Correlates of Change in Cytomegalovirus Viremia in Patients with Advanced Human Immunodeficiency Virus Infection Who Require Transfusion

Michael F. Para, Leslie A. Kalish, Ann C. Collier, Edward L. Murphy, W. Lawrence Drew, and the Viral Activation Transfusion Study Group

The Viral Activation Transfusion Study compared leukocyte-reduced to unfiltered red blood cell transfusions in human immunodeficiency virus (HIV)- and cytomegalovirus (CMV)-coinfected patients. Relationships between serially measured plasma CMV load and clinical and laboratory outcomes over a median of 12 months were examined in 511 subjects. At baseline, subjects had a median of 15 CD4+ cells/mm³, 25% had CMV disease, and 21.5% were viremic. No relationship was found between changes in CMV viremia and changes in HIV RNA. Increased CMV viremia was associated with a concomitant fall in Karnofsky score. Highly active antiretroviral therapy (HAART) led to a decrease in CMV viremia after a 90-day delay. After adjustment for HIV load and CD4+ cell count, CMV viremia remained associated with an increased risk of CMV disease (relative hazard, 5.78). In late-stage HIV-infected patients, CMV viremia was associated with lower functional status and increased risk of CMV disease. HAART suppressed CMV viremia only after a delay of several months.

Cytomegalovirus (CMV) is a common opportunistic pathogen in patients with late-stage human immunodeficiency virus (HIV) infection. Recent studies have demonstrated that a positive CMV qualitative plasma assay and higher levels of viremia, as determined by polymerase chain reaction (PCR), are associated with increased risk for development or progression of CMV disease [1–3]. In addition, by decreasing the frequency of CMV viremia, highly active antiretroviral therapy (HAART) reduces the incidence of disease [4–6].

The Viral Activation Transfusion Study (VATS) Group examined the effects of the first blood transfusion in 531 HIV-infected patients [7]. Medications, functional status, HIV load, and CD4+ cell counts were determined, and CMV viremia was assessed by PCR, before and serially after transfusion. We examined the interrelationships of these parameters in this population of late-stage patients.

Methods

Study population. The VATS, a double-blind trial, examined the clinical and laboratory outcomes of leukocyte-reduced or non–leukocyte-reduced red blood cell transfusion in CMV-seropositive HIV-infected persons [7, 8]. Serious HIV-related complications, HIV- and CMV-associated medications, functional status [9], CD4+ cell counts, and plasma HIV RNA and CMV DNA assays were assessed every 3 months. Patients had dilated eye examinations by an ophthalmologist every 6 months. CMV medication was defined as systemic ganciclovir, foscartern, or cidofovir. A CMV event was new or progressive CMV end-organ disease. Progressive CMV retinitis required an ophthalmologist’s diagnosis and a change in therapy. HAART was defined as the simultaneous use of ≥3 antiretroviral drugs, including ≥1 protease inhibitors or nonnucleoside reverse-transcriptase inhibitors.

Laboratory methods. HIV and CMV assays were done at a central laboratory. For PCR, we used Roche Amplicor CMV assays [10, 11]. The quantitative assay had a lower limit of 400 copies/mL. Qualitative CMV assays were performed in batch through the 24-month visit. If any were positive, a quantitative CMV assay...
was run in batch on the patient’s whole series, but the quantitative results were considered for analysis only if the associated qualitative result was positive. CD4+ cell enumeration was done by flow cytometry [12]. Plasma HIV RNA level was measured by a Roche Amplicor Monitor assay [13], as described elsewhere [14].

Statistical methods. Quantitative CMV and HIV loads were analyzed on a logarithmic scale (base 10) after replacing values below the quantification limit with values at the limit. In some analyses, qualitative negative CMV results were assigned this limit. Since there was no evidence of effect of leukocyte reduction on HIV or CMV viremia other than HAART was the change across adjacent quarterly visits before initiation of HAART. Because many patients had viremia other than HAART was the change across adjacent quarterly visit pairs eligible for these analyses, generalized estimating equations with an autoregressive correlation structure and empirical variance estimates were used to account for correlated observations.

Because of the potential for confounding by anti-CMV therapy, longitudinal changes in CMV viremia were calculated only during periods of stable therapy (i.e., either when these therapies were not given from 30 days before the first visit through the second visit or when these therapies were received on ≥80% of those days). Indicator variables representing the different patterns of qualitative results were used to isolate the quantitative CMV load information from the qualitative.

Analysis of the effect of HAART on CMV viremia was done for patients for whom pre-HAART visit data and ≥1 post-HAART visits were available. If there was ≥1 post-HAART visit, the visit closest to HAART initiation served as the “baseline” visit. Post-HAART visits were categorized according to the time interval since HAART began. CMV and HIV viremia were examined as a function of these intervals.

To examine the relationship between qualitative CMV viremia and CMV events, we used a Cox proportional hazards model, with time from entering the VATS to the first new or progressive CMV event during follow-up as the end point and the most recently measured qualitative CMV result, log_{10} HIV RNA copies, and CD4+ cell count (<10, 10–49, or ≥50 cells/mm³) as time-varying covariates. Additional terms were added to the model to investigate the prognostic value of quantitative CMV load.

Results

Of the 531 subjects enrolled in VATS, 511 were confirmed to be CMV antibody positive and had a baseline CMV assay. The subjects were 79% male, were a median of 37 years old, and were followed up a median of 12 months. Subjects had a median CD4+ cell count of 15 cells/mm³ (interquartile range, 3–72 cells/mm³) and a median HIV RNA level of 4.82 log_{10} copies/mL (interquartile range, 4.03–5.31 log_{10} copies/mL). At entry, 25% of subjects had a history of CMV disease, and 21.5% had a positive qualitative CMV assay. Across all quarterly visits, 11% of 1985 qualitative CMV assays were positive. Of these, 78% had a quantitative CMV result ≥400 copies/mL. The median load was 1470 copies/mL.

<table>
<thead>
<tr>
<th>Qualitative CMV PCR at (visit 1, visit 2)</th>
<th>No. of subjects</th>
<th>Mean log_{10} change in HIV RNA (95% CI)</th>
<th>No. of subjects</th>
<th>Mean change in Karnofsky score (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−, −)</td>
<td>232</td>
<td>−0.05 (−0.15 to 0.05)</td>
<td>252</td>
<td>0.66 (−0.48 to 1.81)</td>
</tr>
<tr>
<td>(−, +)</td>
<td>20</td>
<td>0.006 (−0.18 to 0.30)</td>
<td>21</td>
<td>−6.66 (−14.18 to 0.85)</td>
</tr>
<tr>
<td>(+, −)</td>
<td>20</td>
<td>−0.52 (−0.99 to 0.06)</td>
<td>20</td>
<td>1.54 (−2.70 to 5.79)</td>
</tr>
<tr>
<td>(+, +)</td>
<td>15</td>
<td>−0.03 (−0.36 to 0.30)</td>
<td>15</td>
<td>−5.49 (−11.81 to 0.83)</td>
</tr>
</tbody>
</table>

NOTE. −, Negative finding; +, positive finding; PCR, polymerase chain reaction assay.
Effect of HAART on CMV viremia. The effect of HAART on CMV viremia was examined in 152 patients who had a pre-HAART visit (median, 31 days earlier) and ≥1 (mean, 4.0) post-HAART visits. At baseline (before HAART), the median HIV RNA level was 4.83 log_{10} copies/mL, the CD4+ cell count was 17.5 cells/mm³, and 11.2% of qualitative CMV assays were positive. During the first 90 days after HAART, CMV prevalence was almost identical to the pre-HAART prevalence (table 2), indicating that HAART had not yet had an effect on CMV viremia. After 90 days of HAART, the prevalence of CMV viremia was lower than pre-HAART prevalence in the same patients. Too few subjects to analyze had quantifiable CMV loads at baseline and at post-HAART visits. Parallel analyses that examined the effect of HAART on HIV RNA levels showed much higher prevalences of HIV RNA <200 at all post-HAART intervals, compared with pre-HAART (data not shown). In contrast to CMV, the prevalence of detectable HIV decreased in the first post-HAART month.

Effect of CMV viremia on clinical CMV diagnosis. We investigated the relationship between CMV viremia and CMV disease in 483 subjects with qualitative CMV results. Sixty-one patients had CMV events. The unadjusted relative hazard (RH) of a CMV event associated with a positive qualitative CMV result at the most recent visit was 9.28 (95% CI, 5.47–15.74; \( P < .001 \)). Quantitative CMV loads had no additional prognostic information above that contained in the qualitative CMV results (\( P = .46 \)). In the unadjusted analyses, HIV load and CD4+ cell counts were also significantly associated with a subsequent CMV event.

In a multivariate model that considered the 3 covariates simultaneously, the most recent qualitative CMV result remained highly associated with increased risk of CMV events (RH, 5.66; 95% CI, 3.30–9.71; \( P < .001 \)), even after adjustment for concomitantly measured HIV load and CD4+ cell count. However, HIV load was not significantly associated with an increased risk of CMV events after adjustment for CD4+ cell count (RH, 1.29 per log increment; 95% CI, 0.96–1.72; \( P = .09 \)). Patients with a CD4+ cell count of <10 cells/mm³ (vs. ≥50 cells/mm³) remained at higher risk for a CMV event (RH, 4.15; 95% CI, 1.68–10.23; \( P = .002 \)). Adding HAART exposure to this model as a time-varying covariate did not improve the model (\( P = .69 \)), nor did adding quantitative CMV load.

Discussion

In this large population study of late-stage HIV-infected patients, CMV viremia by qualitative assay was associated with a markedly increased risk of CMV disease and a lower Karnofsky score but not with increased HIV plasma load. Moreover, while HAART was associated with a rapid decrease in plasma HIV, the subsequent decrease in CMV viremia was delayed >3 months.

The finding of increased risk of a CMV event after the development of CMV viremia is consistent with the findings of a number of other studies [1–3]. The size and design of the VATS allowed additional multivariate analyses with adjustments for HIV load and CD4+ cell count. Even after these adjustments, there was still a 6-fold increased risk of CMV disease after a positive qualitative assay. We also found that quantitation of CMV levels added no additional prognostic information. A possible explanation for this is that quantitative assays with low thresholds for CMV loads still have lower sensitivity and lower negative predictive values for CMV disease than qualitative assays have [1]. These results add further support for monitoring CMV viremia and consideration of prophylaxis for patients with CMV viremia.

In our earlier cross-sectional analyses of the baseline characteristics of this cohort, we first noted an inverse relationship between both qualitative and quantitative measures of CMV load and Karnofsky score, even after adjusting for CD4+ cell count, HIV load, and CMV disease history [14]. Our current analysis shows that increased levels of CMV viremia and development of a positive qualitative assay are both associated with a serious concomitant decrease in functional status. Interventions that decrease CMV viremia, even in the absence of CMV disease, may be helpful in improving functional status of late-stage HIV-infected patients.

Before the initiation of HAART, we found that changes in CMV viremia and HIV plasma load were unrelated. Spector et al. [3] also found only a weak correlation (\( r = 0.12 \)) between CMV load and HIV load in 619 AIDS patients. If we extrapolate from our data and the close relationship between CMV viremia and risk of CMV disease, our data seem to indicate that increased HIV load would not correlate with increased risk of CMV disease. In fact, Casado et al. [2] reported no relationship between onset of CMV disease and HIV RNA level in their late-stage patients.

HAART has been reported to suppress CMV viremia and reduce CMV disease [4–6]; however, Jacobson et al. [15] noted the occurrence of CMV disease shortly after initiation of...
HAART. Although some of these occurrences may have represented undiagnosed cases at study entry, immune reconstitution with immunologic response at sites of previously subclinical CMV infection is another explanation. Our findings of delayed control of CMV viremia support the hypothesis that CMV disease developed, or subclinical disease progressed, after the initiation of HAART, before CMV suppression was achieved. Casado et al. [6] reported continued progression of CMV retinitis for 3–6 months after protease inhibitor therapy was started. Furthermore, O'Sullivan et al. [5] reported that CMV plasma levels 30 days after initiation of HAART were similar to pre-HAART levels, but after 3 months of HAART, the levels had decreased significantly. These results support the continued use of CMV therapies or prophylaxis in late-stage AIDS patients for ≥3 months after starting HAART.

In conclusion, our study adds further support for the use of prophylactic anti-CMV therapy when there is a positive qualitative plasma assay for CMV in late-stage HIV-infected patients. Such therapy should be given for ≥3 months after the initiation of HAART and should be continued if CMV viremia (assessed by qualitative PCR) persists. Anti-CMV therapy not only may reduce the risk of CMV disease but also may improve the functional status of patients with advanced HIV infection, even in the absence of CMV disease.

Viral Activation Transfusion Study Group

Locations and researchers of the Viral Activation Transfusion Study Group are as follows. National Heart, Lung, and Blood Institute (NHLBI) contracts for each site are listed in parentheses.


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**References**


