GB virus C and HIV-1 RNA load in single virus and co-infected West African individuals

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Background: Investigations on the impact of GB virus C (GBV-C) co-infection on HIV disease progression relied essentially on clinical follow-up but not on virologic parameters.

Objectives: To detect and quantify GBV-C RNA in West African populations co-infected or not with HIV-1 and to correlate the RNA load of HIV-1 and GBV-C in co-replicating patients with different clinical conditions.

Methods: Three Ghanaian populations (blood donors, pregnant women and HIV-infected patients) were subdivided into six groups according to HIV-1 and clinical status and GBV-C and HIV-1 RNA load was tested by quantitative real time reverse transcriptase-polymerase chain reaction. In one population with HIV-1 disease, CD4+ cell count was also measured.

Results: Prevalence of GBV-C markers in HIV-1-infected groups and HIV-1 non-infected pregnant women were significantly higher than in healthy blood donors. Similar levels and distribution of GBV-C RNA load were found in each population irrespective of HIV-1 status except for a lower GBV-C RNA load in AIDS patients. There was a significant shift of HIV-1 load towards lower value when GBV-C RNA was present and a trend towards an inverse correlation between HIV-1 and GBV-C RNA load. A positive correlation between CD4+ cell count and GBV-C RNA load in symptomatic HIV-1-infected patients was observed.

Conclusions: The moderate impact of GBV-C on HIV-1 viremia is unlikely to entirely account for a favourable clinical outcome of replicating co-infections.

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Keywords: GB virus C, HIV-1, viral load, co-infection

Introduction

GB virus C (GBV-C) is a non-pathogenic flavivirus that is related to hepatitis C virus (HCV) [1,2], but does not appear to be hepatotropic as the virus neither replicates in hepatocytes nor causes hepatitis [3,4]. GBV-C is a lymphotropic virus that replicates in the spleen and bone marrow [5], targeting different peripheral blood mononuclear cells including CD4 positive T cells [6].

GBV-C infection in humans is common and its transmission has similarity with both HCV and HIV including sexual, parenteral and vertical transmission [7]. Recent epidemiological studies strongly suggested an association...
between GBV-C co-infection and delayed mortality in HIV-1-infected individuals [8–13]. In addition to similar target cells, the hypothesis of viral interference was further supported by evidence of GBV-C influencing HIV-1 replication in vitro through induction of chemokines and down-regulation of an HIV co-receptor in vitro [14,15]. However, other studies did not confirm a clinical benefit for HIV-1-infected individuals co-infected with GBV-C [16–20].

Since the clinical outcome of HIV-1 infection is in part correlated with viral load, we formulated the hypothesis that if GBV-C co-infection favourably affected HIV-1 disease progression, it might be reflected by lower HIV-1 load when GBV-C co-replicated. It was also hypothesized that the progressive depletion of CD4+ cells induced by HIV might in turn negatively influence GBV-C replication.

To test these hypotheses, a study was conducted to quantify both GBV-C and HIV-1 RNA load from three West African populations: blood donors, pregnant women and symptomatic HIV-1-infected patients, who were further subdivided according to HIV-1 status for the first two and clinical status for the third population.

**Materials and methods**

**Viral reference samples, plasma specimens and viral standards**

Six extracted GBV-C RNA (G4, G5, G6, G19, G20 and G21) were provided by Prof. J.J. Lefrère, Institut National de Transfusion Sanguine, Paris, France.

The study was approved by the Kwame Nkrumah University of Science and Technology School of Medical Sciences committee for ethics and publication. Patients were informed of the objectives of the study and asked for informed consent. The blood donor study was included in a larger study of emerging viral agents previously approved by the ethics committee as previously indicated [21]. Plasma were collected from blood donors or patients at the Komfo Anokye Teaching Hospital in Kumasi, Ghana in the Departments of Medicine and Obstetrics and Gynecology. All samples were screened and confirmed for antibody to HIV according to previously published procedures [22]. Differential diagnosis between HIV-1 and HIV-2 was performed as previously described [23]. According to HIV infection status and type of population, plasma samples were classified into six groups: (1) HIV-negative blood donor (BD-); (2) HIV-1-positive blood donor (BD+); (3) HIV-1-positive symptomatic patient (SYM); (4) HIV-1 patients with AIDS (AIDS); (5) HIV-1-positive pregnant woman (PW+); and (6) HIV-1-negative pregnant woman (PW-).

For quantification of HIV-1 RNA load by quantitative polymerase chain reaction (QPCR), the first international standard for HIV-1 RNA (97/656 containing 10^6 IU/ml) was provided by the National Institute of Biological Standards and controls (NIBSC; Potters Bar, UK). Results were expressed in IU/ml as previously described [23].

For quantification of GBV-C RNA load, an in-house reference standard was prepared with plasma K759 from a Ghanaian blood donor. After 45 cycles of amplification, the extracted RNA provided a detectable signal when diluted 5 x 10^6 times in water. The intercept between the end point dilution curve of this RNA and the 45-cycle mark of the assay defined 1 arbitrary unit (AU) of GBV-C RNA. The reference sample therefore contained 5 x 10^6 AU/ml. Each quantification run was performed with this reference RNA serially diluted between neat and 5 x 10^6. The standard deviations (SD) of C values from six individual quantification reference curves at four points (50000, 5000, 500, and 50 AU/ml) were 0.35, 0.53, 0.44 and 0.57, respectively.

**Viral RNA extraction and reverse transcription reaction**

Viral RNA was extracted from 200 μl of plasma using High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer’s instructions. The reverse transcription (RT) reaction was optimized as one cycle of 25°C, 10 min; 45°C, 60 min; and 95°C, 3 min, and then cooling to 4°C. Each reaction was in 20 μl of final volume containing 1 x core RT buffer (Stratagene, La Jolla, California, USA), 2 mmol/l dNTPs, 15 ng/μl random primer (Roche Diagnostics GmbH), 1.25 U/μl StrataScript reverse transcriptase (Stratagene), 1 U/μl RNasin Inhibitor (Promega, Madison, Wisconsin, USA) and 10 μl viral RNA extraction.

**Triplex and single quantitative RT-PCR for viral screening and quantification**

GBV-C primers GB-Forward (5’-GCGACCAGGCAAGG-3’), GB-Reverse (5’-GRTGACGAGTTGTTACGACCT-3’) and GB-Probe (5’-CCAACCCCTGTCATCACCCATCCACC-3’) were designed from the 3’ end of the non-coding region (3’ NC) of the GBV-C conserved sequence. The GB-probe was 5’-labelled with Cy5 dye and 3’-labelled with Black Hole Quencher 2 (BH2). The initial screening was performed in the triplex configuration including West Nile and Dengue viruses described elsewhere [24] in which virus-specific probes were labelled with dyes emitting at different wave length enabling direct identification of each virus.

Triplex was performed with either the MX4000 or MX3000 Multiple Quantitative QPCR System (Stratagene). Single virus RT-QPCR assay was used to quantify GBV-C RNA load with the same reagents according to
the same protocol used for the Triplex assay: 45 cycle amplification at 60°C, 1 min; and 95°C, 30 s after an initial of 95°C, 10 min. By using a Brilliant QPCR Core Reagent Kit, each single RT-QPCR reaction was carried out in 50 μl of final volume containing 1 x core PCR buffer, 5 mmol/l MgCl₂, 0.8 mmol/l dNTP, 0.8 μmol/l each primer, 0.1 μmol/l probe, 0.3 μmol/l Rox reference dye, 0.05 U/μl SureStart Tag polymerase and 10 μl viral cDNA template prepared by above method.

The sensitivity of triplex and single RT-QPCR for GBV-C virus did not significantly differ and was similar to the sensitivity of the RT-nested PCR described below.

RT nested-PCR
RT nested-PCR was used as a confirmation test for GBV-C viral RNA positive screened by triplex QPCR. Two pairs of PCR primers (GF1 and GB1, GF2 and GB2) * used in the reaction were as previously described [25].

CDNA was prepared as described above. Two rounds of nested PCR were performed for amplification of GBV-C viral cDNA at each round of 35 cycles of 94°C, 25 s; 48°C, 25 s; and 72°C, 30 s; after an initial of 95°C, 3 min. The amplified DNA products were analysed by agarose gel electrophoresis.

A GBV-C-containing sample was defined as positive with triplex QPCR, single virus QPCR and RT nested-PCR.

GBV-C/HGV anti-E2
Available samples from all six groups were tested for anti-E2 (Roche Diagnostics, Penzberg, Germany) according to the manufacturer's instructions.

CD4+ cell count
CD4+ cell count was performed in Ghana with fresh samples using the Becton Dickinson FACS count instrument (San Jose, California, USA) according to the manufacturer's instructions.

Statistical analysis
The Fisher's exact test was used for the comparison of GBV-C prevalence in the groups (1)–(6) stratified according to HIV-1 infection status. Mann–Whitney test was used for the statistical analysis of viral load median distribution in HIV-1 RNA positive populations according to GBV-C RNA status. Spearman and Pearson tests were used for correlation analyses between GBV-C and HIV-1 RNA loads or GBV-C RNA loads and CD4+ cell counts in HIV-1 RNA positive populations.

Results
Prevalence of GBV-C RNA in HIV-1-infected and non-infected Ghanaian populations
A total of 795 plasma samples from three Ghanaian populations (blood donors, pregnant women and asymptomatic HIV-infected patients subdivided into six groups according to HIV-1 RNA and clinical status) were screened for GBV-C RNA by triplex RT-QPCR. The positive samples for GBV-C RNA by triplex RT-QPCR were further confirmed by single RT-QPCR and RT nested-PCR. The results in Table 1 indicate that the prevalence of detectable GBV-C RNA ranged between 12.9 and 27.9% and of anti-E2 from 3.8 to 18%. The lowest RNA prevalence was found in HIV-non-infected blood donors (12.9%) who also carried the lowest prevalence of anti-E2 (3.8%) and of overall markers of GBV-C (16.5%). These prevalences were significantly lower than found in all other groups, either HIV-infected or non-infected (P < 0.02). Although HIV status significantly affected the prevalence of the GBV-C markers in blood donors, it did not in pregnant women.

<table>
<thead>
<tr>
<th>Population group</th>
<th>Blood donor</th>
<th>HIV+</th>
<th>HIV-</th>
<th>HIV+</th>
<th>HIV-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample tested</td>
<td>HIV+</td>
<td>HIV+</td>
<td>HIV+</td>
<td>HIV+</td>
<td>HIV+</td>
<td>795</td>
</tr>
<tr>
<td>GBV-C RNA</td>
<td>18</td>
<td>21</td>
<td>42</td>
<td>34</td>
<td>62</td>
<td>174</td>
</tr>
<tr>
<td>GBV-C RNA</td>
<td>122</td>
<td>75</td>
<td>161</td>
<td>96</td>
<td>62</td>
<td>621</td>
</tr>
<tr>
<td>% GBV-C RNA</td>
<td>12.9</td>
<td>21.9</td>
<td>20.7</td>
<td>26.2</td>
<td>27.9</td>
<td>25</td>
</tr>
<tr>
<td>GBV-C anti-E2</td>
<td>6/133</td>
<td>12/83</td>
<td>20/7</td>
<td>19/9</td>
<td>10/70</td>
<td>24/133</td>
</tr>
<tr>
<td>% anti-E2</td>
<td>3.8</td>
<td>14.5</td>
<td>10.6</td>
<td>5.9</td>
<td>18.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Nb GBV-C marker +/tested (%)</td>
<td>22/133 (16.5)</td>
<td>31/83 (27.3)</td>
<td>60/199 (30.2)</td>
<td>17/51 (33.3)</td>
<td>27/70 (38.6)</td>
<td>53/133 (39.8)</td>
</tr>
</tbody>
</table>

*SYM: HIV-1 + symptomatic patient; AIDS: AIDS patient (according to Centers for Disease Control definition for Africa).

*GBV-C RNA positive result by triplex-quantitative polymerase chain reaction (QPCR), single-QPCR and reverse transcription-nested PCR.

*By Fisher's exact test, the two-sided P values comparing prevalence of GBV-C RNA are 0.008 (BD HIV+ vs. AIDS), 0.008 (BD HIV+ vs. PW HIV+), 0.014 (BD HIV+ vs. PW HIV+), respectively. Other comparisons did not reach significance.

*For anti-E2, the prevalence between BD HIV+ or HIV+ (P = 0.02), between BD HIV+ and PW HIV+ (P = 0.02), and between BD HIV+ and PW HIV+ (P = 0.001) were significant. The anti-E2 prevalence in AIDS patients was significantly lower than PW HIV+ (P = 0.04).

*One, two, four and one sample in each respective group contained both detectable viral RNA and anti-E2.
Table 2. Distribution of GBV-C RNA positive samples according to HIV status, age and gender.

<table>
<thead>
<tr>
<th>Population group</th>
<th>Blood donor</th>
<th>HIV disease</th>
<th>Pregnant women</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-</td>
<td>HIV+</td>
<td>SYM</td>
<td>AIDS</td>
</tr>
<tr>
<td>Total numbera</td>
<td>70</td>
<td>68</td>
<td>202</td>
<td>40</td>
</tr>
<tr>
<td>Median age</td>
<td>19</td>
<td>31</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>Age distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20 years</td>
<td>40 (7)b</td>
<td>3 (2)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>20–29 years</td>
<td>24</td>
<td>27 (5)</td>
<td>37 (8)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>30–39 years</td>
<td>6 (1)</td>
<td>26 (5)</td>
<td>80 (20)</td>
<td>13 (3)</td>
</tr>
<tr>
<td>40–49 years</td>
<td>0</td>
<td>11 (2)</td>
<td>61 (11)</td>
<td>14 (1)</td>
</tr>
<tr>
<td>≥ 50 years</td>
<td>0</td>
<td>1</td>
<td>23 (3)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>46/24</td>
<td>59/9</td>
<td>67/135</td>
<td>17/23</td>
</tr>
<tr>
<td>GBV-C RNA+ M/F</td>
<td>3/5</td>
<td>14/0</td>
<td>9/33</td>
<td>3/7</td>
</tr>
</tbody>
</table>

aThe number of samples known for age and gender among the samples in Table 1.
bThe number of GBV-C RNA positive samples given in parentheses.

Within the HIV-infected groups, the prevalence of GBV-C detectable RNA did not differ significantly according to the presence or absence of symptoms although patients with AIDS had less anti-E2 than any other HIV-infected group (not significant). In pregnant women, the prevalence of GBV-C RNA, anti-E2 or overall GBV-C markers were similar, irrespective of HIV status (Table 1).

Table 2 shows the distribution of age, gender and GBV-C RNA positivity in individuals from the six groups studied for whom all these parameters were available. The HIV-negative blood donor group was significantly younger than any other groups. The prevalence of GBV-C RNA did not significantly vary with age. The apparently lower prevalence in the two age groups older than 39 years was not significant due to the relatively small number of subjects. Although no significant differences in GBV-C RNA prevalence were found according to gender in individual groups including males and females, the higher prevalence in women approached significance \( P = 0.052 \) when data were aggregated, and was significant when data from pregnant women was added \( P = 0.025 \).

Distribution of GBV-C RNA load according to population and HIV-1 status

A total of 174 GBV-C RNA positive samples from the six groups were quantified by single RT-QPCR against an in-house GBV-C reference standard. GBV-C RNA load was expressed in AU/ml. The distribution of GBV-C RNA loads is shown in Fig. 1. GBV-C RNA load ranged from 2 to 9.47 \times 10^5 AU/ml. Comparing the three

Fig. 1. Distribution of GB virus C (GBV-C) RNA load in six groups of individuals HIV co-infected or not. GBV-C RNA load (arbitrary units/ml) was quantified by single reverse transcriptase-quantitative polymerase chain reaction against a reference standard. RNA load was plotted in each column according to six different groups of three populations. The medians are indicated on each column. By Mann–Whitney test, two-tailed \( P \) values are 0.001 (BD HIV- vs. BD HIV+), 0.042 (SYM/AIDS), 0.683 (PW HIV+ vs. PW HIV–), 0.004 (BD HIV– vs. SYM), 0.371 (BD HIV– vs. AIDS), 0.0001 (BD HIV– vs. PW HIV+), 0.0006 (BD HIV– vs. PW HIV–), 0.005 (AIDS vs. PW HIV+) and 0.004 (AIDS vs. PW HIV–), respectively. BD, blood donor; SYM, HIV-1+ symptomatic patient; AIDS, AIDS patient (according to Centers for Disease Control definition for Africa); PW, pregnant women.
Fig. 2. Distribution of HIV-1 RNA load according to GBV-C RNA status. HIV-1 RNA load (IU/ml) was quantified against an international standard. A total of 501 HIV-1 RNA load were analysed according to GBV-C RNA status (+/-), of which 118 was GBV-C RNA positive and 383 was GBV-C RNA negative. Grey bars indicate HIV-1 RNA load distribution in GBV-C RNA positive and black bars in GBV-C RNA negative samples. For analysis of statistical difference between GBV-C RNA positive and negative populations, HIV-1 viral load (IU/ml) was stratified into lower (< $10^5$ IU/ml) and higher ($\geq 10^5$ IU/ml) groups stratified according to GBV-C RNA status. By Fisher's exact test, the two-sided P-value is 0.006.

In the GBV-C positive populations stratified according to HIV-1 infection and clinical symptoms, there was a statistically significant difference between GBV-C RNA load medians in HIV-1-infected and non-infected blood donors ($2.83 \times 10^4$ and $4.91 \times 10^3$, respectively, $P = 0.001$), between patients with HIV disease and AIDS ($5.11 \times 10^4$ and $1.07 \times 10^5$, respectively, $P = 0.04$), but not between HIV-infected and non-infected pregnant women ($8.77 \times 10^3$ and $1.42 \times 10^5$, respectively, $P = 0.68$). In addition, GBV-C RNA load median in HIV-1 non-infected blood donors was significantly lower than any HIV-infected group except the AIDS group and HIV-1 non-infected pregnant women. The results indicated that GBV-C RNA load appeared higher in HIV-1-infected individuals with the exception of the AIDS or pregnant woman populations than in apparently healthy blood donors.

HIV-1 RNA load according to GBV-C RNA status

HIV-1 RNA load was quantified by RT-QPCR against the first international standard for HIV-1 RNA in 501 plasma samples collected from four HIV-1-infected populations. The median and distribution of HIV-1 RNA load were not significantly affected by GBV-C RNA status in any of the patient groups. When these groups were stratified into asymptomatic (blood donors and pregnant women) and symptomatic (HIV disease and AIDS), higher HIV-1 RNA load was found in the symptomatic group, irrespective of GBV-C RNA status. However, the overall distribution of HIV-1 RNA load in GBV-C RNA positive samples was significantly lower than in GBV-C RNA negative samples ($P = 0.006$) (Fig. 2).

To further explore the potential impact of GBV-C replication on HIV-1 load, the viral load of both the viruses was correlated in 118 co-infected plasma samples. Figure 3 indicates a trend toward an inverse correlation, but this was not statistically significant.

Correlation between GBV-C RNA load and CD4+ cell count in HIV-1-infected symptomatic population

In the group with HIV disease, a CD4+ cell count was available for 37 patients co-infected with GBV-C. A positive correlation ($P = 0.03$) between CD4+ cell count and GBV-C RNA load was found.

Fig. 3. Correlation between GBV-C and HIV-1 RNA load in co-infected individuals. 118 paired GBV-C and HIV-1 RNA loads from four groups of HIV-1-infected populations (BD+, SMY, AIDS and PW+) were plotted. The power trend is indicated by the line on the figure. By Spearman correlation analysis, the one-tailed $P$ value and two-tailed $P$ value with 95% confidence interval are 0.057 and 0.113, respectively.
Discussion

The potential effect of GBV-C replication on the progression and outcome of HIV-1 disease has been a subject of recent controversy. The initial investigators reported a significantly prolonged survival in co-infected patients in comparison with those either single infected or having recovered from GBV-C infection by producing neutralizing anti-E2 [9–11]. More recent reports did not confirm these initial findings and a final answer is still awaited [18,19,26]. The favourable impact of GBV-C replication was supported by data generated in vitro indicating a lower level of HIV-1 replication in CD4+ cultured cells co-infected with GBV-C and by evidence that GBV-C replication induced the production of a chemokine down-regulating an HIV cell surface receptor [14,15]. No in-vivo virological data have been reported that could support or contradict the interference between the two viruses. The present study was intended to provide such data by quantifying the viral load of both viruses and comparing levels and distributions in single infection of both agents and co-infections in various clinical settings.

The first purpose of this study was to determine the prevalence of GBV-C infection as viremia or anti-E2 and to compare levels in populations of asymptomatic individuals co-infected or not with HIV-1. When compared with previously published data, the overall ratio between antibody and infected individual samples was lower and considerably below 1 (0.17–0.46). In addition, antibody S/CO ratios were generally low (26% ≥ 2). It is possible that antibody titres might be lower if infection occurs earlier in life as it is often the case in developing countries. Differences in genotype between capture antigen and prevalent virus might also influence antibody reactivity.

The prevalence of GBV-C markers observed in random Ghanian blood donors was similar to previously published data from Africa [20,27–30]. These prevalences were significantly higher in blood donors carrying antibody to HIV (Table 1). Such a difference can be explained by at least three different mechanisms. First, GBV-C infection may occur more frequently in HIV-1-infected individuals as co-infection or as a result of behaviour considered at high risk of HIV infection. The observation that anti-E2 frequency is significantly higher in HIV-infected than in non-infected donors suggests that the epidemiological circumstances leading to HIV infection also expose these individuals to GBV-C. However, the stability of the prevalence of GBV-C viremia with age while HIV-1 prevalence tends to increase with age does not support such hypothesis (Table 2). Second, GBV-C infection occurring in an HIV-infected individual may tend to persist longer instead of recovering through the development of neutralizing anti-E2 because of the relative immunodeficiency induced by HIV infection at the asymptomatic stage. Third, in individuals infected with GBV-C prior to HIV infection who had recovered, GBV-C reactivation related to HIV-induced immunodeficiency might occur causing a low level of virus hidden in sanctuaries such as lymph nodes to replicate and generate viral levels detectable in circulation. The fact that the ratio of antibody positive versus total number of infected individuals is higher in HIV-infected (0.39) than in non-infected donors (0.27) does not suggest that either of the last mechanisms might play a role. However, the comparison between the data collected from blood donors who are mostly males and pregnant women provided some indirect support to the last hypothesis. In this last group, the prevalence of GBV-C viremia was similarly and significantly higher than in HIV non-infected blood donors, irrespective of HIV-1 status (Table 1). The difference was not attributable to age since this parameter did not significantly affect GBV-C RNA prevalence (Table 2). In addition, the ratio between anti-E2 positive and total infected was similar in HIV-infected donors or pregnant women and in HIV-non-infected pregnant women. The latter group is known to be immunomodulated or to carry a moderate degree of immunodeficiency related to this condition compatible with the reactivation hypothesis [31–35]. In addition, the apparently higher prevalence of GBV-C RNA in females (Table 2) might be more an effect of pregnancy rather than gender. When pregnant women were taken out of the gender comparison, the difference was no longer significant. The high prevalence of GBV-C viremia in the non-HIV-infected pregnant women group is suggestive of reactivation, particularly since samples were collected immediately before delivery [23]. It also suggests that, in pregnant women, being HIV infected does not significantly add to GBV-C prevalence, pregnancy being the primary circumstance responsible for the higher prevalence of GBV-C RNA. The relatively, but not significantly, lower median GBV-C load in HIV-1-infected pregnant women (Fig. 1) may be taken as reflecting an interaction of HIV on GBV-C replication, possibly through lower CD4+ cell count (positive correlation between GBV-C RNA load and CD4+ cell count; \( P = 0.03 \)). A similar effect might be reflected in the lower GBV-C load observed in AIDS patients (Fig. 1) whose CD4+ cell count is consistently below 200/μl [36]. This data is therefore suggestive of some degree of interference of HIV infection with GBV-C replication, possibly through the availability of CD4+ target cells.

According to our working hypothesis, should GBV-C replication have a significant impact on the progression of HIV disease and/or HIV-1 replication, the HIV-1 RNA load in individuals replicating GBV-C was expected significantly reduced. As shown in Fig. 2, none of the populations of HIV-infected individuals, symptomatic or not, showed a difference in median and distribution of HIV-1 RNA load when stratified according to the
presence of GBV-C viremia. However, when all available samples replicating both HIV-1 and GBV-C or only HIV-1 were pooled, a significant shift towards lower HIV-1 RNA load was found in GBV-C replicating individuals ($P = 0.006$). This observation is moderately confirmed by the trend towards an inverse correlation between GBV-C and HIV-1 viral load (Fig. 3). The three HIV-1-infected populations studied covering the whole spectrum of clinical HIV infection were unlikely to be biased. This data is compatible with an impact of GBV-C replication on HIV-1 RNA load but the difference does not seem striking enough to explain a major clinical impact. As mentioned above, the clarity of the viral load approach to assess the interaction between the two viruses might be obscured by the multiplicity of factors that might influence the replication of GBV-C and therefore minimize the differences in the resultant viral load. It is likely that, as previously indicated, the number of available CD4+ cell targets progressively reduced by the HIV-1 infection tends to reduce GBV-C RNA load. At the same time, the progressively more severe immunodeficiency created over time tends to limit the ability of the immune system to control viral replication therefore increasing the GBV-C load. It is difficult to evaluate with any precision the respective weight of these conflicting factors.

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