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Infectious Disease

Cytomegalovirus and Epstein-Barr Virus DNA Kinetics in Whole Blood and Plasma of Allogeneic Hematopoietic Stem Cell Transplantation Recipients

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A B S T R A C T

Currently, no consensus has been reached on the optimal blood compartment to be used for surveillance of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) DNAemia. Although several comparative studies have been performed correlating CMV and EBV DNA loads in whole blood (WB) versus plasma, to our knowledge, no studies to date have analyzed the kinetics of both viruses in the 2 blood compartments. In this retrospective noninterventional multicenter cohort study, the kinetics of CMV and EBV DNA in 121 hematopoietic stem cell transplantation (HSCT) recipients were investigated by analyzing in parallel 569 and 351 paired samples from 80 and 58 sequential episodes of CMV and EBV DNAemia, respectively. Unlike previous studies, this study used a single automated molecular method that was CE-marked and Food and Drug Administration-approved for use in quantifying CMV and EBV DNA in both plasma and WB. Furthermore, the complete viral replication kinetics of all episodes (including both the ascending and the descending phases of the active infection) was examined in each patient. The previously observed overall correlation between CMV DNA levels in WB and plasma was confirmed (Spearman's $\rho = .85$; $P < .001$). However, although WB and plasma CMV DNAemia reached peak levels simultaneously, in the ascending phase, the median CMV DNA levels in plasma were approximately 1 log10 lower than WB. Furthermore, in patients who received preemptive therapy, CMV DNA showed a delayed decrease in plasma compared with WB. A lower correlation between EBV DNA levels in plasma versus WB was found (Spearman's $\rho = .61$; $P < .001$). EBV DNA kinetics was not consistent in the 2 blood compartments, mostly due to the lower positivity in plasma. Indeed, in 19% of episodes, EBV DNA was negative at the time of the EBV DNA peak in WB. Our results suggest a preferential use of WB for surveillance of CMV and EBV infection in HSCT recipients.

INTRODUCTION

Human herpes viruses are considered the most important opportunistic viral agents in terms of their ability to cause diseases after transplantation [1]. In particular, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are major causes

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of post-transplantation viral disease in hematopoietic stem cell transplantation (HSCT) recipients (HSCTR) [2].

These 2 opportunistic infections usually occur in the first 6 months post-transplantation, when the risk for infection depends on the use of antilymphocyte "induction" therapy and the level of immunosuppression [1]. Aggressive CMV infections are characterized by organ diseases, such as pneumonia, hepatitis, gastroenteritis, retinitis, and encephalitis [3]. EBV infection may progress to onset of a post-transplantation lymphoproliferative disorder, an uncommon but frequently fatal complication after HSCT [4].

The quantification of CMV and EBV DNA is a cornerstone for the diagnosis and monitoring of post-transplantation CMV and EBV infection and disease [5,6]. Most centers, especially those in Europe, rely on preemptive treatment strategies as a standard of care, using DNAemia monitoring as a virologic parameter for treatment initiation and interruption [6,7]. However, major discrepancies among transplantation centers in testing methods, blood specimens, frequency of testing, and thresholds for initiating preemptive therapy, as well as treatment duration, are obstacles to a standardized diagnostic and therapeutic approach [5,6,8,9].

In particular, both whole blood (WB) and plasma specimens have been used for early determination of threshold values for CMV preemptive treatment in HSCTR [10]. More recently, efforts have been made to determine a commutability factor for standardizing the DNAemia results obtained using these 2 biological matrices [11]. However, although several comparative studies have correlated CMV DNA loads in WB versus plasma [11-20], no study to date been performed with a single molecular method approved by regulatory agencies for both blood matrices to verify the underlying assumption that CMV DNAemia would follow similar kinetics in the 2 blood compartments. Even less information is available for EBV DNAemia kinetics.

In the present study, we investigated the kinetics of both CMV and EBV DNAemia in WB and plasma using an automated molecular method that was CE-marked and Food and Drug Administration-approved for quantifying CMV and EBV DNA in both plasma and WB, to determine the appropriateness of using WB or plasma polymerase chain reaction (PCR) assays for the surveillance and the clinical management (in terms of optimal initiation of preemptive antiviral therapy and treatment duration) of CMV and EBV infections. In fact, an international consensus on the optimal blood compartments to use is still lacking [2]. This retrospective noninterventional multicenter cohort study simultaneously analyzed the kinetics of CMV and EBV DNAemia in parallel in WB and plasma samples collected from pediatric and adult allogeneic (allo)-HSCTR during positive infection episodes. Unlike in previous studies, for all patients, the entire episode of viral replication (ascending and descending phases), including at least 5 sequential positive samples with a quantitative result preceded and followed by at least 1 negative sample, was studied.

MATERIALS AND METHODS

Study Design

Patients who underwent allo-HSCT at 4 Italian transplantation centers—St. Orsola-Malpighi Polyclinic of Bologna, Foundation IRCCS Polyclinic San Matteo of Pavia, A.O.U. "Città della Salute e della Scienza" of Turin, and Polyclinic Tor Vergata Foundation of Rome—between June 2014 and August 2015 were enrolled in the study. All patients were monitored for CMV and EBV infection during the post-transplantation period. In the 4 transplantation centers, routine virologic surveillance was performed following the same time schedule. Specifically, EDTA-anticoagulated peripheral blood samples were collected weekly for 3 months after transplantation, twice monthly in months 3 to 6, and then monthly in months 6 to 12. Afterward, blood samples were analyzed when clinically indicated. CMV and EBV DNA load were prospectively determined by the molecular assays routinely used at each center to detect positive episodes of CMV and EBV DNAemia. At each time point, WB and plasma sample leftovers were prospectively stored at –80°C until testing for CMV and EBV kinetics determination.

Active CMV and EBV DNAemia episodes were retrospectively selected based on the virologic results obtained during routine clinical practice. The inclusion criteria were as follows: (1) all sequential episodes of CMV and EBV DNAemia detected in the 4 centers were included in the analysis; (2) a minimum of 5 sequential samples with measurable CMV or EBV DNA was required; (3) at least 1 negative sample preceding and following DNA positivity had to be available for each episode; and (4) multiple episodes in a single patients were analyzed independently, as indicated above. The exclusion criteria were the (1) presence of scattered CMV or EBV DNA positivity during follow-up; (2) absence of negative samples preceding and following DNA positivity; (3) absence of paired WB and plasma samples; and (4) absence of clinical information.

To ensure inclusion of a large number of patients in this study, considering the strict virologic inclusion and exclusion criteria, the allo-HSCTR enrolled in all 4 centers showed considerable heterogeneity in transplantation-related characteristics. The selected paired samples were thawed and then tested using a commercial quantitative PCR assay (see below).

Prevention strategies for CMV and EBV infections were adopted based on the results of prospective determinations of CMV and EBV levels in WB performed at each center. In all transplantation centers, adult and pediatric patients were managed with preemptive therapy for CMV infection. CMV and EBV strategies are reported in Tables 1 and 2.

Selected Patients and Clinical Samples

The study cohort comprised 121 allo-HSCTR (30 pediatric and 91 adult) positive for CMV or EBV infection. None of the patients showed CMV- or EBV-related symptoms during routine monitoring. The demographic, clinic, and virologic characteristics of the study population are reported in Table 3.

In detail, 569 paired samples from 80 episodes of CMV infection in 71 allo-HSCTR and 351 paired samples from 58 episodes of EBV infection in 50 allo-HSCTR were retrospectively analyzed. The median number of sequential paired samples tested per infection episode was 7 (range, 7 to 17). In addition, 15 allo-HSCTR negative for CMV or EBV DNAemia over the entire follow-up period were included as the control group. In particular, 54 paired samples from 10 patients without CMV infection and 25 paired samples from 5 patients without EBV infection were retrospectively tested.

Molecular Tests

Detection and quantification of CMV and EBV DNA in paired WB and plasma samples were performed using the commercial automated QIAsymphony RGQ System (QIAGEN, Hamburg, Germany). This system combines the DNA extraction and distribution functions of the QIAsymphony Sample Preparation and Assay Setup modules, respectively, together with the amplification step on a Rotor-Gene Q instrument using the *artus* QS-RGQ Kit (QIAGEN) based on real-time PCR technology. In particular, DNA from WB (200 µL) and plasma (1000 µL) samples were extracted using the QIAsymphony DNA Mini Kit and the QIAsymphony DSP Virus/Pathogen Midi Kits, respectively, according to the manufacturer's instructions. The purified nucleic acid was eluted into a total volume of 90 µL, allowing a minimum accessible volume of 60 µL. CMV and EBV amplification was performed using 5 µL of eluate with the *artus* CMV QS-RGQ Kit and *artus* EBV QS-RGQ Kit, which amplified a region of the major immediate-early gene (MIE) and Epstein-Barr Nuclear Antigen gene (EBNA), respectively. For each protocol, the limit of detection and the limit of quantification were as reported by the manufacturer (Table 4).

Statistical Analysis

Quantitative results were reported as log10 copies/mL of the sample. Positive samples below the lower limit of quantification (LLoQ) were censored with a value corresponding to one-half of the LLoQ (ie, 500 copies/mL in WB and 40 copies/mL in plasma for the CMV assay and 500 copies/mL in WB and 150 copies/mL in plasma for the EBV assay). Negative samples were assigned an arbitrary value of 1 copy/mL of sample. Spearman's correlation was used to compare viral load data between the 2 blood compartments. Median values of viral DNA peak in WB and plasma were compared using the Mann-Whitney test. For categorical data, comparisons between groups were performed using a contingency table analysis with the chi-squared or Fisher's exact test as appropriate.

The kinetics of viral DNAs in the 2 blood specimens were compared following a previous protocol in principle [20]. In detail, CMV and EBV DNA levels in WB served as a reference. For each infection episode, T0 was set as the time of the viral DNA peak in WB. Similarly, the ascending and

Table 1
CMV Prevention Strategies According to Transplantation Center

	Preemptive Strategy			
	Bologna	Pavia	Rome	Turin
CMV cutoff viral DNA levels: drug*, dose, and duration	Pediatric patients: >2000 copies/mL GCV i.v. 5 mg/kg twice daily plus or minus foscarnet 90 mg/kg twice daily, followed by VGCV† Adult patients: >10,000 copies/mL VGCV 900 mg twice daily until at least one WB sample was CMV DNA-negative	Pediatric patients: MUD or HLA-haploidentical related donor: >10,000 copies/mL; HLA-identical related donor: >30,000 copies/mL GCV i.v. 5 mg/kg twice daily plus or minus foscarnet 90 mg/kg twice daily or foscarnet 90 mg/kg twice daily, followed by VGCV† Adult patients: MUD or HLA-haploidentical related donor: >20,000 copies/mL; HLA-identical related donor: >30,000 copies/mL GCV i.v. 5 mg/kg twice daily or foscarnet 90 mg/kg twice daily until at least 2 WB samples were CMV DNA-negative plus CMV immunoglobulins for CMV DNA >100,000 copies/mL 3 administrations, at a dose of 50 U/kg 3 times/wk	Adult patients: Antiviral prophylaxis pre-TX D+/R-, D-/R+, D+/R+ GCV i.v. 5 mg/kg once daily during conditioning regimen from days –7 to –2 Preemptive therapy post-TX 2 CMV DNA positive WB samples: (1) In the first 2 months post-TX: GCV i.v. 5 mg/kg twice daily for 2 wk, followed by GCV i.v. 5 mg/kg once daily (2) After 2 months post-TX: VGCV 900 mg twice daily for 2 weeks, followed by VGCV 450 mg once daily In both strategies → until at least 1 WB sample was CMV DNA-negative	Adult patients: >2000 copies/mL VGCV 900 mg twice daily or GCV i.v. 5 mg/kg twice daily for 2 wk, followed by VGCV 450 mg twice daily for 2 wk or until at least 2 WB samples were CMV DNA-negative

GCV indicates ganciclovir; VGCV, valganciclovir; MUD, matched unrelated donor; D, donor; R, recipient; TX, transplant.
* The dosages of the antiviral drugs during therapy were adjusted for renal function.
† VGCV: 15 mg/kg twice daily until at least 3 consecutive samples were CMV DNA-negative or -positive <500 copies/mL of whole blood.

descending phases of CMV and EBV DNAemia were described indicating the median viral DNA levels in WB and plasma in the 5 weeks before and after T0. To evaluate the rates of increase and decrease in both CMV and EBV DNAemia, median DNA levels in WB and plasma at each time point were expressed as percentages of the median DNA peak value. The decreases in viral loads in WB and plasma samples after 1, 2, 3, 4, and 5 weeks from peak viral load were compared using the Wilcoxon test for paired data. Statistical analyses were performed using GraphPad Prism version 5.00 (GraphPad Software, La Jolla, CA).

Pediatric and adult HSCTR were considered a single population, because a preliminary analysis showed overlapping results regardless of patient age (data not shown).

Ethics

The study was approved by the Ethical Committees of the 4 transplantation centers, and all patients provided informed consent.

RESULTS

Correlation of CMV and EBV DNA Levels in WB and Plasma Samples

With regard to CMV DNA analysis, 38 of 569 samples (6.7%) showed discordant results in WB versus plasma. In particular, 8 samples (21.1%) were positive but below the LLoQ of the assay in WB and negative in plasma, and 30 samples (78.9%) were negative in WB and positive in plasma (29 samples were positive but below the LLoQ of the assay, and 1 sample was positive with a quantitative result). A consistent correlation between CMV DNA levels in WB and plasma was confirmed (Spearman's correlation, $\rho = .85$; $P < .001$) both when including all samples (Figure 1A) and when including only the samples that were positive with a quantitative result in both blood compartments (Spearman's $\rho = .74$; $P < .001$) (Figure 1B).

Concerning EBV DNA analysis, 134 of 351 samples (38.2%) showed discordant results. In particular, 133 of these 134 samples (99.3%) were positive in WB and negative in plasma (99 samples were positive with a quantitative result and 34 were positive but below the LLoQ of the assay), whereas only 1 sample (.7%) was negative in WB and positive in plasma (although below the LLoQ of the assay). Spearman's ρ was .61 ($P < .001$) when evaluating all samples (Figure 2A) and .47

Table 2
EBV Prevention Strategies According to Transplantation Center

	Prevention Strategies			
	Bologna	Pavia	Rome	Turin
EBV cutoff viral DNA level, drug, dose, duration	Adult and pediatric patients: >10,000 copies/mL WB Reduction of immunosuppressive therapy Anti-CD20 monoclonal antibody rituximab*, 4 administrations at a dose of 375 mg/m^2/wk	Pediatric patients: >10,000 copies/mL WB Adult patients: >100,000 copies/mL WB Reduction of immunosuppressive therapy Anti-CD20 monoclonal antibody rituximab*, 4 administrations at a dose of 375 mg/m^2/wk	Adult patients:>10,000 copies/mL WB Reduction of immunosuppressive therapy Anti-CD20 monoclonal antibody rituximab*, 4 administrations at dose of 375 mg/m^2/wk	Adult patients:>10,000 copies/mL WB Reduction of immunosuppressive therapy Anti-CD20 monoclonal antibody rituximab*, 4 administrations or until 2 consecutive WB samples were EBV DNA negative at dose of 375 mg/m^2/wk

* When reducing immunosuppression alone is not sufficient to control EBV DNAemia or when symptoms suggested EBV-related disease.

Table 3
Characteristics of the Study Population with Active CMV or EBV Infection

Characteristic	CMV	EBV
No. of patients (no. of active infection episodes)		
Adult	51 (59)	40 (46)
Pediatric	20 (21)	10 (12)
Total	71 (80)	50 (58)
Antiviral/anti-CD20 monoclonal antibody preemptive treatment, no		
Infection episodes		
Treated	69	0
Not treated	11	58
Samples		
Whole blood	569	351
Plasma	569	351
Total	1138	702
Sex		
Male/female	47/24	31/19
Donor type		
MUD/related	46/25	23/27
Graft origin		
BM	25	11
PBSC	43	37
CB	3	1
Combined (BM + CB)	0	1
Primary disease		
AML	32	17
ALL	14	13
Other disease	25	20
D/R serostatus		
D+/R+	28	11
D-/R+	30	1
D-/R-	1	0
D?/R+	12	38

MUD indicates matched unrelated donor; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; D, donor; R, recipient; +, positive; -, negative; D?, donor not available.
Other diseases included non-Hodgkin lymphoma, chronic myelogenous leukemia, multiple myeloma, myeloid sarcoma, and T cell lymphoma.

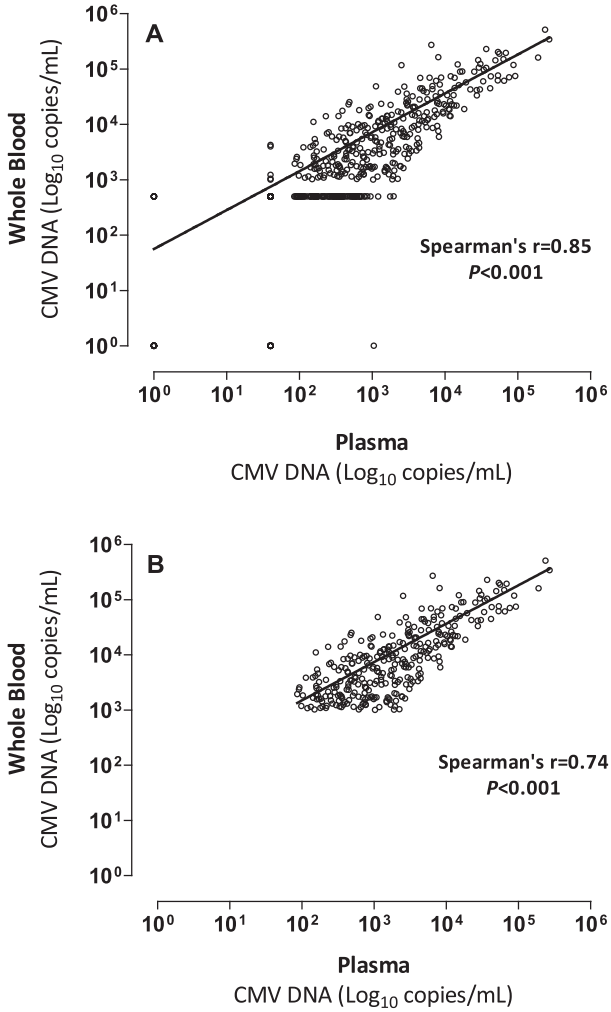

Figure 1. Correlation analysis of CMV DNA in plasma and WB samples of allo-HSCTR. (A) All samples analyzed (n = 1138). (B) Positive samples with a quantitative result (n = 620).

($P < .001$) when comparing only the positive samples with a quantitative result in both blood compartments (Figure 2B). All the paired samples from the patients without CMV or EBV infection tested negative for both CMV and EBV DNA.

Kinetics of CMV and EBV DNA Load in WB and Plasma Samples

As shown in Figure 3, the ascending and descending phases of CMV DNAemia were similar in WB and plasma. Furthermore, CMV DNA peak levels were reached simultaneously in WB and plasma in most cases (56/80, 70.0% active infection episodes). Finally, all plasma samples were CMV DNA positive at the peak of WB DNAemia. At T0, median CMV DNA values were 21,414 copies/mL in WB (range, 1233 to 509,670 copies/mL) versus 3096 copies/mL in plasma (range, 40 to 269,100 copies/mL) ($P < .001$).

As shown in Figure 4A, median CMV DNA values followed similar kinetics in the 2 blood compartments. However, before reaching the peak, median CMV DNA levels in plasma were consistently .83 log10 lower than those in WB ($P < .001$). Furthermore, the majority of the samples that were positive (below the LLoQ) in WB and negative in plasma were detected in the ascending phase.

A significant difference between the 2 groups of data was also observed in the descending phase. In detail, at week +1, 27.4% of WB samples were CMV DNA positive but below the LLoQ or negative, compared with 4.1% of plasma samples ($P < .001$). This significant difference was also observed at weeks +2 and +3 (52.0% versus 24.0%; $P < .001$ and 66.6% vs 36.6%; $P = .002$, respectively).

Evaluation of the kinetics of CMV DNA levels with respect to peak values over time revealed a consistent trend in DNA values in the ascending phase of infection in both compartments. In contrast, a slower decline was observed in CMV DNA levels in plasma samples (Figure 4B); at week +1, CMV DNA levels decreased by 80.5% in WB samples and by 44.0% in plasma samples ($P < .001$). This significant difference was

Table 4
Limits of Detection and Quantification of the Assays

	artus CMV QS-RGQ WB	*artus* CMV QS-RGQ Plasma	*artus* EBV QS-RGQ WB	*artus* EBV QS-RGQ Plasma
LoD, copies/mL	164.6	42.5	288.4	157
LLoQ, copies/mL	1,000	79.4	1,000	316

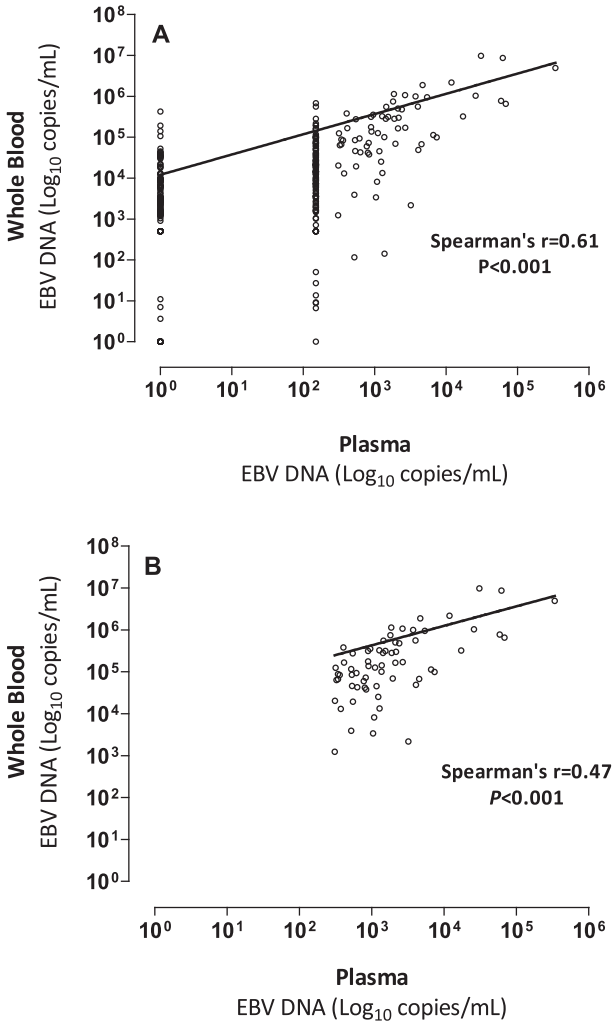

Figure 2. Correlation analysis of EBV DNA in plasma and WB samples of allo-HSCTR. (A) All samples analyzed (n = 702). (B) Positive samples with a quantitative result (n = 130).

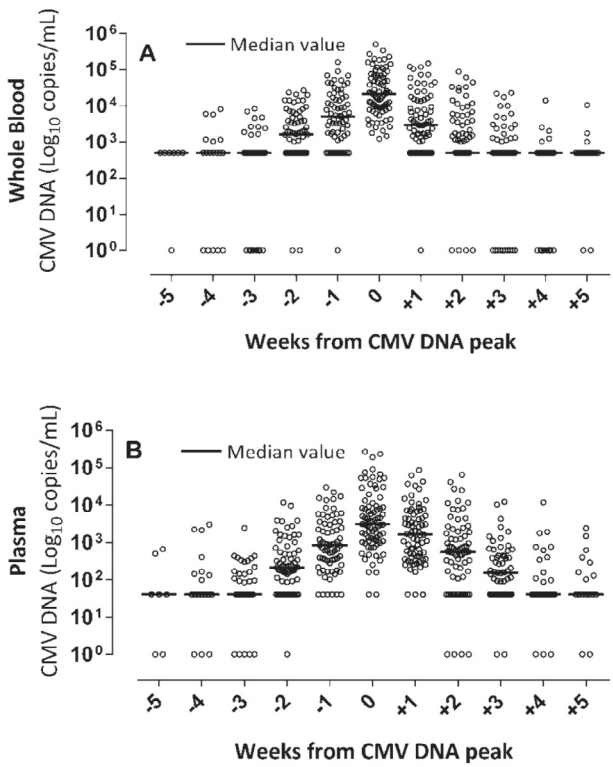

Figure 3. Distribution of CMV DNA values in WB (A) and plasma (B) samples with respect to CMV DNA peak time in WB (T0).

maintained in the rates of WB and plasma decrease at week +2 (93.4% versus 83.6%; $P < .001$) and at week +3 (97.1% versus 94.7%; $P < .001$). Furthermore, 14 of the 30 samples (46.7%) that were negative in WB and positive in plasma were detected at these 3 time points. In particular, 1, 3, and 10 discordant samples were detected at weeks +1, +2, and +3, respectively. At the next time points, no difference in the decline of CMV DNA levels in the 2 blood compartments was seen.

To evaluate whether the antiviral treatment could influence the kinetics of CMV DNA loads in the 2 blood compartments, we evaluated the viral levels in groups of patients who received preemptive therapy and those who did not (Figure 5). In the patients who received antiviral therapy, CMV DNA levels in plasma showed a significantly slower decline after the peak. Specifically, at week +1, a 83.4% decrease in CMV DNA levels was observed in WB, compared with an 35.3% decrease in plasma samples ($P < .001$). A significantly slower decrease in CMV DNA levels in plasma was also observed at week +2 (ie, 93.4% in WB versus 84.8% in plasma samples; $P < .001$) and at week +3 (ie, 97.4% in WB versus 94.2% in plasma samples; $P < .001$) (Figure 5A). In contrast, in patients who did not receive preemptive therapy, the difference in CMV DNA decay in WB versus plasma at weeks +1, +2, and +3 was not statistically significant (72.0% versus 58.0%, $P = .431$; 87.0% versus 83.0%, $P = .637$; and 89.8% versus 87.7% , $P = .297$, respectively) (Figure 5B).

Regarding EBV infection, the ascending and descending phases of EBV DNAemia with respect to WB peak time were dissimilar in the 2 blood compartments (Figure 6A,B).

Plasma samples were EBV DNA-negative at several time points, and in the remaining cases the median values of plasma viral DNA were below the LLoQ. At all time points but 1 (week –5), the median EBV load was at least 2 log10 higher in WB compared with plasma ($P < .001$; Figure 6C). In particular, at T0, the median EBV DNA level was 42,165 copies/mL (range, 1418 to 9,720,000 copies/mL) in WB and below the LLoQ at 150 copies/mL (range, 150 to 58,500 copies/mL) in plasma, a difference of 2.5 log10.

The peak EBV DNA levels in WB (T0) coincided with those in plasma in most of the infection episodes (41 of 58, 70.7%). However, 19% of plasma samples were EBV DNA- negative at T0.

DISCUSSION

Although higher CMV and EBV DNA loads have been shown to be correlated with an increased risk of viral disease in both solid organ transplant recipients and HSCTR [5,8,21-23], the lack of standardization for quantifying viral load has hindered the development of an optimal and universal preemptive therapy trigger point [5,7,22-24]. Among the issues crucial to the successful clinical application of post-transplantation virologic surveillance that remain unclear is a consensus as to which blood compartment is the most adequate for optimal CMV and EBV DNA detection [2,12,25,26]. This is the first large multicenter study analyzing the kinetics

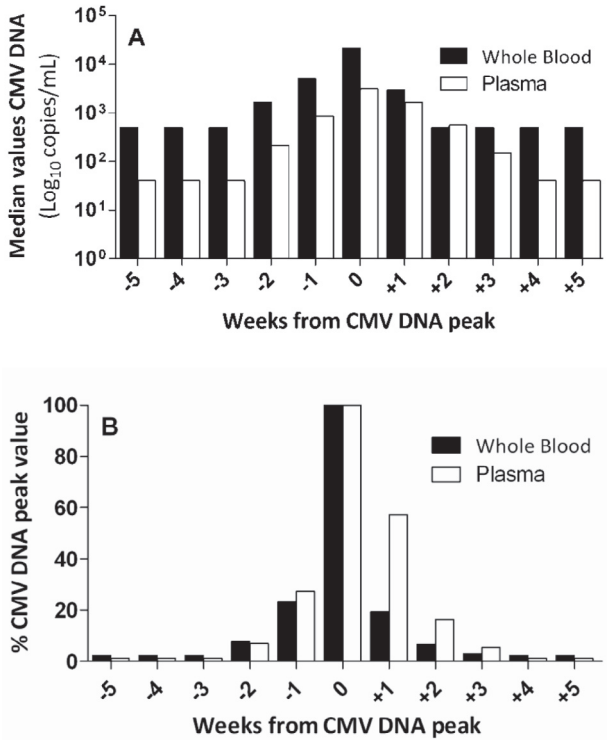

Figure 4. Kinetics of CMV DNA load in WB and plasma samples of allo-HSCTR. (A) Median values of CMV DNA in WB and plasma samples with respect to CMV DNA peak time in WB (T0). (B) Median CMV DNA levels in WB and plasma samples, expressed as percentage with respect to the correspondent median CMV DNA peak value, which was assigned a value of 100.

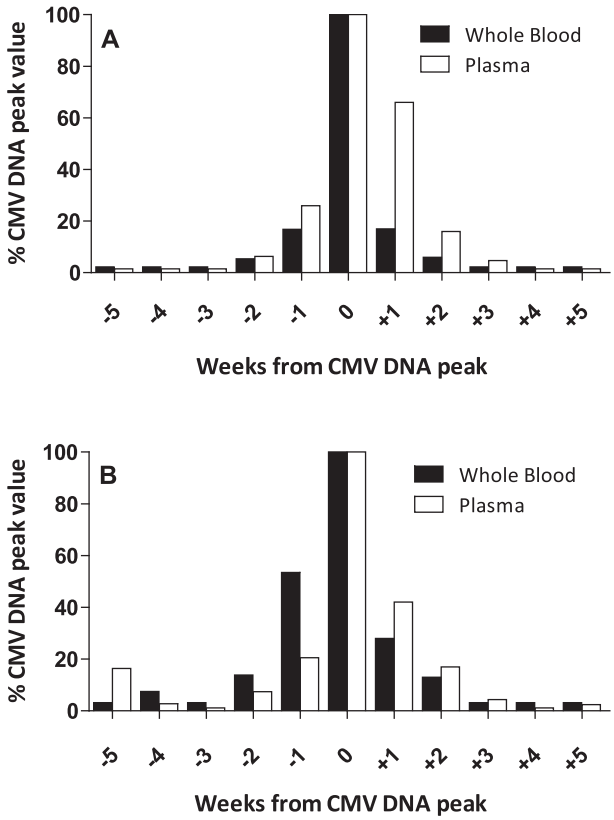

Figure 5. Kinetics of CMV DNA load in WB and plasma samples of allo-HSCTR who received antiviral therapy (A) and who did not (B) with respect to CMV DNA peak time in WB (T0). Median CMV DNA levels in WB and plasma samples expressed as percentage with respect to the correspondent median CMV DNA peak value, which was assigned a value of 100.

of both CMV and EBV DNA in 2 different blood compartments (WB and plasma) using a single automated commercial molecular method, CE-marked and Food and Drug Administration-approved for quantifying CMV and EBV DNA in both biological matrices. In fact, all the studies published at the time of this report correlated the viral load in WB and plasma samples using in-house developed PCR assays [9,13-15,25,27,28] or commercial molecular assays designed to test only plasma matrix [12,17,19] or combinations of different commercial molecular assays or commercial and in-house developed PCR tests [11,16,18]. Furthermore, our present study is the only one comprehensively analyzing episodes of viral replication, following both the ascending and descending phases of both CMV and EBV active infection. In contrast, in previous studies, only the overall correlation was investigated, based on the assumption that no differences would be seen in the different phases of the infection. Finally, no previous studies have ever compared the DNA kinetics of CMV and EBV in WB and plasma using a single experimental approach.

In particular, we performed a comparative evaluation for quantitative detection of CMV and EBV DNA in serial paired WB samples versus plasma samples collected from pediatric and adult HSCTR actively infected. Testing the paired blood samples using a single approved method allowed us to eliminate the variability in interassay quantification and obtain reliable quantitative results. Furthermore, the results for the control group showed 100% specificity of the molecular assay used.

In agreement with previous studies [11,13], a very high correlation between CMV DNA levels in the 2 types of specimens was observed. In addition, as reported by other authors in both the solid organ transplantation and HSCT settings [12,15], median values of CMV DNAemia in WB were overall approximately 1 log10 higher than those in plasma. CMV is highly cell-associated, and both cell-free and intracellular CMV DNA are detected in the WB. However, this biological characteristic can only partly explain the DNAemia differences in the 2 blood compartments. Indeed, CMV DNAemia follows different kinetics in WB and plasma, particularly in treated patients. To analyze the kinetics of CMV DNAemia irrespective of the different absolute amounts of CMV DNA in WB and plasma, values in the 2 blood compartments were normalized with respect to the relevant peak values.

Our analysis showed that in the ascending phase of the infection, CMV DNAemia is detected more frequently in WB than in plasma, whereas in the descendent phase, DNAemia is more often positive in plasma than in WB. However, the peak infection is reached simultaneously in both compartments. These data confirm and extend previous observations by other authors [11], reporting a more rapid initial decline of CMV DNA levels in WB versus plasma. In our study, the difference in the decline of CMV DNA levels in the 2 blood compartments was statistically significant during the first 3 weeks after peak viral load. Of note, a greater number of persistent plasma CMV DNAemia cases were observed at these 3 time points. A statistically significant difference was also observed when the impact of antiviral therapy on viral kinetics

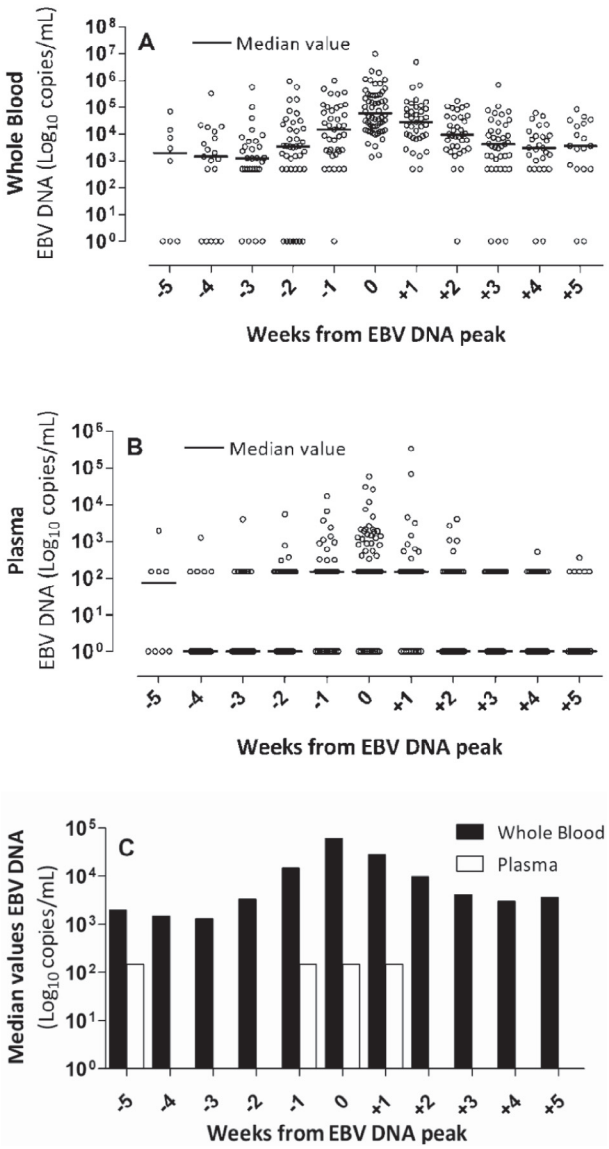

Figure 6. Kinetics of EBV DNA load in WB and plasma samples of allo-HSCTR. Distribution of EBV DNA levels in WB (A) and plasma (B) samples with respect to the time of the EBV DNA peak in WB (T0). (C) Median values of EBV DNA in WB and plasma samples with respect to T0.

was evaluated. As suggested by Lisboa et al. [11], the higher plasma viral loads detected in plasma could represent free CMV DNA released from cells or tissue. After administration of preemptive therapy, the slower CMV DNA decay in plasma, resulting in residual CMV DNAemia, could delay treatment interruption. No difference was observed in the decline of WB and plasma CMV DNA levels in patients who did not receive antiviral therapy. One limitation of this study is the higher number of treated CMV infection episodes than the number of untreated episodes.

Concerning EBV DNA load quantification, a lower correlation between EBV DNA levels in the 2 blood compartments was found. Moreover, a significant difference in EBV DNA values in WB versus plasma was observed. Indeed, whereas EBV DNAemia in WB could be quantified in the majority of samples, the median viral EBV DNA values detected in plasma did not exceed the LLoQ of the molecular assay. The marked discrepancy between EBV DNA levels in the 2 blood compartments relies on the strict cell-associated nature of the infection. This hypothesis also explains the highly different kinetics of EBV DNA in the 2 blood compartments. In WB, it was possible to observe 3 distinct phases of the infection episode: an ascending phase, a peak phase, and a descending phase of EBV DNAemia. In contrast, in plasma, the small number of samples with quantitative viral DNA results precluded recognition of the 3 phases of EBV infection. In addition, 19% of plasma samples were negative at the time of EBV DNA peak in WB samples, indicating poor sensitivity of this analysis. In light of these results, plasma did not prove to be a suitable clinical specimen for monitoring EBV DNA load after transplantation to identify patients at risk for developing EBV-related diseases. These findings are in agreement with and extend previous observations reported by other authors that support the use of WB over plasma for surveillance EBV infection in HSCTR [9,14,27,28].

Our results suggest a preferential use of WB as clinical specimens for post-transplantation CMV infection monitoring, because when using the plasma PCR assay, the results could hinder clinical patient management, particularly when the response to antiviral therapy is being monitored. However, based on the differences observed in CMV DNA values and in the kinetics of viral DNAemia in the 2 blood compartments, the use of only 1 type of specimen is highly recommended when serially surveilling patients, to ensure comparability of results.

Regarding post-transplantation EBV infection, the low sensitivity of plasma for identifying post-transplantation lymphoproliferative disorders limits its clinical usefulness in the management of infection. Moreover, previous studies involving both solid organ transplantation recipients and HSCTR have shown that monitoring of EBV DNAemia in WB is a valuable alternative to measuring EBV DNA load in peripheral blood mononuclear cells, which is an indirect measure of EBV-driven B cell proliferation [20,28]. WB and peripheral blood mononuclear cells are equally useful for assessing the risk for developing an EBV-related post-transplantation lymphoproliferative disorder [4].

The establishment of a preferential CMV and EBV clinical specimen for post-transplantation monitoring will be helpful in identifying the DNA thresholds for preemptive therapy and developing an appropriate virologic monitoring model as part of routine follow-up of HSCTR, to identify and treat patients at the greatest risk of onset of illness and ensure the proper treatment timing.

Strengths of this study include its large sample size, single experimental approach, strict virologic inclusion and exclusion criteria, single method used, and analysis of the complete episode for each viral replication (ascending and descending phases). Finally, because the cohort of HSCTR in this study was significantly heterogeneous, the correlations between transplantation-related characteristics and CMV and EBV reactivations were not investigated, and subgroup analyses according to risk strata were not possible. We maintain that the impact of the transplantation-related characteristics, such as primary disease, donor type, graft origin, conditioning regimen, and use of anti-thymocyte immunoglobulin, on WB CMV and EBV kinetics can be evaluated in prospective clinical trials. These results could be useful in identifying potential risk factors for the development of severe CMV and EBV infections. A tailored anti-CMV and -EBV strategy for each individual patient is a major goal in transplantation.

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