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LETTER TO THE EDITORS

Re-examining the relationship between active cytomegalovirus (CMV) infection and acute graft-versus-host disease in allogeneic stem cell transplant recipients in the era of real-time PCR CMV assays

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Dear Editors,

A bidirectional interaction between active cytomegalovirus (CMV) infection and acute graft-versus-host disease (aGvHD) is pathogenetically feasible given the pro-inflammatory nature of CMV and the immunosuppressive effect directly attributable to aGvHD [1]. Contradictory data have been published on this issue [2–5]. The use of methods for CMV surveillance displaying different sensitivities (pp65 antigenemia assay (AG) vs. real-time PCR assays) may account in part for these discrepancies [6]. In this single-center retrospective study, we re-examined this potential association using highly sensitive real-time PCR assays (limit of detection: approximately 20 copies/ml-[6–8]) (CMV PCR Kit and the New CMV Real*Time* PCR assay; Abbott Molecular, Des Plaines, IL, USA) in plasma specimens. We included 92 nonconsecutive patients undergoing Allo-SCT (January 2010-August 2014) for hematological malignancy.

Pre-emptive antiviral therapy was administered as previously reported [6–8]. Acute aGvHD was defined, graded, and treated as previously described [9].

Seventy-six patients (82.6%) developed CMV DNAemia (median, 29.5 days; range, 4-147 days). Thirty-seven were treated with antivirals. aGvHD developed in 40 patients (43.5%) (median, 61 days; range, 8–180 days). The cumulative incidence (CI) of aGvHD was comparable in patients with or without a preceding episode of CMV DNAemia ($P = 0.33$ for aGvHD irrespective of its severity—CI, 40%; $P = 0.34$ for aGvHD grades II-IV—Fig. 1a; $P = 0.94$ for aGvHD grades III-IV—CI, 13%). Similar results were obtained for CMV DNAemia episodes requiring antiviral treatment (Fig. 1b). The time to antiviral treatment initiation result was comparable regardless of whether aGvHD did subsequently develop ($P = 0.69$, Mann–Whitney *U*-test). The magnitude of viral replication within episodes of active CMV infection, as inferred by the plasma CMV DNA peak load, was also comparable regardless of whether aGvHD did develop later or not (median 3140 IU/ml; range, 1490–6790, and median, 3160 IU/ml; range, 1380–5920, respectively; $P = 0.96$, Mann–Whitney *U*-test). Our data are in line with those of Wang *et al.*[4], who used the pp65 antigenemia assay (AG), which is less sensitive than real-time PCR assays, [6] and those of Gotoh *et al.*[5], who used plasma real-time PCR, but are in contradiction with those published by Cantoni *et al.*[2]. In the latter study, the AG assay was employed for CMV surveillance.

The cumulative incidence of active CMV infection was not significantly different between patients with or without a preceding diagnosis of aGvHD irrespective of aGvHD disease grading ($P = 0.89$; Fig. 1 panel c). The duration of the episodes and the magnitude of CMV DNA peak load within episodes appeared to be comparable regardless of whether these were preceded (median 40 days; range, 7–89 days/median, 3090 IU/ml; range 1490–6800) or not (41 days; range, 7–142/median, 3606 IU/ml; range, 1380–6262) by the occurrence of aGvHD ($P = 0.90$ and $P = 0.94$, respectively, by the Mann–Whitney *U*-test). Cantoni *et al.* [2] found hazard ratios of CMV replication of 1.61 for patients with preceding aGvHD grades II-IV. George *et al.* [3] reported an increased risk of CMV replication only in a subset of Allo-SCT presenting aGvHD, those at intermediate risk for CMV replication according to demographic or pretransplant factors. Given the discrepant data reported in the literature, large, prospective, and adequately powered studies employing sensitive real-time PCR assays for CMV monitoring are needed to elucidate this matter.

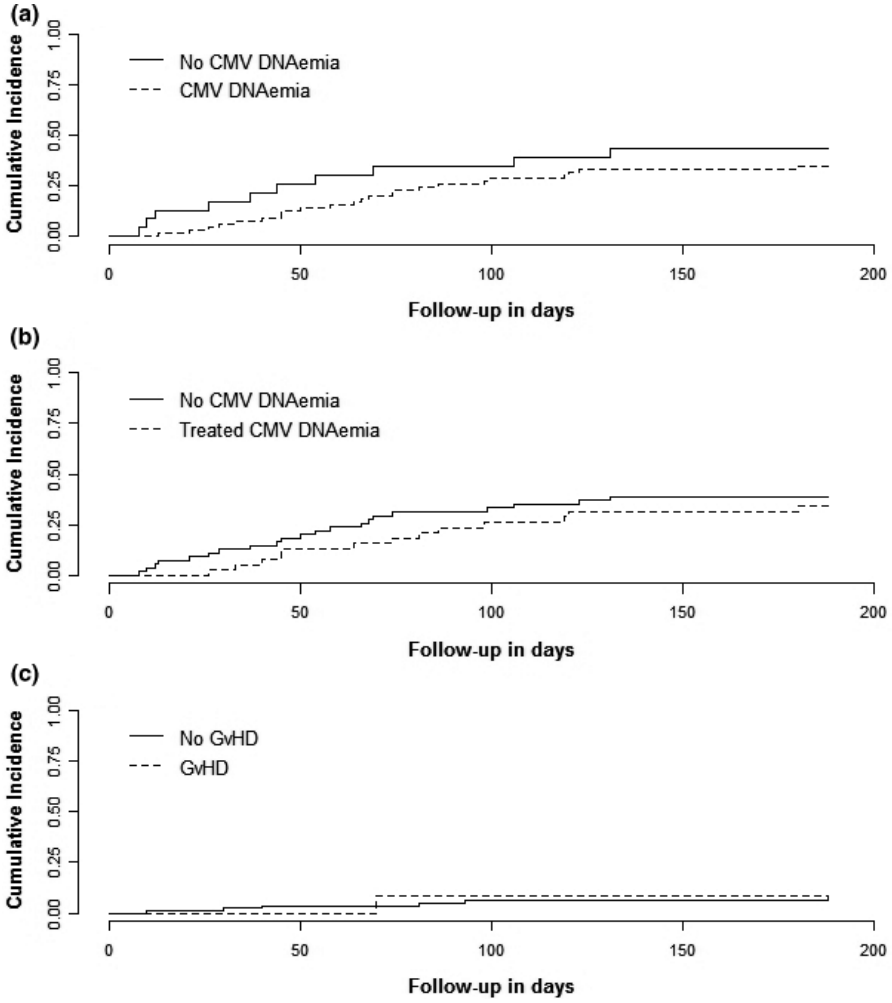

Figure 1 Analysis of the potential interaction between active cytomegalovirus (CMV) infection and acute graft-versus-host disease (aGvHD) in allogeneic stem cell transplant recipients (Allo-SCT). A total of 92 patients were included (median age, 52 years; range, 15–69 years; 55 males and 37 females). The source of the allograft was as follows: peripheral blood (80.4%), umbilical cord blood (15.2%), and bone marrow (4.3%). The type of the allograft was HLA matched and related to 76.1% and 47.8% of patients, respectively. The conditioning regimen was nonmyeloablative in 69.6% of patients; graft vs. host disease prophylaxis was as follows: cyclosporin A/prednisone (10.9%), cyclosporin A/methotrexate (38.0%), cyclosporin A/mycophenolate mofetil (13.0%), tacrolimus/sirolimus (32.6%), and other combinations (5.5%). CMV serostatus of donor (D) and recipient (R) was D+/R+ (56.5%), D−/R+ (37.0%), and D+/R− (6.5%). The protocol of this study was approved by the Institutional Review Board at Hospital Clínico Universitario, Fundación INCLIVA, Valencia, Spain. (a) Cumulative incidence of aGvHD grades II-IV in patients with or without a preceding episode of CMV DNAemia (first, $n = 72$; recurrent, $n = 4$) during the first 180 days after transplant. CMV DNAemia preceded the onset of aGVHD in 24 patients. In 12 of the 24 patients, the diagnosis of aGvHD was made during the episode of CMV DNAemia. In the remaining 12 cases, the diagnosis of aGvHD was made a median of 49 days after CMV DNAemia clearance (range, 5–99 days). In two additional patients, the diagnosis of aGvHD was made at the time of detection of CMV DNAemia. CMV DNAemia episodes developed at a median 30 days (range, 4–140 days) for episodes that preceded the occurrence of aGvHD and at a median 28 days (range 2–147 days) for episodes that did not precede the diagnosis of aGvHD ($P = 0.80$). Relapse (cumulative incidence of 23% at day +180), graft failure, and early death were considered as competitive events for aGvHD. (b) Cumulative incidence of aGvHD grades II-IV in patients with or without a preceding episode of active CMV infection treated pre-emptively with antivirals during the first 180 days after transplant. (c) Cumulative incidence of CMV DNAemia (during the first 180 days after transplant) in patients with or without a preceding episode of aGvHD of any severity (grades I-IV). The diagnosis of aGvHD disease preceded the onset of CMV DNAemia in seven patients (a median of 8 days; range, 1–33 days). In two additional patients, the diagnosis of aGvHD was made at the time of detection of CMV DNAemia. The relapse of the underlying disease before the detection of CMV DNAemia was considered a competitive event for active CMV infection. Active CMV infection and aGvHD were taken as time-dependent covariates, and the analyses were performed by competing risk regression [10] using the statistical software R (http://www.r-project.org/). Two-sided *P*-values <0.05 were deemed to be significant.

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Conflicts of interest

The authors report no potential conflict of interests.

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