Assessment of Cytomegalovirus Hybrid Preventative Strategy in Pediatric Heart Transplant Patients

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Background. Prevention strategies for cytomegalovirus (CMV) in pediatric transplant recipients are sparsely reported. A hybrid strategy that combines prophylaxis with preemptive therapy using serial CMV viral load monitoring is an emerging option. We report our clinical outcomes with a hybrid strategy in pediatric heart transplant recipients.

Methods. A retrospective chart review was performed for pediatric heart transplant recipients who received a hybrid strategy of 2–4 weeks intravenous ganciclovir followed by serial whole blood CMV monitoring from 2002 to 2010. Subject demographics, medications, drug levels, serial CMV viral loads, intravascular ultrasound and angiography reports, and histopathology were collected. Descriptive statistics and patient groups were compared using \( \chi^2 \), Fisher’s exact, and Wilcoxon rank-sum tests.

Results. Twelve females and 13 males, ranging from 4 months to 19 years of age, underwent 26 heart transplants. Mean follow-up was 39 months (range, 5–94 months). Fourteen (54%) subjects were CMV donor (D) + /recipient (R) −, 8 (31%) were D + /R + , and 4 (15%) were D − /R + . Six subjects (23%) died of complications unrelated to CMV. Median prophylaxis duration was 25 days (range, 7–70 days). Ten (38%) subjects developed CMV infection: 1 subject had 2 episodes of CMV syndrome, and 1 subject had 2 episodes CMV. Although 6 of 14 patients with coronary artery vasculopathy had prior CMV, no association was found \( (P = .81) \). Median time to first CMV DNAemia was 2.3 months (range, 9 days to 24.8 months). Median time to viral load clearance was 29 days (range, 4–233 days). In addition, 25 D − /R− patients were transplanted and received no prophylaxis; 2 (8%) patients developed CMV infection.

Conclusions. Pediatric heart transplant recipients who were at risk for CMV and treated with a novel preventative hybrid strategy developed CMV infection, syndrome, and disease at rates similar to those reported in literature for prophylactic strategies.

Key words. Heart Transplantation; Cytomegalovirus; Pediatrics

Primary cytomegalovirus (CMV) infection or reactivation continues to cause significant morbidity in solid organ transplant recipients. CMV has direct effects that include CMV syndrome and tissue invasive diseases; in addition, CMV has been associated with indirect effects that include acute or chronic allograft rejection, transplant vasculopathy, and increased risk for bacterial, fungal, and viral infections. Within the pediatric transplant population, 10%–20% of pediatric liver transplant recipients develop CMV disease [1] and 29%–32% of pediatric lung transplant recipients develop CMV viremia [2]. In pediatric heart transplant patients, clinical CMV infection, defined by the need for oral or intravenous therapy, occurred in up to 18% of CMV mismatches (CMV D + /R −) [3], which is concerning because CMV has been previously associated with coronary artery vasculopathy, graft dysfunction, and graft loss [4, 5].

Historically, CMV prevention efforts have focused on 2 distinct methods: prophylaxis or preemptive therapy. Prophylaxis involves the administration of antiviral medication to all patients or to a subset of at-risk patients, whereas preemptive therapy involves monitoring for
CMV replication and treatment of subclinical infection to prevent progression to clinical disease. In adult kidney transplantations, both strategies have been shown to be equally effective in preventing CMV disease [6]. Although preemptive therapy reduces drug exposure and cost, it requires patient compliance with serial surveillance, increases surveillance costs including coordinator time, and may increase the risk of early-onset CMV [7]. The prophylactic strategy may increase exposure to ganciclovir, increase the development of drug-resistant strains, and is associated with late-onset CMV disease, which occurs after discontinuation of prophylaxis more than 100 days after transplant [7, 8]. The optimal duration of prophylaxis is unknown, but current American Society of Transplantation and Transplantation Society guidelines recommend 3–6 months of prophylaxis for CMV D+/R – adult patients who receive kidney, pancreas, heart, and liver transplants [9, 10]. However, there are few published studies for the pediatric subpopulation, and, thus, even within the Pediatric Heart Transplant Study Group, a wide variety of prophylactic strategies has been used [3].

To address challenges related to prophylactic and preemptive strategies, a hybrid strategy that consists of a shortened course of prophylaxis followed by serial monitoring has been introduced at some centers. In pediatric liver transplant recipients, this strategy was found to be a reasonable alternative to either standard prophylaxis or preemptive therapy [11]. We report our experience with a hybrid CMV prevention strategy that was instituted in 2002 in pediatric heart transplant recipients at the Cleveland Clinic as well as our outcomes and risks associated with the development of CMV infection, syndrome, and disease, and the relationship of CMV to development of coronary artery vasculopathy (CAV).

METHODS

Subject Database
After Institutional Review Board approval, a retrospective chart review was performed on patients who had received orthotopic heart transplantation between 2002 and 2010. All patients who had at least 1 year of follow-up were included, except for patients who did not survive for at least 1 month after the transplant due to their limited risk for CMV infection or disease. Demographics, medications, calcineurin inhibitor levels, serial CMV viral loads, intravascular ultrasound (IVUS) and angiography, and histopathology were collected from medical charts, operative notes, virology, pathology reports, and angiography studies. Before transplantation, CMV serostatus was determined in both donor (D) and recipient (R). Recipient patients <18 months with positive serology but negative urine CMV culture were considered CMV negative, as outlined in the Transplantation Society International Consensus Guidelines on the management of CMV [10].

Induction Protocol
Induction regimen included antithymocyte globulin (1.5 mg/kg per dose for at least 3 doses) and prednisone (15 mg/kg per dose for 3 doses). After transplantation, immunosuppressive therapy included a steroid-sparing regimen with rapid withdrawal of methylprednisolone if possible. Routine immunosuppression included tacrolimus and mycophenolate mofetil.

Hybrid Protocol
CMV D+/R – patients received 5 mg/kg ganciclovir intravenously (iv) every 12 hours for 2 weeks after transplant followed by 5 mg/kg ganciclovir iv once daily for 2 additional weeks. R+ patients received 5 mg/kg ganciclovir iv every 12 hours for 2 weeks. Valganciclovir equivalents of intravenous ganciclovir were administered in place of ganciclovir when the patient was able to take oral medications after at least 1 week of intravenous therapy. Longer durations of prophylaxis were used when clinically indicated, including episodes of rejection in the early posttransplant period, requiring augmented immunosuppression; additional antiviral therapy generally lasted for 2–3 weeks after treatment of the event. Three patients had serial episodes of rejection, resulting in total prophylaxis durations of approximately 8 weeks. Doses were adjusted for renal dysfunction. CMV immunoglobulin was not routinely administered.

Monitoring Regimen
CMV DNAemia was monitored every other week for the first 3 months, and then with routine laboratories for the remainder of the first posttransplant year (averaging every 1–3 months), every 4 months for the second year, and then twice yearly. From 2002 to 2007, the Hybrid Capture CMV Assay® version 2.0 (Digene Corporation, Gaithersburg, MD) was used and modified by including 4 CMV calibrators per each run of the test daily to quantify CMV DNAemia. From 2008 onward, the Artus® CMV™ PCR (QIAGEN, Hilden, Germany) kit was used. The lower limit of quantitation for CMV is 313 copies/mL (313–3.13 × 10⁶ copies/mL). The primer target is the immediate early gene. Angiography and IVUS was performed every other year for patients >15 kg and ≥20 kg, respectively. In addition, IVUS was not performed if the vessel diameter was judged to be <3 mm on angiography. Patients with CAV would have angiography and IVUS surveillance increased to every year.
CMV Treatment
When CMV was detected above 1000 copies/mL whole blood, antiviral therapy was initiated at treatment doses with either ganciclovir or valganciclovir. Serial monitoring occurred once or twice a week until CMV clearance was established. Antiviral therapy at treatment doses was continued for at least 2 weeks after viral loads were undetectable, except in one instance when the patient had persistent low-level viral loads despite clinical stability.

Definitions
CMV infection, syndrome, and disease definitions were based on definitions proposed by the American Society of Transplantation Infection Disease Working Group on Infectious Diseases Monitoring and Ljungman et al [12, 13]. CMV infection is documented CMV DNAemia without any signs or symptoms of infection. CMV syndrome is CMV DNAemia with signs or symptoms such as fever, fatigue, thrombocytopenia, and leukopenia without other infectious etiologies. CMV disease is CMV syndrome with additional histopathologic evidence of CMV tissue invasion seen on biopsy samples.

Outcomes
Acute rejection and antibody-mediated rejection (AMR) were also assessed based on published International Society of Heart and Lung Transplantation criteria [14–16]. CAV was determined from IVUS and angiography. IVUS grading was determined as none, minimal mild, moderate, or severe using the Stanford classification [17]. Stenosis found on angiography was graded as none/very mild (<30% estimated obstruction), mild (30%–49% estimated obstruction), moderate (50%–69% estimated obstruction), or severe (>70% estimated obstruction), which have been used in other studies [18].

Statistical Analysis
Study data were collected and managed using REDCap electronic data capture tools [19]. Descriptive statistics were computed treating each transplant independently. Summary statistics for tacrolimus levels were computed from mean levels within each transplant episode, using only the levels measured prior to the outcome analyzed. The Kaplan-Meier method was used to estimate and plot outcomes rates. The associations between risk factors and outcomes were assessed using Cox proportional hazards models with follow-up censored at death or last visit; posttransplant comorbidities were treated as time-dependent covariates, and tacrolimus levels were modeled on the log scale as time-dependent repeated measures. A robust sandwich covariance matrix estimate was used to adjust for the dependence between multiple transplants on the same patient. All analyses were performed on a complete case basis, at a significance level of 0.05. SAS 9.2 software (SAS Institute, Cary, NC) was used for all analyses, and R 2.11.1 software (The R Foundation for Computing, Vienna, Austria) was used for plots.

RESULTS
Patient Demographics
Between 2002 and 2010, 51 patients underwent orthotopic heart transplantation (Table 1). Twelve females and 13 males underwent 26 heart transplants and were at increased risk for CMV infection due to CMV serostatus. Mean age at transplant was 11 years (range, 4 months to 19 years). Indications for transplant included cardiomyopathy (58%), congenital heart disease (19%), myocarditis (8%), and retransplant secondary to graft failure (15%). Mean length of follow-up was 39 months (range, 4–94 months). Fourteen (54%) subjects were CMV D+/R−, 8 (31%) were D+/R+, and 4 (15%) were D−/R+. In total, 6 subjects (23%) expired: 2 due to posttransplant noninfectious complications, 1 due to posttransplant lymphoproliferative disorder, 1 due to antibody-mediated rejection, 1 due to acute rejection, and 1 due to cardiac arrest at a mean time of 15 months posttransplant (range, 5–35 months posttransplant).

CMV Episodes Posttransplant
Ten (38%) of 26 transplant episodes had detectable CMV DNAemia without symptoms consistent with CMV infection; 1 of these had 3 such occurrences at 5, 9, and 11 months posttransplant. Median time of
Prophylaxis was 25 days (range, 7–70 days) overall; 26 days (range, 9–70 days) for CMV D+/R− and 22 days (7–56 days) for CMV R+. Six patients received prophylaxis for 5 weeks or more due to clinical indications in which increased immunosuppression was required. One patient had renal dysfunction and subsequent delayed initiation of prophylaxis, leading to the development of CMV infection on postoperative day 9.

Median time to first CMV DNAemia was 80 days (range, 9 days to 24.8 months) (Figure 1). CMV viral loads during occurrences of infection ranged from 534 to 84,212 copies/mL at diagnosis (median, 3006), with a peak viral load ranging from 534 to 84,212 (median, 4483) (Table 2). CMV syndrome or disease without prior CMV infection occurred in 2 recipients (each with 2 episodes): 1 patient (D+/R−) received 17 days of prophylaxis and developed CMV syndrome at 2 and 5 months posttransplant with viral loads ranging from 741 to 7859, and another patient (D+/R−) with 29 days of prophylaxis developed CMV disease (1 pneumonitis, 1 gastroenteritis) at 15 and 18 months posttransplant with viral loads ranging from 495 to 24,666. Viral loads for infection compared with syndrome or disease were not substantially different.

Median time to viral load clearance was 29 days (range, 4–233 days). Median and mean time of preemptive treatment was 34 days (range, 4–67 days). The patient with prolonged viral presence resolved clinical symptoms but maintained very low viral load during recovery and therapy was stopped. Twenty-five D−/R− patients were also transplanted and did not receive prophylaxis; 2 (8%) subjects developed CMV infection, but none developed CMV syndrome or disease.

Risks for CMV Infection or Disease
CMV D+ serostatus was associated with development of CMV (P < .001) (Table 3); of the 4 D+/R+ patients, none developed CMV (Table 3). Gender was not associated with development of CMV (P = .19).

All subjects had at least 1 episode of R1 acute rejection, but only 5 (19%) had clinically significant evidence of R2 rejection; 2 of the 5 later developed CMV. No patients had R3 rejection. Grade R2 rejection was not associated with the subsequent development of CMV (P = .36). Three patients (12%) had evidence of AMR, 1 of whom later developed CMV.

Tacrolimus levels were available for 20 transplant episodes (number of measurements per episode ranged from 21 to 134). Lower tacrolimus levels were associated with higher risk of CMV (mean level before CMV vs mean levels in those that did not develop CMV, 7.4 vs 9.6, recovery and therapy was stopped. Twenty-five D−/R− patients were also transplanted and did not receive prophylaxis; 2 (8%) subjects developed CMV infection, but none developed CMV syndrome or disease.

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Table 2. DNA CMV PCR Viral Load Ranges

<table>
<thead>
<tr>
<th></th>
<th>Infection</th>
<th>Syndrome</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial VL</td>
<td>534–84212</td>
<td>1233–1303</td>
<td>495–15293</td>
</tr>
<tr>
<td>Peak VL</td>
<td>534–84212</td>
<td>4835–7859</td>
<td>15293–24666</td>
</tr>
</tbody>
</table>

Abbreviations: CMV, cytomegalovirus; PCR, polymerase chain reaction; VL, viral load.

Table 3. Risk Factors for Development of Cytomegalovirus

<table>
<thead>
<tr>
<th></th>
<th>CMV n = 12</th>
<th>No CMV n = 14</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>.19</td>
</tr>
<tr>
<td>Female, n</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Male, n</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>R2+ rejection</td>
<td></td>
<td></td>
<td>.36</td>
</tr>
<tr>
<td>No, n</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Yes, n</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>AMR</td>
<td></td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>No, n</td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Yes, n</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Average tacrolimus levela (range)</td>
<td>7.4 (6.1, 9.8)</td>
<td>9.6 (5.9, 13.3)</td>
<td>.012</td>
</tr>
<tr>
<td>D/R serostatus</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D+/R−, n</td>
<td>9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>D+/R+, n</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>D−/R+, n</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>D+ vs D−, n</td>
<td>12</td>
<td>10</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>R+ vs R−, n</td>
<td>3</td>
<td>9</td>
<td>.11</td>
</tr>
<tr>
<td>CAVb n</td>
<td>6</td>
<td>8</td>
<td>.81</td>
</tr>
</tbody>
</table>

Abbreviations: AMR, antibody-mediated rejection; CAV, coronary artery vasculopathy; CMV, cytomegalovirus; D, donor; n, number; n/a, not applicable; PCR, polymerase chain reaction; R, recipient; VL, viral load.

aNot available for 6 transplant episodes: 3 that developed CMV and 3 that did not.

bNot available for 2 transplant episodes: 1 that developed CMV and 1 that did not.
CMV DNAemia Not Associated With Increased Risk of CAV

CAV was evaluated in 24 patients and present in 14 (58%). One patient had recurrence of CAV after re-transplantation. Mean time from transplant to CAV detection was 33.6 months (range, 3–88 months) (Figure 1). Diagnosis of CAV was made with IVUS and angiography in 1 patient, IVUS alone in 11 patients, and angiography alone in 2 patients. In the 12 patients with CAV detected by IVUS, 4 (33%) patients showed minimal CAV, 5 (42%) patients had mild CAV, and 3 patients (25%) had moderate disease. In the 3 patients with CAV detected by angiography, 2 patients had mild stenosis and 1 patient had severe stenosis.

Six of 14 patients with CAV had prior CMV, whereas 5 of 10 patients without CAV had prior CMV ($P = .70$). Of the 2 patients with CMV syndrome or disease, 1 later developed CAV and 1 did not; both are still living at the time of last follow-up.

**DISCUSSION**

Prevention of CMV infection in pediatric heart transplant patients decreases CMV-associated morbidity. However, in the current literature, there is a lack of data, discussion, and guidance about optimal treatment strategies. Two more commonly used prevention methods include the prophylactic and the preemptive strategies; however, our institution began a hybrid strategy to minimize unnecessary exposure to ganciclovir while still protecting the allograft through a period of shortened prophylaxis followed by serial CMV DNAemia monitoring in 2002.

Our study reports an incidence of CMV infection of 38% in CMV-seropositive patients, 24% including all transplant recipients, with only 8% of CMV-seropositive patients developing CMV syndrome or disease. The rates are similar to other studies that reported asymptomatic CMV infection in 18%–34.4% [3, 11, 20] and CMV syndrome or disease in 3.6%–9.8% [4, 11]. Although our rate of CMV infection was higher than that previously reported in the Pediatric Heart Transplant Study (PHTS) cohort (18%), this may reflect increased asymptomatic CMV infections with low viral loads that may not have been diagnosed or treated in prior cohorts where viral culture, antigenemia, and clinical symptoms were used for CMV diagnosis. Because the diagnostic methods for cohort within the PHTS study were not separated, there is still a need for an appropriate comparison group that uses similar diagnostic methods. The rate of CMV syndrome and disease in the hybrid cohort at 8% is significantly decreased compared with the PHTS cohort. These prior studies additionally used various CMV prophylaxis agents and strategies for 2–90 days, which is significantly longer than the antiviral exposure in our population [3, 11, 20].

One surprising finding was the association of lower mean tacrolimus levels with CMV. This counterintuitive finding may reflect a statistical anomaly and should be further evaluated in future studies with larger cohorts.

We did not find an association between previous CMV infection and CAV development. Of the 14 subjects who had CAV, 4 (29%) had minimal CAV that was not treated and did not result in increased monitoring. Nine of these patients (64%) had mild to moderate findings in angiography or IVUS that resulted in increased frequency of surveillance from every other year to every year. Atorvastatin was also initiated in patients with mild to moderate CAV findings. In the 1 patient with severe stenosis, sirolimus was added to the immunosuppression regimen. Although other studies have shown 5-year freedom from CAV to be 81%, our increased incidence is likely due to more sensitive detection methods using IVUS that were not used in other studies [3].

In our study, 14 (53.8%) transplant patients were spared further antiviral medications beyond the initial postoperative prophylaxis, compared with a total of 38.5% as seen in the hybrid prophylaxis study in liver transplant patients [11]. However, this benefit must be balanced with the cost of increased surveillance with each CMV polymerase chain reaction, which cost up to $150, and the associated coordinator time to manage serial testing. In addition, only 1 patient developed CMV DNAemia just after 2 years from transplant, and this was not associated with rejection. Because the risk of developing CMV DNAemia after 2 years seems to be lower, less intensive monitoring may be appropriate in asymptomatic patients beyond 2 years posttransplant, decreasing the costs further.

Concerns have also been raised regarding valganciclovir resistance and adverse effects stemming from reliance on the drug in the hybrid protocol regimen [22]. However, within our patient group, no drug resistance was documented and no alternative drugs were required. Although leukopenia may occur with valganciclovir given in combination with other immunosuppressive drugs used in the posttransplant regimen [22], none of our patients developed leukopenia. Furthermore, the 6 patients who died passed because of complications thought to be unrelated to CMV.
Limitations
Our study was limited by its retrospective nature, small sample size, lack of an ideal comparison group, and duration of follow-up. Although the sample size was relatively small, all patients were approached similarly with the surveillance protocol since 2002, which allowed us to assess the hybrid protocol in a fairly large cohort of pediatric heart transplant recipients. Furthermore, diagnosis of CAV using angiography or IVUS was only available for patients greater than 15 kg or 20 kg, respectively, and was never performed in 1 of our patients due to size. In addition, this technique may not be routinely available or performed at all centers. Finally, the ability to perform intense surveillance for either a hybrid or preemptive strategy requires substantial resource investment, which may preclude this strategy from being used routinely in all transplant settings.

Summary and Conclusions
The CMV hybrid preventative strategy was a safe and effective approach for use in at-risk pediatric heart transplant patients. This approach decreased unnecessary exposure to antiviral agents in more than half of the patients in our cohort. Further evaluation of hybrid strategies in heart and other solid organ transplant population is warranted given the currently available data.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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