Medical Microbiology and Immunology (2019) 208:405–414
https://doi.org/10.1007/s00430-019-00594-w

ORIGINAL INVESTIGATION

Kinetics of inflammatory biomarkers in plasma predict the occurrence and features of cytomegalovirus DNAemia episodes in allogeneic hematopoietic stem cell transplant recipients

Alberto Talaya[1] · Estela Giménez[1] · Víctor Vinuesa[1] · Ariadna Pérez[2] · Paula Amat[2] · José Luis Piñana[2] · Eliseo Albert[1] · Juan Carlos Hernández-Boluda[2] · Carlos Solano[2,3] · David Navarro[1,4]

Received: 20 February 2019 / Accepted: 9 March 2019 / Published online: 25 March 2019
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Abstract

Cytomegalovirus (CMV) DNAemia occurs frequently in CMV-seropositive allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients, and usually results from reactivation of latent infection established in the recipient. Predicting the occurrence of CMV DNAemia may be helpful in managing CMV infection in allo-HSCT recipients. Here, the kinetics of several inflammatory biomarkers in plasma were characterized and assessed for their potential value in anticipating the development and features of active CMV infection in allo-HSCT recipients, as documented using real-time PCR assays. The cohort consisted of 46 non-consecutive adult patients who underwent T-cell replete allo-HSCT at our center. Plasma levels of C-reactive protein (CRP), soluble tumor necrosis factor receptor type 2 (sTNF-R2), transforming growth factor-β1 (TGF-β1), and interferon-inducible protein 10 (IP-10/CXCL10) were measured in consecutive specimens obtained from conditioning either by nephelometry (CRP) or by specific immunoassays (the rest). Of the 46 patients, 22 had a first episode of CMV DNAemia at a median of 34 days after allo-HSCT (range, day 19–day 50). We found that both the TGF-β1 area under a curve (AUC) and peak levels were significantly lower in patients who subsequently developed CMV DNAemia than in patients with no CMV DNAemia. Interestingly, CRP but not TGF-β1 AUC and peak levels predicted the occurrence of CMV DNAemia episodes requiring preemptive antiviral therapy. The data presented herein suggest that kinetics of inflammatory biomarkers in plasma might be useful to anticipate post-engraftment CMV DNAemia episodes and predict the need for preemptive antiviral therapy in allo-HSCT recipients.

Keywords Cytomegalovirus (CMV) · Transforming growth factor-β1 (TGF-β1) · C-reactive protein (CRP) · CMV DNAemia · Allogeneic hematopoietic stem cell transplantation (allo-HSCT)

Edited by: Sebastian Voigt.

This article is part of the Special Issue on Immunological Imprinting during Chronic Viral Infection.

✉ David Navarro
david.navarro@uv.es

[1] Microbiology Service, Hospital Clínico Universitario, INCLIVA Research Institute, Av. Blasco Ibáñez 17, 46010 Valencia, Spain

[2] Hematology Service, Hospital Clínico Universitario, INCLIVA Research Institute, Valencia, Spain

[3] Department of Medicine, School of Medicine, University of Valencia, Valencia, Spain

[4] Department of Microbiology, School of Medicine, University of Valencia, Av. Blasco Ibáñez 17, 46010 Valencia, Spain

Introduction

Active cytomegalovirus (CMV) infection develops frequently in the allogeneic hematopoietic stem cell transplantation setting (allo-HSCT) and may lead to end-organ disease if effective antiviral therapy is not initiated in a timely fashion [1]. Precise identification of allo-HSCT recipients particularly prone to developing active CMV infection may become the cornerstone for optimal use of antiviral phrophylaxis strategies with new anti-CMV agents [2, 3], whereby only patients at highest risk would be targeted. CMV reactivation may occur through allogeneic stimulation of peripheral blood mononuclear cells [4]. In addition, pro-inflammatory cytokines, most notably tumor necrosis factor-α (TNF-α), are known to trigger CMV reactivation from its latent state [5, 6]. Increased post-transplantation

TNF-α levels have been shown to be associated with the development of active CMV infection in the allo-HSCT setting [7, 8]. In these studies, the CMV pp65 antigenemia assay was used for active CMV infection surveillance; in this respect we previously showed that a commercially available plasma real-time PCR assay (CMV Real-Time PCR Kit;Qiagen GmbH, Hilde, Germany) was more sensitive than the antigenemia assay (98.9% versus 47.2%) for detection of CMV in blood [9]. Here, we investigated the kinetics of several inflammatory biomarkers in plasma and assessed their potential value in anticipating the occurrence and features of active CMV infection, as diagnosed by means of a highly sensitive real-time PCR assay, in allo-HSCT recipients. Cytokines chosen for the analyses included a general downstream inflammatory marker (C-reactive protein [CRP]), two markers of upstream inflammatory pathways (soluble tumor necrosis factor receptor type 2 [sTNF-R2] and transforming growth factor-β1 [TGF-β1]), and a pro-inflammatory chemokine (interferon-inducible protein 10 [IP-10/CXCL10]). These soluble proteins circulate at much higher levels than conventional inflammatory markers such as TNF-α and IL-6, and may thus provide more reliable information on the net state of inflammation at a given time point [10, 11].

Materials and methods

Patients

This cohort consisted of 46 non-consecutive adult patients who underwent T-cell replete allo-HSCT at the Hematology Service of the Hospital Clínico Universitario of Valencia between May 2011 and June 2014. The only criterion for patient exclusion was lack of available consecutive specimens, arbitrarily defined as less than three, for the analyses described below. The cohort was representative of the entire patient population undergoing allo-HSCT at our center within the study period (not shown). The median age of patients at the time of allo-HSCT was 51 years (range 18–66 years). Patients' demographic, baseline, and post-transplant clinical data are summarized in Table 1. The study period comprised the first 100 days after allo-HSCT. This study was approved by the Hospital Clínico Fundación INCLIVA Ethics Committee. Informed consent was signed by all participants.

Table 1 Patient demographic and clinical characteristics

Parameter	No. of patients (%)
Sex	
Male	29 (63.0%)
Female	17 (37.0%)
Underlying disease	
Acute leukemia	15 (32.6%)
Chronic leukemia	8 (17.4%)
Lymphoma	18 (39.1%)
Myelodysplastic syndrome/myelofibrosis	3 (6.5%)
Multiple myeloma	2 (4.3%)
HLA-matching	
Matched	32 (69.6%)
Mismatched	14 (30.4%)
Donor type	
Related	28 (60.9%)
Unrelated	18 (39.1%)
Stem cell source	
Peripheral blood	37 (80.4%)
Umbilical cord blood	6 (13.0%)
Bone marrow	3 (6.5%)
Conditioning regimen	
Reduced intensity	30 (65.2%)
Standard intensity	16 (34.8%)
Graft-vs-host disease prophylaxis	
Regimen with cyclophosphamide	8 (17.4%)
Regimen with cyclosporin A	25 (54.3%)
Regimen with sirolimus	13 (28.3%)
ATG prophylaxis	
Yes	8 (17.4%)
No	38 (82.6%)
CMV serostatus	
D+/R+	24 (52.2%)
D+/R−	8 (17.4%)
D−/R+	11 (23.9%)
D−/R−	3 (6.5%)
Acute graft-vs-host disease	
Grades 0–I	34 (73.9%)
Grades II–IV	12 (6.1%)

ATG anti-thymocyte globulin, *CMV* cytomegalovirus, *HLA* human leukocyte antigen, *D* donor, *R* recipient

Management of active CMV infection

Plasma CMV DNA load was monitored using the Artus CMV Real-Time PCR Kit (produced by Qiagen GmbH, Hilde, Germany for Abbott Molecular Diagnostics Abbott) until May 2012 and the CMV RealTime CMV PCR (Abbott Molecular, Des Plaines, IL, USA) thereafter. The limit of detection and quantitation of both assays is approximately 20 copies/ml-31 IU/ml-(95% CI) [12]. CMV DNA monitoring was scheduled to be conducted on a weekly basis through day + 100, and twice a week in patients with CMV DNAemia, as per our center protocol. Antiviral therapy with (val)ganciclovir or foscarnet at conventional doses was initiated when the plasma CMV DNA load reached levels of > 500 (until May 2012) or 1000 copies/ml (thereafter)

[13]. Approximately, 500 copies/ml measured by the Artus CMV Real-Time PCR Kit and 1000 copies/ml quantitated by the CMV real-time CMV PCR assay correspond to around 1500 IU/ml [12].

Quantitation of inflammatory biomarkers in plasma

Leftover plasma specimens primarily used for CMV DNA load monitoring obtained from the time of conditioning to day + 50 after allo-HSCT were retrieved (thawed for the first time) for measurements. All plasma specimens from a given patient were analyzed in triplicate (mean values are used throughout the study) in the same run. Analyses were performed in several batches within 2015. CRP was quantitated by nephelometry using the high sensitivity C-reactive protein (hsCRP) assay manufactured by Siemens Healthineers (Erlangen, Germany). The sensitivity of the assay is 0.10 mg/l, the assay range 0.10–50 mg/l, and the reproducibility 6.8% at 1.16 mg/l. sTNF-R2, TGF-β1, and IP-10 were quantitated by a multiplex antibody- and magnetic bead-based system using the Luminex instrument platform (ProcartaPlex immunoassays; Thermo Fisher Scientific, Waltham, USA), following the manufacturer's recommendations. The sensitivity of the sTNF-R2 assay is 0.1 pg/ml, the assay range 1.27–5200 pg/ml and the inter- and intra-assay coefficients of variation less than 10%. The TGF-β1 assay displays a sensitivity of 0.1 pg/ml, the assay range is 1.1–4500 pg/ml and the inter- and intra-assay coefficients of variation less than 10%. Finally, the IP-10 assay has limit of detection of 0.3 pg/ml, the range of quantitation is 1.95–8000 pg/ml and the inter- and intra-assay coefficients of variation 5.5% and 6.6%, respectively.

Blood cell counts

Enumeration of absolute leukocyte and lymphocyte was performed by hemocytometry and flow cytometry using the BD FACSCanto II Flow Cytometer (BD Biosciences, San Jose, CA, USA), respectively. Values for both cell populations determined at the time points selected for plasma biomarkers measurements were taken into consideration for the analyses described below.

Definitions

Active CMV infection and CMV DNAemia are used interchangeably throughout the text. CMV DNAemia was defined as detection of CMV DNA at any level in one or more plasma specimens. The overall duration of a given episode of viral DNAemia was the time elapsed between the day of first detection of viral DNA in plasma and the day of first negative (undetectable) PCR result. Acute graft-versus-host disease (aGvHD) was diagnosed and graded as previously reported [14].

Statistical analysis

Differences between medians were compared using the Mann–Whitney *U* test (for two independent variables). The Spearman rank test was used to assess the correlation between continuous variables. Qualitative variables were compared using the Chi-square test. Two-sided exact *P* values are reported. A P value ≤ 0.05 was considered statistically significant. Univariate and multivariate logistic regression models were built to assess the impact of several clinical and biological factors on the risk of CMV DNAemia. These statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA). The area under the plasma concentration–time curve (AUC) $\log_{10}$ for each cytokine (in mg [CRP] or pg [sTNF-R2, TGF-β1, IP-10] × days/l [CPR] or /ml [sTNF-R2, TGF-β1 and IP-10]) and for absolute leukocytes and lymphocytes (10^6 cells × days/ml) was calculated applying the curve trapezoid rule, using the GraphPad Prism Software (La Jolla, CA, USA), as previously reported [15]. All plasma and blood counts measurements were taken into consideration for AUCs calculations. Receiver operating characteristic (ROC) curve analyses were performed using the same statistical package.

Results

CMV DNAemia episodes in the study population

Of the 46 patients included in the study, 22 had a first episode of CMV DNAemia at a median of 34 days after allo-HSCT (range, day 19–day 50). The initial and peak median CMV DNA loads were 80.5 IU/ml (range 30.5 IU/m–398 IU/ml) and 3141 IU/ml (range 80.5–123,393 IU/ml), respectively. CMV DNAemia episodes lasted a median of 42 days (range 18–142 days). Fourteen of the 22 patients received preemptive antiviral therapy with (val)ganciclovir. There were no significant differences between patients with or without CMV DNAemia regarding most baseline and post-transplant patient characteristics including sex, underlying disease, type of allograft, source of hematopoietic stem cells, conditioning regimen, acute GvHD prophylaxis regimen, CMV serostatus of donors and cumulative incidence of aGvHD (Table 2). Only recipient CMV serostatus was significantly different ($P = 0.003$) across comparison groups, CMV seropositive having a predictably higher prevalence among patients who went on to develop CMV DNAemia.

Table 2 Demographic and clinical characteristics of patients with or without CMV DNAemia

Parameter	No. of patients (%)		
	CMV DNAemia ($n=22$)	No CMV DNAemia ($n=24$)	*P* value
Sex			
Male	14 (48.3%)	15 (51.7%)	0.936
Female	8 (47.1%)	9 (52.9%)	
Underlying disease			
Acute leukemia	8 (53.3%)	7 (46.7%)	0.589
Chronic leukemia	3 (37.5%)	5 (62.5%)	
Lymphoma	9 (50.0%)	9 (50.0%)	
Myelodysplastic syndrome/myelofibrosis	2 (66.7%)	1 (33.3%)	
Multiple myeloma	0 (0.0%)	2 (100.0%)	
HLA-matching			
Matched	14 (43.8%)	18 (56.2%)	0.403
Mismatched	8 (57.1%)	6 (42.9%)	
Donor type			
Related	11 (39.3%)	17 (60.7%)	0.148
Unrelated	11 (61.1%)	7 (38.9%)	
Stem cell source			
Peripheral blood	17 (45.9%)	20 (54.1%)	0.605
Non-peripheral blood	5 (55.6%)	4 (44.4%)	
Conditioning regimen			
Reduced intensity	15 (50.0%)	15 (50.0%)	0.686
Standard intensity	7 (43.8%)	9 (56.3%)	
Graft-vs-host disease prophylaxis			
Regimen with cyclophosphamide	5 (62.5%)	3 (37.5%)	0.215
Regimen with cyclosporin A	9 (36.0%)	16 (64.0%)	
Regimen with sirolimus	8 (61.5%)	5 (38.5%)	
ATG prophylaxis			
Yes	5 (62.5%)	3 (37.5%)	0.361
No	17 (44.7%)	21 (55.3%)	
Donor CMV serostatus			
D+	14 (43.8%)	18 (56.3%)	0.403
D−	8 (57.1%)	6 (42.9%)	
Recipient CMV serostatus			
R+	21 (60.0%)	14 (40.0%)	0.003
R−	1 (9.1%)	10 (90.9%)	
Acute graft-vs-host disease			
Grades 0–I	15 (44.1%)	19 (55.9%)	0.397
Grades II–IV	7 (58.3%)	5 (41.7%)	

ATG anti-thymocyte globulin, *CMV* cytomegalovirus, *HLA* human leukocyte antigen, *D* donor, *R* recipient

Kinetics of inflammatory biomarkers in plasma and occurrence of CMV DNAemia

We investigated whether plasma levels of inflammatory biomarkers measured from the time of conditioning predicted the development of CMV DNAemia. A median of four specimens per patient were available for these analyses (range 3–4). The time points at which samples were drawn from patients who eventually developed CMV DNAemia are shown in Fig. 1a and overlapped with those from patients with no documented CMV DNAemia throughout the study period—Fig. 1b—(range, day − 7 through day 50 from patients with CMV DNAemia, and range, day − 3 through day 50 from patients with no CMV DNAemia). Specifically, a total of 75 and 81 specimens from patients with or without CMV DNAemia were analyzed, respectively. We chose to consider two kinetics parameters, the AUC, which reflects the actual exposure to the selected biomarker, and the peak

A

Day rank	-7 – 0	1 – 10	11 – 20	21 – 30	31 – 40	41 – 50
Patient 1		×		×	× ↑	
Patient 2	×		×		× ↑	
Patient 3		×		×	×	↑
Patient 4	×		×	×	↑	
Patient 5	×		× ×		↑	
Patient 6	×	×	× ×	↑		
Patient 7	×	×	× ×	↑		
Patient 8	×	×	× ×		↑	
Patient 9	×	×	× × ↑			
Patient 10		×	×	× ↑		
Patient 11		×	×		×	↑
Patient 12	×			×		↑
Patient 13		×	×		× ↑	
Patient 14	×	×	×	× ↑		
Patient 15	×			× ×		↑
Patient 16	×	×	×	×	↑	
Patient 17	×	×	×	×	↑	
Patient 18		×	×	×	↑	
Patient 19	×	×	×	×	↑	
Patient 20	×	×	×	×	↑	
Patient 21	×	×	×	× ↑		
Patient 22			×	×	×	↑

B

Day rank	-7 – 0	1 – 10	11 – 20	21 – 30	31 – 40	41 – 50
Patient 23		×	×		×	
Patient 24		×		×	×	
Patient 25		×	×		×	
Patient 26		×		×	×	
Patient 27		×	×		×	
Patient 28		×		×		×
Patient 29	×	×		×		
Patient 30	×		×		×	
Patient 31	×	×	× ×			
Patient 32	×	×	× ×			
Patient 33		×	×		×	
Patient 34	×	×	× ×			
Patient 35	×	×	×	×		
Patient 36		× ×	×	×		
Patient 37	×	×	× ×			
Patient 38	×	×	× ×			
Patient 39			×	×	×	
Patient 40			×	×	×	
Patient 41			×	×	×	
Patient 42			×	×	×	
Patient 43		× ×	×			
Patient 44		× ×		×		
Patient 45		×	×	× ×		
Patient 46		× ×	×	×		

Fig. 1 Time points (x) at which plasma specimen were drawn for analyses in **a** patients subsequently developing active CMV infection (arrows) or not (**b**)

level. We found that both the TGF-β1 AUC_s and peak levels were significantly lower ($P=0.020$ and $P=0.026$, respectively) in patients who subsequently developed CMV DNAemia than in patients with no CMV DNAemia (Tables 3, 4, respectively). Overall, peak levels of this biomarker were reached at a median of 14.5 days (range −6 to 39 days) after allo-HSCT (median of 14; range −6 to 35 days in patients with subsequent CMV DNAemia; median of 16 days; range, −3 to 39 days in patients with no CMV DNAemia; $P=0.890$). Neither AUCs nor peak concentrations of the remaining biomarkers differed significantly across comparison groups. ROC curve analyses were next performed to determine optimal cutoff values for the TGF-β1 AUC and peak levels that best discriminated between patients who did, or did not go on to develop CMV DNAemia (Fig. 2); optimal thresholds were found to be 3.75 pg × days/ml^{-1} (AUC) and 437 pg/ml (peak level), both displaying a sensitivity of 83.3% (95% CI 68.4–98.2%) and an specificity of 50% (95% CI 20.4–79.5%). TGF-β1 AUC and TGF-β1 peak levels below the aforementioned optimal thresholds were found to be independently associated with the occurrence of CMV DNAemia (OR 7.21; 95% CI 1.31–42.7) in multivariate logistic regression models adjusted for sex, donor type, source of hematopoietic stem cells, HLA-matching, conditioning regimen, prophylaxis for aGvHD and donor/recipient CMV serostatus (not shown).

Nevertheless, we found no correlation between either the TGF-β1 AUC or the TGF-β1 peak levels and the duration of CMV DNAemia ($\rho=-0.093$; $P=0.681$, and $\rho=-0.211$; $P=0.345$).

Interestingly, CRP but not TGF-β1 AUC and peak levels predicted the occurrence of CMV DNAemia episodes requiring inception of PET (8 out of 14 episodes). ROC curve analyses (Fig. 2) indicated that the optimal cutoff values for CRP AUC and peak levels best discriminating between patients who did or did not subsequently develop CMV DNAemia were 2.5 mg × days/l and 16.7 mg/l, respectively, with a sensitivity of 100% (95% CI 100%) and a specificity of 50% (95% CI 10–90%).

Of note, patients who developed CMV DNAemia and patients who did not were comparable regarding: (1) the incidence of bacterial infections of any origin (occurring in 10 out of 22 patients with CMV DNAemia and in 12 out of 24 patients without CMV DNAemia; $P=0.40$); (2) the AUC $\log_{10}$ values for absolute leukocytes and lymphocytes, whose calculation took into consideration blood cell counts measured at the same time points than those selected for plasma biomarker measurements (Fig. 3); (3) the incidence

Table 3 Area under a curve (AUC) of pro-inflammatory cytokines in plasma in allogeneic hematopoietic stem cell transplant recipients with or without subsequent CMV DNAemia

Biomarker/outcome	Median AUC $\log_{10}$[a]	AUC $\log_{10}$ range[a]	*P* value
CRP			
CMV DNAemia	2.72	2.25–3.39	0.317
No CMV DNAemia	2.87	2.25–3.41	
CMV DNAemia preemptively treated with antivirals	2.92	2.51–3.39	**0.002**
Self-resolving CMV DNAemia	2.54	2.25–2.74	
IP-10			
CMV DNAemia	2.99	1.43–3.91	0.291
No CMV DNAemia	3.11	2.01–4.12	
CMV DNAemia preemptively treated with antivirals	3.35	1.43–3.76	0.525
Self-resolving CMV DNAemia	2.85	3.39–3.91	
sTNF-R2			
CMV DNAemia	4.03	2.72–4.36	0.226
No CMV DNAemia	4.06	3.36–4.41	
CMV DNAemia preemptively treated with antivirals	4.09	2.72–4.36	0.526
Self-resolving CMV DNAemia	3.78	3.38–4.26	
TGF-β1			
CMV DNAemia	3.81	1.32–4.64	**0.020**
No CMV DNAemia	4.17	1.64–4.92	
CMV DNAemia preemptively treated with antivirals	3.95	1.32–4.64	0.764
Self-resolving CMV DNAemia	3.25	1.32–4.46	

Bold indicates a statistically significant difference with a *p*-value less than 0.05

CMV cytomegalovirus, *CRP* C-reactive protein, *IP-10* interferon-inducible protein 10, *sTNF-R2* soluble tumor necrosis factor receptor type 2, *TGF-β1* transforming growth factor-β1

[a]mg (CRP) or pg (sTNF-R2, TGF-β1, IP-10) × days/l^{-1} (CPR) or /ml^{-1} (sTNF-R2, TGF-β1 and IP-10)

Table 4 Peak levels of pro-inflammatory cytokines in plasma in allogeneic hematopoietic stem cell transplant recipients with or without subsequent CMV DNAemia

Biomarker (units)/outcome	Median peak levels	Peak levels range	*P* value
CRP (mg/l)			
CMV DNAemia	45.85	8.70–294.70	0.636
No CMV DNAemia	42.90	9.40–186.70	
CMV DNAemia preemptively treated with antivirals	76.65	17.50–294.70	**0.005**
Self-resolving CMV DNAemia	21.70	8.70–68.00	
IP-10 (pg/ml)			
CMV DNAemia	66.29	2.52–828.29	0.262
No CMV DNAemia	99.14	8.49–2363.45	
CMV DNAemia preemptively treated with antivirals	119.38	2.52–828.29	
Self-resolving CMV DNAemia	38.46	14.46–527.13	
sTNF-R2 (pg/ml)			
CMV DNAemia	521.84	29.53–1123.91	0.301
No CMV DNAemia	619.49	148.56–1682.93	
CMV DNAemia preemptively treated with antivirals	630.35	29.53–1123.91	0.267
Self-resolving CMV DNAemia	291.26	126.52–781.20	
TG-β1 (pg/ml)			
CMV DNAemia	438.94	1.00–2852.25	0.026
No CMV DNAemia	1423.58	5.57–4965.15	
CMV DNAemia preemptively treated with antivirals	798.89	1.00–2852.25	0.330
Self-resolving CMV DNAemia	107.05	1.00–1630.10	

Bold indicates a statistically significant difference with a *p*-value less than 0.05

CMV cytomegalovirus, *CRP* C-reactive protein, *IP-10* interferon-γ inducible protein 10, *sTNF-R2* soluble tumor necrosis factor receptor type 2, *TGF-β1* transforming growth factor-β1

of aGvHD (Table 2); (4) the immunosuppresion regimen used for the prevention of aGVHD (Table 2).

Discussion

A number of baseline and post-transplant clinical factors, as well as host genetic traits and immunological biomarkers have been recognized to impact on the risk of CMV DNAemia in allo-HSCT recipients [1, 16]. Here, we assessed the predictive value of plasma levels of CRP, sTNF-R2, TGF-β1, and IP-10 in anticipating the occurrence and features of CMV DNAemia in allo-HSCT recipients, which was monitored by means of a highly sensitive real-time PCR assay. The rationale for the selection of these biomarkers in our study was: (1) plasma levels of IP-10 and CRP were found to be independently associated with the presence of cytomegalovirus DNAemia and with tissue-invasive cytomegalovirus disease in the solid organ transplantation setting [11]; (2) the potential role of TGF-β1 in the pathogenesis of CMV-induced organ injury [17]; (3) the reliability of sTNF-R2 as a surrogate marker of inflammation mediated by TNF-α [18], as discussed below.

Here we show that the kinetics of TGF-β1 in plasma could be used as an ancillary parameter to anticipate the occurrence of CMV DNAemia. In effect, both TGF-β1 AUC and peak levels were found to be significantly lower in patients who eventually developed first episodes of CMV DNAemia following engraftment and relatively early after transplantation (up to day 50 after allo-HSCT) in comparison with those who did not. In fact, TGF-β1 AUC and peak levels below optimal thresholds were independently associated with the development of CMV DNAemia. Note that patients with or without CMV DNAemia did not differ significantly in terms of demographics or baseline and post-transplant characteristics, including among the latter the immunosuppresive regimen used for the prevention of aGvHD, and the incidence of aGvHD and bacterial infections of any origin, which may conceivably impact on plasma levels of inflammatory biomarkers. In fact, the AUC $\log_{10}$ values for absolute leukocytes and lymphocytes counts measured at the same time points than those selected for plasma biomarker measurements were comparable across comparison groups.

TGF-β1 is a multifunctional cytokine that participates in the control of cell growth and differentiation, induces fibrosis, suppresses NK and cytotoxic T-cell responses and may counteract the biological activity of certain inflammation mediators [19–21]. Regarding the latter effect, which may account for our finding, TGF-β1 can inhibit the secretion and activity of many cytokines, including TNF-α, and hamper the proliferation of macrophages and monocytes, thus preventing them from producing reactive

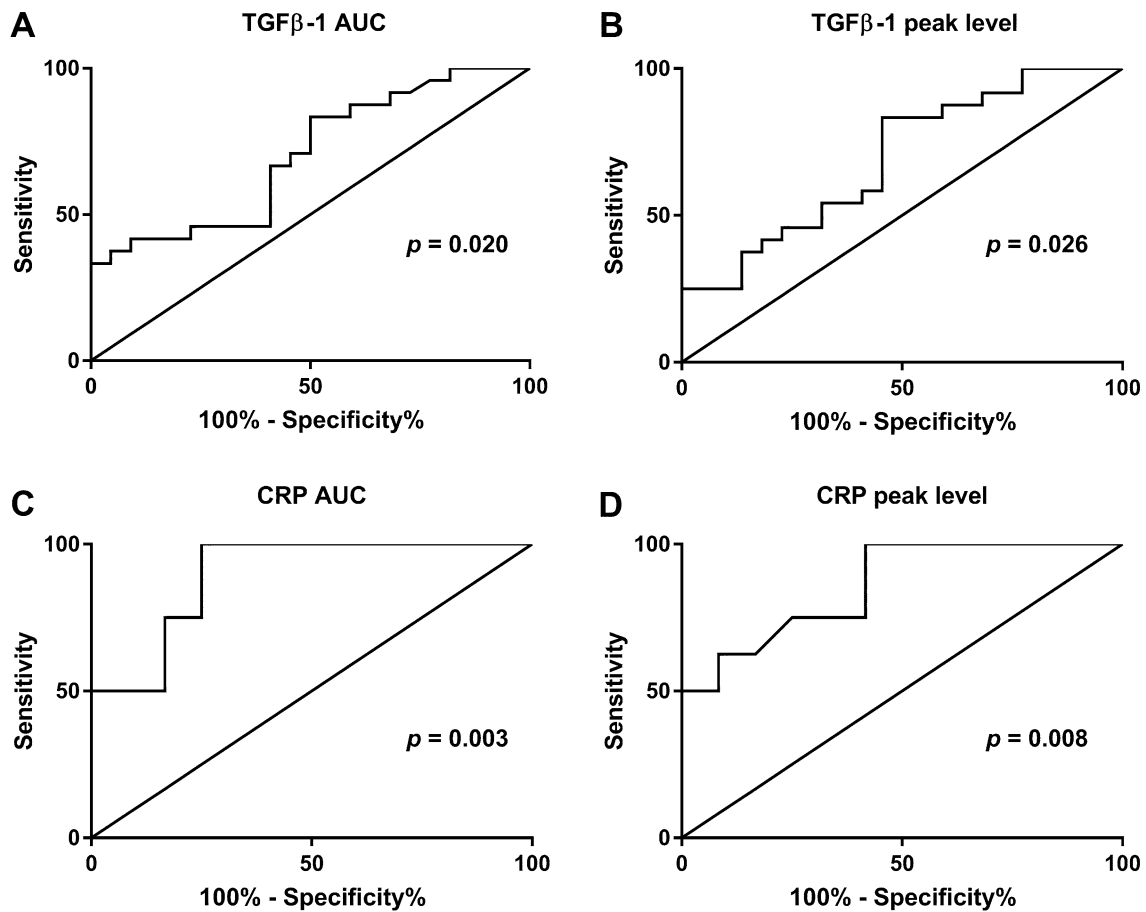

Fig. 2 Receiver operating characteristic (ROC) curves to establish the optimal cutoff of **a** TGF-β1 area under a curve (AUC) and **b** TGF-β1 peak level in plasma discriminating between patients subsequently developing CMV DNAemia from those who did not, and of **c** CRP area under a curve (AUC) and **d** CRP peak level discriminating between patients who eventually developed CMV DNAemia that required preemptive antiviral therapy from those displaying self-resolving episodes

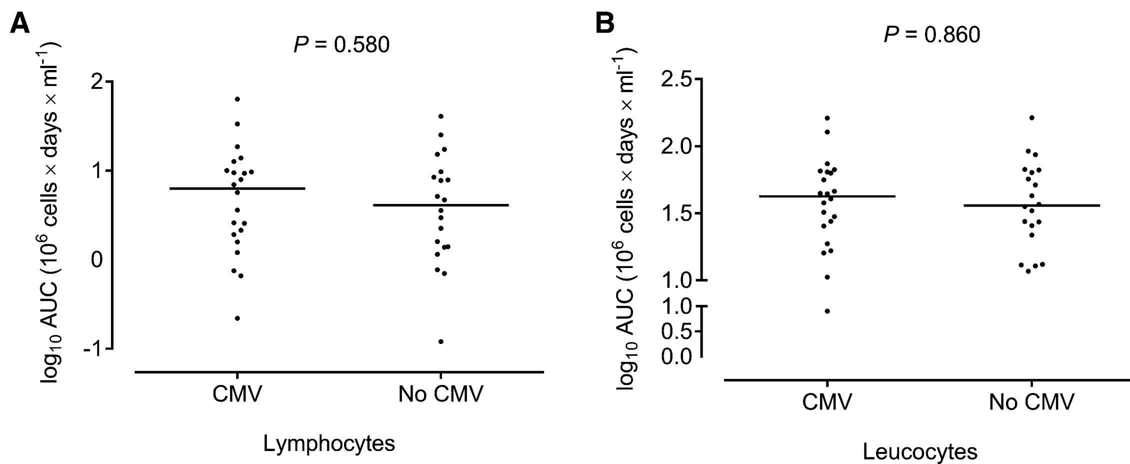

Fig. 3 Area under a curve (AUC) for $\log_{10}$ absolute leukocyte and lymphocyte counts measured at the time points chosen for plasma biomarkers measurements. Comparative *P* values are shown

oxygen [e.g., superoxide (O2−)] and nitrogen [e.g., nitric oxide (NO)] intermediates [19–21]; in this context, both TNF-α and reactive oxygen species levels in plasma have been shown to directly correlate with risk of CMV DNAemia [8, 9, 23].

It was somewhat surprising that sTNF-R2 levels were not predictive of CMV DNAemia, given that concentrations of soluble circulating forms of TNF-α receptors (sTNFRs) are thought to reflect long-term exposure to this pro-inflammatory cytokine [18]. However, it has in fact been shown that assays measuring TNF-α are often of questionable reliability, resulting in inconsistent results. This is likely due to the presence of biological forms of TNF-α in plasma that remain undetectable because of complex formation with sTNFRs, whose dissociation rate constant appears to be assay-dependent, or monomeric conformation [24]. In line with this, failure to detect TNF-α is not uncommon in healthy subjects, whereas sTNF-R2 is systematically quantifiable [25].

CRP is an acute phase protein produced by hepatocytes and behaves as a reliable marker of systemic inflammation [26]. Increased levels of CRP in blood have been shown to occur in association with bacterial infections and aGVHD, and, ultimately, with transplant-related mortality in allo-HSCT recipients [27–29]. Nevertheless, to our knowledge, no previous link has been reported between CRP levels in plasma and active CMV infection in this clinical setting. Here, we found no association between CRP AUCs and peak levels and the occurrence of CMV DNAemia; nevertheless, both parameters appeared to be of higher magnitude in patients who developed first CMV DNAemia episodes that required PET using a relatively conservative threshold for antiviral initiation (1500 IU/ml). The low number of patients in the current cohort of CMV DNAemia requiring PET precludes drawing robust conclusions and invites further speculation as to the underlying pathophysiological mechanism on this issue. In this sense, the net state of inflammation, as inferred by plasma CRP levels, at CMV DNAemia onset may directly influence the viral rate of growth and hence the eventual need of PET.

In our view, the present study has several limitations that deserve comment. First, the low number of patients included precluded performing robust statistical analyses; second, the relative heterogeneity of patients in the cohort; third, cryopreserved rather than fresh specimens were used for analyses; nevertheless, this last is unlikely to have had an impact on our results given the stability of the measured biomarkers in frozen-stored specimens [25]; Fourth, several factors that may impact on the risk of CMV DNAemia (i.e., early CMV-specific T-cell response) [1] were not considered. In addition, the use of two different real-time PCR assays could be viewed as a limitation; however, in our experience both perform comparably in terms of sensitivity and linear range of quantitation [13].

Prospective measurement of biomarkers of inflammation and immune activation in blood have proven useful for predicting the occurrence of CMV end-organ disease in solid organ transplant recipients and guiding antiviral prophylaxis for the prevention of late-onset CMV disease in high-risk solid kidney and liver transplant recipients [10, 11, 30, 31]. In line with the previous studies [8, 9]; the data presented herein suggest that it may also find its spot in the management of CMV infection in the allo-HSCT setting. Precise risk stratification for CMV-related clinical events early after allo-HSCT may be of use in the near future for identification of patients tributary of antiviral prophylaxis with new agents (targeted prophylaxis) [3]. In this sense, peak levels of TGF-β1 were found to be reached within this time frame. Nevertheless, it is unlikely that a single parameter, regardless of its nature may do so; on the contrary, we envision a weighted risk score incorporating immunological (including inflammatory biomarkers), genetic and clinical factors that may improve our capacity to predict CMV-related events and to individualize prevention strategies. Work addressing this issue is currently underway.

Acknowledgements Estela Giménez holds a Río Hortega research contract from the Carlos III Health Institute (Ref. CM16/00200). All authors declare no conflict of interest.

Funding This work was supported by a Grant from Fondo de Investigaciones Sanitarias (FIS) form Carlos III Health Institute (15/0060).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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