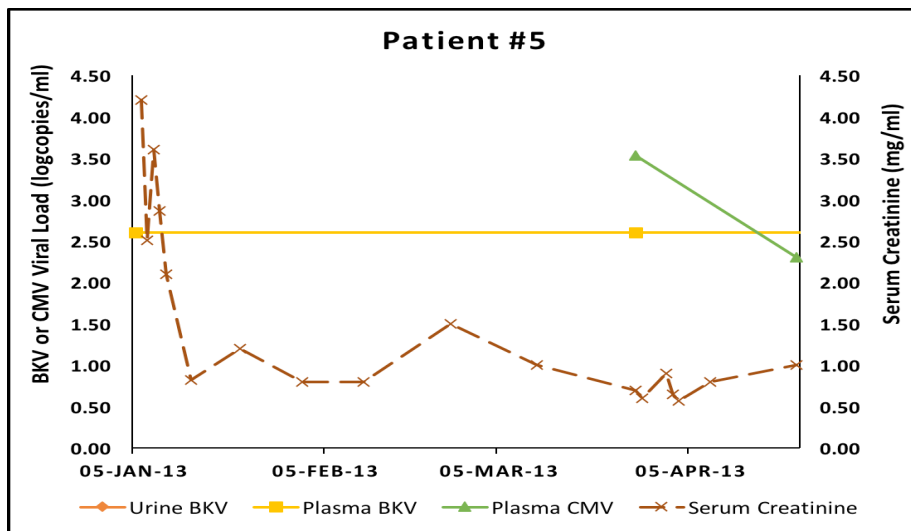
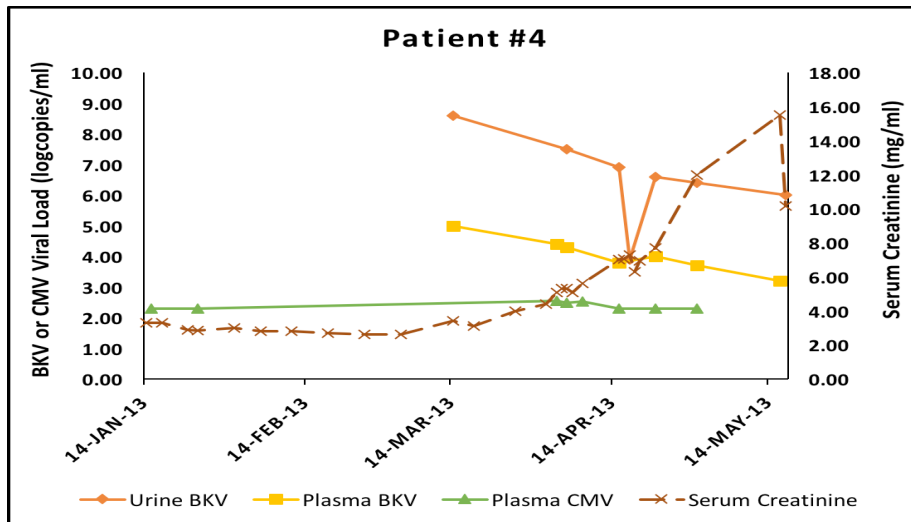
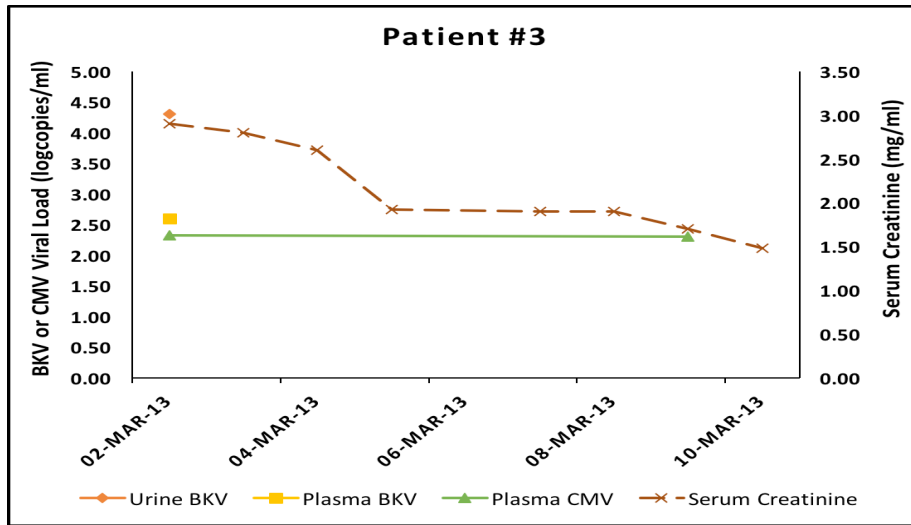
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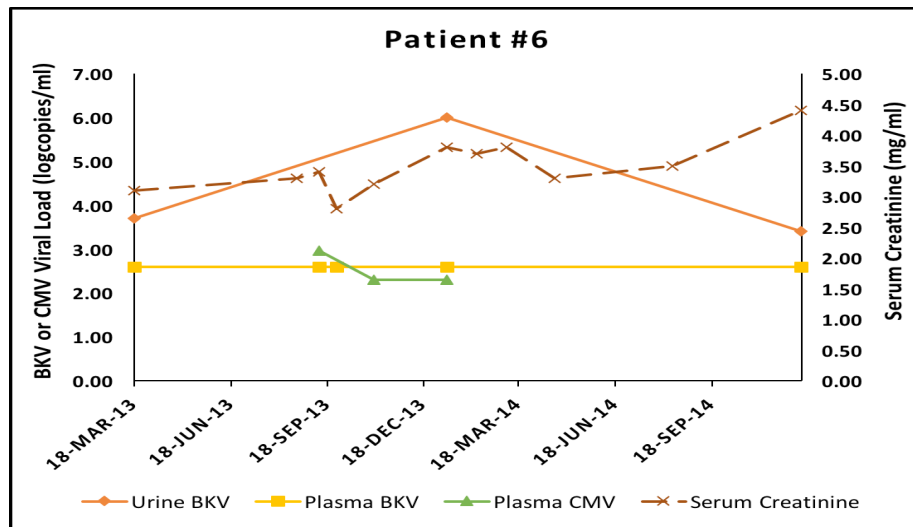


**Fig1.** Relative risk of co-infection with BKV and CMV ( $p < 0.05$ ).



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**Fig2.** Urine BKV, plasma BKV, and CMV viral loads in 6 post-transplant renal allograft patients who demonstrated co-infection of both viruses, along with serum creatinine values (normal range: 0.60-1.25 mg/dl). Viral loads of <2.6 and <2.3 logcopies/ml for BKV and CMV, respectively, were converted to 2.6 and 2.3 logcopies/ml.

**Table1.** Renal-related biochemical characteristics\*

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6
BUN (mg/dL)	154	31	67	100	65	70
sCR (mg/dL)	5.51	3.4	4.3	15.5	4.4	4.28
GFR(non-AA/AA)	19.4/23.5	31.2/37.8	-	-	19./24.1	-

\*Highest values chosen in 6 post-transplant renal allograft patients who demonstrated active BKV-CMV co-infection. BUN (blood urea nitrogen; normal range: 7-23 mg/mdl), sCR (serum creatinine; normal range: 0.60-1.25 mg/dl), GFR (glomerular filtration rate; 90-120 units). Non-AA, non-African American; AA, African-American.

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