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Risk factors for late-onset cytomegalovirus disease in donor seropositive/recipient seronegative kidney transplant recipients who receive antiviral prophylaxis

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Abstract

Background—Cytomegalovirus (CMV) disease occurs frequently after cessation of antiviral prophylaxis in CMV-seronegative kidney transplant recipients from seropositive donors (D+ R−), and the risk factors are incompletely defined.

Methods—We retrospectively assessed the incidence, clinical features, and risk factors for CMV disease in a cohort of D+ R− kidney transplant recipients who received antiviral prophylaxis at a single US transplant center using descriptive statistics and Cox proportional hazards models.

Results—CMV disease developed in 29 of 113 (26%) D+ R− patients at a median of 185 days (interquartile range 116–231 days) post transplant, including CMV syndrome (66%) and tissue invasive disease (34%). The incidence of CMV disease was higher in patients who underwent re-transplantation (57% vs. 24%) and this factor was independently associated with a higher risk of CMV disease in multivariable analysis (hazard ratio, 4.02; 95% confidence interval, 1.3–13; P = 0.016). Other demographic and transplant variables were not independently associated with a risk of late-onset CMV disease.

Conclusions—Despite a comprehensive analysis of patient and transplant variables, only re-transplantation was identified as a risk factor for CMV disease in D+ R− kidney transplant recipients who received antiviral prophylaxis, but had limited clinical predictive value. The development of novel laboratory markers to identify patients at greatest risk for CMV disease should be a priority for future studies.

Keywords

cytomegalovirus disease; kidney transplant; risk factor; retransplantation

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Antiviral prophylaxis has become a commonly used strategy for prevention of cytomegalovirus (CMV) disease in seronegative recipients of an organ from a CMV-seropositive donor (D+ R−) and has typically been given for 3–6 months post transplant (1). Despite significant reductions in the incidence of CMV with this approach, 20–40% of D+ R − patients still develop CMV disease after discontinuation of antiviral prophylaxis. CMV disease in this setting (termed 'late-onset CMV') is an important clinical problem that is associated with significant morbidity (2–4). Optimal approaches for preventing late-onset disease have not been fully defined, but have included extending the duration of prophylaxis, viral monitoring and preemptive therapy after discontinuation of prophylaxis, or enhanced surveillance for clinical symptoms and early treatment.

Although it is well established that D+ R− transplant patients as a group are at significantly higher risk of late-onset CMV disease compared with seropositive (R +) patients, the risk factors within the D+ R− group have not been well defined. If specific clinical or transplant variables predictive of late-onset CMV disease could be identified within this group, it might be possible to design a more rational targeted CMV prevention strategy for those D+ R− patients at greatest risk. We are aware of only 4 previously published studies that analyzed risk factors for late-onset CMV in D+ R− kidney transplant recipients who received antiviral prophylaxis (4–7). Among these, 2 combined results of kidney with other transplant recipients (4, 6), making it difficult to determine which risk factors might specifically be related to kidney transplant without confounding by the other organ transplant. Thus, given the limited data regarding the risk factors for late-onset CMV disease in D+ R− kidney transplant recipients who receive antiviral prophylaxis, we retrospectively assessed the association of multiple demographic and transplant variables with CMV disease in a cohort of adult D+ R− kidney transplant recipients at a single US kidney transplant center.

Patients and methods

Study population and data collection

All CMV-seronegative recipients who received a kidney transplant from a CMV-seropositive donor (D+ R−) at the University of Washington Medical Center between January 1, 2000 and July 9, 2009 were considered for the study. Patients with a previous, concomitant, or subsequent (within 1 year of the kidney transplant) pancreas, liver, heart, or lung transplant were excluded. Patients who received >6 months of antiviral prophylaxis for CMV were also excluded. All data were collected through review of electronic medical records and a clinical transplant database in the context of a quality improvement project designed to optimize CMV prevention strategies in D+ R− kidney transplant recipients at our center.

Immunosuppression and rejection therapy

All patients received induction therapy with anti-thymocyte globulin (ATG), basiliximab, or daclizumab. Routine maintenance immunosuppression regimens varied during the study period, but typically included tacrolimus combined with mycophenolate mofetil (MMF) or sirolimus, with or without corticosteroids. Allograft rejection was diagnosed by kidney allograft biopsy using accepted pathologic criteria (8) and was treated with pulse doses of methylprednisolone. In patients whose allograft rejection failed to respond to pulse doses of corticosteroid therapy, a 5-day regimen of ATG was given.

CMV prevention, diagnosis, and treatment

All patients initially received intravenous (IV) ganciclovir 5 mg/kg daily after transplant. Once oral medications were tolerated, IV ganciclovir was replaced by oral ganciclovir 1 g

three times daily (through July 2002) or valganciclovir 900 mg once daily (starting August 2002), both adjusted for renal function per manufacturer recommendations. Duration of prophylaxis after transplantation was at the discretion of the treating physician, and patients received prophylaxis for approximately either 3 or 6 months. Patients treated with ATG for allograft rejection received concomitant antiviral therapy with valganciclovir 900 mg daily for 1 month, if they were not already receiving prophylaxis. CMV disease was treated either with IV ganciclovir 5 mg/kg twice daily or oral valganciclovir at a dose of 900 mg twice daily, and was continued until all signs and symptoms of disease had resolved and blood CMV viral load as measured by polymerase chain reaction (PCR) was undetectable. CMV viral load in blood was measured by PCR as described previously (9). Quantitative plasma viral load measurement and biopsies were performed at the discretion of the treating physician when clinically indicated, and routine monitoring for CMV viremia in otherwise asymptomatic patients was not performed. CMV disease was defined using American Society of Transplantation consensus definitions, as described previously (10).

Statistical analyses

Patient characteristics and outcomes were summarized using proportions, medians, and interquartile ranges (IQRs). The primary endpoint was the time to occurrence of CMV disease during the first year after transplant. Univariable and multivariable Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals. The candidate risk factors included recipient and donor age, recipient sex, re-transplantation, donor origin, organ match, induction therapy, delayed graft function, maintenance immunosuppression agents, type of antiviral used, antiviral prophylaxis duration, and acute rejection. Acute rejection was modeled as a time-dependent variable. Variables with a *P*-value <0.3 in the univariable analysis were entered into the multivariable model. Induction therapy was forced into the multivariable model. All reported *P*-values are 2-sided and considered significant at $\alpha < 0.05$. Data were analyzed using SAS software, version 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Study population and CMV disease

During the study period, 826 patients received a kidney transplant. A total of 166 patients (20%) were CMV-seronegative recipients of a transplant from a CMV-seropositive donor (D + R−). We excluded 40 patients who received a previous, concomitant, or subsequent (within 1 year of the kidney transplant) non-kidney organ transplant. Five patients who received >6 months of antiviral prophylaxis and 8 patients who were lost to follow-up shortly after transplant were also excluded. Thus, 113 patients were included in the final study population and their characteristics are shown in Table 1. The incidence of CMV disease at 1 year was 26% (29 of 113) (Table 2) and developed at a median of 185 days post transplant (IQR, 116–231 days), and tended to occur earlier in those who received 3 vs. 6 months of prophylaxis (166 days [IQR, 114–191 days] vs. 254 days [IQR, 144–312 days], respectively, $P = 0.07$). Overall graft survival (96%) and patient survival (99%) in the cohort at 1 year were excellent.

Risk factors for CMV disease

The association of patient and transplant characteristics with CMV disease in univariable and multivariable analyses are shown in Tables 3 and 4, respectively. The incidence of CMV disease trended toward being higher in those who underwent re-transplant compared to those who underwent a first transplant (57% vs. 24%, respectively, $P = 0.07$), and this factor was independently associated with an increased risk of CMV disease in multivariable analysis. The 1-year incidence of CMV disease was lower in recipients >50 years old as

compared with younger patients (16% vs. 34%, $P = 0.03$), and older age was marginally associated with a lower risk of CMV disease in multivariable analysis (Table 4). The risk of CMV disease decreased with each decade of age from 40 to 60 years in a multivariable model, although the results were not statistically significant (data not shown). The incidence of CMV disease among the 16 patients who did not receive MMF was lower than among those who received MMF (0% vs. 30%, $P = 0.01$). All patients who did not receive MMF received tacrolimus and sirolimus without prednisone as their maintenance immunosuppression regimen. The HR for MMF use could not be calculated owing to the absence of events among patients who did not receive MMF, and consequently could not be included as a variable in the multivariable analysis.

Discussion

This study assessed the association of a broad range of clinical and transplant variables with CMV disease in a cohort of D+ R− kidney transplant patients who received anti-viral prophylaxis, and found that only re-transplantation was an independent risk factor for CMV disease in this population. As only a relatively small proportion of patients in the cohort had received a re-transplant, the clinical utility of this factor as a marker for targeting enhanced CMV prevention strategies is likely to be limited.

Relatively few data are available regarding risk factors for CMV disease in D+ R− kidney transplant recipients. We are aware of only 4 previous studies that assessed risk factors for late-onset CMV disease in D+ R− kidney transplant patients (4–7), and in 2 of these studies (4, 6), kidney transplant patients were pooled with patients who received other organ transplants, making it too difficult to define kidney transplant-specific risk factors. One study reported an increased risk of CMV disease associated with delayed graft function (5) while 2 previous reports did not find any association between this variable and CMV disease (6, 7). One study, which included pancreas transplant recipients, found an increased risk of CMV disease associated with use of ATG induction as compared with no induction (6). In our analysis and in the other study that included a population who received induction with either ATG or anti-interleukin (IL)2 (4), the lower HR for CMV disease associated with anti-IL2 induction was not statistically significant. Arthurs et al. (4) reported an association between a high Charlson comorbidity index and early-onset bacterial and fungal infection after transplantation with an increased risk of CMV disease, although multivariable analyses were not undertaken to confirm those findings.

Despite the relatively small number of patients who underwent re-transplantation in this cohort, we found that this variable was independently associated with an increased risk of CMV disease. The association of re-transplantation with an increased risk of CMV disease is biologically plausible because of the longer duration of immunosuppression in patients undergoing a second transplant. We are unaware of prior studies that specifically addressed re-transplantation as a potential risk factor for CMV disease in D+ R− kidney transplant recipients. This finding is important because the number of patients undergoing kidney re-transplantation has increased by 40% during the last decade (11). Thus, given the significantly increased risk for CMV disease seen in D+ R− patients undergoing kidney re-transplantation, this is a specific group that should be targeted for enhanced CMV prevention strategies, such as longer durations of prophylaxis, monitoring and preemptive therapy after discontinuation of prophylaxis, or more frequent monitoring for clinical symptoms of CMV disease.

Some, but not all, prior studies have shown that a longer than the standard 3-month duration of antiviral prophylaxis is associated with a decreased incidence of CMV disease (5–7, 12–14). In our cohort, we did not find that a 6-month duration of antiviral prophylaxis (as

compared with 3-month duration) was associated with a lower incidence of CMV disease. We emphasize that the present study was retrospective, not randomized, and not designed specifically to assess the duration of prophylaxis on the incidence of CMV disease, and thus this result should be interpreted with caution. One potential explanation for our results is the possibility of a selection bias toward longer prophylaxis regimens specifically in patients deemed to be at highest risk for developing CMV disease. In addition, dose reduction and compliance were not systematically monitored and differences between the groups could possibly explain the results obtained. Also, the overall incidence of CMV disease in our cohort was lower than in a previous randomized trial (26% vs. 36%), making it more difficult to detect a true difference in this smaller cohort (12). And finally, other unmeasured variables might have confounded the association between antiviral prophylaxis duration and CMV disease.

Our study has strengths and limitations. The retrospective nature and single-center analysis may have introduced unintended biases. We used multivariable models to adjust for confounding factors, but the impact of unmeasured factors cannot be excluded. Our results may not be applicable to other kidney transplant populations with different patient characteristics and immunosuppressive regimens. As an exploratory analysis that included multiple variables, there is a possibility that associations could be discovered by chance. However, we used standardized consensus definitions for the primary endpoint of CMV disease, used systematic statistical analyses, included only biologically plausible variables in the multivariable models, and had 100% follow-up of all patients at the 1-year timepoint.

In summary, despite a comprehensive analysis of clinical and transplant variables, we identified only re-transplantation as an independent risk factor for late-onset CMV disease in D+ R− kidney transplant recipients who received antiviral prophylaxis, but the predictive value of this finding is limited based on the small proportion of patients who underwent re-transplantation. Given the limited predictive value of clinical and transplant characteristics, the development of novel laboratory markers to identify D+ R− patients at greatest risk should be a priority for future studies, and might allow for more rational targeting of CMV prevention strategies to reduce the incidence and morbidity of late-onset CMV disease in patients who receive antiviral prophylaxis.

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Table 1

Patient characteristics

Variables	*n* = 113
Recipient age, median years (IQR)	47 (35–55)
Recipient sex, male	79 (70)
Cause of renal failure	
Glomerulonephritis	30 (27)
Polycystic kidney disease	20 (18)
Diabetic nephropathy	20 (18)
Hypertensive nephrosclerosis	9 (8)
Others	34 (30)
Re-transplant	7 (6)
Living donor transplant	31 (27)
Donor age, median years (IQR)	39 (24–46)
Number of HLA mismatches, median (IQR)	
HLA-DR	1.1 (1–2)
Total (HLA-A, B, and DR)	4 (3–5)
Induction therapy	
Anti-thymocyte globulin	98 (87)
Anti-IL-2 agent	15 (13)
Delayed graft function	32 (28)
Maintenance immunosuppression	
Mycophenolate mofetil	97 (86)
Calcineurin inhibitor	
Tacrolimus	112 (99)
Cyclosporine	1 (1)
Sirolimus	29 (26)
Prednisone	45 (40)
Prophylactic antiviral	
Oral ganciclovir	29 (26)
Valganciclovir	84 (74)
Antiviral prophylaxis duration	
3 months	79 (70)
6 months	34 (30)
Acute rejection within 1 year	30 (27)

Data are number of patients (%) unless otherwise indicated.

IQR, interquartile range; HLA, human leukocyte antigen; DR, donor/recipient; IL-2, interleukin-2.

Table 2

Incidence of cytomegalovirus (CMV) disease by 1 year post transplant (n = 113) in D+ R− kidney transplant recipients who received antiviral prophylaxis

Outcome	No. (%)
CMV disease	29 (26)
Syndrome	19 (66)
Invasive disease	10 (34)
Gastrointestinal tract	8 (80)
Pneumonia	1 (10)
Hepatitis	1 (10)

D+ R−, donor CMV seropositive, recipient CMV seronegative.

Table 3

Risk factors for cytomegalovirus (CMV) disease in D+ R− kidney transplant recipients who received antiviral prophylaxis: univariable analysis

Variable	HR (95% CI)	*P*-value
Recipient age ≥ 50 years	0.40 (0.2–0.9)	0.029
Recipient sex, male	0.96 (0.4–2.1)	0.919
Donor age ≥ 40 years	1.17 (0.5–2.7)	0.699
Re-transplant	2.71 (0.9–7.8)	0.065
Living donor transplant	0.65 (0.3–1.6)	0.341
Total HLA mismatches		
>1 HLA-DR mismatch	1.60 (0.7–3.6)	0.259
≥ 3 total HLA mismatches	1.17 (0.5–2.7)	0.723
Induction with anti-IL2 agent (vs. ATG)	0.46 (0.1–1.9)	0.284
Delayed graft function	0.98 (0.4–2.2)	0.970
Maintenance immunosuppression		
Mycophenolate mofetil	N/A[1]	0.018[1]
Sirolimus	0.76 (0.3–1.9)	0.543
Steroid	0.87 (0.4–1.9)	0.726
Six months prophylaxis	1.18 (0.5–2.5)	0.675
Acute rejection[2]	1.51 (0.6–3.6)	0.346
Oral ganciclovir (vs. valganciclovir)	0.96 (0.4–2.2)	0.924

[1] Unable to calculate hazard ratio due to the absence of CMV disease in the group of patients who did not receive mycophenolate mofetil as maintenance immunosuppression. *P*-value was calculated by log-rank test.

[2] As a time-dependent variable.

D+ R−, donor CMV seropositive, recipient CMV seronegative; HR, hazard ratio; CI, confidence interval; HLA, human leukocyte antigen; DR, donor/recipient; IL-2, interleukin-2; ATG, anti-thymocyte globulin.

Table 4

Risk factors for cytomegalovirus (CMV) disease in D+ R− kidney transplant recipients who received antiviral prophylaxis: multivariable analysis

Variable	HR (95% CI)	*P*-value
Recipient age ≥ 50 years	0.40 (0.2–1.0)	0.053
Re-transplant	4.02 (1.3–13)	0.016
>1 HLA-DR mismatch	2.20 (0.9–5.1)	0.068
Induction with anti-IL2 agent (vs. ATG)	0.51 (0.1–4.0)	0.525

D+ R−, donor CMV seropositive, recipient CMV seronegative; HR, adjusted hazard ratio; CI, confidence interval; IL-2, interleukin-2; HLA, human leukocyte antigen; DR, donor/recipient; ATG, anti-thymocyte globulin.