No Observed Effect of GB Virus C Coinfection on Disease Progression in a Cohort of African Woman Infected with HIV-1 or HIV-2

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We studied mortality among subjects with human immunodeficiency virus (HIV)–1 and HIV–2 infection in relation to GB virus (GBV)–C coinfection. No significant differences in mortality were seen between subjects with and subjects without GBV-C coinfection who also had either HIV-1 or HIV-2 infection. No association between GBV-C and HIV plasma virus loads or CD4 cell count was observed.

In 1988 [1], it was observed that patients with HIV-1 and GB virus type C (GBV-C) coinfection may exhibit a slower rate of disease progression than patients not coinfected with GBV-C. This observation has been confirmed in subsequent studies [2, 3], although some investigators have failed to observe the effect [4, 5]. Studies reporting beneficial effects have described delays in disease progression to AIDS or death and have associated these delays with lower plasma virus RNA loads and higher CD4 cell counts.

Mechanisms through which chronic GBV-C coinfection may influence disease progression have been investigated and reported. Generally, such studies have not specifically investigated the influence of GBV-C coinfection on HIV-1 infection. They include post-entry inhibition of replication [6], alteration of T helper cytokine profiles, and changes in chemokine receptor display [7]. Since the reported effects of coinfection with GBV-C are not specific to HIV-1 infection, one might make the prediction that such effects would also apply to infection with HIV-2, a closely related retrovirus that predominantly infects the same cell population. Therefore, in this report we discuss the effects of GBV-C coinfection on HIV-2 disease progression in a cohort of women enrolled in a perinatal transmission study [8]. We also discuss the rate of disease progression in women with HIV-1 infection from the same cohort. Active GBV-C infection was detected by PCR amplification of GBV-C plasma RNA. Quantitative PCR was used to determine GBV-C RNA loads, and these were related to HIV RNA loads and CD4 cell counts.

Methods and materials. Subjects in this study included women aged 14–42 years (median, 25 years) enrolled in a cohort study of perinatal HIV transmission. All women were HIV-infected at recruitment. Time of seroconversion was unknown, but most women were asymptomatic. Stored plasma samples were available for 91 women with HIV-1 infection and 159 women with HIV-2 infection. The baseline characteristics of these women did not differ significantly from those detailed in the main cohort study [8]. No dually infected subjects were included in this study. Women who participated were counselled, and they gave informed consent before participation. The study was approved by the Gambian Government and the Medical Research Council Joint Ethics Committee.

RNA was extracted from plasma by the Boom method and PCR, which was amplified using a single-step RT-PCR method (Qiagen), according to the manufacturer’s instructions. The primers amplified a 294-base-pair product from the 5′ untranslated region, as has been described elsewhere by Linnen et al. [9]. Products were detected by solid-phase hybridization. HIV plasma RNA loads (PVL) were assayed, as has been described elsewhere [10]. The lower limit of detection of the HIV-1 and HIV-2 virus load assays was 500 RNA copies/mL plasma, and for analysis, sample levels below the lower limit were assigned a value of 250 RNA copies/mL plasma. GBV-C PVLs were quantified with a real-time thermal cycler (Opticon, MJ Research) using the same primers as the screening assay and the QuantiTect SYBR Green RT-PCR assay (Qiagen), according to the manufacturer’s instructions. A standard curve was generated from dilutions of a pool of plasma containing GBV-C, which was identified during screening. The top standard was quantified by end point dilution PCR. Sufficient plasma was available to assay the PVL in all 18 subjects coinfected with GBV-C and HIV-1 and in 29 of 30 subjects coinfected with GBV-C and HIV-2.

Data analysis was done with Stata software, version 6 (Stata). Person-years of observation were calculated from the date of enrollment until 1 July 2001, or until the date of death or last
date the patient was known to be alive, whichever came first. Mortality rates were calculated as deaths per 1000 person-years of observation, with 95% CIs. Poisson regression analysis was used for multivariable analysis of mortality rates.

**Results.** Data were available for 250 HIV-infected women. GBV-C RNA was detected in 18 (19.8%) of 91 subjects with HIV-1 infection and 30 (18.9%) of 159 subjects with HIV-2 infection (P = .86). The median GBV-C PVL was 3.2 × 10^6 RNA copies/mL (range, 7800–3.1 × 10^10 copies/mL). There was no significant difference in GBV-C PVL between subjects with HIV-1 infection (median PVL, 7.0 × 10^6 RNA copies/mL) and subjects with HIV-2 infection (median PVL, 2.9 × 10^6 copies/mL; ranksum test, P = .12). There was no significant difference in CD4 cell percentage between subjects with GBV-C coinfection and those without it (P = .24 for subjects with HIV-1 infection, and P = .68 for subjects with HIV-2 infection). Coinfection with GBV-C did not affect HIV-1 PVLs (ranksum test, P = .22) or HIV-2 PVL (P = .83). Linear regression analysis revealed no association between CD4 cell percentage and GBV-C PVL (P = .96) or between HIV-1 PVL and GBV-C PVL (P = .46) among subjects with HIV-1 and GBV-C coinfection. Similarly, no association was found between CD4 percentage and GBV-C PVL (P = .82) or HIV-2 PVL and GBV-C PVL (P = .49) among subjects with HIV-2 and GBV-C coinfection.

Twenty-six (10%) of 260 women were lost to follow-up; this proportion was similar among women with HIV-1 infection and those with HIV-2 infection (P = .29). Observation continued until the last date the patient was known to be alive. Twenty-eight (30.8%) of 91 subjects with HIV-1 infection died, and 12 (7.6%) of 159 subjects with HIV-2 infection died (P < .0005) (table 1). There was no difference in the proportion of subjects who died among those with and those without GBV-C coinfection (P = .79). There was no difference in GBV-C PVL among subjects who subsequently died and those who survived (P = .35). The crude mortality rate was not different between subjects with and those without GBV-C coinfection (P = .66). The mortality rate was not associated with GBV-C PVL among coinfected subjects (P = .67). A multivariate Poisson analysis showed that, after adjustment for age, HIV-1 PVL, and CD4 cell percentage, there was no significant association between GBV-C infection and mortality rate (P = .40). Similarly, among subjects with HIV-2 infection there was no difference in mortality between those with and those without GBV-C coinfection (P = .33). There was no difference in GBV-C PVL among subjects who subsequently died and those who survived (P = .12). The crude mortality rate was not different between those with and those without GBV-C coinfection (P = .45). The mortality rate was not associated with the GBV-C PVL among subjects with HIV and GBV-C PVL coinfection (P = .41). After adjustment for age, HIV-2 PVL, and CD4 cell percentage, a multivariate Poisson analysis showed that there was no significant association between GBV-C infection and mortality rate (P = .65).

**Discussion.** We have looked at the influence of GBV-C coinfection on mortality, CD4 cell percentage, and PVL in cohorts of subjects with HIV-1 and with HIV-2 infection. Previous studies are almost evenly divided between those that describe a beneficial effect on the patient (a slower disease progression to AIDS or death, a lower PVL, and a higher CD4 cell count) and those that describe no benefit. Unlike other viral coinfections, GBV-C infection has not been reported to have a harmful effect on HIV disease.

Our results did not confirm previous findings of slower disease progression associated with GBV-C coinfection for either subjects with HIV-1 or HIV-2 infection. There are no previous reports that detail the effects of GBV-C infection on HIV-2 disease progression or its influence on the HIV PVL or the CD4 cell count. The reasons why we did not find an association between GBV-

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**Table 1. Follow-up data and mortality for African women with HIV-1 or HIV-2 infection, according to status of GB virus type C (GBV-C) infection status**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women with HIV-1 infection</th>
<th>Women with HIV-2 infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not coinfected with GBV-C</td>
<td>Confected with GBV-C</td>
</tr>
<tr>
<td></td>
<td>(n = 73)</td>
<td>(n = 28)</td>
</tr>
<tr>
<td>Refusal of or loss to follow-up, no. (%) of patients</td>
<td>5 (6.9)</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Deaths, no. (%) of patients</td>
<td>22 (30.1)</td>
<td>6 (33.3)</td>
</tr>
<tr>
<td>Total observation time, PYO</td>
<td>432.7</td>
<td>96.5</td>
</tr>
<tr>
<td>Duration of follow-up, median years (IQR)</td>
<td>6.7 (4.4–7.8)</td>
<td>5.4 (3.1–7.5)</td>
</tr>
<tr>
<td>Mortality rate (95% CI per 1000 PYO)</td>
<td>50.8 (33.5–77.2)</td>
<td>62.2 (27.9–138.5)</td>
</tr>
<tr>
<td>Crude mortality rate ratio (range)a</td>
<td>…</td>
<td>1.22 (0.50–3.02)</td>
</tr>
<tr>
<td>Adjusted mortality rate ratio (range)b</td>
<td>…</td>
<td>1.51 (0.58–3.91)</td>
</tr>
</tbody>
</table>

**NOTE.** PYO, person-years of observation; IQR, interquartile range.

a Comparison of women with GBV-C infection and those without.

b Determined using Poisson multivariable analysis after adjustment for age at baseline, HIV plasma viral load, and CD4 cell percentage.

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C coinfection and HIV-1 disease progression, when other investigators have done so, are unclear. Our cohort was exclusively composed of black African women of child-bearing age, and although sex has rarely been linked to differences in disease progression, ethnicity may have contributed to our findings. It is possible that HIV-1 clade differences may account for the divergent findings. All studies that describe a beneficial effect of GBV-C coinfection have been conducted in the developed world where the predominant HIV clade within the infected community is clade B. We have not subtyped HIV in the present study, but previous studies in the region suggest that most subjects would be infected with HIV subtypes A or A/G [11]. None of the studies that discuss the effects of GBV-C infection have described a mechanism of inhibition of HIV-1 replication that is likely to be specific to the clade or the subtype of HIV.

Findings of previous studies suggesting that GBV-C infection may have effects that are not specific to the HIV clade or subtype prompted us to look at the effects of GBV-C infection on HIV-2 disease progression. Again, no such effects were found. The effects that may be seen in persons with HIV-2 infection are likely to be more difficult to observe, because most persons with HIV-2 infection have low or undetectable virus loads and near normal CD4+ T lymphocyte counts, and most die from causes unrelated to HIV-2 infection. The number of deaths among women with HIV-2 infection in this study was small (n = 12), so the power to detect an effect of GBV-C infection on mortality among women with HIV-2 infection was limited. However, apart from the lack of effect on mortality, there was also no trend towards alterations in CD4+ cell percentage or PVL in subjects with GBV-C and HIV-2 coinfection.

It is tempting to think there may be an influence of GBV-C on the in vivo replication of HIV because both viruses can infect CD4+ T lymphocytes. It is possible that the divergent findings of studies on GBV-C coinfection could be due to strain differences in the strains of GBV-C circulating in different populations. Genotypic strain variations have been described previously [12], but whether such variations translate to altered biological characteristics is yet to be determined.

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