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ORIGINAL ARTICLE

Lack of evidence for a reciprocal interaction between bacterial and cytomegalovirus infection in the allogeneic stem cell transplantation setting

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SUMMARY

Pathogenic interactions between bacteria and cytomegalovirus (CMV) may potentially occur early after allogeneic stem cell transplantation (allo-SCT). This possibility nevertheless has not been investigated in depth. This was a retrospective study that included 170 consecutive patients who underwent 173 allo-SCTs. Both bacterial infection (most of which were bacteremic) and CMV DNAemia were detected in 78 allo-SCTs (62.9%). In total, 51 and 32 episodes of bacterial infection preceded or occurred after CMV DNAemia detection, respectively. Both events were diagnosed concurrently in four allo-SCTs. The cumulative incidence of bacterial infection (of any type) over the study period was comparable in patients with or without a preceding episode of CMV DNAemia ($P = 0.321$). Cox proportional hazards regression analysis failed to identify CMV DNAemia as a significant risk factor for bacterial infection. Likewise, the cumulative incidence of CMV DNAemia within the study period was not significantly different in patients with or without a preceding episode of bacterial infection ($P = 0.189$). Furthermore, the occurrence of bacterial infection within episodes of active CMV infection had no apparent impact on the kinetics of CMV DNAemia. Our data, thus, do not support the existence of a bidirectional synergistic effect between bacterial infection and active CMV infection in the allo-SCT setting.

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Key words
allogeneic stem cell transplantation, bacteremia, bacterial infection, CMV DNAemia, cytomegalovirus

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Introduction

Recovery of innate and adaptive immune cell types after allogeneic stem cell transplantation (Allo-SCT) follows different kinetic profiles [1]. Neutrophils, which are depleted as a result of the conditioning regimen, recover at 14–30 days depending upon the source of stem cells. During the neutropenic phase, extracellular bacteria are mainly responsible of infectious complications [2]. The use of high doses of parenteral corticosteroids for the treatment of severe acute graft-versus-host disease (aGvHD) further increases the risk of local or systemic bacterial infection [2]. In turn, reconstitution of adaptive T- and NK-cell immunity is crucial for the control of virus infection. The first 100 days after allo-SCT are characterized by immune deficiencies affecting these cell

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subsets [1] that render the patient highly susceptible to infection caused by viruses, particularly those establishing chronic-persistent infections such as cytomegalovirus (CMV) [3]. In this scenario, pathogenic interactions between bacteria and viruses may conceivably occur. The potential role of bacterial infection in promoting active CMV infection in the allo-SCT setting has not been thoroughly investigated. In turn, CMV readily infects macrophages *in vivo*, impairing their ability to recognize and eliminate bacteria by phagocytosis [4]. Furthermore, infection of endothelial cells by CMV may facilitate bacterial invasion across mucosal barriers [5]. Thus, CMV replication may potentially increase the risk of organ-specific or systemic bacterial infection in the allo-SCT setting. This possibility, nevertheless, is mainly supported by indirect evidence [6–8]. The current study aimed to investigate whether a synergistic interaction between bacteria and CMV does occur early following allo-SCT. As an advantage over previous studies directly or indirectly addressing this issue, highly sensitive real-time PCR assays were employed for CMV surveillance.

Subjects and methods

Patients

This retrospective study included 170 nonconsecutive patients who underwent 173 allo-SCT at the Hematology Unit of the Hospital Clínico Universitario of Valencia from February 2006 to April 2014. CMV-seronegative patients receiving an allograft from CMV-seronegative donors were excluded from the study, as the incidence of CMV DNAemia in this group is negligible. The median age of patients was 48 years (range, 18–70 years). Relevant clinical and demographic data of the patients are summarized in Table 1. The study period comprised the first 60 days following transplantation, a time frame at which both bacterial infection and CMV DNAemia occur more frequently. The study was approved by the local review board and ethics committee. All patients gave written informed consent prior to participation in the study.

Table 1. Demographic characteristics of the patients.

Parameter	No. of patients (%)*
Sex	
Male	106 (62.3)
Female	64 (37.7)
Underlying disease	
Acute myeloid leukaemia	64 (37.7)
Non-Hodgkin's lymphoma	41 (24.1)
Myelodysplastic syndrome	8 (4.7)
Acute lymphocytic leukaemia	14 (8.2)
Chronic lymphocytic leukaemia	13 (7.7)
Multiple Myeloma	6 (3.5)
Hodgkin's lymphoma	8 (4.7)
Aplastic Anemia	1 (0.6)
Others	15 (8.8)
HLA-matching	
Matched	132 (76.3)
Mismatched	41 (23.7)
Donor type	
Related	87 (50.3)
Unrelated	86 (49.7)
Stem cell source	
Peripheral blood	131 (75.7)
Umbilical cord blood	35 (20.2)
Bone marrow	7 (4.1)
Conditioning regimen	
Myeloablative	68 (39.3)
Nonmyeloablative	105 (60.7)
Graft-versus-host disease prophylaxis	
Cyclosporin A/methotrexate	70 (40.5)
Cyclosporin A/mycophenolate mofetil	31 (17.9)
Other combinations	72 (41.6)
CMV serostatus	
D+/R+	99 (57.2)
D−/R+	64 (37)
D+/R−	10 (5.8)
Acute Graft-versus-host disease developing during the study period (days 0–60)	
0–I	147 (85.0)
II–IV	26 (15.0)

CMV, cytomegalovirus; D, donor; HLA, human leukocyte antigen; R, recipient.

*A total of 170 patients undergoing 173 allogeneic stem cell transplants were included in the study.

Definition and management of bacterial infection

All patients were attended in HEPA-filtered positive air pressure rooms until engraftment and harbored an indwelling central venous catheter of the Hickman type. All patients received antibacterial prophylaxis with fluoroquinolones. Two sets of blood cultures (BD BACTEC™ Plus Aerobic/F and BD BACTEC™ Plus Anaerobic/F; Becton, Dickinson and Company, Sparks, MD, USA) were drawn upon occurrence of fever (>37 °C) and/or the presence of clinical signs or symptoms of infection. Empirical antibacterial treatment was then initiated, in most cases with a third-generation cephalosporin and aminoglycoside or carbapenem. Antibiotic treatment was adapted to antimicrobial susceptibility testing results when deemed appropriate.

Blood cultures were obtained on a daily basis until resolution of the episode. A bloodstream infection was defined as: (i) the isolation of one or more recognized pathogenic bacteria (e.g., *Staphylococcus aureus*, *Enterobacteriaceae* species, *Enterococcus* species, *and Pseudomonas aeruginosa*) from one or more blood cultures, and (ii) the isolation of the same potential contaminant (e.g., coagulase-negative *Staphylococcus* species, *Streptococcus* species) from two or more blood cultures drawn on separate occasions within a 48-h period [9,10]. Urinary tract, gastrointestinal, lower respiratory tract, skin and soft tissue and skeletal infections were defined following Infectious Diseases Society of America (IDSA) criteria (http://www.idsociety.org/Organ_System/) and were diagnosed on the basis of the 2013 recommendations of the IDSA and the American Society for Microbiology [11]. Primary and recurrent episodes (those occurring after negative cultures from the site of infection) of bacterial infection were considered for the analyses reported herein.

Management of CMV infection

From February 2006 to May 2010, patients were monitored for CMV infection by the pp65 antigenemia assay (AG) and the CMV real-time PCR Kit (Abbott Molecular, Des Plaines, IL, USA) (once a week), although the administration of preemptive antiviral therapy was guided solely by the AG assay (>1 positive pp65 cells/200 000 polymorphonuclear leukocytes). From May 2010 to May 2012, CMV surveillance was exclusively performed by real-time PCR (CMV real-time PCR Kit) and antiviral therapy was initiated when the plasma CMV DNA load reached >500 copies/ml (Abbott CMV real-time PCR, Abbott Molecular, Des Plaines, IL, USA) [12]. Since May 2012, the CMV DNA load threshold for the initiation of antiviral therapy was set at 1000 copies/ml, as determined by the new CMV real-time PCR (Abbott Molecular, Des Plaines, IL, USA) [13]. Preemptive antiviral therapy was administered following previously detailed protocols [12]. CMV DNAemia (active CMV infection) was defined by the detection of any level of CMV DNA in plasma. The duration of a given episode of CMV DNAemia was defined as the interval between the day of the first positive PCR result and the day of the first negative (undetectable) result.

Statistical analysis

The relationship between bacterial and active CMV infection was assessed by treating these post-transplant events as time-dependent covariates using competing risk regression [14]. These analyses were run using the statistical software R (http://www.r-project.org/). The relapse of underlying disease and early death were considered as competitive events for both active CMV infection and bacterial infection. Cox proportional hazards regression reporting hazard ratios (HR) and 95% confidence intervals (CI) were used in the univariate analyses and the multivariate analysis of potential risk factors for the development of active CMV infection and bacterial infection, including baseline parameters such as the type of allo-SCT (related versus unrelated/HLA-matched versus HLA-mismatched), the source of stem cells, the conditioning regimen, and the GvHD prophylaxis regimen. All post-transplant events including aGvHD, neutropenia after the engraftment, bacterial infection, and CMV DNAemia were entered as time-dependent covariates. For multivariate analyses, only variables with parameter estimates showing a P value ≤ 0.10 in the univariate analyses were included. Median values (days, CMV DNA loads) were compared by means of the Mann–Whitney U-test. Two-sided P-values <0.05 were deemed to be significant. The latter statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

Incidence of bacterial infection and CMV DNAemia

Bacterial infection was documented in 98 of 173 allo-SCTs (56.6%) within the study period, at a median of 8 days (range, 0–59 days) after transplant. Nine patients had a second bacterial infection episode at a median of 43 days after transplant (range, 7–58 days); thus, in total, 107 episodes were registered. All but one episode were monomicrobial. Seventy-one were caused by Gram-positive bacteria and 35 by Gram-negative rods. Eighty episodes (74.7%) were bloodstream infections. The type of infection and the spectrum of bacteria isolated are shown in Table 2.

CMV DNAemia was detected in 124 of 173 allo-SCTs (71.7%), at a median of 26 days after transplant (range, 6–60 days). Preemptive antiviral therapy was administered in 86 of these episodes (69.4%). CMV DNAemia lasted a median of 42 days (range, 2–259 days). The median CMV DNA load peak level during episodes was 1715 IU/ml (range, 35–1 431 756 IU/ml). No recurrent episodes of CMV DNAemia were detected within the study period.

As shown in Table 3, both bacterial infection and CMV DNAemia were detected in 78 allo-SCTs (62.9%).

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Table 2. Type of bacterial infection and spectrum of bacteria isolated (monomicrobial infections).

Type of infection	No. (%)
Bloodstream infections	76 (71.0)
Coagulase negative *Staphylococcus* spp.	48 (63.1)
Enterobacteriaceae	12 (15.8)
Enterococcus spp.	7 (9.2)
Streptococcus spp.	3 (4.0)
Pseudomonas aeruginosa	2 (2.6)
Other Gram-negative bacteria	3 (4.0)
Anaerobials	1 (1.3)
Urinary tract infections	15 (14.0)
Enterobacteriaceae	11 (73.3)
Enterococcus spp.	3 (20.0)
Other Gram negatives	1 (6.7)
Respiratory tract infections	2 (1.9)
Staphylococcus aureus	1 (50.0)
Moraxella catarrhalis	1 (50.0)
Gastrointestinal infections	4 (3.7)
Salmonella spp.	3 (75.0)
Clostridium difficile	1 (25.0)
Skin, soft-tissue or skeletal infections	10 (9.4)
Coagulase-negative *Staphylococcus*	5 (50.0)
Enterococcus spp.	1 (10.0)
Streptococcus anginosus	2 (20.0)
Pseudomonas aeruginosa	1 (10.0)

In 42 patients, bacterial infection occurred prior to CMV DNAemia, while in 23 patients, CMV DNAemia preceded bacterial infection, and in four patients, both events developed concomitantly (these episodes were analyzed bidirectionally). The remaining nine patients had two episodes of bacterial infection, one occurring prior to the diagnosis of CMV DNAemia and another developing after it. Thus, in total, final figures taken for analyses shown below were 55 (for episodes of bacterial infection occurring prior to CMV DNAemia) and 36 (for episodes occurring after CMV DNAemia). Cumulative incidence curves of both CMV DNAemia and bacterial infection within the study period are shown in Figure S1.

Table 3. Occurrence of bacterial infection and CMV DNAemia in the study cohort.

	No. of Allo-SCT (%)
No CMV DNAemia	49 (28.3)
No bacterial infection	29 (59.2)
Bacterial infection	20 (40.8)
CMV DNAemia	124 (71.7)
No bacterial infection	46 (37.1)
Bacterial infection	78 (62.9)
Prior to CMV DNAemia	42 (53.8)
After CMV DNAemia	23 (29.4)
Concurrent to CMV DNAemia	4 (5.1)
Bacterial infection prior and after CMV DNAemia	9 (11.5)

CMV, cytomegalovirus.

Six patients died within the study period (at days +10, $n = 2$, +24, +26, +54, +58). The cause of death was severe aGvHD ($n = 1$), septic shock ($n = 2$), and nosocomial pneumonia due to *Pseudomonas aeruginosa*, and it was not ascertained in the remaining two cases.

DNAemia as a risk factor for bacterial infection

CMV DNAemia preceded the occurrence of bacterial infection in 36 allo-SCTs (75% of which were bacteremic infections). Gram-positive bacteria were involved in 27 of these episodes (all but one monomicrobial), and Gram-negative rods were responsible for the remaining cases. The median time from CMV DNAemia detection to the documentation of bacterial infection was 25.5 days (range, 0–59 days). As shown in Fig. 1a, the cumulative incidence of bacterial infection (of any type) over the study period (from the day of cell infusion to day +60 after transplant) was overall comparable in patients with or without a preceding episode of CMV DNAemia ($P = 0.552$). Likewise, the cumulative incidence of bacterial infection was not significantly different ($P = 0.271$) when the analysis was restricted to those bacterial infection episodes occurring near the time of documentation of CMV DNAemia (within 15 days; $n = 26$) (Fig. 1b). We further assessed the potential effect of CMV DNAemia on the development of bacterial infection by conducting a landmark time-point analysis. For this analysis, the baseline time point was set at day +30, as this was the median time from transplant to documentation of CMV DNAemia. Again, the incidence of bacterial infection was similar irrespective of whether or not a preceding episode of CMV DNAemia did occur ($P = 0.390$) (Figure S2).

The median CMV DNA peak load within episodes of active CMV infection was comparable ($P = 0.248$) irrespective of whether bacterial infection did (2215 IU/ml) or did not (860 IU/ml) develop subsequently. As shown in Table 4, the occurrence of CMV DNAemia was not found to be a risk factor for bacterial infection in the univariate Cox regression analysis. In our cohort, none of the pre- or post-transplant parameter included in the univariate models was found to associate significantly

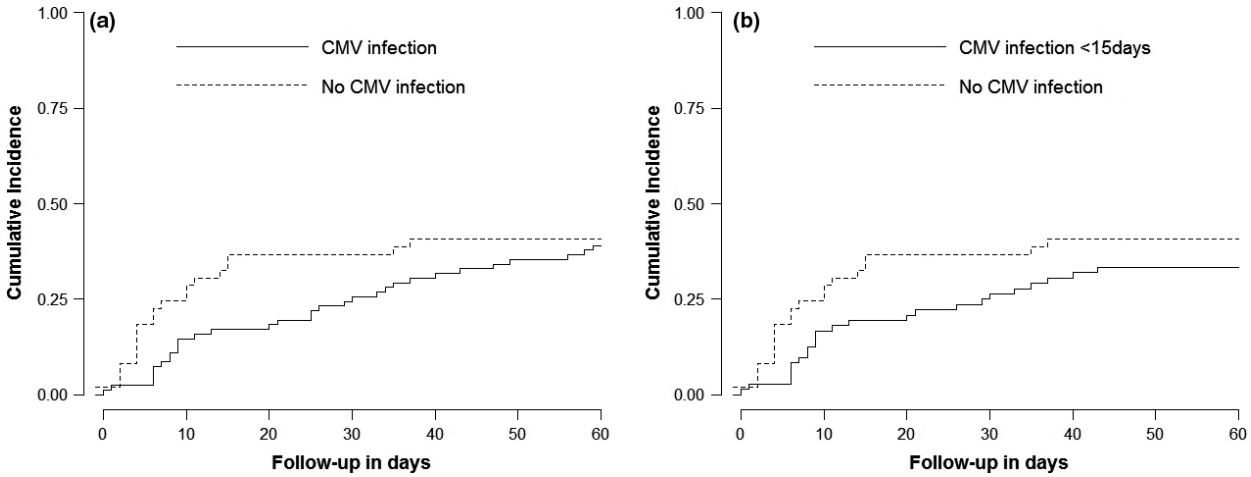

Figure 1 Impact of CMV DNAemia on the risk of subsequent bacterial infection in allogeneic stem cell transplant recipients (allo-SCT). (a) Overall cumulative incidence of bacterial infection from the day of hematopoietic stem cells infusion (day 0) to day +60 after transplant in patients with or without a preceding episode of CMV DNAemia. (b) Cumulative incidence of bacterial infection in patients with or without a preceding episode of CMV DNAemia (this occurring a maximum of 15 days earlier).

with the occurrence of bacterial infection in multivariate models (Table 4).

Occurrence of bacterial infection and risk of subsequent CMV DNAemia

The potential effect of bacterial infection in promoting CMV DNAemia was investigated next. Bacterial infection preceded CMV DNAemia in 55 allo-SCTs. These infections were caused by Gram-positive organisms in 38 cases and by Gram-negative rods in the remaining 17 cases and were diagnosed at a median of 7 days after transplant (range, 0–41 days). Of these episodes, 40 were bacteremic. As shown in Fig. 2a, the cumulative incidence of CMV DNAemia within the study period was not significantly different in patients with or without a preceding episode of bacterial infection ($P = 0.207$). This was also the case when bacterial infections caused by Gram-positive and Gram-negative bacteria were considered separately ($P = 0.156$ and $P = 0.319$, respectively; Fig. 2b), or when only bloodstream infections were included in the analysis ($P = 0.342$; Fig. 2c). Likewise, the cumulative incidence of CMV DNAemia was also comparable ($P = 0.586$; Fig. 2d) when only episodes developing closely after bacterial infection (within 15 days; $n = 25$) were taken into consideration for the analysis. Landmark time-point analyses seemed to confirm the above

Table 4. Risk factors for the development of bacterial infection in a cohort of allogeneic stem cell transplant recipients.

	Univariate		Multivariate	
Factor	HR (95% CI)	*P* value	HR (95% CI)	*P* value
HLA-matching	2.12 (1.37–3.29)	0.001	1.87 (0.60–5.78)	0.277
Donor type (unrelated versus related)	1.12 (0.74–1.70)	0.577		
Stem cell source				
Umbilical cord blood versus Peripheral blood	1.96 (1.23–3.12)	0.005	1.84 (0.54–6.25)	0.327
Conditioning regimen (myeloablative versus nonmyeloablative)	1.50 (0.99–2.27)	0.053	0.57 (0.21–1.54)	0.270
Graft-versus-host disease prophylaxis				
CyA-MTX versus Others	0.72 (0.46–1.14)	0.160		
CyA-MMF versus Others	0.84 (0.47–1.50)	0.568		
Acute graft-versus-host disease (II–IV versus 0–I)	2.64 (1.07–6.53)	0.035	0.54 (0.24–1.23)	0.973
CMV DNAemia	1.00 (0.57–1.75)	0.993		
Neutropenia post engraftment (after day +21 after Allo-SCT)	2.24 (0.91–5.52)	0.080	1.85 (0.68–5.02)	0.229

CyA, cyclosporin A; CMV, cytomegalovirus; MTX, methotrexate; MMF, mycophenolate mofetil.

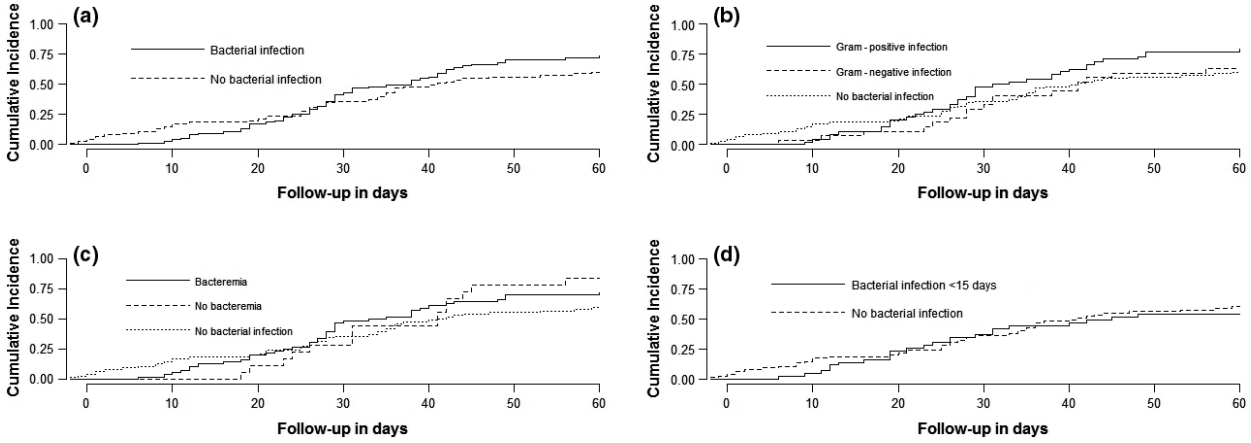

Figure 2 Analysis of the potential effect of bacterial infection on the risk of subsequent CMV DNAemia in allogeneic stem cell transplant recipients (allo-SCT). (a) Overall cumulative incidence of CMV DNAemia in patients with or without a preceding episode of bacterial infection from the day of hematopoietic stem cells infusion (day 0) to day +60 after transplant. (b) Cumulative incidence of CMV DNAemia in patients with or without a preceding episode of bacterial infection caused by either Gram-positive or Gram-negative bacteria. (c) Cumulative incidence of CMV DNAemia in patients with or without a preceding episode of a bloodstream bacterial infection. (d) Cumulative incidence of CMV DNAemia in patients with or without a preceding episode of bacterial infection (this occurring a maximum of 15 days earlier).

observations. For these analyses, two base line time points were chosen: day +7, time at which the incidence of bacterial infection is high, and day +30 to mimic the time point selected for evaluating the impact of CMV DNAemia on the occurrence of bacterial infection. In effect, the cumulative incidence of CMV DNAemia was comparable in patients with or without a preceding episode of bacterial infection ($P = 0.151$ at day +7, and $P = 0.164$ at day +30). (Figure S3).

The median CMV DNA peak load within episodes of active CMV infection was comparable ($P = 0.407$) irrespective of whether these were preceded (1883 IU/ml) or not (860 IU/ml) by a bacterial infection. Neither bacterial infection nor other pre-or post-transplant factors were found to be a significant risk factor for CMV DNAemia in our cohort (Table 5).

Effect of bacterial infection on the kinetics of ongoing episodes of CMV DNAemia

We next investigated whether the occurrence of a bacterial infection within the episodes of active CMV infection ($n = 25$) had any effect on the kinetics of plasma CMV DNAemia. The data are shown in Table 6. Both the plasma CMV DNA peak load within episodes of CMV DNAemia and the duration of CMV DNAemia were comparable regardless of whether or not a bacterial infection occurred. Likewise, the number of episodes that required the administration of antiviral therapy was similar in both comparison groups, regardless of the criteria used for triggering the inception of therapy (pp65 antigenemia or real-time PCR results; $P = 0.22$ for pp65 antigenemia and $P = 0.389$ for real-time PCR).

Discussion

It has long been suggested that CMV may promote bacterial superinfection in allo-SCT recipients [6–8]. Although mechanistically plausible [4,5], mostly indirect evidence supports this assumption. In this sense, Broers *et al.* [6] showed that CMV-seropositive allo-SCT patients receiving T-cell-depleted allografts had an increased risk of bacterial infection (mostly pulmonary infection) as compared to CMV D-/R- patients. Likewise, Craddock *et al.* [7] reported a similar phenomenon in the unrelated T-cell-depleted setting. Furthermore, Nichols *et al.* [8] observed a high risk of death due to bacterial and fungal infection in CMV-seronegative patients receiving an allograft from CMV-seropositive donors that could not be linked to the occurrence of ganciclovir-induced neutropenia. Notably, these authors found no association between the presence of pp65 antigenemia and these infectious complications. In contrast, Capellano *et al.* [15] identified positive pp65 antigenemia as a significant risk factor for bloodstream bacterial infection in multivariate models. Nevertheless, the antigenemia assay is markedly less sensitive than real-time PCR assays [16], so it may fail to detect episodes of active CMV infection in which the virus replicates at a low level and is cleared without the need for antiviral therapy. This could be of relevance,

Table 5. Risk factors for the development of cytomegalovirus DNAemia in a cohort of allogeneic stem cell transplant recipients.

	Univariate		Multivariate	
Factor	HR (95% CI)	*P* value	HR (95% CI)	*P* value
HLA-matching	1.87 (1.25–2.80)	0.002	1.70 (0.97–2.97)	0.066
Donor type (unrelated versus related)	1.81 (1.24–2.65)	0.002	1.65 (0.96–2.86)	0.072
Stem cell source				
Umbilical cord blood versus Peripheral blood	1.94 (1.26–2.97)	0.002	0.80 (0.37–1.69)	0.562
Conditioning regimen (myeloablative versus nonmyeloablative)	1.71 (1.18–2.49)	0.005	1.45 (0.87–2.43)	0.154
Graft-versus-host disease prophylaxis				
CyA-MTX versus Others	0.65 (0.43–0.99)	0.045	0.99 (0.56–1.77)	0.964
CyA-MMF versus Others	1.18 (0.74–1.90)	0.483		
Acute graft-versus-host disease (II–IV versus 0–I)	1.38 (0.69–2.73)	0.361		
CMV serostatus				
D+/R+ versus D+/R−	1.83 (0.73–4.55)	0.194		
D−/R+ versus D+/R−	2.15 (0.85–5.41)	0.105		
Bacterial infection	0.76 (0.51–1.13)	0.169	0.87 (0.58–1.35)	0.572

CyA, cyclosporin A; CMV, cytomegalovirus; MMT, methotrexate; MMF, mycophenolate mofetil; D, donor; R, recipient.

Table 6. Effect of bacterial infection occurring within episodes of active CMV infection on the dynamics of CMV DNAemia.

	Bacterial infection		
Parameter	Yes (n = 25)	No (n = 48)	*P* value*
Need of antiviral treatment, no. (%)			
Yes	18 (72)	30 (62.5)	0.324
No	7 (28)	18 (37.5)	
CMV episode duration, median days, (range)	44 (8–155)	49 (4–257)	0.508
Median CMV DNA peak load in log IU/ml (range)	3.41 (1.47–4.80)	2.84 (0.89–6.15)	0.252

CMV, Cytomegalovirus.

*Differences between medians were compared using the Mann–Whitney *U*-test. Frequency comparisons for categorical variables were carried out using the chi-square test (Fisher's exact test). Two-sided exact *P* values are reported. A *P* value <0.05 was considered statistically significant.

inasmuch as low-level CMV replication may potentially exert immunomodulatory effects. In this context, we sought to examine this pathogenic possibility in a cohort of allo-SCT recipients who were systematically monitored for the presence of CMV in blood by means of highly sensitive real-time PCR assays. Our data do not support the idea that active CMV infection is a promoting factor for bacterial infection early after transplant. First, the cumulative incidence of bacterial infections, most of which were bacteremic, was similar in patients with or without a preceding episode of CMV DNAemia. Second, the magnitude of CMV replication within episodes of active CMV infection, as inferred by the plasma CMV DNA peak load, had no apparent impact on the risk of subsequent bacterial infection. Third, Cox regression models failed to identify CMV DNAemia as a significant risk factor for bacterial infection. In support of our view, Boeckh *et al.* [17] recently reported that valganciclovir prophylaxis, which abrogates CMV replication, was not superior in reducing the incidence of complications of late cytomegalovirus infection (composite end point of CMV disease, invasive bacterial or fungal disease, or death) when compared with polymerase chain reaction-guided preemptive therapy. Bacterial components such as lipopolysaccharide as well as inflammatory mediators such as tumor necrosis factor-α, or interleukin-1β, trigger the reactivation of latent murine CMV infection [18]. Furthermore, sepsis of bacterial origin has been identified as a risk factor for active CMV infection in

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solid organ transplant recipients [19,20] and in critically ill patients [21]. We found, nevertheless, no evidence pointing to bacterial infection as being a relevant risk factor for the occurrence of CMV DNAemia. Neither the cumulative incidence nor the dynamics of CMV DNAemia within episodes of active CMV infection appeared to be influenced by the presence or absence of a preceding episode of bacterial infection. Furthermore, the occurrence of bacterial infection within episodes of active CMV infection had no apparent impact on the kinetics of CMV DNAemia. In fact, the number of episodes that required the inception of preemptive antiviral therapy was similar regardless of whether bacterial infection either developed or not.

The major limitations of the present study are the relatively scarce number of patients experiencing both bacterial and active CMV infections in the cohort and its retrospective nature. Other limitations that deserve comment are the following: (i) the heterogeneity of the patient cohort, (ii) the inclusion of different types of bacterial infections (most of them were nevertheless bloodstream infections), (iii) the fact that local interactions between bacteria and CMV potentially occurring in tissues or organs (and not leading to viremia) were inevitably missed, and (iv) the use of antibacterial prophylaxis, which may have masked naturally occurring interactions between bacteria and CMV. In summary, our data do not support the existence of a bidirectional synergistic effect between bacterial infection and active CMV infection in the allo-SCT setting. Nevertheless, prospective and more homogeneous studies involving larger cohorts are needed to conclusively settle this issue.

Authorship

VV and EG: performed PCR analyses and assisted in the analysis of the data. CS, JLP, JCH-B and PA: contributed to the analysis of the data and attended the patients. DN: designed the study, analyzed the data, and wrote the manuscript. All authors critically reviewed the manuscript.

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Conflict of interest

The authors have declared no conflicts of interest.

Ethical approval

This study was approved by the Ethics Committee of Hospital Clínico Universitario de Valencia, Fundación INCLIVA.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Overall cumulative incidence of bacterial infection and CMV DNAemia from the day of hematopoietic stem cells infusion (day 0) to day + after transplant.

Figure S2. Cumulative incidence of bacterial infection from day +30 to day +60 after allogeneic stem cell transplantation in patients with or without a prior episode of CMV DNAemia.

Figure S3. Cumulative incidence of CMV DNAemia from day +7 to day +60 after transplant (A), and from day +30 to day +60 after transplant (B) in allogeneic stem cell transplant patients with or without a preceding episode of bacterial infection.

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