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GB Virus C Coinfection and HIV-1 Disease Progression: The Amsterdam Cohort Study

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Background. The effect that GB virus C (GBV-C) coinfection has on human immunodeficiency virus type 1 (HIV-1) disease progression is controversial and therefore was studied in 326 homosexual men from the prospective Amsterdam Cohort Studies who had an accurately estimated date of HIV-1 seroconversion and were followed up for a median period of 8 years.

Methods. A first plasma sample, obtained shortly after HIV-1 seroconversion, and a last plasma sample, obtained before 1996, were tested for GBV-C RNA and envelope protein–2 antibodies. The effect that GBV-C has on HIV-1 disease progression was studied by use of time-dependent Cox proportional-hazards models with adjustment for baseline variables and time-updated HIV-1 RNA and CD4+ cell count.

Results. Men who lost GBV-C RNA between collection of the first sample and collection of the last sample had a nearly 3-fold-higher risk of HIV-1 disease progression than did men who had never had GBV-C RNA. This effect became much smaller after adjustment for time-updated CD4+ cell count.

Conclusion. Rather than a positive effect of GBV-C RNA presence, a negative effect of GBV-C RNA loss on HIV-1 disease progression was found, which disappeared after adjustment for time-updated CD4+ cell count. We therefore hypothesize that GBV-C RNA persistence depends on the presence of a sufficient number of CD4+ cells—and that the CD4+ cell decrease associated with HIV-1 disease progression is a cause, not a consequence, of GBV-C RNA loss.
unclear, but it may involve either interaction with chemokine-receptor mutations [17], inhibition of HIV-1 replication [10], or GBV-C–mediated maintenance of an intact T-helper–1 cytokine profile [18].

Whether GBV-C coinfection is an independent prognostic factor or secondary to HIV-1 disease progression remains to be elucidated. We therefore prospectively studied the effect that GBV-C coinfection had on HIV-1 disease progression in a large cohort of homosexual men with an established date of HIV-1 seroconversion.

SUBJECTS AND METHODS

Study population. Data were obtained from subjects in the prospective Amsterdam Cohort Studies (ACS) of homosexual men [19]. Included in the present study were (1) men who seroconverted for HIV-1 during follow-up before January 1996 and (2) men who, when they entered the ACS during October 1984–May 1985, were infected with HIV-1. Of the 364 men meeting these inclusion criteria, 33 declined further participation in the ACS and 5 others were excluded because no blood samples were available for GBV-C testing, resulting in a total of 326 homosexual men who were included in the present study. All men signed an informed consent form. We calculated the HIV-1 seroconversion date for each man by using ACS-specific estimates of the cumulative HIV-1 seroincidence over calendar time [20].

Routine diagnostics. All men infected with HIV-1 visited the ACS every 3 months. Clinical and epidemiological data were collected by means of standardized questionnaires and physical examinations. Blood samples were taken for virological and immunological testing, and viable cells were stored in liquid nitrogen and serum or plasma at −70°C. For samples taken since June 1996, HIV RNA load has routinely been measured; samples obtained before that time were tested for all HIV-1 seroconverters and, for others, on request. HIV-1 RNA load was tested by use of NASBA (Organon), Nuclisens (Organon), and the bDNA test (Chiron). CD4+ and CD8+ cells were counted by flow cytofluorometry. Every 3 months until 2001 and every year thereafter, peripheral-blood mononuclear cells (PBMCs) were cocultivated with MT2 cells, to detect the presence of syncytium-inducing (SI) HIV-1 variants—that is, those using CXCR4 for entry into cells [21]. Parallel coculturing of patient PBMCs and healthy donor PBMCs always resulted in virus isolation (data not shown), indicating that the absence of syncytia in the MT2 cocultures was due to the presence of only non-SI HIV and not to the total absence of replication-competent HIV. Data on the occurrence of SI variants were available for 297 (91%) of 326 individuals. We estimated the time of first emergence of SI HIV-1 variants as being the midpoint between the date of the last sample negative for SI and the first sample positive for SI.

Men in whom an AIDS event developed during follow-up were referred to the Academic Medical Center in Amsterdam. In February 1999, follow-up of all men infected with HIV-1 was transferred to the Jan van Goyen Clinic in Amsterdam, through a project of the HIV-1 Monitoring Foundation (HMF) in The Netherlands; since then, the HMF has provided regular updates concerning clinical (e.g., diagnosis of AIDS, death, and treatment), virological (HIV-1 RNA), and immunological (CD4+ cell count) data, most recently in July 2003. We based diagnosis of AIDS on the Centers for Disease Control and Prevention 1993 criteria [22]. Until December 2000, we ascertained new AIDS cases by cross-reference with the Amsterdam AIDS Registration; after this date, information on diagnosis of AIDS was provided by the HMF.

GBV-C testing. For each man in the study, serum samples obtained at ACS entry and the last serum sample available before January 1996 were all tested for the presence of both GBV-C RNA and envelope protein–2 (E2) antibodies. According to existing definitions, the presence of GBV-C RNA is considered to reflect present active infection, whereas the presence of E2 antibodies is considered to reflect past infection [6, 8, 15, 16]. For HIV-1 seroconverters, a sample obtained immediately after HIV seroconversion was also tested, in case of a change in GBV-C status between these 2 tests; if a man’s GBV-C status changed and his follow-up exceeded 8 years, a sample obtained at the midpoint of the follow-up also was tested. GBV-C serology was determined by an ELISA (μPLATE Anti-HGenv; Roche Diagnostics) for the qualitative determination of IgG antibodies against the GBV-C E2 antigen. Serum samples were diluted 1:21. Positive or borderline results were confirmed by an appropriate confirmation test, as described by the manufacturer. The presence of GBV-C RNA was determined by polymerase chain reaction (PCR) using cDNA and a primer set that anneals specifically to the 5 untranslated region (UTR). Nucleic acid was extracted from 100 μL of serum by use of the Boom method [23] and the NucliSens Isolation Kit (Bioxel; bioMérieux). RNA was subsequently reverse-transcribed with random prime labeling and was amplified by PCR, as described elsewhere [24]. For amplification in the 5 UTR, primers NC1 (5′-CGGCCAAAGGGTTGGATG-3′) and NC4 (5′-CCAACACCTGTGGACGGTTC-3′) were used, and the product was detected by oligomer hybridization using probe NC3 (5′-GTAGCACATATAGGTGGG-3′). Both negative- and positive-testing serum samples were used and were diluted as much as 1:1000.

Statistical analysis. All men were censored either 1 year after their last visit or, if continuing in follow-up, on 31 December 2002. Characteristics of men who, at baseline, tested either positive or negative for GBV-C were analyzed by 1-way
analysis of variance or Kruskal-Wallis test. Using the STATA program (STATA), we created Cox proportional-hazards models, allowing for late entry for the seroprevalent cases, to study the effect that GBV-C RNA and E2 antibody have on HIV-1 disease progression (i.e., time between HIV-1 seroconversion and SI conversion, first CD4+ cell count <200/μL, AIDS, or death; and time between diagnosis of AIDS and death). We analyzed the effect of GBV-C RNA and E2 antibody in the first sample available after seroconversion, both separately (i.e., GBV-C RNA positive vs. GBV-C RNA negative; E2 antibody positive vs. E2 antibody negative) and combined (i.e., active or past infection vs. no evidence of active or past infection), as was done in previous studies [6, 8, 15, 16]. Because a change in either GBV-C RNA status or antibody status cannot be included as baseline variables [25], we studied GBV-C RNA and E2 antibody as time-dependent variables in a Cox proportional-hazards model. If a change in GBV-C status was detected, we assumed that it had occurred at the midpoint between the 2 consecutive test dates. To differentiate between GBV-C gain or loss and persistently negative or positive status, we used 4 categories for the 2 covariates; for example, if an individual’s GBV-C status changed from RNA positive to RNA negative over time, the characterization of the covariate was changed from RNA persistence to RNA loss. In multivariate analysis, the effect of GBV-C was adjusted for age at seroconversion, CCR5 genotype, highly active antiretroviral therapy (HAART) status as a calendar period (as a time-dependent covariate, with its value allowed to change, over time and for each person, from “pre-HAART era” [i.e., before 1996] to “HAART era”) and both CD4+ cell count and HIV-1 RNA load 1 year after the estimated date of seroconversion. If the CD4+ cell count or HIV-1 RNA load was not available, we substituted the closest CD4+ cell count or HIV-1 RNA load available 6 months–3 years after seroconversion. To explore possible causal pathways, we adjusted for updated (i.e., change occurring during follow-up) CD4+ cell count and HIV-1 RNA as time-dependent covariates. Because HIV-1 RNA was routinely tested after June 1996 and, before then, was routinely tested only in HIV-1 seroconverters, we used fitted values of HIV-1 viral load, which were obtained from a random-effects model for the joint development of CD4+ cells and viral load [26], in place of the missing pre–June 1996 values. Both CD4+ cell count and HIV-1 RNA load were transformed for statistical analyses (by square-root and log_10 transformations, respectively). Data on SI conversion were unavailable for 9% of the men; these men were not excluded from multivariate analyses but were entered into the model as a separate category (i.e., “unknown”). To exclude any confounding effect that HAART might have had on GBV-C and HIV-1 disease progression, we repeated the above analyses, using 1 January 1996, instead of 31 December 2002, as the censoring date. P < .05 was considered to be statistically significant.

RESULTS

Study population. Of the 326 homosexual men in the study, 203 (62%) were infected with HIV-1 when they entered the ACS (i.e., before 1986), and 123 (38%) seroconverted for HIV-1 antibodies during follow-up before 1996. The median age at entry was 34.6 years (SD, 7.1 years), and the majority were white. Median follow-up time after HIV-1 seroconversion or first positive HIV-1 test in the ACS was 8.0 years (interquartile range [IQR], 4.9–12.5 years). The median time between the estimated date of HIV-1 seroconversion and the first test for GBV-C was 1.9 years (IQR, 0.2–2.1 years). Of the 326 men, 129 (40%) received monotherapy or combination therapy not including protease inhibitors or nonnucleoside reverse-trancriptase inhibitors; of the 127 still in follow-up after 1996, 96 (76%) received HAART.

For the first sample tested, 137 (42%) men tested positive for GBV-C RNA, and 134 (41%) had detectable E2 antibodies; for the last sample tested before 1996, these numbers were 69 (21%) and 126 (39%), respectively (table 1). Interesting is that, of the 137 men positive for GBV-C RNA at their first test, 78 tested negative for GBV-RNA at their last test; and only 14 (18%) of these 78 had detectable E2 antibodies at their last GBV-C test.

Effect of GBV-C measured early during the course of HIV-1 infection. In univariate analyses, men who tested positive for GBV-C RNA in their first sample had an increased risk of progression to CD4+ cell count <200/μL (hazard ratio [HR], 1.59 [95% confidence interval [CI], 1.21–2.10]), AIDS (HR, 1.37 [95% CI, 1.02–1.81]), or death (HR, 1.44 [95% CI, 1.06–1.96]) (table 2). Adjustment for age at HIV-1 seroconversion, CCR5Δ32 genotype, HAART, CD4+ cell count, and HIV-1 RNA load 1 year after seroconversion did not substantially lower the HR, indicating that these covariates do not have a strong effect on the association between GBV-C RNA and HIV-1 disease progression (table 2). However, the HR for GBV-C’s effect on HIV-1 disease progression decreased substantially, toward 1, after adjustment for updated CD4+ cell count. Univariate analysis revealed that GBV-C viremia early during the course of HIV-1 infection had no statistically significant effect on either the period between diagnosis of AIDS and death or the rate of conversion toward SI HIV-1 variants (table 2).

The presence of E2 antibodies in the first sample tested after HIV-1 seroconversion did not influence subsequent progression to either AIDS (HR, 0.81 [95% CI, 0.61–1.07]) or death (HR, 0.87 [95% CI, 0.64–1.19]), in either univariate or multivariate analysis. For the other 2 end points (i.e., first CD4+ cell count <200/μL and SI conversion), the only finding was a univariate association between E2 antibody and slower progression to a CD4+ cell count <200/μL (HR, 0.68 [95% CI, 0.51–0.91]), which became weaker (HR, 0.77 [95% CI, 0.57–1.06]) after adjustment for age at seroconversion, CCR5 Δ32 genotype,
Table 1. GB virus C (GBV-C) RNA status and envelope protein–2 antibody (E2-Ab) status shortly after HIV-1 seroconversion, in 326 homosexual men positive for HIV-1.

<table>
<thead>
<tr>
<th>GBV-C status at last sample tested, no. (%)</th>
<th>Negative for GBV-C RNA</th>
<th>Positive for GBV-C RNA</th>
<th>P \textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative for E2-Ab</td>
<td>(n = 68)</td>
<td>Positive for E2-Ab</td>
<td>(n = 121)</td>
</tr>
<tr>
<td>Age at seroconversion, median (IQR), years</td>
<td>34.4 (30.1–42.2)</td>
<td>36.3 (29.8–40.5)</td>
<td>32.1 (27.6–35.7)</td>
</tr>
<tr>
<td>Follow-up since HIV-1 seroconversion or first positive test, median (IQR), years</td>
<td>8.3 (4.8–13.4)</td>
<td>8.4 (4.8–12.5)</td>
<td>7.8 (5.2–11.7)</td>
</tr>
<tr>
<td>Time of first GBV-C test after seroconversion, median (IQR), years</td>
<td>1.9 (0.6–2.1)</td>
<td>1.5 (0.1–2.1)</td>
<td>2.0 (0.3–2.1)</td>
</tr>
<tr>
<td>Time between first and last GBV-C test, median (IQR), years</td>
<td>8.4 (5.6–10.1)</td>
<td>6.6 (4.0–10.4)</td>
<td>7.4 (4.6–10.1)</td>
</tr>
<tr>
<td>CD4\textsuperscript{+} cell count 1 year after seroconversion, median (IQR), cells/\muL</td>
<td>600 (390–780)</td>
<td>595 (457–822)</td>
<td>490 (360–710)</td>
</tr>
<tr>
<td>HIV-1 RNA 1 year after seroconversion, median (IQR), log\textsubscript{10} copies/mL</td>
<td>4.2 (3.1–4.7)</td>
<td>4.3 (3.8–4.7)</td>
<td>4.4 (3.6–7.8)</td>
</tr>
<tr>
<td>CD4\textsuperscript{+} cell count at last GBV-C testing, median (IQR), cells/\muL</td>
<td>250 (115–365)</td>
<td>285 (40–530)</td>
<td>145 (40–295)</td>
</tr>
<tr>
<td>GBV-C status at last sample tested, no. (%)</td>
<td>6 (9)</td>
<td>4 (3)</td>
<td>58 (47)</td>
</tr>
<tr>
<td>Positive for E2-Ab</td>
<td>10 (15)</td>
<td>102 (84)</td>
<td>5 (4)</td>
</tr>
</tbody>
</table>

\textbf{NOTE.} IQR, interquartile range.

\textsuperscript{a} By 1-way analysis of variance or Kruskal-Wallis test.
HAART, CD4+ cell count, and HIV-1 RNA load 1 year after HIV-1 seroconversion (data not shown).

The men in the present study were categorized into 3 groups, according to their GBV-C status early during the course of HIV-1 infection: men with active infection, men with past infection, and men with no evidence of either active or past GBV-C infection; 13 men were positive for both active and past GBV-C infection. The 3 groups showed no significant difference in HIV-1 disease progression, in either univariate and multivariate analysis, irrespective of the end points used (data not shown).

Effect of GBV-C coinfection as a time-dependent variable. Because GBV-C viremia can be both gained and lost over time, we analyzed, as a time-dependent variable, the effect that GBV-C coinfection had on HIV-1 disease progression. At first glance, compared with men who had never tested positive for GBV-C RNA, GBV-C RNA persistence was associated with a decreased risk of death, whereas GBV-C RNA loss was associated with an increased risk of death (table 3, models 1 and 2). However, when adjusted for updated CD4+ cell count, both HRs approached 1 and became nonsignificant (table 3, model 4). GBV-C RNA persistence was not a significant predictor of HIV-1 disease progression to any other end point. In univariate analyses, men who had lost GBV-C RNA had a significantly increased risk of AIDS, compared with men who had never tested positive for GBV-C RNA (HR, 2.91 [95% CI, 1.93–4.01]) (table 3, model 1); this difference in the risk of AIDS remained significant after adjustment for age at seroconversion, HAART, CCR5 genotype, and either updated HIV-1 RNA (table 3, model 3) or updated CD4+ cell count (table 3, model 4). In men who lost GBV-C RNA, the time of the first CD4+ cell count <200/µL was significantly earlier when the data were adjusted for age at seroconversion, HAART, CCR5 genotype, and updated HIV-1 RNA (table 3, model 3); these men also had an earlier appearance of SI HIV-1 variants (table 3, models 3 and 4). Survival analysis using E2 antibodies as a time-dependent variable revealed that only E2-antibody persistence was significantly associated with more-rapid appearance of SI HIV-1 variants (HR, 1.55 [95% CI, 1.02–2.30]), an association that remained after the data were adjusted for age at seroconversion, HAART, CCR5 genotype, and either updated HIV-1 RNA or updated CD4+ cell count (data not shown).

Similar results were obtained when the same analyses were restricted to the group of 123 seroconverters (data not shown). Finally, we evaluated the effect that GBV-C coinfection had on the clinical course of HIV-1 infection in the absence of HAART by using 1 January 1996 (the year in which HAART was generally introduced in The Netherlands) as the censoring date; the finding of similar HRs indicated that HAART did not influence the effect that GBV-C coinfection had on HIV-1 disease progression (data not shown).

Because we made only 2 or 3 measurements, the exact time of GBV-C RNA loss remains unknown. We therefore assessed the robustness of our findings by varying the time of GBV-C RNA loss, from 1 and 7 years after HIV-1 seroconversion. The effect became smaller—but remained significant—when we fixed the time of GBV-C RNA loss at 1 year after HIV-1 seroconversion, and, conversely, the effect increased when we fixed the time of loss at 7 years after seroconversion; these results show the robustness of our results when various times of GBV-C RNA loss are used.

**DISCUSSION**

To clarify conflicting findings as to the effect that GBV-C co-infection has on HIV-1 disease progression [6–16], we studied the effect that GBV-C had in 326 homosexual men in the ACS

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**Table 2.** Cox proportional-hazard models for progression from HIV-1 seroconversion to first CD4+ cell count <200/µL, syncytium-inducing (SI) conversion, AIDS, and death and for progression from AIDS to death, by GB virus C (GBV-C) RNA status measured shortly after HIV-1 seroconversion in 326 homosexual men positive for HIV-1.

<table>
<thead>
<tr>
<th>Progression from HIV-1 seroconversion to</th>
<th>First CD4+ cell count &lt;200/µL</th>
<th>SI conversion</th>
<th>AIDS</th>
<th>Death</th>
<th>Progression from AIDS to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted: model 1</td>
<td>1.59 (1.21–2.10)</td>
<td>1.19 (0.81–1.76)</td>
<td>1.37 (1.02–1.81)</td>
<td>1.44 (1.06–1.96)</td>
<td>1.22 (0.89–1.67)</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1a</td>
<td>1.36 (1.01–1.80)</td>
<td>1.20 (0.80–1.80)</td>
<td>1.32 (0.97–1.80)</td>
<td>1.35 (0.97–1.88)</td>
<td>1.22 (0.86–1.70)</td>
</tr>
<tr>
<td>Model 2c</td>
<td>1.25 (0.93–1.69)</td>
<td>1.15 (0.77–1.72)</td>
<td>1.21 (0.89–1.66)</td>
<td>1.27 (0.92–1.76)</td>
<td>1.18 (0.85–1.67)</td>
</tr>
<tr>
<td>Model 3c</td>
<td>...</td>
<td>1.16 (0.78–1.74)</td>
<td>1.10 (0.80–1.50)</td>
<td>1.04 (0.75–1.44)</td>
<td>1.07 (0.76–1.49)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are hazard ratio (96% confidence interval).

a Adjusted for age at seroconversion, highly active antiretroviral therapy, CCR5 genotype, square root CD4+ cell count, and log10 HIV-1 RNA 1 year after seroconversion.
b Adjusted for age at seroconversion, highly active antiretroviral therapy, CCR5 genotype, square root CD4+ cell count 1 year after seroconversion, and updated log10 HIV-1 RNA.
c Adjusted for age at seroconversion, highly active antiretroviral therapy, CCR5 genotype, log10 HIV-1 RNA 1 year after seroconversion, and updated square root CD+ cell count.
Table 3. Cox proportional-hazard models for progression from HIV-1 seroconversion to first CD4\(^+\) count <200/\(\mu\)L, SI conversion, AIDS, and death and for progression from AIDS to death, with GBV-C RNA as a time-dependent variable, in 326 homosexual men positive for HIV-1.

<table>
<thead>
<tr>
<th>GBV-C RNA status, HR (95% CI)</th>
<th>Unadjusted: model 1</th>
<th>Adjusted: Model 2(^b)</th>
<th>Adjusted: Model 3(^c)</th>
<th>Adjusted: Model 4(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistence</td>
<td>1.19 (0.87–1.63)</td>
<td>1.02 (0.71–1.45)</td>
<td>0.98 (0.70–1.43)</td>
<td>0.92 (0.58–1.47)</td>
</tr>
<tr>
<td>Gain</td>
<td>0.34 (0.08–1.41)</td>
<td>0.74 (0.18–3.08)</td>
<td>1.24 (0.29–5.30)</td>
<td>...</td>
</tr>
<tr>
<td>Loss</td>
<td>3.26 (2.20–4.80)</td>
<td>2.36 (1.53–3.61)</td>
<td>2.17 (1.23–3.85)</td>
<td>...</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.

\(^a\) Significance of GBV-C RNA status.
\(^b\) HR (95% CI) adjusted for age at seroconversion, highly active antiretroviral therapy, CCR5 genotype, square root CD4\(^+\) cell count, and log\(_{10}\) HIV-1 RNA 1 year after seroconversion.
\(^c\) HR (95% CI) adjusted for age at seroconversion, highly active antiretroviral therapy, CCR5 genotype, square root CD4\(^+\) cell count 1 year after seroconversion, and updated log\(_{10}\) HIV-1 RNA.
\(^d\) HR (95% CI) adjusted for age at seroconversion, highly active antiretroviral therapy, CCR5 genotype, log\(_{10}\) HIV-1 RNA 1 year after seroconversion, and updated square root CD4\(^+\) cell count.

who were positive for HIV-1. Their accurately estimated dates of seroconversion, long-term follow-up, and cryopreserved samples allowed us to measure the presence of GBV-C RNA and E2 antibodies early and late during HIV-1 infection. In this setting, we found no evidence that GBV-C RNA or E2 antibodies had a protective effect with regard to HIV-1 disease progression. However, compared with the continuous absence of GBV-C RNA, GBV-C RNA loss was associated with more-progressive HIV-1 disease, irrespective of the end point of the analysis. This effect seemed to correlate most with changes in CD4\(^+\) cell count, because the effect became weaker in multivariate analyses in which adjustment was made for this covariate. Gain or loss of GBV-C–specific E2 antibodies had no observed effect on HIV-1 disease progression, but E2-antibody persistence was an independent predictor for more-rapid emergence of SI HIV-1 variants.

The discrepancies between previous studies most likely reflect differences in the clinical stage of subjects’ HIV-1 infection at baseline and a potential misclassification of GBV-C status. Our study design provided interesting insights into the dynamics of both GBV-C infection and GBV-C–specific antibody response in relation to HIV-1 infection. Contrary to previous studies’ assumptions, the present study found that GBV-C viremia was not necessarily followed by seroconversion for E2 antibodies; GBV-C RNA loss was not necessarily associated with E2-antibody persistence, and E2 seroconversion did not necessarily result in the clearance of GBV-C RNA. These findings also imply that the current definition of active and past GBV-C infection should be reconsidered, because the absence or presence of E2 antibodies does not exclude past or active infection, respectively. Another difference between the present study and previous studies is that 38% of our subjects seroconverted for
HIV-1 during follow-up, so their seroconversion dates were available; previously, seroconversion dates had been accurately imputed for the other 62% [20]. Findings for the total cohort were similar to the results obtained for the group of HIV-1 seroconverters alone. Of the previous studies suggesting that GBV-C infection at baseline had a positive effect on the clinical course of HIV-1 infection, all but one [7] used subjects for whom the duration of HIV-1 infection was unknown [6, 8–11]. In 4 of these previous studies [6, 8–10], the mean CD4+ cell count initially recorded was relatively low (range, 170–346/µL), suggesting an advanced stage of HIV-1 disease. The present study has demonstrated that GBV-C RNA loss, which occurred primarily without evidence of E2-antibody production, is associated with faster HIV-1 disease progression. Previous reports of a protective effect of GBV-C coinfection might therefore be due to misclassification, in which individuals who have lost GBV-C RNA and lack E2 antibodies are erroneously assumed to have been persistently GBV-C negative. We used our own data to simulate these baseline analyses, by fixing the GBV-C RNA loss at 3, 5, and 7 years after HIV-1 seroconversion. When GBV-C RNA loss was fixed at 3 or 5 years after HIV-1 seroconversion, there was no survival difference between men positive for GBV-C RNA and men negative for GBV-C RNA; however, when it was fixed at 7 years after HIV-1 seroconversion, with a median CD4+ cell count of 320/µL, there was a significantly decreased risk of death in men who were positive for GBV-C RNA. These results imply that both (1) the time point, during the clinical course of HIV-1 infection, selected for measurement of GBV-C coinfection and (2) the follow-up period used for survival studies have a great impact on the outcome of the analysis. Indeed, in a recent study by Williams et al. [15], GBV-C status was determined 12–18 months and 5–6 years after HIV-1 seroconversion; in agreement with the results of the present study, they found that GBV-C RNA loss and persistence were associated, respectively, with an increased and a decreased risk of AIDS-related death. In the present study, the latter association disappeared after we adjusted for updated CD4+ cell count, an analysis not performed by Williams et al.—a finding that suggests that the protective effect of GBV-C RNA persistence, like the effect of GBV-C RNA loss, is related to changes in CD4+ cell count during follow-up. In addition, we found that GBV-C RNA persistence had no effect on any of the other end points tested, whereas an association between GBV-C RNA loss and faster HIV-1 disease progression was consistently found for all end points tested.

What could be a plausible explanation for the observation that GBV-C RNA loss, not its continuous absence, is associated with accelerated HIV-1 disease progression? Because GBV-C RNA can replicate in CD4+ cells [27], the decrease in CD4+ cells during the course of HIV-1 infection implies a loss of target cells for GBV-C RNA. This might explain why GBV-C RNA loss is associated with an increased risk of death in HIV-1–infected individuals. The absence of evidence of GBV-C RNA can be considered to be normal during the course of HIV-1 disease progression, and, consequently, persistence and loss of GBV-C RNA can be considered to be surrogate markers for high and low CD4+ cell counts, respectively. These inferences are supported by the fact that most of the effect that GBV-C RNA loss has on HIV-1 disease progression disappeared after the data were adjusted for changes in CD4+ cell count. We therefore hypothesize that GBV-C RNA loss is due to CD4+ cell loss, not vice versa. Interestingly, in 1 man who lost GBV-C RNA in the absence of E2 antibodies, GBV-C RNA reappeared when initiation of HAART increased the CD4+ cell count from 130/µL to 450/µL and, subsequently, to 760/µL (data not shown), suggesting that, during the course of HIV-1 disease progression, GBV-C RNA loss is secondary to CD4+ cell loss. Serum samples from only 2 other men who lost GBV-C RNA were available for GBV-C RNA testing after initiation of HAART; the absence of GBV-C RNA in these samples may have been due to the presence of E2 antibodies.

In conclusion, although GBV-C infection does not seem to protect against either CD4+ cell loss or HIV-1 disease progression and most likely persists only when a sufficient number of CD4+ cells are present, it has been seriously considered as a potentially protective agent in the fight against HIV-1 disease progression. In light of the great significance of such findings, firm conclusions should not be drawn until any factor that may influence HIV-1 infection has been studied—in large, well-defined cohorts of HIV-1–infected individuals whose dates of seroconversion are documented and from whom serum samples can be obtained shortly after HIV-1 infection.

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