High incidence of clinically significant cytomegalovirus infection in CMV D+/R+ lung transplant recipients receiving 3 months of antiviral prophylaxis

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Abstract
Background: Universal antiviral prophylaxis is the preferred preventive strategy for lung transplant recipients (LTRs) at risk of CMV infection. We compared the risk of CMV infection between CMV D+/R+ and D−/R+ LTRs after 3 months of prophylaxis.

Methods: This was a retrospective review of CMV R+ LTRs transplanted between 2005 and 2013. Patients dying before completing 3 months, or receiving >180 days of prophylaxis were excluded. The primary outcome was proportion of LTRs who developed CMV infection and clinically significant CMV infection defined as CMV infection leading to preemptive therapy or CMV disease.

Results: We analyzed 90 D+/R+ and 72 D−/R+ with a median follow up of 730 days. CMV infection and disease was more common in D+/R+ compared to D−/R+ (CMV infection 66% vs 40%; P = 0.001; CMV disease 13% vs 4% P = 0.045). Fifty-nine patients developed at least one episode of clinically significant CMV infection (41/90 [46%] D+/R+ and 18/72 [25%] D−/R+; P = 0.007) with recurrence occurring in 29 LTRs (49% of patients with previous CMV infection), of which 22 (76%) were CMV D+/R+.

Thirty percent had side effects related to CMV therapy.

Conclusion: Three months prophylaxis in D+/R+ LTRs was associated with high rates of clinically significant CMV infection and recurrences.

Keywords: antiviral prophylaxis, CMV infection, lung transplant

Abbreviations: Anti-IL-2, Interleukin-2 inhibitor; aHR, Adjusted hazard ratio; BOS 3, Bronchiolitis Obliterans Syndrome stage 3; CI, Confidence interval; CMV, Cytomegalovirus; CMV D+/R+, Cytomegalovirus IgG antibodies donor positive and recipient positive; CMV D−/R+, Cytomegalovirus IgG antibodies donor negative and recipient positive; CMV D+/R−, Cytomegalovirus IgG antibodies donor negative and recipient negative; CMV D−/R−, Cytomegalovirus IgG antibodies donor positive and recipient negative; COPD, Chronic Obstructive Pulmonary Disease; DNA, Deoxyribonucleic acid; G-CSF, Granulocyte-colony stimulating factor; HHV-6, Human Herpes Virus-6; HHV-7, Human Herpes Virus-7; HR, Hazard Ratio; ILD, Interstitial Lung Disease; IQR, Interquartile range; LTRs, Lung transplant recipients; PCR, Polymerase chain reaction.
Despite significant improvement in the diagnosis, prevention and treatment of CMV infection over the past two decades, it remains a major cause of morbidity and mortality post-lung transplantation.\(^1\)\(^-\)\(^4\) The CMV serostatus of the donor and the recipient remains the most important risk factor for the development of CMV infection and disease.\(^5\) CMV-seronegative lung transplant recipients (LTRs) from seropositive donors (D+/R-) carry the highest risk of development of CMV infection and disease due to their lack of CMV-specific immunity.\(^5\)\(^-\)\(^7\) CMV-seropositive recipients, (R+) are considered at moderate risk, whereas CMV-seronegative LTRs receiving seronegative donors (D-/R-) have negligible risk of CMV infection.\(^8\) Schoeppler et al demonstrated that among R+ LTRs, transplantation of a D+ organ was associated with a higher risk for CMV infection and disease than transplantation from a seronegative donor.\(^8\) Other risk factors include the use of antilymphocyte antibodies for induction or treatment of rejection, use of high-dose steroids, concomitant infection with HHV-6 and HHV-7, and shorter courses or the absence of antiviral prophylaxis.\(^9\)\(^-\)\(^11\)

Although, universal prophylaxis is the mainstay of CMV prevention strategies, there is still a significant burden of disease due to late CMV infection.\(^2\)\(^,\)\(^12\)\(^,\)\(^13\) Antiviral prophylaxis with valganciclovir is associated with toxicity, primarily neutropenia that may increase the risk of developing infection and lead to significant morbidity and mortality.\(^2\)\(^,\)\(^14\)\(^,\)\(^15\) While there is consensus on universal prophylaxis, its optimal duration remains controversial, especially among CMV-seropositive recipients. Palmer et al demonstrated in their randomized control trial that extending prophylaxis to 12 months compared to 3 months effectively reduced the occurrence of CMV infection and disease.\(^11\) However, it is unclear from this study if a shorter duration of prophylaxis might be appropriate for R+ LTRs. In our center, over the past 10 years, R+ LTRs have received universal prophylaxis for 3 months. This duration is shorter than that recommended in the International Consensus Guidelines and shorter than the prophylaxis duration used in the majority of lung transplant centers included in a recent survey, which is usually 6 months.\(^13\)\(^,\)\(^16\)

The aim of our retrospective study was to compare the incidence of CMV infection and disease between D+/R+ and D-/R+ LTRs receiving only 3 months of antiviral prophylaxis, and evaluate the adverse effects related to CMV prevention and treatment.

2 | METHODS

2.1 | Study design

We performed a retrospective review of all LTRs transplanted at the University of Alberta Hospital between September 2005 and December 2013. Eligible patients were adult CMV-seropositive LTRs. We excluded LTRs who died before completing 3 months of antiviral prophylaxis, or received more than 180 days of antiviral prophylaxis, as to evaluate the efficacy of 3 months of antiviral prophylaxis. The study follow-up was 24 months (730 days) post-lung transplantation.

This study was approved by the University of Alberta Health Research Ethics Board (HREB_Pro00064703).

2.2 | Definitions

CMV infection, and CMV disease were defined using standard definitions previously described.\(^17\) In brief, CMV infection was defined as the detection of CMV DNA in plasma samples. Clinically significant CMV was defined as CMV infection leading to preemptive therapy or CMV disease, using criteria described in clinical trials.\(^18\) CMV disease included CMV syndrome and end organ disease. CMV syndrome was defined as CMV infection in addition to 2 of the following: Fever ≥38°C, new or increased fatigue, neutrophil counts of <1500 cells/μL or a decrease of >20% (if the neutrophil count before development of CMV infection was <1500 cells/μL), a platelet count of <115,000 cells/μL or a decrease of >20% (if the platelet count prior to development of CMV infection was <115,000 cells/μL) or elevation of hepatic aminotransferases to two times the upper limit of normal. For tissue-invasive CMV disease, positive histopathology findings were required for diagnosis. Treatment success was defined as achieving a negative CMV PCR on therapy, and secondary CMV prophylaxis when antivirals were continued after treatment success for at least 2 weeks. Neutropenia was defined as an absolute neutrophil count (ANC) of ≤1500 cells/μL. Bronchiolitis Obliterans Syndrome Stage 3 (BOS 3) was defined as 50% or less of the baseline FEV1, which is the two greatest FEV1 values more than 3 weeks apart.\(^19\)

2.3 | Immunosuppressive medications

Lung transplant recipients received induction therapy with antithymocyte globulin (rATG) or IL-2 receptor inhibitor (basiliximab), followed by maintenance immunosuppression with mycophenolate mofetil (1 to 1.5 g twice daily) and tacrolimus or cyclosporine if intolerant to tacrolimus. Methylprednisolone was initiated at 0.5 mg/kg post-transplant and was then tapered to 5 mg of prednisone by 1 year. From 2006-2007, rATG was used for induction for all patients, mid 2007 the protocol was switched to IL-2 receptor antagonists and this became standard induction for unsensitized patients. rATG was still used for patients in which there was a positive virtual cross match or actual cross match at the time of transplantation, to reduce the risk of antibody mediated rejection. Standard therapy for rejection was pulse methylprednisolone 10 mg/kg/day for 3 days. Steroid-resistant rejection was treated with anti-thymocyte globulin.

2.4 | Other anti-infectious prophylaxis

The standard peri-operative antimicrobial regimen consisted of intravenous cefazolin that was substituted by vancomycin in cases of penicillin allergy or Methicillin Resistant Staphylococcus aureus colonization. Antibiotics were tailored thereafter, according to intraoperative donor and recipient bronchial swab culture results. Universal voriconazole for fungal prophylaxis was given for the first
3 months post-transplant. Lifelong trimethoprim-sulfamethoxazole (TMP-SMX) was administered to all patients for prevention of Pneumocystis jiroveci pneumonia (dapsone or inhaled pentamidine were used for patients with an allergy to sulfonamides).

2.5 | CMV monitoring and prevention

Two molecular assays were used for quantitative CMV DNA measurement in plasma during the study period. Molecular testing began at our center in October 2005, using an in-house quantitative real-time PCR assay, with results expressed in CMV genome copies per mL. In March 2012, the RealStar CMV PCR (Altoma Diagnostics), which measures the viral load in International Units per mL, was implemented, with an average conversion factor of 2-fold between the former and the RealStar assay. All samples below 250 IU/mL were considered negative for the purposes of this analysis.

CMV prophylaxis consisted of intravenous Ganciclovir for 2 weeks followed by oral Valganciclovir 900 mg daily, with dose adjustment for creatinine clearance for a total of 12 weeks. CMV DNA in plasma was monitored weekly for 8 weeks post-prophylaxis and then at discretion of treating physician. The decision to treat CMV infection after prophylaxis and the use of secondary prophylaxis relied on the attending physician and was not based on a pre-specified threshold in our center.

2.6 | Statistical analysis

All statistics were calculated with SPSS statistical package (Chicago, IL) version 23. Categorical variables are summarized as percentages. Continuous variables are summarized as median and interquartile range. Cox-regression analysis was performed to identify independent variables associated with CMV infection. Variables with \( P < 0.2 \) in the univariate analysis were used in the multivariate analysis and these included age, thymoglobulin use, and CMV donor serostatus. Kaplan-Meier survival analysis was used to represent the risk of CMV infection according to CMV serostatus. A two-sided \( P < 0.05 \) was considered to be statistically significant.

3 | RESULTS

During the study period, 174 R + LTRs were transplanted, of which 12 were excluded (11 died before completing 3 months of prophylaxis and one had prolonged prophylaxis due to recurrent episodes of rejection) for a final study cohort of 162 LTRs (90 D+/R+ and 72 D-/R+) (Figure 1). The baseline characteristics are summarized in Table 1. Patients were followed for a median

**TABLE 1** Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>D-/R+ (n = 72)</th>
<th>D+/R+ (n = 90)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at transplant (SD)</td>
<td>52.2 (13.5)</td>
<td>54.6 (11.3)</td>
<td>0.207</td>
</tr>
<tr>
<td>Male sex</td>
<td>53 (74%)</td>
<td>58 (64%)</td>
<td>0.212</td>
</tr>
<tr>
<td>Reason for transplant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>9 (13%)</td>
<td>9 (10%)</td>
<td>0.543</td>
</tr>
<tr>
<td>COPD</td>
<td>32 (44%)</td>
<td>33 (37%)</td>
<td></td>
</tr>
<tr>
<td>ILD</td>
<td>27 (37%)</td>
<td>39 (43%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>4 (6%)</td>
<td>9 (10%)</td>
<td></td>
</tr>
<tr>
<td>Pre-transplant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia during prophylaxis</td>
<td>14 (19.4%)</td>
<td>18 (20%)</td>
<td>0.930</td>
</tr>
<tr>
<td>CMV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any CMV DNAemia</td>
<td>29 (40%)</td>
<td>59 (66%)</td>
<td>0.001</td>
</tr>
<tr>
<td>DNAemia resulting in treatment</td>
<td>18 (25%)</td>
<td>41 (46%)</td>
<td>0.007</td>
</tr>
<tr>
<td>CMV disease</td>
<td>3 (4%)</td>
<td>12 (13%)</td>
<td>0.045</td>
</tr>
<tr>
<td>Median days to peak CMV DNAemia (IQR)</td>
<td>155 (129-212)</td>
<td>148 (129-176)</td>
<td>0.436</td>
</tr>
<tr>
<td>BOS 3 at 5 y</td>
<td>31 (44%)</td>
<td>29 (33%)</td>
<td>0.166</td>
</tr>
</tbody>
</table>

Abbreviations: BOS 3, Bronchiolitis Obliterans Syndrome stage 3; CMV, Cytomegalovirus; COPD, Chronic Obstructive Pulmonary Disease; ILD, Interstitial Lung Disease; IQR, Interquartile range.
of 730 days. The median duration of CMV antiviral prophylaxis was similar for D+/R- and D-/R+ (88.0 [80.0-98.0] vs 87.5 [79.0-100.5] days, P = 0.783). Neutropenia during prophylaxis occurred in 20% of LTRs. 24 D+/R- and 23 D-/R+ patients had to stop prophylaxis prior to 88 days due to toxicity and two developed CMV DNAemia prior to 88 days. CMV infection and disease was more common in D+/R- compared to D-/R+ (CMV infection 66% vs 40%; P = 0.001; clinically significant CMV 46% vs 25%; P = 0.007; CMV disease 13% vs 4% P = 0.045). Invasive gastrointestinal disease was documented in four LTRs; three had clinical suspicion for gastrointestinal disease in addition to CMV syndrome and eight had CMV syndrome. Secondary prophylaxis was used in 12 LTRs with clinically significant CMV (9 D+/R- and 3 D-/R+). Acute rejection was documented in 28 (31%) D+/R- and 22 (31%) D-/R+; P = 0.939 with a median of 94 days IQR (49-258) vs 75 days IQR (36-253), respectively. Figure 2 shows the probability of clinically significant CMV infection according to donor-recipient CMV serostatus.

When analyzing the impact of CMV serostatus on the risk of clinically significant CMV infection, CMV D-/R+ had a protective role, after adjusting for confounders (aHR = 0.472, 95% CI [0.270-0.827]; P = 0.009) (Table 2). Adjusting for age and CMV serostatus, the use of antilymphocyte globulin agents was not associated with increased risk (aHR = 1.46, 95% CI [0.901-2.365]; P = 0.124).

Fifty-nine patients developed at least one episode of clinically significant CMV infection (41/90 [46%] D+/R- and 18/72 [25%] D-/R+ P=0.007). The majority of these infections occurred between 90 and 180 days post-transplantation (D+/R- 30/41 [73.2%] and D-/R - 8/18 [44.4%] P = 0.034). Of these 59 patients, 29 and 10 LTRs had one and two recurrences of clinically significant CMV infection, respectively (Table 3). Clinically significant CMV infection was not associated with BOS (HR = 1.24 CI [0.73-2.12]; P = 0.42). 17 D+/R- and 10 D-/R+ had immunosuppression decreased or modified after the initial episode. We observed no significant difference in recurrence of clinically significant CMV with modification of immunosuppression (16/27 [59%] vs 13/32 [41%]; P = 0.154).

Side effects related to therapy were seen in 30% of the patients. Nineteen percent of LTRs with CMV infection required granulocyte-colony stimulating factor (G-CSF) for neutropenia and three had complications from neutropenia (fever, sepsis). The dose of Valganciclovir was adjusted for creatinine clearance but not reduced for neutropenia. Ganciclovir-resistant CMV was suspected (no genotypic testing for resistance was performed) in only one patient and the dose of antiviral was increased for management with good outcome.

![FIGURE 2](image)

**FIGURE 2** Kaplan-Meier analysis of the probability of CMV clinically significant infection according to CMV donor-recipient serostatus. Vertical dash lines represent the median duration of antiviral prophylaxis in the R+ LTRs cohort. DNAemia observed prior to 88 d corresponds to early cessation of prophylaxis and not breakthrough infections.

| TABLE 2 Cox-regression analysis of the risk factors for clinically significant CMV infection |
|-----------------|--------|--------|--------|--------|--------|--------|--------|
|                 | HR     | 95% CI       | P      | aHR   | 95% CI       | P      |
| Age             | 1.033  | 1.077-1.060   | 0.011  | 1.031 | 1.004-1.058   | 0.022  |
| Pre-transplant immunosuppression | 1.374  | 0.813-2.321   | 0.235  | —     | —             | —      |
| Thymoglobulin for induction       | 1.373  | 0.860-2.191   | 0.184  | 1.460 | 0.901-2.365   | 0.124  |
| CMV D-/R+         | 0.472  | 0.271-0.222   | 0.008  | 0.472 | 0.270-0.827   | 0.009  |

Abbreviations: aHR, Adjusted hazard ratio; CI, Confidence interval; CMV, Cytomegalovirus; HR, Hazard ratio.
TABLE 3 Main characteristics of the episodes of clinically significant CMV infection after prophylaxis in R + LTRs

<table>
<thead>
<tr>
<th></th>
<th>Episode 1</th>
<th>Episode 2</th>
<th>Episode 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>59</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>Donor/recipient CMV serology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV D+/R+</td>
<td>41 (70%)</td>
<td>22 (76%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>CMV D-/R+</td>
<td>18 (30%)</td>
<td>7 (24%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Median days of treatment</td>
<td>26 (15-40)</td>
<td>17 (8.5-35.5)</td>
<td>20 (11-31)</td>
</tr>
<tr>
<td>Incidence of adverse events</td>
<td>19 (32%)</td>
<td>9 (31%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Treatment with G-CSF neutropenia</td>
<td>12 (20%)</td>
<td>6 (21%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>CMV resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspected</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Confirmed</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Episodes refer to the first episode of CMV infection and first and second recurrence (episodes 2 and 3 respectively). Adverse events: include neutropenia, febrile neutropenia, sepsis, and thrombocytopenia. Abbreviations: CMV D+/R+. Cytomegalovirus Ig G antibodies donor positive and recipient positive; CMV D-/R+. Cytomegalovirus Ig G antibodies donor negative and recipient positive; G-CSF, Granulocyte-colony stimulating factor; LTRs, Lung transplant recipients.

4 | DISCUSSION

The optimal length of CMV prophylaxis in R + LTRs is not well defined. Most of the studies focused on the length of CMV prophylaxis in lung transplantation include LTRs with seropositive and seronegative donor and recipients. In a recent prospective observational multicenter study on 92 R + LTRs, CMV disease incidence was higher in patients with 90 days prophylaxis than in those with 180 day prophylaxis, and the duration of prophylaxis was an independent risk factor for CMV disease. Although they studied a more homogenous cohort by excluding high risk LTRs (D+/R-), they did not distinguish between seropositive and seronegative donors. We analyzed 162 R + LTRs (90 D+/R + and 72 D-/R+) and found a significantly higher incidence of CMV infection, clinically significant CMV infection and disease in D+/R + compared to D-/R + after 3 months of prophylaxis. Although our CMV infection rates are higher compared to centers where the duration of prophylaxis for R + LTRs is 6 months, CMV disease rates are similar.

Our results reinforce the findings of Schoeppler et al., who found that among R + LTRs, D-serostatus was associated with less CMV infection and absence of CMV disease after 6 months of prophylaxis. In D+/R+, infecting CMV strains originate from the organ donor, or are endogenous latent virus strains that have reactivated whereas in D-/R + CMV infection is only related to reactivation from latent virus. This has also been shown in renal transplant recipients, where CMV seropositive kidney transplant recipients who receive a CMV--seropositive organ in which the antibodies against CMV strain-specific neutralizing epitopes of glycoprotein H are mismatched, suggesting re-infection with a different CMV strain, had more risk of CMV infection, disease and acute cellular rejection.

Clinically significant CMV infection was common and recurrent in our cohort. Recent studies have demonstrated that recurrent CMV was common after treatment of CMV infection/disease (19%-30.5%) in solid organ transplant recipients, and that lung transplantation and CMV D+/R- were independent predictors of recurrence. In our 59 LTRs that had clinically significant CMV infection, 29 and 10 LTRs had one and two recurrences resulting in a need for further antiviral therapy.

THERAPY OF CLINICALLY SIGNIFICANT CMV INFECTION

Therapy of clinically significant CMV infection was prolonged and associated with side effects, mainly neutropenia. The degree of neutropenia during therapy was clinically relevant; 19% of those LTRs required G-CSF. Although neutropenia is a common side effect during antiviral prophylaxis, and can lead to early cessation of antiviral use, higher doses of antivirals required during treatment makes it more profound. Neutropenia also increases the risk of other infections, acute cellular rejection, and is associated with increased mortality. It can also lead to dose decreases or holding certain drugs such as antimetabolite drugs, and TMP-SMX.

A 3-month antiviral prophylaxis for D+/R + was associated with high rates of CMV infection and relevant side effects arising from the treatment of CMV infection post-prophylaxis in our study. Although our study did not assess whether longer duration of prophylaxis decrease the rates of CMV infection or just delay the occurrence of infection post-prophylaxis; our rates of infection are higher compared to centers where R + LTRs receive 6 months of prophylaxis. Thus, although our data does not define the optimal duration of prophylaxis, it demonstrates that 3 months of prophylaxis is suboptimal in D+/R+, as 46% of these patients are requiring therapy for CMV infection post-prophylaxis.

Our study has limitations. This is a retrospective, single center study. We included the largest R + LTRs cohort to date in a long period span, which comprised of different era of induction therapy use. Although 60% of our cohort received thymoglobulin for induction, a rate much higher than common practice in 2018, its use was not associated with a significant increase in clinically significant CMV infection. Finally, the treatment of CMV infection was not based on a pre-specified threshold. This might explain the high morbidity related to therapy since if initiated too late, longer courses of anti-viral drugs may be required to clear CMV infection, exacerbating the combined marrow effects of CMV itself and the antiviral drug.

Donor serology had a strong influence on the occurrence of CMV infection and disease among our cohort of R + LTRs, with 3 months antiviral prophylaxis in D+/R + LTRs being associated with high rates of recurrent clinically significant CMV infection. Future studies should clarify if a 3-month prophylaxis strategy is appropriate for the management of D-/R + LTRs.

DISCLOSURES

The authors have no conflicts of interest to disclose.
CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Dr Kabbani, Dr Cervera, Dr Hirji, and Dr Hernandez participated in Research Design. Harjot Malhi, and Sanjaya Chandrarathne participated in data collection. Dr Kabbani, Dr Cervera, Curtis Mabilangan participated in data analysis. Dr Kabbani, Dr Cervera, Dr Hirji, Dr Preiksaitis, and Dr Halloran participated in manuscript preparation. All authors participated in critically revising the manuscript.

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