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# Early Kinetics of Plasma Cytomegalovirus DNA Load in Allogeneic Stem Cell Transplant Recipients in the Era of Highly Sensitive Real-Time PCR Assays: Does It Have Any Clinical Value?

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**We report that in a population of allogeneic stem cell transplant recipients, determination of the viral doubling time (dt) of the cytomegalovirus (CMV) DNA plasma load predicted the eventual need for inception of preemptive antiviral therapy, whereas the level of the initial plasma CMV DNA load did not. The data thus indicated that determination of the dt of CMV DNA may be useful in the therapeutic management of CMV infection in this clinical setting.**

Preemptive antiviral therapy based on virological monitoring is currently the standard of care for the prevention of cytomegalovirus (CMV) disease in allogeneic stem cell transplant (Allo-SCT) recipients (1–3). Quantitative real-time PCR (qRT-PCR) tests have largely replaced the pp65 antigenemia (AG) assay for guiding antiviral inception, with satisfactory clinical results (1, 2, 4, 5). While in the AG test era, the positivity of the assay triggered the initiation of antiviral therapy, in the qRT-PCR era, the deployment of treatment is delayed until the CMV DNA load in the blood reaches a predetermined threshold. This is meant to avoid overtreatment, as qRT-PCR tests are more sensitive than the AG assay (3, 4). The fact is that the limits of detection (LOD) and quantitation (LOQ) of most "in-house" and commercially available qRT-PCRs are well below the plasma CMV DNA load thresholds that are widely accepted for triggering the initiation of preemptive antiviral therapy (between 500 and 1,000 copies/ml at most centers, in the setting of no risk-adapted strategies) (1). In our experience (6), CMV DNA loads at the beginning of episodes of active CMV infection (first and even second positive qRT-PCR results) are frequently of low magnitude (below 500 copies/ml); this seems to be the experience of other groups as well (7, 8). However, whether information on clinical utility could be derived from these early measurements remains to be determined. In the present study, we investigated whether the analysis of early kinetics of plasma CMV DNA load, as measured by highly sensitive qRT-PCR assays, allowed us to predict which episodes of active systemic CMV infection would eventually need to be treated in the context of a preemptive antiviral therapy strategy triggered by widely accepted plasma CMV DNA load cutoffs (1).

The patients included in this study ($n = 82$) underwent Allo-SCT at the Hematology Unit of University Clinic Hospital of Valencia between May 2010 and February 2013. The median age of the patients was 46 years (range, 15 to 71 years). The most relevant characteristics of the patients are shown in Table 1. From May 2010 to May 2012, patients were treated preemptively, as described previously (9), when the plasma CMV DNA load reached >500 copies/ml, as determined by the Abbott CMV PCR kit (Abbott Molecular, Des Plaines, IL) (4). Since May 2012, the CMV DNA load threshold for the initiation of antiviral therapy has been set at 1,000 copies/ml, as determined by the new Real*Time* CMV PCR (Abbott Molecular, Des Plaines, IL). This cutoff approximately equates to that employed earlier, as the new Real*Time* PCR assay yields CMV DNA values of higher magnitude than the CMV PCR kit (10). The limits of detection and quantitation of both qRT-PCR assays are approximately 20 copies/ml (95% confidence interval [CI]) (4, 11). Data from 17 patients guided by the Abbott CMV PCR kit had been previously reported (6).

Fifty-five of the 82 patients developed an episode of active CMV infection within the first 100 days following Allo-SCT, at a median of 32 days after transplant (range, 2 to 56). Only the first episodes of active CMV infection were considered for analysis in this study. Of these, 32 patients were preemptively treated with antivirals, whereas the remaining 23 patients spontaneously cleared the episode. Only one patient developed CMV end-organ disease (intestinal disease). Initial CMV DNA loads were below the threshold for the inception of antiviral therapy in all episodes, regardless of whether these were ultimately treated or not (data not shown); in fact, in 41 of 55 episodes, they were below 100 copies/ml. We first asked whether the initial CMV DNA load in episodes that were eventually treated with antivirals differed from that in episodes that were not. For this analysis, CMV DNA load values obtained by the respective qRT-PCR assay were normalized to the *1st WHO International Standard for CMV for Nucleic Acid Amplification-Based Assays* (National Institute for Biological Standards and Control, Hertfordshire, United Kingdom) and expressed in IU/ml (12). The conversion factors (from copies/ml to IU/ml) for the qRT-PCR assays employed in this study were previously defined (11). Overall, plasma CMV DNA loads were higher in the former group, although the differences did not reach statistical significance (Table 2). This was irrespective of both the qRT-PCR assay employed and the individual risk of patients for CMV end-organ disease (1–3). Thus, no reliable initial CMV DNA load threshold predicting the eventual need for antiviral therapy could be established by this study. This is in accordance

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**TABLE 1** Demographic and clinical data for the patients in this study

| Parameter | No. (%) of patients with result |
|---|---|
| Sex | |
| Male | 53 (64.6) |
| Female | 29 (35.4) |
| Stem cell source | |
| Bone marrow | 4 (4.9) |
| Peripheral blood | 57 (69.5) |
| Cord blood | 21 (25.6) |
| Type of donor | |
| Related | 36 (43.9) |
| Unrelated | 46 (56.1) |
| HLA match | |
| Identical | 57 (69.5) |
| Mismatched | 25 (30.5) |
| CMV serostatus[a] | |
| $D^-/R^-$ | 11 (13.4) |
| $D^-/R^+$ | 31 (37.8) |
| $D^+/R^-$ | 5 (6.1) |
| $D^+/R^+$ | 35 (42.7) |
| Conditioning regimen | |
| Nonmyeloablative | 42 (51.2) |
| Myeloablative | 40 (48.8) |
| CMV end-organ disease | |
| No | 81 (98.8) |
| Yes | 1 (1.2) |
| Acute graft vs host disease | |
| Grades 0-I | 49 (59.8) |
| Grades II-IV | 33 (40.2) |
| Underlying disease | |
| Acute lymphatic leukemia | 8 (9.8) |
| Acute myeloid leukemia | 31 (37.8) |
| Aplastic anemia | 3 (3.7) |
| Chronic lymphatic leukemia | 12 (14.6) |
| Hodgkin's disease | 4 (4.9) |
| Multiple myeloma | 2 (2.4) |
| Myelodysplastic syndrome | 2 (2.4) |
| Non-Hodgkin's lymphoma | 19 (23.2) |
| Plasma cell leukemia | 1 (1.2) |

[a] D, donor; R, recipient.

**TABLE 2** Early kinetics of plasma CMV DNA load in active CMV infection developing in allogeneic stem-cell transplant recipients who required or did not require preemptive antiviral therapy

| Parameter/group of patients(no. of patients)[a] | Median (range) result: With preemptive therapy | Median (range) result: Without preemptive therapy | *P* value[b] |
|---|---|---|---|
| Initial CMV DNA load, IU/ml | | | |
| Overall (55) | 75 (31–9,641) | 36 (31–1,090) | 0.222 |
| High risk (35) | 32 (31–3,920) | 31 (31–1090) | 0.693 |
| Low risk (20) | 232 (31–9,641) | 59 (31–596) | 0.153 |
| CMV dt, days[c] | | | |
| Overall (36) | 1.91 (0.81–7.05) | 4.21 (2.13–11.52) | 0.001 |
| High risk (25) | 2.12 (0.81–3.91) | 3.26 (2.13–11.35) | 0.012 |
| Low risk (11) | 1.73 (1.42–7.05) | 7.87 (4.21–11.52)[d] | 0.099 |

[a] High-risk patients for CMV end-organ disease were those meeting one or more or the following conditions: (i) unrelated or (ii) HLA-mismatched allograft, (iii) CMV seropositive receiving an allograft from a CMV-seronegative donor, and (iv) treatment with high-dose corticosteroids (>1 mg/kg body weight/day) for acute graft versus host disease (1). Twenty of 35 high-risk patients were preemptively treated with antivirals. Low-risk (and intermediate-risk) patients for CMV end-organ disease were those meeting one or more of the following conditions: (i) sibling and HLA-matched allograft, (ii) CMV seropositive receiving an allograft from a CMV-seropositive donor or CMV seronegative receiving an allograft from a CMV-seropositive donor, and (iii) absence of acute graft versus host disease or treatment with high corticosteroid doses (1). Twelve of the 20 low-intermediate-risk patients were preemptively treated with antivirals.

[b] Differences between medians were compared using the Mann-Whitney U test. A *P* value of <0.05 was considered statistically significant.

[c] The CMV doubling time was given by $dt = (t_2 - t_1) \times [\log_2/\log(q_2/q_1)]$, with $q_1$ and $t_1$ representing the CMV DNA (copies/ml) at the time of the first positive qRT-PCR (in days), respectively, and $q_2$ and $t_2$ representing CMV DNA at the time of the second positive qRT-PCR. Only episodes in which there was an increase between the first and second CMV DNA load values of >3-fold were considered for analysis ($n = 36$). Twenty of the 25 high-risk patients and 9 of the 11 low- to intermediate-risk patients were preemptively treated with antivirals.

[d] Mean of two values.

with data published previously by our group for a cohort of patients in which the initiation of antiviral therapy was triggered either by the AG assay or by a qRT-PCR assay (6). CMV DNA loads measured in the second positive qRT-PCR tests were below the cutoff for the initiation of antiviral therapy in 41 of 47 episodes. (A single positive qRT-PCR result was seen in 8 episodes.) Second qRT-PCR analyses were performed at a median of 6 days (range, 4 to 8 days) after the first. This allowed us to calculate the viral doubling time (dt) in the absence of antiviral treatment (6). These calculations were only performed for episodes in which there was an increase between the first and second CMV DNA load values of >3-fold (>0.5 $\log_{10}$ copy/ml, which is above the interassay coefficient of variation of both PCR assays) ($n = 36$) (2, 13). Twenty-nine of these 36 episodes were treated with antivirals. As shown in Table 2, patients who were eventually treated displayed dt values that were significantly shorter than those who did not. Receiver operating characteristic analyses (14) were performed to determine the optimal cutoff value for predicting the need of treatment. Overall, a dt threshold of 2.41 days displayed a sensitivity of 81.3% and a specificity of 71.6%. For the high-risk group, the dt was 2.17 days (sensitivity, 81.8%; specificity, 59.4%), and for the low- to intermediate-risk group, the optimal dt was 3.6 days (sensitivity, 80.0%; specificity, 66.7%). Overall, a dt cutoff value of 2.0 days predicted the need for antiviral treatment with maximum sensitivity (100%), albeit with a modest specificity (51%); that is, all patients with dt values of ≤2 days (9 high risk and 6 low to intermediate risk) would have been treated approximately 1 week earlier, whereas patients with dt values of >2 days that eventually required the inception of antiviral therapy (11 high-risk and 3 low- to intermediate-risk patients) would have been treated at the time they had reached the established CMV DNAemia cutoff for initiation of preemptive therapy.

The clinical significance of the early kinetics of CMV DNA load in blood in transplant recipients was first postulated by Emery et al. (10). They showed that the rate of viral load increase was an independent risk factor for CMV disease in a mixed population of solid organ transplant and Allo-SCT recipients. In the referred study, nevertheless, an endpoint quantitative PCR assay display-

ing a higher LOD than that in the present study was employed. In the era of highly sensitive qRT-PCRs, our data lend support to the clinical utility of these analyses. In our setting, dt values, but not initial CMV DNA load values, allowed the prediction of the need for antiviral therapy in both high-risk and low- to intermediate-risk patients. According to current practice at most transplant centers (1–3), the implementation of antiviral therapy upon reaching dt values of ≤2.0 days would have prompted the inception of antiviral therapy approximately 1 week earlier in 15 patients. However, whether this would have resulted in a clinical benefit for patients (shorter duration of treatments and less toxicity) is unknown. We are currently in the process of designing a controlled clinical trial aimed at evaluating the safety and clinical efficacy of this strategy.

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