Clinical Infectious Diseases

BRIEF REPORT

Association of Cytomegalovirus (CMV) DNAemia With Long-Term Mortality in a Randomized Trial of Preemptive Therapy and Antiviral Prophylaxis for Prevention of CMV Disease in High-Risk Donor Seropositive, Recipient Seronegative Liver Transplant Recipients

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In a post-hoc analysis of the association of CMV DNAemia with long-term mortality in a randomized trial of CMV preemptive therapy vs. antiviral prophylaxis in D+R- liver transplant recipients, post-intervention CMV DNAemia was associated with increased mortality after adjusting for study arm.

Keywords. liver transplant; immunocompromised host; viral infections; cytomegalovirus; antiviral therapy.

In a National Institutes of Health–sponsored, multicenter, randomized trial (Cytomegalovirus [CMV] Antiviral Prevention Strategies in D + R– Liver Transplant [CAPSIL]), we recently demonstrated that preemptive therapy (PET) was superior to antiviral prophylaxis (AP) for prevention of CMV disease in high-risk donor-seropositive, recipient-seronegative (D + R–) liver transplant recipients (LTxRs) [1]. Other clinical outcomes by 12 months, including overall long-term survival, were similar between groups at a median follow-up of 3.2 years after transplant. All analyses in the primary trial were conducted within the intent-to-treat population. To further explore long-term mortality among those who completed the primary trial intervention of PET or AP (per-protocol analysis), we performed a post hoc landmark analysis at 100 days post-transplant, which is the duration of the primary study intervention excluding participants who did not complete their assigned intervention for any reason. We further assessed the association between late-onset CMV DNAemia at 6 and 12 months and long-term mortality using a landmark analysis among survivors by 12 months post-transplant.

METHODS

Study Design

This study was a post hoc analysis of the CAPSIL study that compared PET and AP in D + R– LTxRs. The full study details are provided in the primary study [1]. Patients randomized to the PET arm were monitored weekly for CMV DNAemia using CMV polymerase chain reaction (PCR) through day 100 and treated for any level of DNAemia until resolution. The prophylaxis arm consisted of 100 days of prophylaxis dose valganciclovir (900 mg daily). Post-intervention CMV monitoring and treatment were done at clinical discretion. Plasma specimens were prospectively collected at 6 and 12 months post-transplant for all patients and cryopreserved for future analysis.

Post Hoc Sensitivity Analysis of Long-Term Mortality With PET vs AP Among Survivors by Day 100 Post-Transplant

Post hoc Kaplan–Meier survival analyses were conducted in the per-protocol population from the CAPSIL trial [1] using a landmark of 100 days for inclusion, the duration of the CMV prevention intervention. A total of 12 participants were excluded from this analysis population (Supplementary Figure 1). Differences in mortality between arms were assessed using the log-rank test, with a P value $< .05$ considered significant.

Association Between CMV DNAemia and Long-Term Mortality Among Survivors by 12 Months Post-Transplant

Plasma CMV DNAemia at 6 and 12 months post-transplant was retrospectively measured in stored samples from participants who survived 12 months post-transplant using quantitative PCR. The proportion of patients with CMV DNAemia at each time point was compared between the 2 arms using a 2-sample χ^2 test. The distribution of CMV viral loads was compared at each point using the Mann–Whitney U test. Kaplan–Meier curves were constructed and analyzed for participants who survived to 12 months post-transplant. Since previous work has shown that Cox proportional hazards (PH) models incorporating internal longitudinal measurements as time-dependent covariates are mathematically unsound and may

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produce spurious correlations [2, 3], we used time-independent Cox PH models to determine the association of maximum CMV viral load with mortality in this population. Covariates included in the Cox PH model were the maximum viral load from the 6- and 12-month post-transplant samples calculated as log10 (maximum CMV viral load + 1) and treatment arm coded as a binary variable with AP as the referent group (0). Multiple imputation using chained equations was used to impute missing CMV DNAemia values for Cox PH analysis, and a sensitivity analysis using a complete case approach was conducted. Analyses were conducted using the tidyverse, mice, survival, and survminer packages in R (v 4.2.3).

RESULTS

Comparison of Survival Between PET and AP Arms Using Landmark Kaplan–Meier Analyses

Of the 205 randomized participants in the original trial, 193 (94%) completed the 100-day intervention. In this group, estimated survival at 5 years post-transplant was 87% in the PET group vs 74% in the AP group (log-rank $P = .07$; Figure 1*A*). There were 179 of 205 participants (87%) who completed their assigned intervention and survived to 12 months post-transplant; estimated survival was 92% in the PET group vs 79% in the AP group by 5 years post-transplant. Survival was significantly higher among participants in the PET group compared with the AP group (log-rank $P = .021$; Figure 1*B*). The reported causes of death for patients who survived 1-year post-transplant are provided in Supplementary Table 1.

CMV DNAemia at 6 Months and 12 Months Post-Transplant Between PET and AP Arms

The proportion with CMV DNAemia was significantly lower in the PET vs AP arm at both 6 months ($P = .02$) and 12 months ($P = .04$) post-transplant (Figure 1*C*). The distribution of CMV viral loads was significantly different at both time points between the 2 arms ($P = .02$ at 6 months post-transplant and $P = .03$ at 12 months post-transplant, Figure 1*D*). Further details are provided in Supplementary Table 2.

Association of CMV DNAemia With Mortality Among 12-Month Survivors Between PET and AP

Maximum viral load at either 6 or 12 months was significantly associated with mortality after 1 year (adjusted hazards ratio [aHR], 1.55; 95% confidence interval [CI], 1.09–2.2; $P = .03$), while the study arm was not (aHR, 0.33; 95% CI, 0.09–1.23; $P = .12$). The results using a complete case approach were similar for both maximum viral load (aHR, 1.57; $P = .01$) and study arm (aHR, 0.36; $P = .12$).

DISCUSSION

In this post hoc landmark analysis of long-term survival in the CAPSIL trial, long-term mortality was significantly lower in the PET arm compared with the AP arm among 12-month survivors. Both the proportion with late-onset CMV DNAemia and the CMV viral load at 6 and 12 months post-transplant was lower in the PET group, and CMV viral load was associated with increased long-term mortality even when controlling for CMV prevention strategy.

In 2 meta-analyses of PET vs AP in LTxRs, no difference in survival was reported, but both included few studies with follow-up beyond 1 year post-transplant [4, 5]. In contrast, our results that demonstrate improved survival with PET compared with AP are consistent with the long-term follow-up results from a prior randomized trial of PET and AP in kidney transplant recipients [6]. Though our findings from the CAPSIL study mirror the results of the kidney transplant trial, there were significant differences between the 2 studies (type of organ transplant, CMV serostatus, duration of follow-up), so results of our post hoc analyses should be interpreted cautiously and considered hypothesis-generating. These preliminary findings provide compelling rationale for future studies to assess long-term survival in comparative trials of PET and AP.

We identified a quantitative relationship between CMV DNAemia at 6 and 12 months post-transplant and increased long-term mortality. Every 10-fold increase in maximum CMV viral load was associated with a 55% increased risk of subsequent mortality, even after controlling for CMV prevention strategy (PET or AP). This raises the hypothesis that CMV DNAemia may potentially mediate long-term mortality in D + R– LTxRs through previously proposed mechanisms [7, 8], as reported in observational studies in kidney and hematopoietic stem cell transplant settings [9, 10]. If this association is confirmed, characterizing the underlying mechanisms should be a priority of future studies.

In the CAPSIL study, PET was associated with increased CMV-specific immunity compared with AP at the end of the 100-day intervention [1]. The finding of decreased viremia in the PET arm at 6 and 12 months post-transplant is compatible with the hypothesis that the improved CMV-specific immunity with PET may better control CMV DNAemia long-term. CMV DNAemia has been linked to increased inflammation, alloimmune responses, and immune senescence [7], which may lead to increased risk of mortality and underlie the improved long-term survival with PET compared with AP that we identified.

Strengths of the study include the prospective, randomized design of the trial and the analytic approaches used. We acknowledge potential limitations. Although limiting the analysis to the per-protocol population provides an estimate of the difference in outcomes between successful treatments, it can introduce attrition bias and complicate generalization of

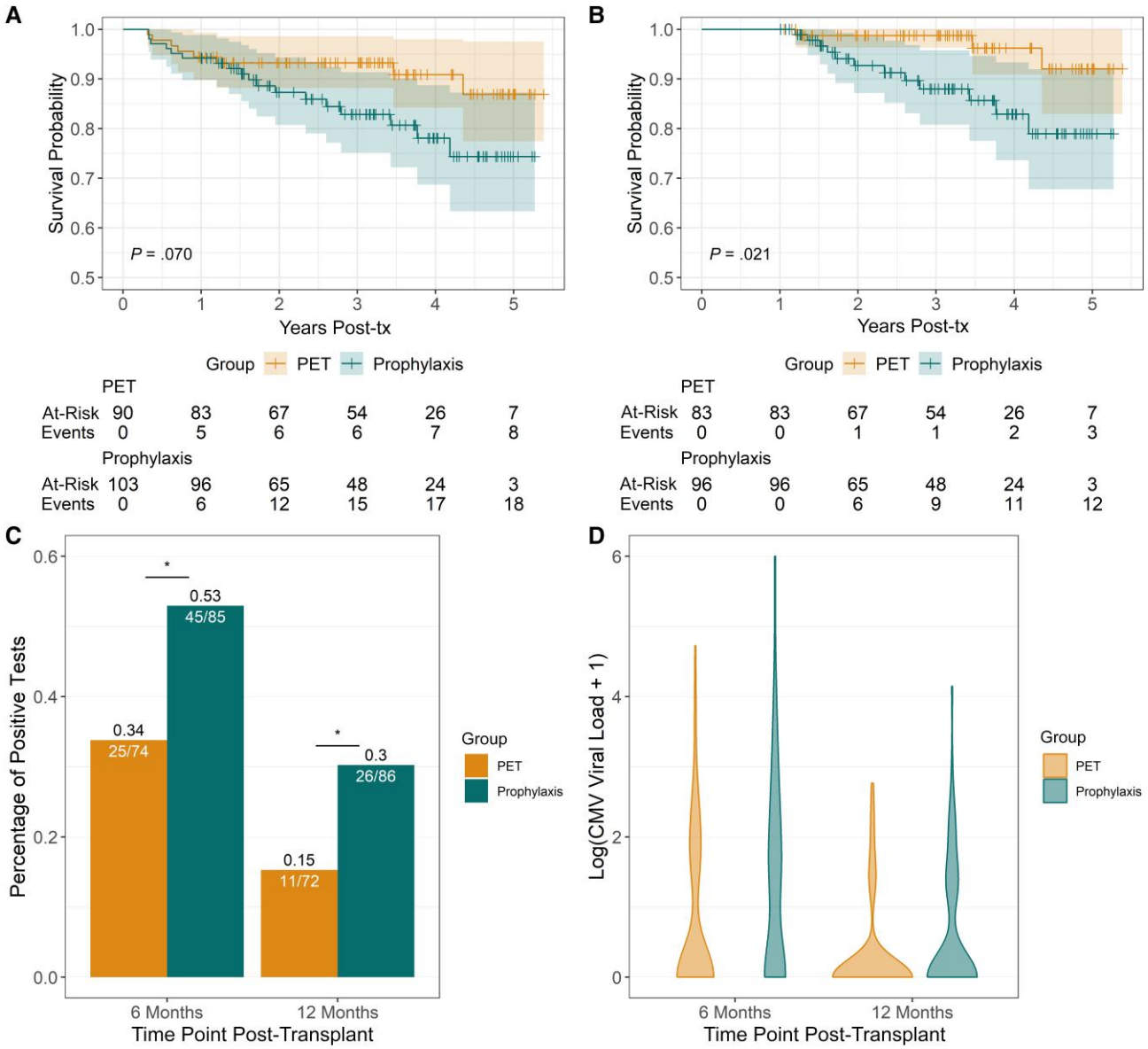

Figure 1. Comparisons of mortality in participants who completed the intervention period and late-onset CMV DNAemia in donor-seropositive, recipient-seronegative liver transplant recipients. *A*, Kaplan–Meier survival curve of participant who survived and completed the assigned intervention. *B*, Kaplan–Meier survival curve of participants who survived to 12 months post-transplant. *C*, Comparison of percent of participants who developed CMV DNAemia at 6 months and 12 months post-transplant. Analysis was limited to participants who survived to 12 months post-transplant. *D*, Violin plots of CMV DNAemia showing distribution of $\log_{10}$ (CMV viral load) at 6 and 12 months post-transplant in participants who survived to 12 months post-transplant. Abbreviations: CMV, cytomegalovirus; PET, preemptive therapy; tx, treatment.

findings to real-world situations where differences in feasibility of the treatment may impact success. There was a significant loss to follow-up over time, limiting confidence around survival estimates at later time points. Further, the overall number of deaths was relatively low. Because of the stochastic nature of CMV replication, episodes of DNAemia could have been missed, leading to misclassification since CMV DNAemia measurements were limited to 2 time points. However, because of the randomized trial study design, there would have had to be differential misclassification between arms to affect the results, which seems unlikely. Additionally, these results in liver transplant may not be generalizable to other solid organ transplant recipients, although similar findings have been reported in other organ transplant settings [9–11].

In summary, this post hoc analysis of a multicenter, randomized trial of PET vs AP for CMV disease prevention demonstrated improved long-term survival with PET in high-risk CMV D + R– LTxRs and an association of CMV DNAemia with worse long-term survival. These findings identify testable hypotheses and provide compelling rationale for a large

head-to-head trial of PET vs AP that includes long-term follow-up for mortality.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. A. P. L. is a consulting and/or site investigator for and has received institutional payments from Merck & Co, GlaxoSmithKline, Moderna, and Takeda Pharmaceuticals; has received institutional support via consulting fees from AiCures and Vera Therapeutics; reports consulting fees from Merck & Co, Moderna, AlloVir, and GlaxoSmithKline; and was reimbursed for participation on a data and safety monitoring board for Novartis, Syneos, and NobelPharma. C. E. F. is a consulting and/or site investigator holding grants or contracts unrelated to the study for Moderna and Amplyx Pharmaceuticals, Inc; has received support from National Institutes of Health (NIH); reports honoraria from NIH for serving as a VIDD study section ad hoc member; has received travel reimbursement from NIH; and was an unpaid participant of the United Network for Organ Sharing Disease Transmission Advisory Committee. C. M. K. has received institutional funding support unrelated to this article from the NIH, Patient-Centered Outcomes Research Institute (PCORI), and University of New Mexico; was reimbursed for travel expenses for participation on a clinical trials advisory panel for PCORI; and has received honoraria for leadership positions as chair for the PCORI Clinical Trials Advisory Panel and co-chair for the Membership Committee of the Society for Clinical Trials. S. D. has received institutional support from NIH (National Cancer Institute/National Institute of Allergy and Infectious Disease), University of Washington, Gilead Sciences, Inc, and Merck & Co and employee travel support from the Fred Hutchinson Cancer Center. All other authors report no potential conflicts.

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