TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Evaluation of clinical outcomes of prophylactic versus preemptive cytomegalovirus strategy in liver transplant recipients

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Keywords
Cytomegalovirus, liver transplantation, preemptive strategy, prophylactic strategy.

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Conflicts of interest
None.

Received: 10 October 2012
Revision requested: 30 October 2012
Accepted: 18 March 2013
Published online: 16 April 2013

doi:10.1111/tri.12101

Summary

Cytomegalovirus (CMV) is a major cause of morbidity and mortality following solid organ transplantation (SOT). Two strategies, prophylactic, and preemptive have emerged for the prevention of CMV infection and disease after SOT. This retrospective chart review of two liver transplant cohorts: prophylactic and preemptive, compares the clinical impact of transitioning from prophylactic to preemptive strategy. The primary outcome is the incidence of CMV viremia at 3-and 6-months post-transplant. Secondary outcomes include: incidence of CMV tissue-invasive disease, acute cellular rejection, leukopenia and neutropenia, opportunistic infection rates, hospital readmission rates, and mortality at 3-and 6-months post-transplant. A total of 109 patients were included in the analysis. The incidence of CMV viremia was 4.9% and 50.0% ($P < 0.001$) in the prophylactic versus preemptive cohort, respectively, at 3 months post-transplant. The incidence of CMV viremia was 24.6% and 8.3% ($P = 0.026$) in the prophylactic versus preemptive cohort, respectively, at 6 months post-transplant. There were no statistical significant differences in the secondary outcomes between both cohorts. In conclusion, there is a statistical significant difference in time to onset of CMV viremia; however, the use of either prophylactic or preemptive strategy was not associated with significant negative clinical outcomes of CMV.

Introduction

Cytomegalovirus (CMV) is a major cause of morbidity and mortality among solid organ transplant (SOT) recipients [1,2]. CMV mediates direct and indirect effects on both the allograft and transplant recipient. Direct effects of CMV include CMV syndrome (fever, myelosuppression, weakness, myalgia, and arthralgia) and tissue-invasive disease including hepatitis, nephritis, colitis, retinitis, and pneumonitis [3]. Indirect effects mediated by CMV include increased incidence of opportunistic infection, allograft rejection, and reduction in allograft and patient survival [4].

Two strategies – prophylactic and preemptive – have emerged for the prevention of CMV disease [5]. Efficacy superiority has not been demonstrated for either strategy [6,7]. Each strategy has benefits and risks. The prophylactic strategy has been associated with decreased incidence of CMV disease, reduced bacterial and fungal infection, and death, but it is associated with higher incidence of

neutropenia and late-onset CMV disease [6–8]. The preemptive strategy, on the other hand, has been shown to decrease the incidence of CMV disease and late-onset CMV disease, but did not produce significant reduction in bacterial and fungal infection and death as seen with the prophylactic strategy [6–8].

Valganciclovir is the most commonly used drug for CMV disease prevention in all the SOT recipients, including liver transplant (LT) recipients [5,9]. The widespread use of valganciclovir in LT recipients comes as a surprise, because valganciclovir was denied approval by the Food and Drug Administration in LT recipients following results from the Valganciclovir SOT Study Group study which noted a higher rate of tissue-invasive CMV infection in LT recipients who received valganciclovir prophylaxis compared with ganciclovir prophylaxis [10, 11]. A subsequent study found that the risk of CMV tissue-invasive disease in LT recipients receiving valganciclovir prophylaxis was 4.5 times the risk seen with ganciclovir prophylaxis ($P = 0.04$) [5]. This finding confirmed that the increased risk of developing CMV tissue-invasive disease in LT recipients who received valganciclovir prophylaxis is not merely a chance-finding.

On January 14 2011, the LT team transitioned from a prophylactic strategy to a preemptive strategy. The CMV disease prevention strategy was switched based on the following: a retrospective chart review showed a high incidence of leukopenia (73%) in our LT recipients who received valganciclovir prophylaxis (unpublished data); and results from the study by Kalil *et al.* showed that valganciclovir prophylaxis was significantly associated with the increased risk of CMV tissue-invasive disease in LT recipients and an increased risk of absolute neutropenia in all SOT recipients including LT recipients [5].

Research evidence supporting the efficacy of prophylactic and preemptive strategies for CMV disease prevention arises primarily from meta-analyses with few direct head-to-head comparative studies in distinct SOT populations [6,7,12]. The majority of head-to-head trials exist in renal transplantation, with limited data for other SOT populations – heart, liver, and lung transplantation [4,8,13]. To date, there is no direct head-to-head (prospective or retrospective) study comparing prophylactic strategy to preemptive strategy in an all-inclusive cohort of CMV donor (D) and recipient (R) serologic status (D+/R−, D+/R+, D−/R+, D−/R−) LT recipients.

This study aims to compare the clinical impact of transitioning from prophylactic to preemptive strategy in LT recipients. The primary outcome of this study is the incidence of CMV viremia between both strategies at 3- and 6- months post-transplant. Secondary outcomes include: incidence of CMV tissue-invasive disease, acute cellular rejection (ACR), leukopenia, neutropenia, opportunistic infection rates, hospital readmission rates, and mortality rates at 3- and 6- months post-transplant.

Patients and methods

Study design

This is a single-center retrospective chart review of two LT cohorts: prophylactic and preemptive. The prophylactic cohort includes LT recipients from January 14, 2010 to August 31, 2010 and the preemptive cohort includes LT recipients from January 14, 2011 to August 31, 2011. Patients were included if they were 18 years of age or older at the time of transplant. Patients were excluded if they received combined liver/kidney transplants. This study was approved by the Institutional Review Board.

Data collection

The study outcomes and patients' baseline information such as age, gender, race, reason for transplantation, CMV donor (D) and recipient (R) serologic status, induction immunosuppression, and maintenance immunosuppression at discharge from the transplant admission were obtained from individual patient's electronic medical record. The absolute neutrophil count (ANC) was calculated from the white blood cell count (WBC), and granulocytes or bands and segmented cells.

Outcomes

The primary outcome was the incidence of CMV viremia. CMV viremia was defined as the evidence of CMV replication measured by the CMV DNA quantitative testing [Artus CMV TM Polymerase Chain Reaction (PCR) by Qiagen; Sensitivity: 100 copies/mL = 100 international units/mL] using plasma samples. Episodes of CMV viremia separated by two or more consecutive negative specimens were considered as a new episode. Late-onset CMV viremia was defined as first onset of CMV viremia occurring beyond 3 months post-transplant.

The secondary outcomes include incidence of CMV tissue-invasive disease, ACR, leukopenia, neutropenia, rates of opportunistic infection, hospital readmission rates, and mortality. CMV tissue-invasive disease was defined as an annotation of viral cytopathic effect or disease on a tissue biopsy specimen report. ACR was defined as documentation of ACR on a tissue biopsy specimen report. Leukopenia was defined as WBC <3000 cells/mm^3. Neutropenia was defined as ANC <500 cells/mm^3. Opportunistic infection was defined as positive microbiologic cultures that were treated with a minimum of 3 day antibiotics for which the organism was sensitive to. Opportunistic infections were categorized based on the organ system infected and type of microorganism.

CMV surveillance protocol

All LT recipients (prophylactic and preemptive cohorts) followed a post-transplant pathway protocol, which required outpatient transplant clinic attendance for weekly CMV PCR immediately post-transplant until 3 months, and then every other week during months 4 through 6; then at the 9 and 12 months post-transplant visits and when clinically indicated (LT patients with fever, leukopenia, diarrhea, malaise, altered mental status).

CMV prophylaxis protocol

Patients under the prophylactic cohort whose CMV donor and recipient serologic status were: D+/R−, D+/R+, D−/R+ received valganciclovir 900 mg daily orally for 3 months following LT for CMV prophylaxis. Patients unable to tolerate oral medications were protocolized to receive ganciclovir 5 mg/kg IV daily until able to tolerate oral medications. Valganciclovir and ganciclovir were adjusted renally for patients with creatinine clearance <70 mL/min. During the CMV surveillance protocol, all patients with detectable CMV in their CMV PCR were provided induction and maintenance therapy as described in the section below on CMV Preemptive Protocol.

CMV preemptive protocol

During the CMV surveillance protocol, valganciclovir 900 mg twice daily orally (induction therapy) was initiated on any LT recipient with detectable CMV in their CMV PCR (CMV DNA levels $\geq$ 100 international units/mL were detectable) for 21 days. Beginning day 22, patients were placed on valganciclovir 900 mg daily orally (maintenance therapy) until 2 CMV PCRs could not detect CMV. Ganciclovir 5 mg/kg IV twice daily or Ganciclovir 5 mg/kg IV daily were used as an induction therapy or maintenance therapy, respectively, for patients who were unable to tolerate oral medications. Valganciclovir and ganciclovir were adjusted renally for patients with creatinine clearance <70 mL/min. During induction and maintenance therapy, CMV PCR testing was performed weekly.

Statistical analyses

Statistical comparisons of patient demographics were performed using chi-square test or Fisher exact test for categorical variables and with Student's *t*-test for continuous variables. Primary and secondary outcome variables were analyzed with Fisher exact test or chi-square tests. Fisher Exact and chi-square test analyses were performed with the JavaStat – 2-way Contingency Table [14]. Student's *t*-test and Pearson product moment correlation coefficient, *r*, were analyzed with Microsoft Excel™ 2007. A significance level of 0.05 was set for all tests.

Results

Patient characteristics

A total of 119 patients met the inclusion criteria: 64 patients in the prophylactic cohort and 55 patients in the preemptive cohort. Ten patients with combined liver/kidney transplant were excluded from the study (3 patients in the prophylactic cohort and 7 patients in the preemptive cohort). A total of 109 patients were eligible for the analysis: 61 in the prophylactic cohort and 48 in the preemptive cohort (Fig. 1).

Patient demographics

There was no statistical significant difference in the demographic variables between cohorts as presented in Table 1. There were more CMV D+/R− patients in the prophylactic cohort compared with the preemptive cohort. Although not statistically significant, more patients in the preemptive cohort compared with the prophylactic cohort received basiliximab induction immunosuppression.

Primary outcome

At 0–3 months post-transplant period, CMV viremia occurred in 4.9% (n = 3) of patients in the prophylactic cohort and in 50% (n = 24) of patients in the preemptive cohort ($P < 0.001$). During the 3–6 months post-transplant period, CMV viremia occurred in 24.6% (n = 15) of patients in the prophylactic cohort and in 8.3% (n = 4) of patients in the preemptive cohort ($P = 0.026$). One of the fifteen patients (6.7%) in the prophylactic cohort and all four patients (100%) in the preemptive cohorts with CMV viremia at 3–6 months post-transplant had prior episodes of CMV viremia at 0–3 months. The percentage of patients with late-onset CMV viremia was 22.9% (n = 14) in the prophylactic cohort versus 0% (n = 0) in the preemptive cohort ($P < 0.001$). The average number of days to the first CMV viremia episode was

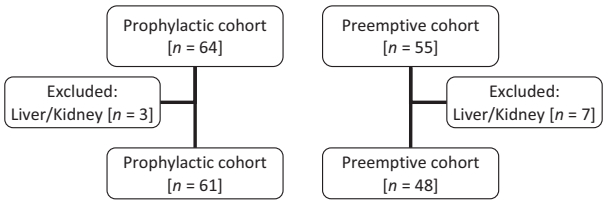

Figure 1 Flow chart of inclusion criteria to study cohorts: prophylactic and preemptive.

Table 1. Patient Demographics.

	Prophylactic (n = 61)	Preemptive (n = 48)	P-value
Age in years, mean (range)	54.2 (21–72)	53.6 (28–66)	0.742
Gender, *n* (%)			
Male	42 (68.9)	33 (68.7)	0.991
Female	19 (31.1)	15 (31.3)	
Race, *n* (%)			
White	47 (77.0)	37 (77.1)	0.997
Black	9 (14.8)	8 (16.6)	0.785
Asian	4 (6.6)	1 (2.1)	0.382
Hispanic	1 (1.6)	2 (4.2)	0.582
CMV serostatus, *n* (%)			
D+/R−	15 (24.6)	7 (14.6)	0.196
D+/R+	26 (42.6)	26 (54.2)	0.231
D−/R+	16 (26.2)	13 (27.1)	0.920
D−/R−	4 (6.6)	2 (4.2)	0.693
Induction immunosuppression, *n* (%)			
Basiliximab	28 (45.9)	30 (62.5)	0.085
Methylprednisolone	56 (91.8)	44 (91.7)	0.979
Hydrocortisone	0	1 (2.1)	0.440
Maintenance immunosuppression, *n* (%)			
Tacrolimus	59† (96.7)	44* (91.7)	0.251
Cyclosporine	0	3 (6.3)	0.082
Mycophenolate mofetil	59† (96.7)	46 (95.8)	0.807
Prednisone	44 (72)	32 (66.7)	0.538
Transplant indication, *n* (%)			
Hepatitis C	10 (16.4)	9 (18.8)	0.747
Hepatitis C and hepatocellular carcinoma (HCC)	10 (16.4)	8 (16.7)	0.970
Hepatitis C and Laennec's cirrhosis	0	3 (6.3%)	0.082
Nonalcoholic steatohepatitis (NASH)	10 (16.4)	3 (6.3)	0.105
Primary sclerosing cholangitis	8 (13.1)	1 (2.1)	0.075
Laennec's cirrhosis	4 (6.6)	6 (12.5)	0.331
Cryptogenic cirrhosis	4 (6.6)	7 (14.6)	0.208
HCC	3 (4.9)	0	0.254
Autoimmune hepatitis	3 (4.9)	2 (4.2)	1.000
Hemochromatosis	2 (3.3)	0	0.503
Primary biliary cirrhosis	1 (1.6)	0	1.000
Hepatitis B and HCC	1 (1.6)	0	1.000
Hepatitis B	0	2 (4.2)	0.192
Other	5 (8.2)	6 (12.5)	0.531

*One patient died prior to discharge.
†Two patients died prior to discharge.
CMV, cytomegalovirus; D, donor; R, recipient; HCC, hepatocellular carcinoma; NASH, nonalcoholic steatohepatitis.

129.7 days in the prophylactic cohort and 36.2 days in the preemptive cohort. The log average CMV viral load per week between cohorts is shown in Fig. 2. Primary outcomes descriptive data are presented in Table 2.

Secondary outcomes

All secondary outcomes data are presented in Tables 3–6. No biopsy-proven CMV tissue-invasive disease occurred in either cohort during the first 6 months post-transplant.

There was no significant difference between the groups for episodes of ACR at 0–3 or 3–6 months post-transplant. During the 0–3 months post-transplant period, ACR occurred in 4.9% (n = 3) in the prophylactic cohort and 6.3% (n = 3) in the preemptive cohort (P = 1.0). At 3–6 months post-transplant period, ACR occurred in 3.3% (n = 2) in the prophylactic cohort and 0% (n = 0) in the preemptive cohort (P = 0.503). The average number of days to the first ACR episode was 70.4 days in the prophylactic cohort and 30 days in the preemptive cohort. The days to development of first ACR did not correlate with days to development of first CMV viremia (Pearson r = 0.423, P = 0.296).

During the 0–3 months post-transplant period, the outcome of leukopenia was similar between the cohorts [57.4% (n = 35) prophylactic cohort versus 58.3% (n = 28) preemptive cohort, P = 0.920]. However, at the 3–6 months post-transplant period, there was a trend toward higher incidence of leukopenia in the prophylactic cohort compared to the preemptive cohort [49.2% (n = 30) prophylactic cohort versus 35.4% (n = 17) preemptive cohort, P = 0.130].

The incidence of neutropenia was low and comparable between both cohorts at 3 and 6 months post-transplant period. Neutropenia occurred in 4.9% (n = 3) of patients in the prophylactic cohort and 8.3% (n = 4) of patients in the preemptive cohort (P = 0.697) at 0–3 months post-transplant. During the 3–6 months post-transplant period, neutropenia occurred in 6.6% (n = 4) of patients in the prophylactic cohort versus 10.4% (n = 5) in the preemptive cohort (P = 0.503).

Both the incidence of opportunistic infections and patients with opportunistic infections were similar between cohorts. The incidence of opportunistic infections at 0–3 months post-transplant was 25 in the prophylactic cohort and 26 in the preemptive cohort (P = 0.354). At 3–6 months post-transplant, the incidence of opportunistic infections was 10 in the prophylactic cohort and 8 in the preemptive cohort (P = 0.973). The percentage of patients with opportunistic infections at 0–3 months post-transplant was 22.9% (n = 14) in the prophylactic cohort and 22.9% (n = 11) in the preemptive cohort (P = 0.920). At 3–6 months post-transplant, the percentage of patients with opportunistic infections was 9.8% (n = 6) in the prophylactic cohort and 12.5% (n = 6) in the preemptive cohort (P = 0.659). The most common infections by organ system were bacteremia (29%), urinary tract infections (18.8%), intra-abdominal infections (17.4%), and respira-

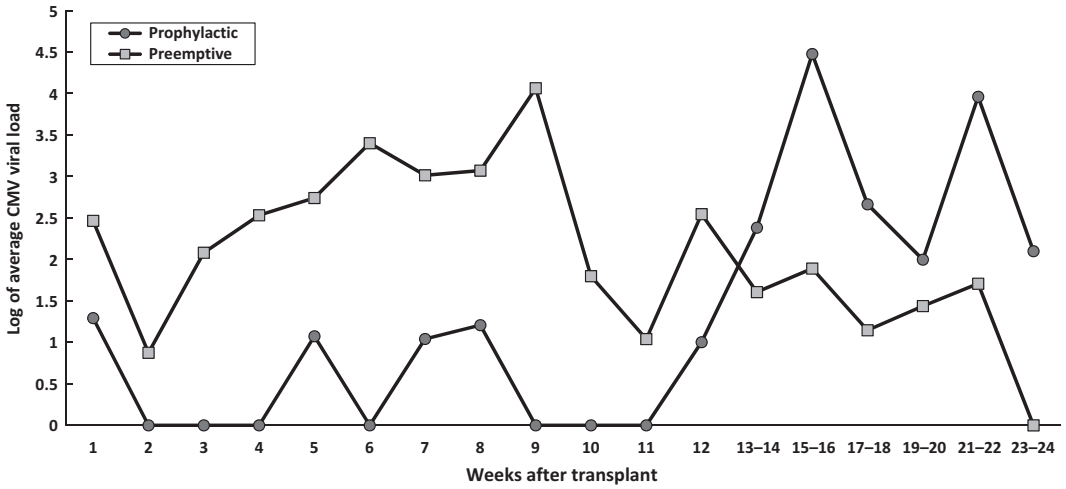

Figure 2 Average CMV viral load per week after transplant in the prophylactic and preemptive cohort. Levels are shown as base 10 logarithms. Circles represent the prophylactic cohort and squares represent the preemptive cohort.

Table 2. Primary outcomes.

Outcome	Prophylactic (n = 61)	Preemptive (n = 48)	P-value
CMV viremia (0–3 months), n (%)	3 (4.9)	24 (50)	<0.001
CMV viremia (3–6 months), n (%)	15 (24.6)	4 (8.3)	0.026
Average days to first CMV viremia (Range)	129.7 days (5–181)	36.2 days (1–90)	

Table 3. Secondary outcomes at 0–3 months.

Outcome	Prophylactic (n = 61)	Preemptive (n = 48)	P-value
CMV tissue-invasive disease, n	0	0	
Acute cellular rejection, n (%)	3 (4.9)	3 (6.3)	1.000
Leukopenia, n (%)	35 (57.4)	28 (58.3)	0.920
Neutropenia, n (%)	3 (4.9)	4 (8.3)	0.697
Incidence of opportunistic infections, n	25	26	0.354
Patients with opportunistic infections, n (%)	14 (22.9)	11 (22.9)	0.920
Readmissions, n	42	23	0.198
Patients with readmissions, n (%)	23 (37.7)	14 (29.2)	0.350
Mortality, n (%)	2 (3.3)	1 (2.1)	1.000

ACR, acute cellular rejection; CMV, cytomegalovirus.

Table 4. Secondary outcomes at 3–6 months.

Outcome	Prophylactic (n = 61)	Preemptive (n = 48)	P-value
CMV tissue-invasive disease, n	0	0	
Acute cellular rejection, n (%)	2 (3.3)	0	0.503
Leukopenia, n (%)	30 (49.2)	17 (35.4)	0.130
Neutropenia, n (%)	4 (6.6)	5 (10.4)	0.503
Incidence of opportunistic infections, n	10	8	0.973
Patients with opportunistic infections, n (%)	6 (9.8)	6 (12.5)	0.659
Readmissions, n	20	21	0.383
Patients with readmissions, n (%)	12 (19.7)	13 (27.1)	0.361
Mortality, n (%)	1 (1.6)	0	1.000

ACR, acute cellular rejection; CMV, cytomegalovirus.

tory tract infections (14.5%) (Table 5). Bacterial infections accounted for 80% (n = 56) of all infections based on microorganism classification (Table 6).

During the 0–3 months post-transplant period, 37.7% (n = 23) of patients in the prophylactic cohort and 29.2% (n = 14) in the preemptive cohort (P = 0.350) were readmitted to the hospital. At the 3–6 months post-transplant period, 19.7% (n = 12) of patients in the prophylactic cohort and 27.1% (n = 13) in the preemptive cohort (P = 0.361) were readmitted to the hospital. Patients were readmitted for a heterogeneous list of medical diagnoses that encompassed post-surgical complications, infections, acute renal failure, etc. In some cases, patients had multiple diagnoses in a single readmission. Of all readmissions, only three patients had CMV infection as reason for readmission.

There was low incidence of mortality in both cohorts. The mortality rate at 0–3 months post-transplant was 3.3% (n = 2) and 2.1% (n = 1) in the prophylactic and preemptive cohort, respectively (P = 1.0). At 3–6 months post-transplant, the mortality rate was 1.6% (n = 1) and 0% (n = 0) in the prophylactic and preemptive cohort, respectively (P = 1.0). The causes of death for the three patients in the prophylactic cohort were allograft failure, myocardial

Table 5. Opportunistic infections by organ system (0–6 months).

Organ system	Prophylactic (n = 61)	Preemptive (n = 48)
Urinary tract	7	6
Bloodstream	11	9
Catheter-related bloodstream infections	0	4
Respiratory tract infections	2	8
Intra-abdominal infections*	8	4
Clostridium difficile infections	6	3
Other infections†	1	0
Total	35	34

*Intra-abdominal infections include hepatobiliary and peritoneal space infections.
†Oral herpes infection.

Table 6. Opportunistic infections by microorganism classification (0–6 months).

Organism classification	Prophylactic (n = 61)	Preemptive (n = 48)
Bacterial	28	28
Viral	5	4
Fungal	3	2
Total	36*	34

*Total organism is 36 compared to infection incidence of 35 because one patient grew out 2 microorganism class (bacterium, and fungus) from one culture sample.

infarction, and unknown cause (patient's electronic medical record revealed death as an outpatient), whereas the one death that occurred in the preemptive cohort was due to septic shock.

Given that some patients experienced outcomes, with the exception of ACR and mortality, at both the 0–3 months and 3–6 months post-transplant period, we summarized results of the proportion of patients who experienced any of the primary or secondary outcomes during the entire 6-month post-transplant period in Table 7.

CMV surveillance results

Each LT recipient, based on the post-transplant pathway protocol, should have had 18 CMV PCR results within the first 6 months post-transplant. Of the 59 of 61 patients in the prophylactic cohort who had at least one CMV PCR result reported (2 patients died during the first week and had no CMV PCR result), the mean CMV PCR results reported out of the targeted 18 CMV PCR results was 66% complete (11.88 out of 18) versus 63.9% complete (11.5 out of 18) in all 48 patients in the preemptive cohort ($P = 0.587$). All patients (prophylactic and preemptive cohorts) who developed CMV viremia received valganciclovir prescription.

Table 7. Patients with any outcome at 6 months.

Outcome	Prophylactic (n = 61)	Preemptive (n = 48)	P-value
CMV viremia, n (%)	17 (27.9)	24 (50)	0.018
CMV tissue-invasive disease, n	0	0	
Acute cellular rejection, n (%)	5 (8.2)	3 (6.3)	1.000
Leukopenia, n (%)	42 (68.9)	32 (66.7)	0.808
Neutropenia, n (%)	7 (11.5)	8 (16.7)	0.435
Opportunistic infections, n (%)	18 (29.5)	14 (29.2)	0.969
Readmissions, n (%)	30 (49.2)	19 (39.6)	0.317
Mortality, n (%)	3 (4.9)	1 (2.1)	0.629

ACR, acute cellular rejection; CMV, cytomegalovirus.

Discussion

In this retrospective study, prophylactic and preemptive strategies were compared in two consecutive cohorts of LT recipients. Our transition from a prophylactic strategy to a preemptive strategy allowed us to study the direct effects of CMV (CMV viremia & CMV tissue-invasive disease), indirect effects of CMV (opportunistic infections, ACR, and mortality), leukopenia, neutropenia, and hospital readmissions. All outcomes were evaluated at 0–3 months and at 3–6 months post-transplant. The 0–3 month post-transplant period is the time period when the risk of developing CMV is highest in LT recipients without prophylaxis and also correlates with our prophylactic valganciclovir duration, which is common in many LT centers [9,15]. The 3–6 month post-transplant period follows the prophylactic valganciclovir duration and this period has been associated with late-onset CMV disease [15].

A difference in the time to onset of CMV viremia was observed between the prophylactic and preemptive cohort. At 0–3 months post-transplant, a statistically significant higher incidence of CMV viremia was observed with the preemptive strategy and as expected, there was statistical significant increase in the incidence of CMV viremia with the prophylactic strategy during the 3–6 months post-transplant period. Figure 2 provides a graphical trend of the average CMV viral load per week between both cohorts. This finding is consistent with prior studies which showed that prophylaxis with valganciclovir delays the onset of CMV viremia compared with preemptive therapy [8,16,17].

Late-onset CMV disease has been associated with increased mortality [18]. In this study, we found a significant difference in the proportion of patients with late-onset CMV viremia in the prophylactic cohort compared with the preemptive cohort: 22.9% (14/61) versus 0%, respectively ($P < 0.001$). In a study by Khoury *et al.*, all patients with late-onset CMV disease had CMV viremia and an association was found with CMV disease and mean higher

peak CMV viral load [8]. This leads us to deduce that preventing late-onset CMV viremia may provide benefit in preventing late-onset CMV disease. Extended valganciclovir prophylaxis or frequent surveillance monitoring after antiviral prophylaxis discontinuation has been proposed for preventing late-onset CMV disease [19,20]. In two sequential studies led by Humar *et al.,* CMV disease occurred in significantly less patients who received a 200-day versus a 100-day valganciclovir prophylaxis at 1-year and 2-years post-transplant [21,22]. Although the Humar *et al.* [21,22] findings provide evidence for extended valganciclovir prophylaxis in kidney transplant patients, its extrapolation to LT recipients may be controversial as valganciclovir prophylaxis is associated with increased risk of CMV tissue-invasive disease among LT recipients [5]. Frequent surveillance monitoring, with initiation of preemptive antiviral therapy in patients with asymptomatic CMV replication, may present a reasonable option for LT centers using valganciclovir prophylaxis, but may present some logistical challenges in coordinating outpatient CMV PCR laboratory tests [19,20].

In our study, we noted a two times increase in the relative risk of developing CMV viremia in the CMV D+/R− serostatus compared with other serostatus (relative risk, 2.051; 95% confidence interval [CI], 1.19 to 3.04; $P = 0.005$), however, no benefit was seen with using either strategy in preventing CMV viremia in the CMV D+/R− patients [percentage of CMV D+/R− patients with viremia during 6-month post-transplant: 10/15 (66.7%) prophylactic cohort versus 4/7 (57.1%) preemptive cohort, $P = 1.0$). Other non-CMV D+/R− serostatus had no statistical significant difference in their relative risk of developing CMV viremia. A recent single-center study evaluating the preemptive strategy in 689 liver and kidney transplant supports our finding and noted that the CMV D+/R− group have a greater risk of developing CMV viremia compared with patients with non-CMV D+/R− serostatus [23]. This study also reported low levels of CMV disease with preemptive approach, further emphasizing the effectiveness of preemptive strategy in preventing CMV disease [23].

There was no documented incidence of CMV-tissue-invasive disease in this retrospective study. Previous studies comparing prophylactic and preemptive strategies have generally documented low incidence of CMV tissue- invasive disease. Khoury *et al.* reported one incidence (1.02%) of CMV target-organ disease [8] and van der Beek *et al.* reported no CMV end-organ disease in their studies respectively [16]. This indicates that preemptive and prophylactic strategies are both equally effective in preventing CMV tissue-invasive disease.

Our assessment of CMV tissue-invasive disease, which is a component of CMV disease, is a limitation to the study. CMV disease consists of CMV syndrome (CMV viremia with fever, myelosuppression, weakness, myalgia, and arthralgia) and CMV tissue-invasive disease (hepatitis, nephritis, colitis, retinitis, pneumonitis, etc.). The unintentional omission of CMV syndrome evaluation in this study limits our ability to assess the effect of prophylactic or preemptive strategies on CMV disease holistically. Inclusion of CMV syndrome is important in assessing the burden of CMV disease as CMV syndrome accounts for a significant proportion of CMV disease. In two studies by Humar *et al.*, 93.75% (90/96) of all cases of CMV disease (CMV syndrome and CMV tissue-invasive disease) were exclusively CMV syndrome without CMV tissue-invasive disease [21,22]. In future studies, evaluating the effect of prophylactic or preemptive strategy on CMV infection and disease, we recommend studying the total burden of CMV disease: CMV syndrome and CMV tissue-invasive disease, as defined by Ljungman *et al.* [24].

There was no statistical significant difference in any secondary outcomes analyzed in this chart review as presented on Tables 3, 4, and 7. The incidence of ACR was low in both the cohorts and no statistical significant difference was seen. We found no correlation in the days for development of first ACR and days for development of first CMV viremia. Indeed, out of the eight patients who developed ACR during the 6 months post-transplant period, 62.5% ($n = 5$) of these patients did not develop CMV viremia during this time period. Several studies have reported no differences in acute rejection when comparing prophylactic versus preemptive groups in adult renal transplant patients [4,8,25]. Although it is postulated that CMV mediates ACR through its immune modulating abilities, the role of CMV in ACR is debatable as studies are consistently showing low incidence of ACR. Moreover, relationship between CMV disease and acute graft rejections has not been shown in all studies [26].

The incidence of leukopenia and neutropenia was not significantly different between cohorts at 0–3 months post-transplant. This was an unexpected finding as we anticipated higher incidences of leukopenia and neutropenia in our prophylactic cohort as they received valganciclovir (myelosuppressive agent) during the 0–3 month post-transplant period. This insignificant finding may be explained by the sheer fact that 50% of patients in the preemptive cohort developed CMV viremia at 0–3 months and received valganciclovir. At 3–6 months post-transplant, the incidence of leukopenia and neutropenia was also not significantly different between cohorts. The difference not seen between cohorts in the incidence of leukopenia and neutropenia may be confounded by other myelosuppressive producing factors such as CMV, mycophenolate mofetil, and trimethoprim-sulfamethoxazole. The lower incidence of neutropenia may be because of our definition of neutropenia as ANC < 500 cells/mm^3 instead of a higher ANC count.

The incidence and percentage of patients with opportunistic infections at 0–3 and 3–6 months post-transplant was not significantly different between the prophylactic and preemptive cohorts. There was a higher incidence of opportunistic infections in both cohorts at 0–3 months post-transplant compared with the 3–6 months post-transplant period. This suggests that the 3-month following LT is the period of greater risk for the development of infections. Analogous to our study, trials by Khoury *et al.* and Witzke *et al.* reported no significant difference in opportunistic infections between the prophylactic and preemptive groups in renal transplant recipients [8,25]. However, the meta-analysis by Kalil *et al.* found that prophylactic strategy is associated with reduced bacterial and fungal infection when compared with the preemptive strategy [6]. These conflicting findings leave us with an inconclusive answer on the effect of prophylactic or preemptive strategies on opportunistic infections.

Hospital readmissions and mortality rates at 0–3 months and at 3–6 months post-transplant was not significantly different between both cohorts. The reason for readmissions was very heterogeneous between cohorts, with very few patients readmitted with CMV infection as readmission diagnosis. No death was directly attributed to the CMV infection in this study.

Some limitations were applied to this study. The retrospective nature of this study made us rely only on the data available on our institutional patient's electronic medical record. The omission of CMV syndrome evaluation likely prevented us from assessing CMV disease holistically. The single-center aspect of this study weakens its external validity. The small sample size ($N = 109$) limits the generalizability of this study results, and may contribute to the lack of statistical significant difference seen in the secondary outcomes.

Conclusion

This is the first head-to-head study comparing the clinical outcomes of prophylactic and preemptive strategy in an all-inclusive cohort of CMV donor and recipient serologic status LT recipients. The time to onset of CMV viremia varies significantly with the use of prophylactic versus preemptive CMV strategy. However, the use of either prophylactic or preemptive strategy was not associated with negative clinical outcomes of CMV such as ACR, opportunistic infections, leukopenia, neutropenia, hospital readmissions, and mortality.

Authorship

IO: participated in research design, acquisition of data, data analysis and interpretation, and preparation of manuscript. ST: had the original idea for the study, and participated in research design, data analysis and interpretation, and preparation of manuscript. EM: participated in review of literature, data analysis, and critical review of manuscript. SP: participated in research statistics testing, data analysis and interpretation, and critical review of manuscript. AM: participated in research design, data analysis, and critical review of manuscript. GML: participated in data analysis and critical review of the manuscript. SK: participated in data analysis and critical review of manuscript. SH: had original idea for the study, and participated in research design, data analysis and interpretation, and critical review of manuscript.

Funding

The authors have declared no funding.

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