Cytomegalovirus Seropositivity and Human Immunodeficiency Virus Type 1 RNA Levels in Individuals with Hemophilia

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The effects of cytomegalovirus (CMV) seropositivity on the course of human immunodeficiency virus (HIV) type 1 RNA levels and HIV disease progression were assessed in a cohort of 109 hemophilic men infected with HIV-1 for a median of 12.7 years. There was no evidence of higher HIV RNA levels in the first year after HIV seroconversion (P = .88) or faster rates of increase over infection (P = .20) in the 59 CMV-seropositive individuals than in the CMV-seronegative individuals. In univariate analyses, CMV seropositivity was associated with significantly faster progression to AIDS and death (relative hazards of 1.58 and 2.22, respectively). These effects were unchanged after adjusting for the RNA level, but they were reduced after adjusting for the CD4 cell count, age at seroconversion, and calendar year of follow-up. Thus, the effect of CMV seropositivity on clinical progression remains significant in this cohort but does not appear to be mediated through an increase in HIV RNA levels.

Infection with cytomegalovirus (CMV) is common among persons infected with human immunodeficiency virus (HIV), although the impact of CMV infection on HIV disease progression remains unclear. By using data from a cohort of hemophilic men infected with HIV, we have reported that CMV-seropositive individuals experienced faster progression to AIDS and shorter survival than those who were CMV seronegative [1, 2]. Although CD4+ T cell loss was more rapid in CMV-seropositive patients, this did not fully explain the faster progression experienced by these individuals. Other studies have found no such relationship between CMV infection and HIV disease progression in hemophilic patients [3–5], whereas studies of male homosexuals [6] and HIV-infected infants [7] who acquired CMV infection in the first 18 months of life reported that CMV-infected individuals had a significantly higher rate of disease progression than those infected with HIV alone. Furthermore, 2 studies have reported a significant association between CMV virus load and death [8, 9].

It is now possible to assess the relationship between HIV-1 RNA levels and CMV infection in our cohort because we have retrospectively measured HIV RNA levels over the course of infection in the patients. The aim of this paper is to analyze the impact of CMV seropositivity on the course of HIV RNA levels and to consider whether any effect of CMV seropositivity on disease progression can be explained by differences in HIV RNA levels between persons who are seropositive and seronegative for CMV.

Methods

Patients. One hundred eleven hemophilic men who were registered at the Royal Free Haemophilia Centre became infected with HIV after treatment with unsterilized clotting-factor concentrates. Because of the availability of stored serum samples, it was possible to estimate dates of seroconversion for each patient. These men have been described [10] and have been followed for up to 20 years since HIV seroconversion. Patients are seen every 3–6 months, when possible, for clinical and laboratory review. All patients are coinfected with hepatitis C virus (HCV).

Laboratory methods. Antibodies to CMV were measured in an early stored serum sample for each individual by IgG RIA, as described elsewhere [1]. Since 1982, lymphocyte subsets have been performed routinely on all fresh samples at the hospital by use of an Ortho Cytoron-Absolute (Ortho Diagnostics, High Wycombe, UK) as described elsewhere [11]. All measurements were monitored in the UK National External Quality Assurance Scheme. During the period of follow-up, the study patients have had a median of 26 (range, 1–85) CD4 cell counts for analysis: CMV-seronegative patients have had
a median of 30 (range, 1–85) and CMV-seropositive patients have had a median of 26 (range, 1–76) CD4 cell counts for analysis.

As part of a study on the natural history of virus load throughout the course of HIV infection, HIV RNA levels were retrospectively measured on samples collected yearly after HIV-1 seroconversion. HIV-1 RNA was measured in serum by use of a reverse-transcriptase polymerase chain reaction assay (Amplicor HIV-1 Monitor v1.0 assay plus add-in non-B primers; Roche Diagnostic Systems, Branchburg, NJ) according to the manufacturer’s instructions. Since 1996, HIV-1 RNA has been measured prospectively on fresh plasma samples by use of the Amplicor Monitor v1.5 assay (Roche) and the ultrasensitive version of this assay. During follow-up, the study patients had a median of 9 (range, 1–26) RNA levels for analysis: CMV-seronegative patients had a median of 11 (range, 1–26) and CMV-seropositive patients had a median of 8 (range, 1–26) RNA levels for analysis.

Treatments. Since the introduction of antiretroviral therapy, patients have been offered treatment according to guidelines in place at the time. From 1987, patients were enrolled in the Medical Research Council/INSERM Concorde trial of early-versus-deferred use of zidovudine. Subsequently, patients have received monotherapy with zidovudine, dual combination therapy with nucleoside reverse-transcriptase inhibitors, and, more recently, highly active antiretroviral therapy (HAART), including either protease inhibitors or non-nucleoside reverse-transcriptase inhibitors (or both).

In addition, patients with low CD4 cell counts were offered prophylactic treatment against Pneumocystis carinii pneumonia, candidiasis, and mycobacterium infections, as treatment became available. Prophylaxis is now withdrawn if patients respond to HAART.

Statistical methods. The median yearly HIV RNA level was plotted for CMV-seronegative and CMV-seropositive patients. For these plots, the “last observation carried forward” method was used; thus, if patients dropped out of the study, either through death or loss to follow-up, the last available RNA level was retained in the plot.

To further investigate the impact of CMV infection on the evolution of HIV RNA levels within individuals, random-effects models [12] were used to consider whether the HIV RNA level in the first year after seroconversion or the changing RNA level during infection was related to CMV status. Previous analyses of this cohort [13] have shown, after taking a log transformation of the data, that RNA levels in these patients increase linearly over infection. The effect of CMV was considered to be a fixed effect in these analyses, whereas the intercept (HIV RNA level in the year after HIV seroconversion) and slope (rate of change of RNA) were assumed to vary between patients. All analyses were adjusted to take account of age at seroconversion. For these analyses, any RNA levels measured in the first year after HIV seroconversion were excluded to avoid any possible biasing effect of particularly high viral loads at the time of seroconversion; thus, the time scale was rescaled so that the intercept that was calculated related to the RNA level at 1 year after seroconversion.

The impact of CMV serology on progression to AIDS and death, after adjusting for other factors, was studied by use of Cox proportional hazards regression models. For analyses of survival, patient follow-up was considered from the time of seroconversion to the date of death and was right-censored if a patient was alive on the analysis cutoff date, 30 April 1999. For progression to AIDS, patient follow-up was additionally right-censored on the date of death if the individual died before developing AIDS. CMV infection and age at seroconversion were included in the regression models as fixed covariates; the changing CD4 cell count (after square-root transformation) and HIV RNA level (after log transformation) were included in the model as time-updated covariates so that they were allowed to change in the model as new values became available. To control for the impact of HAART and the increasing use of prophylaxis over time, calendar year of follow-up (before 1990, 1990–1995, and 1996 and later) was included in the model as a time-updated covariate.

Random-effects models were performed by use of Mln software (Institute of Education, University of London, United Kingdom). All survival analyses were performed by use of the PHREG procedure (SAS software; SAS Institute, Cary, NC).

Results

All but 2 of the 111 patients in the cohort could be tested for CMV. Of the 109 patients who could be tested for CMV, 59 (54%) were CMV seropositive. The patients have been followed for a median of 12.7 years (range, 0.8–19.5 years); over this time, 57 (52.3%) of the 109 patients have developed AIDS, and 65 (59.6%) have died. The median age at seroconversion was 22.6 years (range, 1.9–77.8 years; 25.6 years [range, 4.0–77.8 years] for those who were CMV seropositive; 18.7 years [range, 1.9–72.9 years] for those who were CMV seronegative).

Plots of the median HIV RNA level yearly after seroconversion, according to CMV serostatus, are shown in figure 1. The lack of an apparent difference in RNA trajectories was confirmed by random-effects models. These models showed that the RNA level 1 year after seroconversion did not differ between the 2 groups. The mean (95% confidence interval [CI]) for a 22-year-old patient at seroconversion was 3.39 log_{10} copies/mL (range, 3.18–3.59 log_{10} copies/mL) for CMV-seropositive patients and 3.37 log_{10} copies/mL (range, 3.17–3.58 log_{10} copies/mL) for CMV-seronegative patients.

Figure 1. Median yearly human immunodeficiency virus RNA levels stratified by cytomegalovirus (CMV) serostatus of study patients.
Table 1. Impact of cytomegalovirus (CMV) infection on progression to AIDS and death in 109 human immunodeficiency virus (HIV) type 1–infected men when data are unadjusted and adjusted for other factors.

<table>
<thead>
<tr>
<th>Model, adjusted factors</th>
<th>Progression to AIDS</th>
<th>Progression to death</th>
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<tr>
<td></td>
<td>RH</td>
<td>95% CI</td>
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<tr>
<td>Unadjusted model</td>
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<tr>
<td>CMV seropositive</td>
<td>1.58</td>
<td>0.93–2.69</td>
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<td>Adjusted for HIV RNA</td>
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<tr>
<td>CMV seropositive</td>
<td>1.82</td>
<td>1.05–3.16</td>
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<td>HIV RNA, per log_{10} higher</td>
<td>2.77</td>
<td>2.02–3.79</td>
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<tr>
<td>Adjusted for all factors</td>
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<td></td>
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<tr>
<td>CMV seropositive</td>
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<td>0.93–2.91</td>
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<tr>
<td>HIV RNA, per log_{10} higher</td>
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<td>1.08–2.13</td>
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<tr>
<td>CD4 T cell count, per cells/mm$^3$ lower</td>
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<tr>
<td>Age at seroconversion, per 5 years</td>
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<td>0.97–1.18</td>
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<td>Year of follow-up</td>
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<td>1990–1995</td>
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<td>0.12–0.78</td>
</tr>
<tr>
<td>1996 or later</td>
<td>0.13</td>
<td>0.02–0.77</td>
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</table>

NOTE. RH, relative hazard; CI, confidence interval.

mL) for CMV seronegative patients ($P = .88$). The yearly rate of increase in RNA levels was 0.12 log_{10} copies/mL (range, 0.08–0.15 log_{10} copies/mL) in CMV-seropositive patients and 0.09 log_{10} copies/mL (range, 0.05–0.12 log_{10} copies/mL) in CMV-seronegative patients ($P = .20$).

Table 1 shows the impact of CMV on progression to AIDS and death when unadjusted; adjusted for the changing HIV RNA level only; and adjusted for CD4 cell count, age at seroconversion, calendar year of follow-up, and changing HIV RNA level. When unadjusted for other factors, individuals infected with CMV appeared to have a 58% increased risk of AIDS (relative hazard [RH], 1.58; 95% CI, 0.93–2.69; $P = .09$) and a 122% increased risk of death (RH, 2.22; 95% CI, 1.32–3.71; $P = .003$). After adjusting for the changing RNA level over time, RHs for progression to AIDS and death increased slightly and remained significant. After adjusting for all factors, RHs for both AIDS and death remained >1 and were of borderline significance. In particular, CMV-seropositive patients, compared with CMV-seronegative patients, were estimated to have a 65% increased risk of progressing to AIDS ($P = .09$) and a 77% increased risk of death ($P = .05$).

Discussion

In this cohort of hemophilic men, CMV seropositivity continues to be associated with a more rapid rate of progression to AIDS and death. This effect does not appear to be mediated by the changing HIV RNA level or CD4 cell count, suggesting that some other mechanism is responsible. These results are consistent with our earlier findings; however, patients in the cohort have now been followed for an additional 6–7 years, and many have also received HAART. Therefore, the natural course of HIV infection has been altered in these patients. Despite this, these findings are remarkably similar to our earlier findings, which suggests that treatment with HAART has not greatly modified the impact of CMV infection in these patients.

In addition, we have confirmed recent findings by Kovacs et al. [7], who found that the effect of CMV infection on HIV disease progression in infants is not mediated through more rapid HIV RNA increase. In patients enrolled in an oral ganciclovir prophylaxis study [9], the predominant risk factor for death was CMV load at baseline rather than HIV RNA load, adding further support to the suggestion that the poor prognosis associated with CMV infection is not explained by an effect on HIV RNA levels. Until recently, HIV RNA levels were not available for analysis in our cohort. However, the availability of stored serum samples in this cohort has now allowed us to consider the impact of CMV infection on the course of HIV RNA over the entire period of infection.

There are a number of reasons why CMV infection may be expected to have an impact on HIV disease progression (reviewed in [14]). Most methods of interaction between the 2 viruses would be expected to up-regulate HIV, leading to an increase in plasma HIV load. However, it is possible that local effects, such as up-regulation of CD4 or chemokine coreceptors, reduction of alternative cellular receptors, or pseudotype formation within tissues could expand the tropic range of HIV without necessarily increasing the plasma HIV virus load. Clearly, our findings and those from Kovacs et al. [7] provide information on the likely importance of the different methods of interaction between CMV and HIV in vivo.

Hemophilic patients are one of the few groups of individuals in whom it is possible to study the effect of CMV seropositivity, because a substantial proportion of patients have not been infected by CMV. However, some caveats should be noted. First, HIV virus loads have been measured retrospectively in stored serum samples. Such measurements are expected to lead to lower values than those measured in fresh blood samples; however, we do not believe that this has resulted in substantial bias.
(discussed in [13]). Second, in our study, all patients were co-infected with HCV; however, it is not clear whether there is any interaction between CMV and HCV infections in HIV-infected individuals. Last, only early samples were tested for CMV infection. Thus, we may have missed a number of CMV seroconversions in this analysis. However, as the prevalence of CMV infection in hemophilic men is similar to that in the general population (CMV is not transmitted through clotting-factor concentrates), it is unlikely that more than 2 or 3 seroconversions would occur in this group, a number that is unlikely to greatly affect our results.

It is not clear why our results continue to differ from those of other studies [3–5], in which no independent effect of CMV on HIV disease progression is seen, although nonhemophilic populations have reported such an association. Clearly, further work is needed to clarify the role of CMV seropositivity on HIV disease progression.

References


