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Factors influencing cytomegalovirus DNA load measurements in whole blood and plasma specimens from allogeneic hematopoietic stem cell transplant recipients

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ABSTRACT

We assessed the impact of several parameters, including the nature of the episode of Cytomegalovirus (CMV) DNAemia, the use of preemptive antiviral therapy, and the blood cell content in CMV DNA loads measured in whole blood (WB) and plasma (PL). CMV DNA load was quantified in 245 paired specimens collected within 43 postengraftment episodes of CMV DNAemia by using the CMV Real*Time* CMV PCR (Abbott Molecular). Concordant categorical results were obtained for 78.4% of paired specimens (Kappa index, 0.385; $P = <0.001$). Overall, CMV DNA loads in PL were higher than those in WB (mean bias, +0.115 log IU/mL) in both initial and recurrent episodes; this was so in post-antiviral treatment but not in pretreatment paired specimens. Median CMV DNA doubling time values in both compartments were not significantly different. Leukocyte counts had a significant impact on the comparability of CMV DNA loads measured in both matrices.

1. Introduction

Cytomegalovirus (CMV) infection is a major threat following allogeneic hematopoietic stem cell transplantation (allo-HSCT), as it may lead to end-organ disease and increase nonrelapse mortality (Pérez Romero et al., 2015). Preemptive therapy (PET) is the first-choice strategy for the prevention of CMV-related complications in this transplant modality (Pérez Romero et al., 2015). Systematic and frequent monitoring of CMV DNA load in the blood compartment is a cornerstone for the success of this therapeutic approach, most commonly using whole blood (WB) and plasma (PL) as matrices. Current consensus guidelines for CMV infection management in allo-HSCT describe both as equally suitable for that purpose (Emery et al., 2013; 7th ECIL, 2017). Nevertheless, a number of studies indicate that CMV DNA loads measured in both compartments are not similar (Babady et al., 2015; Costa et al., 2016; Dioverti et al., 2017; Garrigue et al., 2008; Jones et al., 2016; Lazzarotto et al., 2018; Lisboa et al., 2011; Razonable et al., 2002; Suganda et al., 2016; von Müller et al., 2007). Unfortunately, only a few of these studies were conducted using molecular methods approved by regulatory agencies (either CE, FDA, or both) for the 2 matrices (Jones et al., 2016; Lazzarotto et al., 2018), of paramount importance for a rigorous comparative analysis.

Here we assessed the impact of several parameters, including the nature of the episode of CMV DNAemia (initial vs. recurrent), the use of antiviral therapy, and the blood cell content on CMV DNA loads measured in paired WB and PL specimens from allo-HSCT recipients.

2. Patients and methods

2.1. Patients and clinical specimens

In this retrospective single-center study, we included a total of 245 paired PL and WB specimens (total number, 490 samples) from 33 nonconsecutive allo-HSCT recipients (baseline characteristics of patients are shown in Supplementary Table 1) submitted to our clinical microbiology laboratory within the period February–September 2014. PL specimens were used for routine CMV DNA load quantitation as per laboratory guidelines. WB samples were requested for the current study and were scheduled to be collected with the same frequency as the PL

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specimens. Leftover PL specimens and unmanipulated WB samples were cryopreserved at −70 °C within 24 h of receipt, until used. The availability of at least 2 paired specimens/episode of CMV DNAemia was the only criterion for episodes inclusion.

2.2. CMV DNA quantitation in whole blood and plasma

CMV DNA load in both matrices was quantified using the CMV Real*Time* CMV PCR (Abbott Molecular, Des Plaines, IL, USA) (Clari et al., 2013). This assay targets the UL34 and UL80.5 ORFs of the CMV genome and has been validated for CMV DNA quantitation in both PL and WB specimens (CE label for both matrices and FDA approval for EDTA-plasma specimens). According to the manufacturer, both the limits of detection (LOD) and quantitation (LOQ) are 31.2 IU/mL for PL and 62.4 IU/mL for WB. DNA extraction from both matrices was performed with the Abbott mSample preparation system DNA Kit on the 2000sp instrument. The starting volume for DNA extraction was 500 μL for PL and 300 μL for WB, and the final elution volumes were 70 μL and 110 μL, respectively. An internal control is supplied to check the overall process including DNA extraction and possible PCR inhibition. Amplification and real-time detection of CMV DNA were performed on the m2000rt platform. For both matrices, the total PCR volume was 60 μL (35 μL eluate and 25 μL master mix). Cryopreserved whole blood and plasma specimens were retrieved and analyzed in parallel within 24 h of thawing. The specimens were assayed in singlet in several consecutive runs. Paired specimens from individual patients were analyzed in the same run.

2.3. CMV infection management

CMV DNA load monitoring was conducted once or twice a week (at physician discretion) during CMV DNAemia episodes. Antiviral therapy with (val)ganciclovir or foscarnet at conventional doses was initiated when the PL CMV DNA load reached levels of 1000 copies/mL (>1500 IU/mL) in a single specimen and was interrupted upon documentation of 1 or 2 consecutive negative PCR results (Solano et al., 2013).

2.4. Blood cell counts

The absolute leukocyte, lymphocyte, and mononuclear cell counts were enumerated with either the ADVIA 120 Hematology System (Siemens Healthineers, Erlangen, Germany) or the XN-9000™ Hematology Analyzer (Sysmex Co., Kobe, Japan).

2.5. Kinetic analyses of CMV DNA load

The CMV DNA doubling time (dt) and half-life were estimated as previously reported (Giménez et al., 2014; Muñoz-Cobo et al., 2011). The CMV DNA dt was estimated using CMV DNA loads measured in 2 successive specimens (usually the 2 first specimens yielding detectable CMV DNA loads) in the absence of antiviral therapy. The dt (in days) is given by $dt = (t2 - t1) \times \ln(2)/\ln(q2/q1)$, with q1 and t1 being the CMV DNA load at the time of the first measurement, respectively, and q2 and t2 the CMV DNA load at the time of the second one, respectively. CMV dt calculations were performed only for episodes in which the increase in CMV DNA load between the 2 specimens drawn no longer than 10 days apart was ≥3-fold. The kinetics of CMV DNA load clearance follows a logarithmic decay curve in episodes treated with antivirals, expressed by the equation $yt = y0e^{-kt}$, where y0 is the CMV DNA load at the time of treatment inception, *t* is time from initiation of antiviral therapy, and *k* is the decay constant. A nominal value of 10 IU/mL was arbitrarily ascribed for specimens with undetectable CMV DNA load. CMV DNA load half-life was then calculated using the equation $\ln 2/k$.

2.6. Statistical methods

CMV DNA loads in IU/mL were transformed to $\log_{10}$ prior to performing comparisons. The qualitative agreement between PL and WB results was estimated using Kappa statistics. Differences between median values were analyzed using either the Wilcoxon rank test (for paired samples) or the Mann–Whitney *U* test for independent measurements, as appropriate. Quantitative correlations between the CMV DNA loads were assessed using the Spearman correlation test. The Bland and Altman method was used to evaluate the agreement between CMV DNA loads measured in both matrices. Sensitivity was calculated considering all detectable CMV DNA results in either specimen as true positives. Statistical calculations were performed using the SPSS 17.0 program (SPSS Inc. Chicago, IL); *P* values of ≤0.05 were considered statistically significant.

3. Results

3.1. Features of CMV DNAemia episodes

In all, 245 paired PL and WB specimens collected within 43 episodes of CMV DNAemia occurring following engraftment in 33 allo-HSCT recipients were tested. A median of 5 paired specimens/episode (range, 2–23) was available. Out of the 43 episodes, 24 were initial episodes and 19 were recurrences. According to routine PCR testing results using fresh plasma specimens, initial episodes developed at a median of 30 days (range, day 17–50) after allo-HSCT, and recurrences were documented at a median of 193 days after transplantation (range, day 35–599). Median CMV DNA peak levels in initial and recurrent episodes of CMV DNAemia were 3.26 log IU/mL (range, 1.75–6.09 log IU/mL) and 2.92 log IU/mL (range, 2.22–3.65 log IU/mL), respectively. Overall, 19 out of the 43 episodes were preemptively treated with antivirals (12 initial episodes and 7 recurrences).

3.2. CMV DNA loads in plasma and whole blood specimens

In total, 192 out of the 245 paired specimens (78.4%) yielded concordant categorical results (positive, including detectable and quantifiable, or negative [not detectable]) (Kappa index, 0.385; $P = <0.001$). The data are shown in Table 1. CMV DNA was detectable in 163 paired specimens (66.5%), whereas negative results were recorded for 29 (11.8%). Discordant results were obtained for 53 paired specimens (21.6%). In most cases ($n = 33$), these yielded negative results in WB but positive in PL (median, 1.71 $\log_{10}$ IU/mL; range, 1.48–2.92 $\log_{10}$ IU/mL). The opposite was seen in the remaining 20 paired specimens (median CMV DNA load in WB, 1.79 log IU/mL; range, 1.79–2.39 log IU/mL).

Table 1
Categorical concordance between CMV DNA detection in paired PL and WB specimens from allo-HSCT recipients.

CMV DNA in plasma[a]	CMV DNA in whole blood (no. of specimens)		
	Quantifiable (>LOQ)	Detectable but not quantifiable (<LOQ)	Not detectable
Quantifiable (>LOQ)	132	28	29
Detectable but not quantifiable (<LOQ)	0	3	4
Not detectable	5	15	29

[a] According to the manufacturer, both the LOD and LOQ are 31.2 IU/mL (95% confidence) for PL and 62.4 IU/mL (95% confidence) for WB. Nevertheless, the assay is capable of detecting CMV DNA levels below this threshold in both matrices, although with a lower confidence (75%). In our experience, these very low CMV DNA loads are not usually bleeps but eventually increase in subsequent specimens to become quantifiable (unpublished data). Thus, specimens meeting this characteristic were categorized here as having detectable but not quantifiable CMV DNA.

Thus, in our series CMV DNAemia detection was achieved more frequently using PL than WB (89.0% vs. 84.0%, respectively).

Overall, CMV DNA loads quantified in PL were significantly higher ($P = <0.0001$) than those in WB (Fig. 1 panel A), as shown by a mean bias of +0.115 log IU/mL (range, −0.660 to 0.890) (Fig. 1, panel B). This trend was documented for specimens containing both low (arbitrarily defined as ≤1000 IU/mL) and high (>1000 IU/mL) CMV DNA levels. Nevertheless, the difference was not statistically significant for low–CMV DNA content specimens (Table 2).

Overall, CMV DNA loads measured in whole blood and plasma correlated significantly (Fig. 2, panel A). The degree of correlation was notably lower, yet significant, for paired specimens with low CMV DNA content (Fig. 2, panel B) than for those with high CMV DNA load (Fig. 2, panel C).

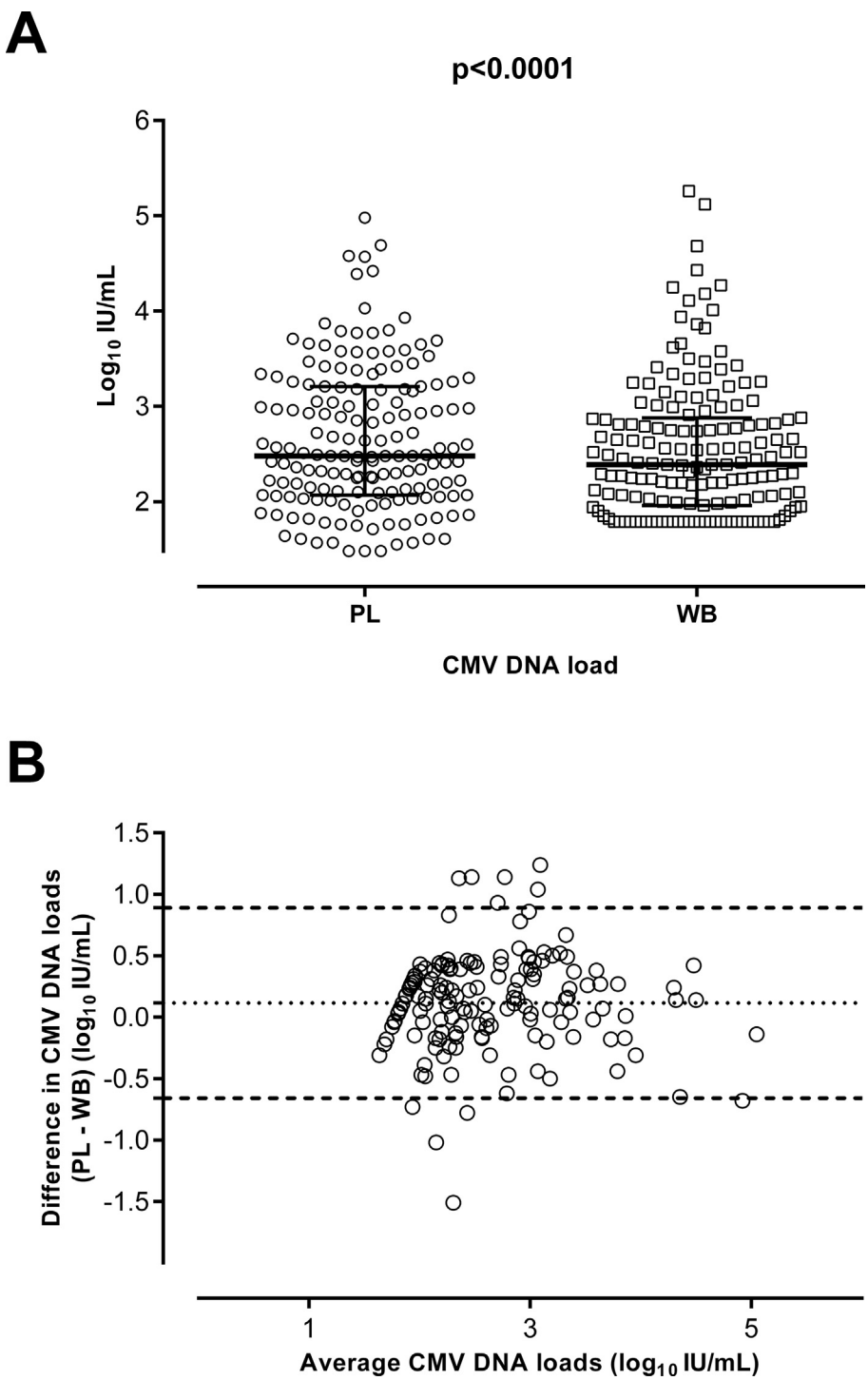

Fig. 1. CMV DNA loads measured in PL and WB specimens from allo-HSCT recipients. (A) CMV DNA loads (log IU/mL) in 163 paired PL and WB yielding positive (detectable) PCR results in both matrices. PL and WB specimens with detectable CMV DNA but below the LOQ were arbitrarily ascribed a nominal value of 1.48 log IU/mL or 1.79 log IU/mL (0.01 log IU/mL lower than the LOQ in these matrices), respectively. The *P* value is shown. (B) Bland–Altman analysis of the results shown in panel A.

Table 2
CMV DNA loads in paired PL and WB specimens from allo-HSCT recipients.

Categories (no. of specimens)[a]	CMV DNA load in PL in log IU/mL[b]		CMV DNA load in WB in log IU/mL[b]		*P* value
	Median	Range	Median	Range	
Total (163)	2.48	1.48–4.98	2.39	1.79–5.26	<0.0001
≤3 log IU/mL (113)	2.25	1.48–3.0	2.18	1.79–3.43	0.058
>3 log IU/mL (50)	3.46	3.02–4.98	3.22	1.9–5.26	<0.0001

[a] Categories were arbitrarily established according to CMV DNA loads measured in PL at the time of routine testing. Overall, these figures differed by <0.11 log IU/mL from PL CMV DNA loads measured in cryopreserved specimens.

[b] The dynamic range of quantitation of the assay is 1.48 to 8.19 $\log_{10}$ IU/mL and 1.79 to 8.19 $\log_{10}$ IU/mL for PL and WB, respectively, and the intra-assay and interassay coefficients of variation for CMV DNA loads within the linear range of quantitation are <0.25 $\log_{10}$ according to the manufacturer and our own experience (unpublished results). PL and WB specimens with detectable CMV DNA but below the LOQ were arbitrarily ascribed a nominal value of 1.48 log IU/mL or 1.79 log IU/mL (0.01 log IU/mL lower than the LOQ in these matrices), respectively.

The above observations were consistently reproduced when initial and recurrent episodes of CMV DNAemia were considered separately for analysis (Supplementary Table 2).

3.3. The effect of antiviral treatment on CMV DNA loads in whole blood and plasma

Out of the 163 paired specimens with detectable CMV DNA, 73 (44.8%) were obtained while patients were under antiviral treatment. CMV DNA loads in pretreatment paired samples were not significantly different across specimen types (PL: median, 2.3 log IU/mL; range, 1.48–4.58 log IU/mL; WB: median, 2.26 log IU/mL; range, 1.79–2.56 log IU/mL; $P = 0.740$), whereas CMV DNA loads in PL were significantly higher than in WB ($P = <0.0001$) in posttreatment specimens (median 3.0 log IU/mL; range, 1.61–4.98 log IU/mL in PL and median, 2.65 log IU/mL; range 1.79–5.12 log IU/mL, in WB).

3.4. The effect of blood cellularity on CMV DNA loads in whole blood and plasma

Contemporary blood cell counts were available for a total of 158 out of 163 paired PL and WB specimens with detectable CMV DNA. As shown in Fig. 3, total leukocyte counts were significantly higher in blood samples in which CMV DNA loads in WB exceeded (any level) those in PL specimens ($n = 57$) than in those in which the opposite was observed (panel A). In contrast, the absolute numbers of lymphocytes (panel B) and monocytes (panel C) were not significantly different.

3.5. Kinetics of CMV DNA load in plasma and whole blood

We compared the CMV DNA dt_s in paired PL and WB specimens. A total of 28 episodes out of the 43 episodes of CMV DNAemia included in the study fulfilled the criteria for dt calculation; the remaining 15 episodes were excluded because either the difference between q1 (initial CMV DNA load) and q2 (CMV DNA load at the time of the second PCR positive result) was <3-fold ($n = 9$) or the time elapsed between q1 and q2 measurements (t1 and t2) was longer than 10 days ($n = 6$) (Giménez et al., 2014; Muñoz-Cobo et al., 2011). Nominal values of 30 IU/mL and 61 IU/mL were considered for PL and WB specimens with detectable–not quantifiable results. Overall, median CMV DNA dt values in both compartments were comparable (median 2.01 days, range 0.93–4.77 days in PL and median 2.32 days, range 0.92–5.28 days in WB; $P = 0.260$) and did correlate significantly ($P = <0.0001$). Categorical agreement for CMV dt_s (≤2 days vs. >2 days) was observed in 20 out of the 28 episodes (Kappa, 0.429; $P = 0.004$). CMV DNA clearance following inception of PET, as inferred by its half-life, appeared faster in WB than in PL (median 4.93 days, range 1.30–14.81 days in PL and median 2.43 days, range 1.17–13.57 days in WB; 12 episodes were included for analyses), although the difference did not reach statistical significance ($P = 0.180$), yet the first undetectable PCR result was observed in PL or WB for a comparable ($P = 0.51$) number of episodes subjected to analysis (not shown).

4. Discussion

Several studies have compared CMV DNA loads measured in WB and PL samples drawn from allo-HSCT recipients (Babady et al., 2015; Costa et al., 2016; Dioverti et al., 2017; Garrigue et al., 2008; Lisboa et al., 2011; Razonable et al., 2002; Suganda et al., 2016; von Müller et al., 2007), but aside from ours, only 2 (Jones et al., 2016; Lazzarotto et al., 2018) employed a real-time PCR assay approved by international agencies for use in both matrices: CE for the Abbott assay (Jones et al., 2016) and FDA for the artus® CMV QSR GQ Kit (QIAGEN, Hamburg, Germany) (Lazzarotto et al., 2018). There is currently no consensus on which specimen should be used for CMV DNA load monitoring in this setting. In fact, recent consensus guidelines for managing CMV infection in this transplant modality are cautious on the matter and do not favor the use of one over the other (Emery et al., 2013; 7th ECIL, 2017). There are a number of issues on this topic that remain unsettled. First, which specimen is better for documentation of CMV DNAemia? In our experience, PL specimens were slightly better than WB; this is in line with data obtained with the COBAS® AmpliPrep/COBAS® TaqMan® CMV test from Roche Diagnostics (Indianapolis, IN) (Babady et al., 2015; Dioverti et al., 2017; Suganda et al., 2016) and the artus® CMV QSR GQ Kit (QIAGEN, Hamburg, Germany) (Lazzarotto et al., 2018) and is presumably due, at least in part, to the increased sample volume used for PL specimens (Babady et al., 2015; Dioverti et al., 2017; Suganda et al., 2016). In contrast, 2 studies reported the opposite results; in 1 of them (Costa et al., 2016), 2 different real-time PCR assays were used for CMV DNA monitoring in PL and WB; the other employed a substantially less sensitive end-point PCR assay, the COBAS AMPLICOR CMV MONITOR (Roche Diagnostics) (von Müller et al., 2007).

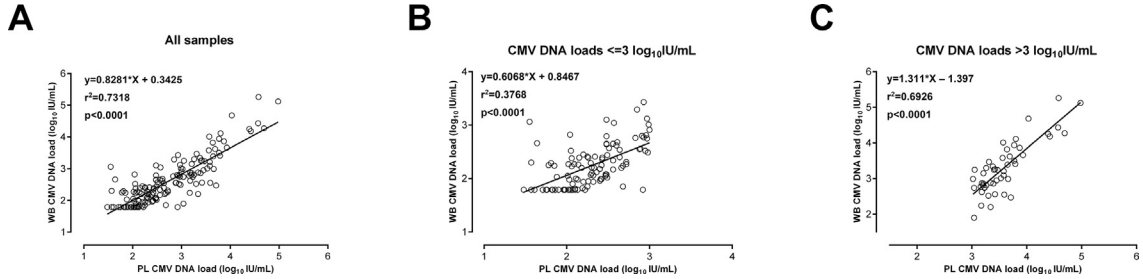

Fig. 2. Linear correlation between CMV DNA loads quantified in PL and WB from allo-HSCT recipients. (A) Correlation for all paired PL and WB specimens included in the analyses. PL and WB specimens with detectable CMV DNA but below the LOQ were arbitrarily ascribed a nominal value of 1.48 log IU/mL or 1.79 log IU/mL respectively. Correlation between CMV DNA loads measured in PL and WB according to the CMV DNA content measured in PL at the time of routine testing: (B) low content (CMV DNA load ≤1000 IU/mL) and (C) high content (CMV DNA load >1000 IU/mL). Spearman rank correlation and *P* values are shown.

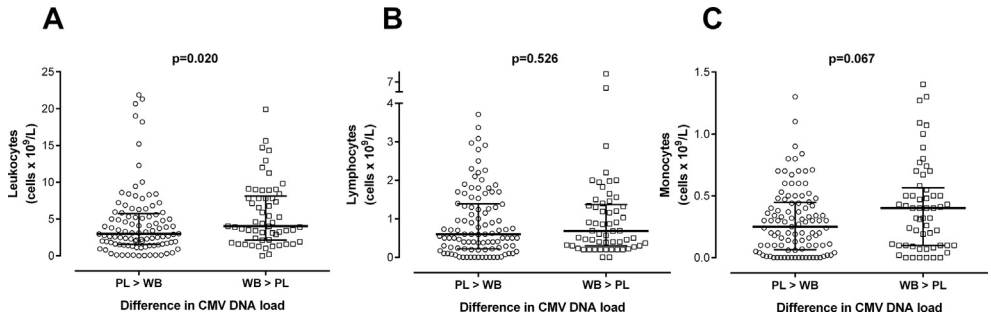

Fig. 3. Impact of blood cell counts on CMV DNA loads measured in PL and WB paired specimens from allo-HSCT recipients. The number of cases in which the CMV DNA load was higher (any increase) in WB than in PL or vice versa was plotted against the total number of leukocytes (A), lymphocytes (B), and monocytes (C) in contemporary blood specimens. *P* values are shown for comparisons.

Second, how do CMV DNA loads quantified in both compartments compare? There is consensus that CMV DNA loads measured in WB and PL correlate significantly in allo-HSCT recipients (Babady et al., 2015; Costa et al., 2016; Dioverti et al., 2017; Lazzarotto et al., 2018; Suganda et al., 2016; von Müller et al., 2007), although the extent to which varies widely across studies (R^2 from 0.489 to 0.9575). In our series, the degree of correlation was overall fairly good ($R^2 = 0.73$) but was notably lower ($R^2 = 0.376$) for paired specimens with low CMV DNA content (arbitrarily defined as ≤1000 IU/mL). Historically, transplant recipients have been reported as having higher magnitude CMV DNA loads in WB than in PL (Garrigue et al., 2008; Lisboa et al., 2011; Razonable et al., 2002; von Müller et al., 2007), attributed in part to the highly cell-associated nature of CMV (Pérez Romero et al., 2015). This trend is also seen in more recent studies targeting allo-HSCT recipients (Babady et al., 2015; Costa et al., 2016; Dioverti et al., 2017; Lazzarotto et al., 2018; Suganda et al., 2016). Our data indicated otherwise. In fact, overall CMV DNA loads quantified in PL were higher than in WB, this proving true over the entire range of CMV DNA values measured, both in initial and in recurrent episodes. Several non–mutually exclusive reasons may account for this discrepancy: i) The degree of commutability of the CMV WHO IS (to which CMV PCR assays are calibrated nowadays) in both matrices may differ across commercially available PCR assays; in addition, careful validation of conversion factors provided by the manufacturers for the WHO IS in WB has been shown to be advisable (Schnepf et al., 2013). ii) The use of PCR assays with large amplicon sizes (i.e., the Roche assay with amplicon size 340 nt vs. 95 nt-UL80.5 and 105 nt-UL34 for the Abbot assay) may infraestimate to a greater extent CMV DNA loads in PL than in WB. This assumption is based on 2facts: (i) CMV DNA in PL is almost exclusively free DNA, highly fragmented, and not virion associated (Boom et al., 2002; Tong et al., 2017), whereas WB contains infectious virions (intact genomes) in addition to fragmented viral DNA (Pérez Romero et al., 2015), and (ii) PCR assays with small amplicon sizes yield significantly higher CMV DNA loads in PL compared to assays with larger amplicon sizes (Preiksaitis et al., 2016; Tong et al., 2017).

Our data provide further clues for interpreting the above studies. We showed that after engraftment, variations of blood leukocytes but not monocytes or lymphocytes counts have a notable impact on viral loads measured in both matrices, as previously hinted (Bressollette-Bodin et al., 2009). Specifically, total leukocyte counts were significantly higher in blood samples in which CMV DNA loads in WB exceeded those in PL specimens. This is consistent with the role of neutrophils as major reservoirs of CMV DNA within episodes of active CMV infection and with the fact that CMV accessing the blood compartment from organ and tissues may be a quantitatively relevant source of viral DNA in plasma (Pérez Romero et al., 2015). We also observed that the difference between CMV DNA load in PL and WB specimens reached statistical significance for post–antiviral treatment paired specimens but not for pretreatment ones. This could be explained by the fact that CMV DNA levels in PL appeared to decline at a slower pace than in WB, as reflected by a higher (not significant) half-life, in agreement with a previous study (Lazzarotto et al., 2018). Despite this, first undetectable PCR results were obtained in both matrices at a comparable frequency. Accordingly, in our setting, the use of WB instead of PL would not have led to longer antiviral treatment courses.

We previously showed that the CMV DNA dt, as calculated herein, is relatively unaffected by the inherent variability in CMV DNA loads provided by different CMV real-time PCR assays, thus permitting comparison (Vinuesa et al., 2016). CMV DNA dt_s ≤2 days were found to anticipate the need for antiviral therapy according to our local criteria (Giménez et al., 2014). Here, we found reasonable agreement between CMV DNA dt_s in both matrices; thus, this parameter may be used as a trigger to start PET when CMV DNA load is monitored in WB. In line with our observation, Babady et al. (2015) reported comparable trends (similar slopes) of CMV DNA load in paired PL and WB specimens from a number of allo-HSCT recipients.

The current study has 2 main limitations. First, despite having the same collection schedule as the PL specimens, a number of WB specimens were unavailable. Second, cryopreserved specimens were used for side-by-side PCR analyses in both matrices; nevertheless, it is uncertain to what extent (if any) this could have had an impact on our observations.

In our view, the data presented herein add to the current body of knowledge on this issue and may assist transplant centers to choose between WB and PL for CMV DNA monitoring in allo-HSCT recipients.

5. Conclusion

Blood cell content and presence or absence of antiviral treatment have a major impact on the magnitude of CMV DNA loads quantified in paired whole blood and plasma specimens obtained from allo-HSCT recipients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2018.11.012.

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Conflicts of interest

The authors declare no conflicts of interest.

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