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Donor CMV Reactivation as a Novel Risk Factor for CMV Replication in Seropositive Liver Transplant Recipients

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Background. Risk factors for cytomegalovirus (CMV) viremia in CMV seropositive liver transplant recipients are incompletely defined and have focused primarily on recipient factors. We hypothesized that active CMV replication (CMV viremia) in seropositive donors might increase the risk for CMV viremia in recipients, as reported for other viruses in organ transplantation. **Methods.** From January 3, 2009, to July 27, 2015, stored plasma from consecutive CMV seropositive liver donors was retrospectively tested for CMV viremia by PCR. From April 20, 2012, to July 27, 2015, CMV seropositive recipients of a liver transplant from the donors during this time period received preemptive therapy for CMV prevention (valganciclovir therapy for CMV viremia ≥250 IU/mL). The association of recipient factors and donor CMV viremia with viremia in recipients was assessed. **Results.** Among 317 CMV-seropositive donors, CMV viremia was detected in 11 (3.5%) and was associated with longer time to collection after admission and bacteremia. Among 115 CMV-seropositive liver recipients, 5 (4.3%) received an organ from a donor with CMV viremia. Donor CMV viremia was independently associated with higher incidence of CMV viremia ≥250 IU/mL and shorter time to onset of CMV viremia ≥250 IU/mL in recipients: 4 (80%) versus 26 (23.6%), $P=0.02$, and hazard ratio 8.55 (2.60–28.10), $P=0.003$, respectively. **Conclusion.** Donor CMV reactivation is associated with CMV viremia in seropositive orthotopic liver transplant recipients receiving preemptive therapy, identifying a novel potential risk factor for CMV infection in seropositive liver transplant recipients. Future studies should independently validate and assess these findings in other organ transplant settings.

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INTRODUCTION

Cytomegalovirus (CMV) remains an important pathogen in solid organ transplant recipients. Preemptive therapy (PET, CMV monitoring and initiation of antiviral drug only at the time of detection of CMV at a predefined threshold) is an accepted prevention strategy both for high-risk donor seropositive, recipient seronegative (D+R–), and seropositive (R+) recipients.[1] Better identification of risk factors for significant CMV viremia in R+ orthotopic liver transplantation (OLT) recipients might allow for refinement of current PET strategies, but prior studies in R+ liver transplant patients failed to consistently identify specific recipient risk factors.[2]

There is increased recognition that CMV-seropositive adults with critical illness due to a broad range of etiologies (including sepsis, acute myocardial infarction, trauma, or thermal injury) may develop CMV reactivation during the course of illness.[2-4] Additionally, reactivation of certain viruses in the donor have been associated with an increased risk for concordant reactivation in the recipient.[5-8] Based on experience with CMV reactivation in seropositive adults with critical illness, and by analogy to that reported for reactivation of certain viruses (eg, BK virus) in donors and increased risk for viral replication in the recipient, we hypothesized that CMV reactivation might be present in seropositive donors at the time of organ donation and that donor reactivation would increase the likelihood for significant CMV infection in the recipient.

The goals of this study were to assess the prevalence and associated factors for CMV viremia among CMV-seropositive liver donors and to determine whether donor CMV replication was associated with an increased risk for CMV replication in recipients.

MATERIALS AND METHODS

Stored serum samples from consecutive CMV seropositive liver donors were retrospectively tested for CMV viremia at the University of Washington Medical Center (UWMC) Liver and Kidney Transplant Program from January 3, 2009, to July 27, 2015. We aimed to address 2 major questions in this study:

1. What was the prevalence of and associated factors for CMV viremia among liver transplant donors?
2. Was CMV viremia in donors associated with risk for CMV viremia in liver recipients?

To address no. 1 above, we retrospectively assessed CMV viremia in 317 consecutive CMV seropositive donors. To address no. 2 above, we assessed the subset of these 317 donors whose livers were transplanted into CMV-seropositive patients (N = 115).

This cohort included patients (N = 160) who were included in a separate study of preemptive therapy, from 2012 to 2015.[2] Stored donor plasma samples and recipient samples assessed as part of a prospective preemptive therapy protocol were both similarly tested for CMV by personnel blinded to clinical data using a previously published quantitative CMV PCR assay.[9] The CMV preemptive therapy protocol used for all recipients included in the study was as previously described.[2] Donor data collected included CMV reactivation, number of days from admission to the day the serum was collected and stored, gender, age in years, cause of death (anoxia, CVA, other, and trauma), blood infection, if steroids were given, body mass index (BMI), and last recorded blood urea nitrogen (BUN).

Consecutive CMV seropositive liver recipients from April 20, 2012, to July 27, 2015, of the UWMC Liver Transplant Program who received PET for CMV prevention (as previously described[2]) were retrospectively studied. These recipients were prospectively monitored and treated for CMV viremia using a PET strategy with valganciclovir administered for CMV viremia levels ≥250 IU/mL. Recipient data collected included age, gender, laboratory Model for End-Stage Liver Disease score (lab-MELD), if pretransplant dialysis was required, and if antithymocyte globulin (ATG) was given for induction immunosuppressive therapy. CMV viremia (at any level) was the donor variable assessed as a potential risk factor for CMV viremia in recipients. The outcome measures were recipient CMV viremia at a level ≥250 IU/mL and time to onset of CMV viremia ≥250 IU/mL (the threshold for initiation of antiviral therapy in the PET protocol) and peak CMV virema levels.

Approval for this study was obtained from the University of Washington's Human Subjects Division, and waivers of consent and HIPAA authorization were granted. Continuous variables are depicted as mean and SD or median and interquartile range (IQR). Categorical variables are presented as percentages. Student's *t*-test or the Mann-Whitney test was used as appropriate for the distribution to compare continuous variables, and chi-square analysis was used to compare categorical variables. Multivariable logistic regression was used to assess variables in donors that were associated with CMV reactivation and for recipient variables associated with developing CMV viremia at a level ≥250 IU/mL. Cox proportional hazards analysis was used to assess the variables associated with time to onset of CMV viremia levels ≥250 IU/mL. Linear regression was used to assess variables associated with recipient peak CMV viremia levels. All results with a $P < 0.05$ were considered significant. All statistics were performed using JMP-Pro version 14.3.0 (SAS Institute, Inc., Cary, NC).

RESULTS

Overview of Study Population of Donors and Recipients

An overview of the donors and their respective recipients, stratified by donor CMV reactivation, is shown in Figure 1. Among 317 consecutive CMV seropositive liver donors, 11 (3.5%) had CMV viremia (ie, any detectable level). From these CMV seropositive donors, 115 livers were transplanted into 115 seropositive CMV recipients. Of these recipients, 110 received organs from donors without CMV reactivation, and 5 received organs from donors with CMV reactivation.

Prevalence and Risk Factors for CMV Viremia Among Donors

The prevalence of CMV viremia (at any level) among donors was 3.5% (11 of 317). The donor CMV viremia levels had a mean of 21 IU/mL (IQR, 10–69 IU/mL; range 6–460 IU/mL). The donor factors associated with active CMV reactivation are shown in Table 1. A series of bivariate logistic regression analyses (not to overfit the model) that included all 2 variable combinations were constructed. Number of days to plasma sample collection (odds ratio [OR], 1.15; confidence interval [CI], 1.05-1.26) and blood infection (OR, 5.26; CI,

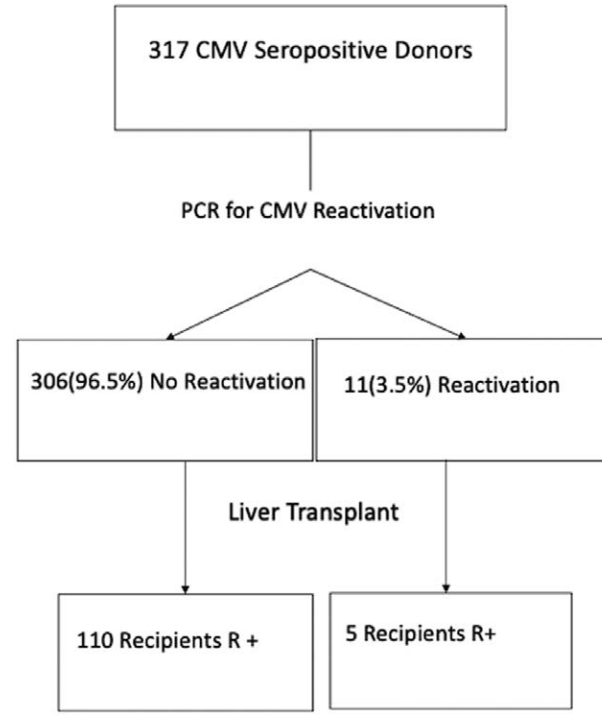

FIGURE 1. Summary of study population. CMV, cytomegalovirus.

1.11-20.22) were the only significant predictors of donor CMV viremia in these analyses.

Risk Factors for CMV Viremia in Recipients

A multivariable logistic regression algorithm was used to assess variables associated with recipient CMV viremia at a level of ≥250 IU/mL (Table 2). Of the 6 variables entered in the model (CMV reactivation in the donor, recipient age, recipient female gender, laboratory MELD, ATG used for induction immunosuppressive therapy, and dialysis at the time of transplantation), only CMV reactivation in the donor (OR, 12.3 [CI, 1.67-215]) was significantly associated (P = 0.01) with recipient CMV viremia ≥250 IU/mL.

Association of Donor CMV Viremia With Time to Onset of Viremia in Recipients

Cox proportional hazards analysis was used to assess the factors associated with time to onset of CMV viremia (at a level ≥250 IU/mL) in recipients. Only donor CMV reactivation was significantly associated with shorter time to recipient viremia onset (Table 2).

Viral Loads in Recipients From Donors With or Without CMV Viremia

Recipients from donors with CMV viremia trended toward developing a higher mean peak CMV viral load compared with recipients from donors without CMV viremia: 458.0 ± 350.6 versus 142.8 ± 336.6, P = 0.11, respectively. In a multivariable linear regression analysis of factors associated with peak CMV viremia in recipients, only donor CMV viremia was significant (P = 0.046). The other variables were not significant, including lab-MELD P = 0.08, female gender P = 0.38, age P = 0.54, ATG P = 0.69, and predialysis P = 0.87.

DISCUSSION

We found that CMV reactivation (assessed as viremia) was present in a small proportion of CMV seropositive donors and was associated with specific donor factors, including time of assessment after admission and presence of bacteremia, both of which are compatible with studies of CMV reactivation in CMV seropositive adults with critical illness.[3] Additionally, we found that CMV reactivation in the donor was associated with earlier time to onset and increased incidence of viremia requiring PET among recipients, compatible with viral replication in the donor liver as the likely source of CMV viremia in the recipient. These findings extend to CMV (data from prior studies linking reactivation of BK virus in donors to an increased risk for BK virus reactivation in the recipient[5-8]) and identify a novel potential risk factor for CMV replication in seropositive recipients.

The finding that CMV reactivation in the donor was associated with increased risk for recipient CMV replication is biologically plausible, if replication was already present in the donor organ. CMV latency occurs in multiple cell types and tissues, including the liver, and recent studies have shown that ~85% of CMV seronegative recipients will acquire donor organ-transmitted CMV, among liver transplant recipients.[1] Donor CMV reactivation was generally associated with greater severity of illness in the donor, and it is theoretically possible that factors instead of, or in addition to, donor CMV reactivation might have increased the risk for active CMV replication in the recipient. In the absence of well-defined recipient risk factors for CMV reactivation among CMV-seropositive OLT recipients,[2] assessment of donor CMV viremia might be a useful means of identifying those at risk and tailoring preventive strategies accordingly. These results should be confirmed in future studies. However, given the relatively low prevalence of CMV viremia in donors, additional studies should be done to define specific donors in whom CMV reactivation is more likely, to target donor testing strategies accordingly.

We acknowledge strengths and potential limitations of our study. Strengths included a relatively large cohort, use of a standard and protocol-driven CMV PET prevention strategy, and assessment of donor CMV viremia by blinded personnel to minimize bias. Although a higher incidence of CMV viremia and earlier time to onset was found among recipients from donors with CMV reactivation, it was not possible to differentiate donor versus recipient source of the viremia that was detected (ie, genotypic strain typing was not performed). Because only liver donors were assessed in the current study, whether similar impacts of donor CMV reactivation on recipient CMV infection risk would occur for other organs

TABLE 1.

Factors associated with CMV viremia in the donor

	No CMV reactivation	CMV reactivation	P
Number	306 (96.5%)	11 (3.5%)	
Days from admission until testing	2.3 ± 3.0	4.9 ± 6.1	0.04
Female gender	140 (45.8%)	6 (54.5%)	0.76
Age, y	37.1 ± 14.5	32.6 ± 13.9	0.33
Cause of death			
Anoxia	60 (19.6%)	5 (45.5%)	0.053
CVA	91 (29.7%)	1 (9.1%)	0.19
Other	7 (2.3%)	2 (18.2%)	0.03
Trauma	148 (48.4%)	3 (27.2%)	0.22
Bacteremia	20 (6.5%)	3 (27.3%)	0.04
Steroids given	105 (34.3%)	6 (54.6%)	0.20
BMI	26.3 ± 5.6	24.6 ± 4.2	0.21
BUN	16 ± 10.3	20.5 ± 10.9	0.12

BMI, body mass index; BUN, blood urea nitrogen; CMV, cytomegalovirus; CVA, cerebrovascular accident.

TABLE 2.

Risk factors for CMV viremia in recipients

	Outcome: CMV viremia (≥250 IU/mL)[a]	Outcome: time to viremia onset (≥250 IU/mL)[b]
Age, y	OR 0.99 (0.96–1.04) P = 0.84	RR 1.01 (0.97–1.04) P = 0.75
Female gender	OR 0.81 (0.31–2.03) P = 0.65	RR 1.01 (0.45–2.24) P = 0.98
Laboratory MELD	OR 1.03 (0.99–1.08) P = 0.16	RR 1.03 (0.99–1.07) P = 0.10
ATG induction	OR 1.21 (0.46–3.36) P = 0.71	RR 1.31 (0.57–2.99) P = 0.53
No pretransplant dialysis	OR 0.37 (0.05–.70) P = 0.22	RR 0.40 (0.09–1.76) P = 0.22
Donor CMV reactivation (at any level)	OR 12.31 (1.67–251.88) P = 0.01	RR 8.55 (2.60–28.10) P = 0.003

[a]Multivariable logistic regression.
[b]Multivariable cox proportional hazards.
ATG, antithymocyte globulin; CMV, cytomegalovirus; IQR, interquartile range; MELD, model for end-stage liver disease; OR, odds ratio; RR, relative risk.

is unknown and should be assessed in future studies. The number of donors with CMV viremia was small, and it was therefore not possible to control for all potential confounders. The timing of samples for CMV viremia testing from donors was not uniform across all donors, and samples drawn specifically on the day of donation might have been more informative regarding risk for viremia in recipients. Additionally, assessment of this observation (association of donor viremia with recipient viremia) was made feasible because recipients received preemptive therapy. This effect might not have been detected among patients receiving antiviral prophylaxis, with its greater effect on suppressing viremia.

In summary, we have identified a novel potential risk factor (donor CMV replication) for recipient CMV infection in seropositive recipients receiving PET. Future studies to confirm these findings, extend to other organ types, and to assess the impact of donor replication as a risk factor for transmission of CMV infection among other CMV serogroups (eg, D+R–) are warranted.

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