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Cytomegalovirus and Epstein-Barr Virus Infections Among Pediatric Kidney Transplant Recipients at a Center Using Universal Valganciclovir Prophylaxis

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Abstract

Background: Cytomegalovirus (CMV) is associated with adverse effects in renal transplant recipients. The objective of this study was to characterize the incidence and timing of CMV and Epstein-Barr virus (EBV) infections in relation to valganciclovir (valGCV) prophylaxis in a pediatric renal transplant cohort.

Methods: Retrospective cohort of pediatric renal transplant patients given universal valGCV prophylaxis and universal viral surveillance was evaluated. Demographics, prophylaxis, acute rejection and CMV, EBV infections were abstracted.

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Results: 92 pediatric renal allograft recipients, 2008–2013, were included. One or more viral infections developed in 77/92 (83.7%) of the patients. EBV was the most common, in 62/92 (67%) patients; irrespective of valGCV (82% of episodes occurring on valGCV). CMV DNAemia occurred in 30/92 (33%) patients; 14 episodes (47%) occurring on valGCV. Incidence of breakthrough CMV on prophylaxis was 15%, and was associated with persistent DNAemia (OR 7.8, CI:1.6–32.9, $p<0.02$). CMV tissue-invasive disease was not seen. CMV syndrome occurred in 10% of the cohort, only in CMV D+ patients, and only one symptomatic breakthrough infection required treatment. 21/92 (23%) had simultaneous co-infections with 2–3 viruses.

Conclusions: Viral infections in pediatric renal transplant recipients receiving universal valGCV prophylaxis were common. EBV infections were not reduced by valGCV prophylaxis, and nearly half of CMV infections occurred on valGCV. Symptomatic CMV infection while on prophylaxis was rare. Valganciclovir prophylaxis may prevent symptomatic CMV infection but not EBV infection, and frequent CMV surveillance in pediatric renal transplant recipients on prophylaxis may not be necessary.

Keywords

Pediatrics; antiviral prophylaxis; cytomegalovirus; renal transplantation

Introduction

Cytomegalovirus (CMV) infection is common in renal transplant recipients and is associated with direct effects, such as CMV disease, and indirect effects, such as increased risk of co-infections and acute rejection^{1–3}. CMV seronegative recipients who receive a graft from CMV seropositive donors (D+/R-) have the highest risk for CMV infection and disease, although any CMV positive serostatus confers increased risk compared to D-/R- serostatus. For this reason, CMV antiviral prophylaxis is often recommended for 3 to 6 months after kidney transplantation for patients with any CMV positive serostatus to prevent CMV infection and disease^{4–7}. Studies supporting these recommendations for antiviral prophylaxis were conducted in adult kidney transplant patients, with limited data for pediatric patients.

Similar to adults, pediatric renal transplant patients with CMV D+/R- serostatus are at increased risk for CMV disease⁸. Other risk factors associated with pediatric CMV and other viral infections include younger age, EBV-negative serostatus, biopsy-proven acute rejection, and MMF dose >500 mg/m²^{8,9}. Several single center studies have investigated the impact of valganciclovir (valGCV) prophylaxis on outcomes among pediatric renal transplant recipients. In one single-center cohort, 35 pediatric kidney transplant patients received valGCV prophylaxis for 5–12 months, of whom 14 patients developed CMV infection after cessation of prophylaxis¹⁰. CMV surveillance was not performed during the period of prophylaxis. In another single center retrospective analysis, 101 pediatric renal transplant patients received a mean of 24 weeks of valGCV prophylaxis, dosed at 15 mg/kg/day (max dose 900 mg/day)¹¹. CMV infection occurred in 27% of the patients, 30% of which occurred during valGCV prophylaxis for an incidence rate of 9% on appropriate prophylaxis. Additionally, 4.5% of those patients had CMV disease, and 24% developed leukopenia attributed to valGCV¹¹. Thus, valGCV prophylaxis did not fully prevent the occurrence of CMV infection or disease, and had substantial hematologic morbidity. In

another study, 73 pediatric kidney transplant patients receiving valganciclovir (valGCV) at 10–12 mg/kg/day for 6 or 12 months were found to have similar rates of CMV infection, CMV disease, anemia, leukopenia, and acute rejection, suggesting that extended 12-month duration valGCV is safe but not more effective than a 6-month duration.¹² That study also reported an incidence of CMV viremia while on prophylaxis of 2.7%. Many pediatric centers utilize a hybrid, or surveillance after prophylaxis approach to CMV disease prevention, providing antiviral prophylaxis followed by a period of monitoring for pre-emptive therapy, but data to support this approach are lacking^{6,13}. Monitoring for CMV infection while on prophylaxis is not standardized and most publications report the practice at their center, which varies considerably between reports.^{1,11,12} While many publications report routine surveillance for CMV infection while on prophylaxis, the most recent guidelines on CMV in SOT recommend against routine monitoring for CMV DNAemia in adults on standard dose CMV prophylactic therapy⁷. Recommendations for pediatric SOT recipients are similar to those for adults, with an additional note that some experts perform surveillance during prophylaxis due to concern for breakthrough DNAemia.⁷ The impact of this type of breakthrough DNAemia remains unclear.

Among adult renal transplant patients, CMV infection is associated with viral co-infections, acute rejection, and late graft dysfunction^{3,14}. The impact of valGCV prophylaxis upon these outcomes has not been well studied in children. Among pediatric renal transplant patients, CMV D+/R- serostatus was a risk factor for opportunistic infections⁹. In a small cohort of 28 pediatric high risk EBV D+/R- kidney transplant patients, valGCV prophylaxis was associated with reduced incidence of EBV primary viremia in the first year post-transplant¹⁵. Among pediatric and adult renal transplant patients, a higher rate of blood CMV and BKV co-infection (50% among 12 patients) was reported compared to CMV infection without BKV DNAemia (5.3% among 57 patients). Based on this finding, the authors suggested that detection of one virus by surveillance should trigger testing for the other virus¹⁶. In another study, BKV nephropathy (BKVN) was associated with CMV positive serostatus (71.4% of patients with BKVN compared to 41.1% without BKVN) among 260 adult renal transplant patients¹⁷. However, antiviral drugs against CMV have no activity against BKV, and it is unknown whether disrupting CMV replication has any impact upon BKVN. Biopsy-proven acute rejection was not increased with CMV infection within 12–18 months post-transplant in two pediatric studies^{12,18}. Among a cohort of 242 pediatric renal transplant patients receiving either prophylactic or pre-emptive antiviral treatment, prophylaxis given to high or moderate CMV risk patients was associated with better preservation of transplant function at 3 years (as measured by estimated glomerular filtration rate) compared to patients receiving pre-emptive treatment¹⁹.

In this study, we retrospectively analyzed a cohort of pediatric renal transplant patients at a single transplant center who received universal valGCV prophylaxis regardless of CMV serostatus. The patients were monitored by surveillance for CMV, EBV, and BKV viremia at scheduled clinic visits per institutional clinical protocol, permitting analysis of viral infection incidence and rates of co-infections in the entire cohort. The objective of this study was to characterize the incidence and timing of CMV and EBV infections in relation to valGCV prophylaxis in a pediatric renal transplant cohort. Secondary objectives were to

determine incidence of CMV disease, rates of viral co-infections (CMV, EBV, BKV), and association of acute rejection with episodes of viral infection.

Methods

Definitions

CMV infection was defined as any detectable CMV DNAemia by PCR testing at any time-point. CMV disease was defined as tissue invasive CMV disease with biopsy confirmation. CMV syndrome was defined as CMV DNAemia with concurrent fever (temp $>38.0^{\circ}\text{C}$), malaise, white blood cell count $<4,500/\text{mcL}$ or platelet count $<135,000/\text{mcL}$.²⁰ Patients with leukopenia and/or thrombocytopenia preceding documented CMV DNAemia were not diagnosed with CMV syndrome. Neutropenia was defined as an ANC $<1000/\text{mcL}$. Transient viremia was defined as a single positive PCR result. Persistent viremia was defined as 2 or more consecutively positive PCR results (any detectable CMV DNAemia) and viral clearance was defined as 2 or more consecutively negative PCR results. An Immunosuppressive Score (IS) modified according to Vasudev et al. was used for assessment of the immunosuppressive load by adjusting a previously reported adult score for body surface area (see Supporting Information Table 1).^{15,21}

Study Population

Records from 92 pediatric kidney allograft recipients transplanted at the University of Alabama at Birmingham and Children's of Alabama between 2008–2013 were reviewed. Patients were monitored outpatient by the pediatric transplant nephrology group according to the following schedule: daily for the first week post-transplant, twice weekly through weeks 2–4, weekly through months 1–3, every two weeks through months 4–6, monthly through months 7–24, and every 1–3 months beyond 24 months. Surveillance allograft biopsies were obtained at 3, 6, and 12 months post-transplant per institutional clinical protocol, and additionally for clinical suspicion of rejection. The following clinical data were collected from the medical records: date of transplant, age, race, sex, CMV serostatus of donor and recipient, EBV donor and recipient serostatus (if available), reason for transplant, induction immunosuppression, antiviral prophylaxis, antiviral treatment, immunosuppressive therapy, weight, height, allograft biopsy results, and clinical symptoms. The study was approved by the Institutional Review Board of the University of Alabama at Birmingham.

Viral Monitoring

Surveillance CMV, EBV, and BKV blood viral loads were obtained per institutional clinical protocol at weekly intervals for the first month post-transplant, then every 2 weeks through months 2–6, and thereafter during every scheduled clinic visit. Children were monitored for any clinical symptoms and additional viral testing was performed at the discretion of the treating clinician. Patient records and pathology reports were reviewed for clinical symptoms and histologic findings to identify CMV syndrome and/or end organ disease.

PCR Methods

All viral PCR testing was performed in the clinical laboratory at The Children's Hospital of Alabama as ordered by the transplant physicians as part of institutional clinical care protocols. Quantitative CMV PCR was performed in the clinical laboratory using primers and probes for the HCMV UL55, encoding glycoprotein B, throughout the study time period as previously described²². From 2009 onward, a second CMV primer/probe (p/p) set encompassing a highly conserved region of the HCMV immediate-early 2 open reading frame was added to the clinical testing to account for a newly described UL55 mutation not recognized by the gB p/p set²³. The addition of the second p/p set did not change the assay limit of detection, and the combination p/p sets were used during the majority of this study. Quantitative PCR for EBV and BKV were performed in the clinical laboratory using an in-house real-time TaqMan assay (see Supporting Information Table 2 for primers and probe sequences) and quantitated against plasmid standards. The limit of detection for all PCR assays was 100 genomic equivalents/ml blood, with results below the limit of detection being reported as negative.

Antiviral Prophylaxis and Treatment

ValGCV prophylaxis was administered to all patients regardless of CMV serostatus per institutional clinical protocol. Standard prophylaxis duration was 12 weeks; total duration was modified for each patient according to the clinician's judgement. ValGCV dosing was adjusted according to body surface area and estimated glomerular filtration rate calculated according to the modified Schwartz formula.^{24,25} For each documented CMV infection, syndrome or disease, follow-up clinic data were assessed to determine if CMV immunoglobulin, ganciclovir, valGCV, acyclovir, and/or valacyclovir were given.

Statistical analyses

Simple bivariate analyses were conducted using the Chi-square test and one-way analysis of variance to compare demographic and transplant related clinical information of patients in the different serostatus groups, those with CMV infection on antiviral prophylaxis and those not on prophylaxis. Continuous variables were analyzed with one-way analysis of variance and Student's t-test, and dichotomous variables with chi square and Fisher exact tests. Analyses were performed using GraphPad Prism version 7.04 for Windows (GraphPad Software, La Jolla California, USA). Two-tailed P-values <0.05 were considered statistically significant.

Results

Study Population Characteristics

The study population consisted of 92 pediatric renal transplant recipients (Table 1) with a total of 1,631 clinic visits recorded. Patients were followed for a mean of 120 ± 75 weeks ($\pm$ SEM) post-transplant with a mean number of 18 ± 7 follow up clinic visits with viral testing performed. The study population was divided into four CMV serostatus donor/recipient groups (D+/R+, D+/R-, D-/R+, D-/R-). The average age at transplant was 11 ± 5.6 years. No differences in transplant age were observed among the four CMV D/R serostatus groups.

Among the patients, 44.6% of the patients were African American, 48.9% were Caucasian, and 6.5% were of other ethnicities. There were 47.8% female and 52.2% male transplant recipients. Living donor transplants comprised 35.9% of the transplants, with 64.1% of deceased donor origin. The reason for transplant was genetic or structural anomalies for most patients (40.2%), followed by immunologic diseases (15.2%), and other reasons (22.8%) (Table 1). Most patients (84.8%) received induction immunosuppression with either daclizumab or basiliximab, followed by a combination of tacrolimus, mycophenolate mofetil, and corticosteroids.

CMV Infection, Serostatus and Timing

Among the 92 renal transplant recipients, 30 (33%) had one or more documented episodes of CMV DNAemia by PCR. CMV infection by serostatus is shown in Table 2. Patients that were CMV D-/R- were less likely to have one or more episodes of DNAemia (OR 0.05, CI: 0.01–0.31, $p<0.0001$). Patients that were CMV D+ were more likely to have a CMV infection (OR 10.24, CI: 2.89–34, $p<0.0001$) than those patients that were CMV D-. No difference was found in the rate of CMV infection between the CMV R+ and R-groups (12 vs. 18 patients, $p=ns$).

The mean intervals between transplant and first documented CMV infection among the four serostatus groups are shown in Table 3. Patients with CMV positive serostatus (D+ and/or R+) had a mean time to first positive CMV PCR of 160 days, whereas the one D-/R- patient with CMV infection had viremia at 299 days post-transplant, presumably a primary infection. Persistent CMV DNAemia was seen in 12/30 (40%) and 11 of those twelve patients were D+/R-. Excluding the D-/R- patient with CMV infection on day 299, and the patients with only transient CMV DNAemia; CMV R+ patients had a shorter duration of CMV DNAemia until clearance compared to CMV R- patients (14 days versus 147 days).

CMV Infection and Antiviral Prophylaxis

All patients received valGCV prophylaxis regardless of CMV D/R serostatus, according to institutional protocol, with target minimum prophylaxis duration of 12 weeks. Actual valGCV duration ranged between 5–74 weeks (mean 20.6 ± 13). Seventeen patients received less than 12 weeks of prophylaxis. In addition, 13 of the 17 patients underwent transplantation between 2008–2010; prior to the publication of the international consensus guidelines on CMV management in SOT²⁶. There were no differences seen in the duration of valGCV prophylaxis in those patients with CMV DNAemia ($n=30$) and those patients without DNAemia ($n=62$) (Figure 1A). Additionally, there was no significant difference in duration of valGCV prophylaxis in those patients with or without CMV DNAemia, when stratified by CMV donor/recipient serostatus (Figure 1B).

Neutropenia occurred in 39 of the 92 (42.4%) patients included in this cohort. Twenty-four of the 39 (61.5%) patients with neutropenia were receiving valGCV prophylaxis. In the overall cohort, the incidence of neutropenia while on valGCV was 26% (24/92). Ten patients remained on valGCV per protocol with no changes, and the neutropenia resolved once they completed routine prophylaxis. Eight patients stopped valGCV early, in temporal association with neutropenia ($ANC < 1000$). Four patients resolved their neutropenia without stopping

valGCV prophylaxis. Two patients with neutropenia while receiving valGCV had no subsequent follow up. The 15 remaining neutropenic patients were not receiving valGCV or other antiviral drugs at the time.

Of the 30 patients with CMV infection, 14 (46.7%) episodes occurred while receiving valGCV prophylaxis; overall incidence of 15% CMV breakthrough on prophylaxis. All episodes of breakthrough CMV DNAemia occurred in D+ recipients. Sixteen patients developed CMV infection following completion of prophylaxis. CMV infection following prophylaxis occurred an average of 17 ± 4.7 weeks after stopping valGCV (median 10 weeks, IQR:5–18). Patients with breakthrough CMV infection had a significantly earlier onset of DNAemia than those with infection following prophylaxis (mean $\pm$ SEM, 9.4 ± 1.5 versus 34.4 ± 4.8 weeks post-transplant, $p < 0.0001$) (Figure 1C). By definition, patients with breakthrough CMV DNAemia were on valGCV prophylaxis at the time of DNAemia, with median duration of preceding prophylaxis of 9 weeks, IQR 6.5–11.0. Patients with breakthrough CMV were also more likely to have persistent rather than transient viremia (OR 7.8, CI:1.6–32.9, $p = 0.02$). 64% (9/14) of patients with breakthrough CMV infection had persistent DNAemia while only 19% (3/16) of CMV DNAemia after completion of prophylaxis was persistent. While persistent viremia itself was more common for those with breakthrough CMV, when comparing median duration of persistent DNAemia in those patients with breakthrough ($n = 9$) versus non-breakthrough ($n = 3$) DNAemia, there was no significant difference (90 days, IQR 45–161 versus 190 days, IQR 80–444, $p = 0.157$). Overall, 81% (13/16) of the viremia that occurred following prophylaxis was transient. Degree of DNAemia, or greatest median CMV viral load (VL) per patient, was also not significantly different between those with DNAemia during prophylaxis versus after prophylaxis (3,400 IU/ml, IQR 686–55335 versus 2,638 IU/ml, IQR 1462–16469, $p = 0.403$, respectively). Antiviral resistance testing was not ordered by clinicians for any of these patients.

Median immunosuppressive load (as determined by the modified Vasudev IS score) at time of initial CMV DNAemia was greater, but not significantly different, in those patients with breakthrough CMV compared to those with CMV occurring after completion of prophylaxis (12.4, IQR 8.2–16.8 versus 5.7, IQR 5–9.3, $p = 0.104$).

Nine out of 30 (30%) patients met criteria for CMV syndrome and the remaining were asymptomatic infections; 4 episodes of CMV syndrome occurred on valGCV prophylaxis. Three out of these 4 episodes resolved with no intervention, other than scheduled reduction in immunosuppression. One patient was changed from valGCV prophylaxis to treatment valGCV and subsequently had resolution of CMV viremia. Of the 5 remaining patients with CMV syndrome following completion of valGCV prophylaxis, 4 were placed on treatment valGCV. Median duration of treatment was 9.5 weeks (IQR 3.5–36.5). In the overall cohort, 9/92 (9.8%) had CMV syndrome at one point. There were no documented episodes of CMV tissue-invasive disease. Of the 9 patients with CMV syndrome, 4 were diagnosed based on fever and the remaining 5 had leukopenia alone. There was a non-significant trend of CMV syndrome occurring more often in CMV infected D+/R- patients compared to D+/R+ (OR 2.8, CI:0.5–15.7, $p = 0.4$). All episodes of CMV syndrome occurred in D+ recipients; there were no episodes of CMV syndrome in D-recipients.

Of the remaining patients with asymptomatic CMV DNAemia, 10 were on valGCV prophylaxis and 11 had completed prophylaxis. Of those 21 patients with asymptomatic DNAemia 10 (48%) were actively treated with antiviral therapy. Five patients already on valGCV prophylaxis were subsequently changed to treatment dose valGCV; 4 patients had completed prophylaxis and were started on treatment valGCV. Median duration of valGCV treatment was not significantly different between those on valGCV prophylaxis versus those that had completed valGCV prophylaxis (16 weeks, IQR 8–43 versus 9.5 weeks, IQR 4.5–13.8 weeks, $p=0.315$). A single patient, on valGCV prophylaxis, received 2 doses of CMV hyperimmune globulin as treatment for asymptomatic CMV DNAemia.

EBV & BK Infections

Pre-transplant EBV serostatus was not documented in the electronic medical record for 36 (39%) patients, so analysis of EBV DNAemia by EBV serostatus was not feasible. Sixty-two of the 92 (67%) patients had documented EBV DNAemia at any time point. Of those, 51/62 (82%) episodes occurred while on valGCV prophylaxis; 45/62 (73%) episodes were persistent and the remaining 17 (27%) were transient. Of the patients with persistent EBV DNAemia, 86.6% (39/45) developed DNAemia while on valGCV prophylaxis. In those patients with persistent DNAemia ($n=45$), comparison between those with DNAemia during valGCV prophylaxis versus after prophylaxis showed no significant differences in the median initial EBV viral load (16,800 GE/ml, IQR 4760–45000 versus 15,300 GE/ml, IQR 11063–91500, $p=0.660$). The median peak EBV viral loads trended toward higher values for episodes during valGCV prophylaxis compared to episodes after prophylaxis, but this was not statistically significant (70,500 GE/ml, IQR 18268–192000 versus 162,900 GE/ml, IQR 55,435–307,500, $p=0.6361$). Similarly, there was no difference in the duration of valganciclovir prophylaxis between those patients with or without EBV DNAemia (Figure 2).

Pre-transplant BKV serostatus was not ordered clinically for any patients in this cohort. There were 27 patients with BK DNAemia (29%) at any time during the collection period, and 19/27 (70%) episodes occurred while on valGCV prophylaxis. The median peak VL was not significantly different between those with a peak VL while on valGCV versus those not on valGCV (3,507 GE/ml, IQR 825–25050 versus 6,300 GE/ml, IQR 2100–9520, $p=0.4953$, respectively). There were no signs or symptoms reported of symptomatic BK infection. Three patients had evidence of BK infection seen on biopsy, concurrent with blood BK DNAemia; none were associated with rejection.

Viral co-infections

In the overall study population, 77 patients (83.7%) had one or more documented viral infection with CMV, EBV and/or BK from blood PCR testing, leaving only 15 (16.3%) patients with no viral infection documented. The majority of patients had one viral infection (56/92, 60.9%) at any given time, while 18 patients (19.6%) were co-infected with 2 viruses and 3 patients (3.3%) had simultaneous CMV, EBV and BK co-infections.

There were 20 episodes of viral co-infections among 18 patients. Two of the 18 patients had two separate co-infection episodes with 2 different viruses. Fourteen out of the 20 (70%)

total co-infections were with EBV & BK and 5/20 (25%) were with EBV & CMV. CMV with BK co-infection occurred in 1 patient.

Acute Rejection and PTLT

Twenty-eight patients (30.4%) had histologic evidence of acute rejection on biopsy at any time point. Twenty-five of those (89%) had acute cellular rejection, 1 with acute humoral rejection and the remaining 2 patients had mixed cellular/humoral rejection. Fifteen out of 28 (53.6%) patients had a documented DNAemia with EBV, CMV and/or BK either at the time of diagnosis (n=3) or immediately preceding diagnosis (n=12). Four patients had CMV DNAemia at the time of, or immediately following rejection. One patient had a transient, one time only episode of CMV DNAemia (VL 1500 IU/ml) at the time of rejection and one patient had low-level CMV DNAemia for 2 weeks prior to rejection (VL 255 IU/ml) followed by 12 weeks of CMV DNAemia (max VL 576 IU/ml) after the rejection diagnosis. The two remaining patients had either new onset CMV DNAemia following rejection (duration of 80 weeks) or a clear increase in CMV DNAemia at the time of rejection (CMV VL 212,500 IU/ml). Patients with viral DNAemia were less likely to be simultaneously diagnosed with acute rejection than those without active DNAemia (OR 0.16, CI:0.06–0.41, $p<0.02$). Mean duration of DNAemia prior to rejection was 69 days. Five patients with rejection had a prior episode of resolved DNAemia at time of diagnosis; with mean interval between last DNAemia and acute rejection was 179 days. Eight patients had no EBV, CMV or BK DNAemia at any time prior to rejection. No relationship between DNAemia with any virus and acute rejection was found by χ^2 analysis.

PTLT was infrequent in this cohort. Three patients (3.3%) developed PTLT during the 6-year study period, one of whom was on valgCV prophylaxis at the time of diagnosis. Two patients had tonsillar PTLT, and one patient had polymorphic PTLT. Two had prolonged EBV DNAemia prior to PTLT diagnosis; 383 days and 825 days, respectively. The other patient had 33 days of EBV DNAemia, which had resolved approximately 180 days prior to PTLT diagnosis. Two patients were EBV D+/R- and the remaining was EBV donor unknown, recipient negative. No patient with PTLT had BKV DNAemia.

Discussion

Viral infections were common in this cohort treated with universal CMV prophylaxis for an average of 20 weeks post-transplant. This cohort is unusual in that all transplant patients underwent frequent initial routine surveillance for viral infections that transitioned later to less frequent, but continued surveillance over time. All patients were also given valgCV prophylaxis regardless of CMV donor and recipient serostatus.

Overall, 84% of the patients included had a viral infection at one or more time points, and 23% had simultaneous co-infections with 2 or 3 viruses. The vast majority of these infections were asymptomatic, with only 10% of the cohort developing CMV syndrome and no episodes of tissue-invasive disease. In the entire cohort, only one episode of breakthrough CMV syndrome while on prophylaxis required treatment with directed therapy. Asymptomatic CMV DNAemia occurring while on CMV prophylaxis was treated 60% of the time (6 out of 10 cases), primarily by increasing valgCV from prophylaxis to treatment

for a median duration of 16 weeks. In total, 50% of the episodes of CMV DNAemia, symptomatic or asymptomatic, that occurred while on prophylaxis were treated with directed therapy. All other episodes of CMV DNAemia on prophylaxis were asymptomatic and/or required no specific therapy to resolve.

Consistent with previous reports, CMV infection was more likely to occur in recipients of a CMV donor positive graft, and CMV syndrome was only seen in CMV donor positive patients as well.^{8,9} We found an incidence rate of breakthrough CMV infection while on appropriate prophylaxis of 15%. Breakthrough CMV was also associated with prolonged viremia, compared to CMV infection after completion of prophylaxis. While >80% of the episodes of CMV DNAemia following completion of prophylaxis were transient (at one time point only), this is somewhat difficult to interpret since 40% of those patients were treated with valganciclovir. Our rate of breakthrough CMV is greater than the 3–9% incidence recently reported in the literature, with no definitive explanation.^{11,12} Routine viral monitoring occurred weekly for the first 4 weeks following transplant, then twice a month through 6 months, which is a greater frequency of surveillance than that reported in recent publications and could account for the higher observed rate of breakthrough infections in this study.^{11,12}

Our overall incidence rate of biopsy proven acute rejection was 30%, during the 6 years of the study, which is consistent with that reported by other institutions.^{11,12} One of the goals of immunosuppression is to balance risk of rejection and risk of infection; with our observed incidence of biopsy-proven rejection consistent with that of other centers, it appears less likely that the explanation for our rate of breakthrough CMV can be attributed to a relatively over-immunosuppressed population. If that were the case, the expectation would be for a reduced incidence of rejection. Overall, our incidence of symptomatic CMV infection, with CMV syndrome or disease, was low and in line with other reports. Although CMV DNAemia did not correlate with acute rejection in this cohort, an association might not be detected by this sample size and could be examined in future for larger populations.

Asymptomatic, or subclinical, CMV and EBV viremia has also been associated with increased risk of rejection, but that was not observed in this cohort.¹ This may be due to the fact that >80% of our patients had at least one viral infection, and the majority of those episodes were asymptomatic, likely due to the intense viral surveillance protocol at our institution compared to reports at other institutions. With the overall high frequency of asymptomatic viremia, there was no associated risk of rejection and active EBV, CMV or BKV DNAemia. This lack of clear association with rejection, taken together with our findings of frequent, asymptomatic CMV DNAemia while on valganciclovir prophylaxis lend additional weight to the consideration that CMV surveillance of patients on appropriate prophylaxis may not be necessary. This is in agreement with the recent consensus guidelines for CMV in adult SOT recipients and provides additional evidence that a similar approach may be reasonable for pediatric renal transplant recipients⁷.

As previously stated, one uncommon aspect of this cohort is that patients at low-risk (CMV D-/R-) for CMV infection were given prophylaxis. Rates of CMV infection in D-/R- pediatric renal transplant recipients are low and not frequently published.²⁷ In our cohort,

results were similar: there was only one D-/R- patient that developed CMV, which occurred off prophylaxis, 299 days after transplantation. From that perspective, it may be reasonable to exclude CMV D-/R- patients from routine surveillance for CMV DNAemia. In general, the, optimal surveillance strategy for primary CMV infection among D-/R- pediatric patients is not well defined and deserves further study.

In addition to cost and pill-burden, medication associated side effects are reasonable considerations when assessing the use of antimicrobial prophylaxis. Neutropenia is a well-known side effect of valganciclovir, and was seen in 26% of our patients on valganciclovir. Valganciclovir was discontinued in 20% of those patients with neutropenia (9% of the entire cohort), with subsequent resolution. Therefore, neutropenia requiring dose modification of valganciclovir was seen in 9% of the cohort, which is consistent with other reports of valganciclovir side effects.¹²

While symptomatic and asymptomatic CMV infections remain clinically important, EBV was the most commonly observed viral infection, seen in 67% of all patients. There was no apparent impact of valganciclovir prophylaxis on EBV infection; 82% of all episodes of EBV DNAemia occurred in patients on valganciclovir. BK DNAemia was the least frequently observed at 29% of all patients, and similar to EBV, occurred irrespective of valganciclovir prophylaxis. This is not surprising, given the lack of antiviral activity of valganciclovir against BKV. Overall, valganciclovir prophylaxis showed little utility in decreasing the incidence of EBV or BKV infection following pediatric renal transplant. PTLD was infrequent in the cohort with only 3 patients diagnosed during the collection period.

The relationship between CMV and BKV co-reactivation is unclear, with some studies indicating high rates of co-reactivation, but other studies showing no relationship between reactivations^{16,17,28}. In-vitro, CMV infection enhances BKV growth rate in co-infected cells, suggesting a possible mechanism for co-reactivations²⁹. However, in this cohort, CMV and BKV co-infection occurred in only one patient and did not suggest that CMV-BKV co-reactivations were a frequent event in this population.

A number of potential limitations were present in this study. It is a retrospective cohort study that lacked a comparator group not given valganciclovir prophylaxis following transplant, making it difficult to directly compare the utility of providing CMV prophylaxis between high, intermediate and low risk patients. Also, as this study only analyzed data obtained in the outpatient setting, hospitalizations for CMV treatment were not well captured. However, it is likely that inpatient antiviral treatments would occur after, and not before, detection of viral DNAemia in the outpatient setting, and therefore inpatient treatments would be unlikely to confound the analysis performed for this study. In addition, although in this cohort there was no association between acute rejection and CMV infection, our study did not evaluate the effect of CMV infection upon long term renal function (such as the estimated glomerular filtration rate, eGFR) as an outcome measure. Hocker et al. found that patients treated with antiviral prophylaxis had better eGFR at 3 years post-transplant compared to those treated with pre-emptive antiviral drugs¹⁹. In this cohort, all of the patients received antiviral prophylaxis, so we had no comparator group for eGFR. A final limitation of this study is that the low occurrence of PTLD prevents any meaningful associations with viral DNAemia.

In summary, despite universal valgCV prophylaxis, CMV and EBV infections are frequently observed and CMV infections continue to be seen even in those patients on appropriate prophylaxis. While symptomatic and asymptomatic CMV infections occur on prophylaxis, and are associated with persistent viremia, there was no apparent increased risk of rejection in this population. The majority of CMV infections were asymptomatic and only a single episode of symptomatic breakthrough CMV required a switch from prophylaxis to antiviral treatment, therefore frequent CMV surveillance in pediatric renal transplant recipients on valgCV prophylaxis may not be necessary in all cases. In contrast to EBV and BK, the majority of CMV DNAemia occurred late, following the cessation of prophylaxis and provide further support of a sequential CMV prophylaxis strategy consisting of prophylaxis followed by viral surveillance. The value of CMV prophylaxis in low-risk (CMV D-/R-) patients was not demonstrated in this study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BKV	BK virus
BKVN	BK virus nephropathy
CMV	cytomegalovirus
D/R	donor/recipient
EBV	Epstein-Barr virus
eGFR	estimated glomerular filtration rate
IS	Immunosuppressive Score
MMF	mycophenolate mofetil
PCR	polymerase chain reaction
p/p	primer/probe
PTLD	post-transplant lymphoproliferative disease
SOT	solid organ transplant
valgCV	valganciclovir

VL viral load

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Figure 1. CMV Infection and Valganciclovir prophylaxis duration.

1A. Duration of valganciclovir prophylaxis in patients with CMV infection (open squares, n=30) and patients without infection (dark squares, n=62).

1B. Duration of valganciclovir prophylaxis in patients with (open bars) or without (gray bars) CMV infection, stratified by CMV D/R serostatus.

1C. Week of CMV DNAemia onset is shown for patients with CMV infection that developed on prophylaxis (open squares) or after prophylaxis (dark squares).

Figure 2.
Duration of valganciclovir prophylaxis between those patients with EBV DNAemia (circles, n=62) or without EBV DNAemia (squares, n=30).

Table 1.

Study Population Characteristics by CMV Serostatus

Patient Factors	ALL	CMV D+/R+	CMV D+/R-	CMV D-/R+	CMV D-/R-
N	92	19 (20.7%)	37 (40.2%)	9 (9.8%)	27 (29.3%)
Mean age at transplant, years (SEM)	11 (5.6)	13.7 (5.4)	11.3 (5.5)	12.6 (3.2)	10.9 (6.2)
RACE					
African American	41 (44.6%)	5	20	6	10
Caucasian	45 (48.9%)	10	16	3	16
Other	6 (6.5%)	4	1	0	1
SEX					
Female	44 (47.8%)	7	13	5	19
Male	48 (52.2%)	12	24	4	8
DONOR TYPE					
Living	33 (35.9%)	9	9	3	12
Deceased	59 (64.1%)	10	28	6	15
REASON FOR TRANSPLANT					
Genetic/Structural	37 (40.2%)				
Posterior Urethral Valves (PUV)	12 (13%)	2	5	0	5
Obstructive Uropathy	0	0	0	0	0
Hypoplasia/Dysplasia	13 (14.1%)	4	6	2	1
Congenital Nephrotic Syndrome	6 (6.5%)	2	2	1	1
Autosomal Recessive Polycystic Kidney Disease	3 (3.3%)	1	0	1	1
Alport Syndrome	3 (3.3%)	0	2	1	0
Immunologic	14 (15.2%)				
Focal Sclerosing Glomerulosclerosis	10 (10.9%)	4	5	0	1
Membranoproliferative Glomerulonephritis (MPGN)	2 (2.2%)	1	0	0	1
IgA Nephropathy	2 (2.2%)	0	2	0	0
Other[#]	21 (22.8%)	3	8	2	8
Unknown	7 (7.6%)	1	2	0	4
More than one etiology[*]	13 (14.1%)	1	5	2	5
INDUCTION IMMUNOSUPPRESSION					
BAS, TAC, MMF, CST	54 (58.7%)	9	21	7	17
DZB, TAC, MMF, CST	24 (26.1%)	7	7	2	8
TAC, MMF, CST	6 (6.5%)	1	5	0	0
Other[†]	8 (8.7%)	2	4	0	2

[#] Other reasons for transplant include: calcineurin toxicity, cystinosis, neurogenic bladder, hemolytic uremic syndrome, Jeune syndrome, Wilms' tumor, systemic lupus erythematosus, chronic glomerulonephritis, familial hypoplastic glomerulocystic kidney, Henoch-Schonlein purpura.

^{*} Multifactorial reasons for transplant included: PUV + Hypoplasia/Dysplasia, IgA Nephropathy + Prune Belly Syndrome, PUV + Obstructive Uropathy, IgA Nephropathy + Hypoplasia/Dysplasia, MPGN + IgA Nephropathy, Obstructive Uropathy + Hypoplasia/Dysplasia.

[†]Other induction immunosuppression regimens: BAS or DZB with a combination of AZA, TAC, MMF, CST and/or CSA; MMF and CST; CST only.

Abbreviations: CMV = Cytomegalovirus, D = Donor, R = Recipient, BAS = Basiliximab, DZB = Daclizumab, TAC = Tacrolimus, MMF = Mycophenolate Mofetil, CST = Corticosteroids, CSA = Cyclosporine A, AZA = Azathioprine, SEM = Standard Error of Mean

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Table 2.

Serostatus of Patients by CMV Infection

Serostatus	Patients with CMV infection (n = 30)	Patients without CMV infection (n = 62)
CMV D+/R+	10	9
CMV D+/R-	17	20
CMV D-/R+	2	7
CMV D-/R-	1	26

Abbreviations: CMV = Cytomegalovirus, D = Donor, R = Recipient

Table 3.

Characteristics of CMV infection by CMV Serostatus

	CMV D+/R+ (n = 10)	CMV D+/R- (n = 17)	CMV D-/R+ (n = 2)	CMV D-/R- (n = 1)
Days from Transplant to CMV DNAemia, Mean (SEM)	171 (54)	140 (34)	169 (120)	299
Persistent CMV DNAemia, N (%)	1 (10%)	11 (65%)	0	0
Days of CMV DNAemia, Mean (SEM)	14 (N/A)	147 (35)	N/A	N/A
CMV Syndrome, N (%)	2 (20%)	7 (41%)	0	0
CMV Disease	0	0	0	0

Abbreviations: CMV = Cytomegalovirus, SEM = Standard Error of Mean