GB Virus C/Hepatitis G Virus Infection: A Favorable Prognostic Factor in Human Immunodeficiency Virus–Infected Patients?


To investigate a possible influence of GB virus C (GBV-C) in immunocompromised patients, the prevalences of GBV-C RNA and anti–E2 antibody in 197 human immunodeficiency virus (HIV)–infected patients and in 120 control blood donors were studied. GBV-C RNA was detected in 33 of 197 HIV-infected patients (16.8%) compared with 1 in 120 blood donors (0.8%) (P < .001). Previous exposure to GBV-C (anti–E2 antibody–positive) was shown in 56.8% of HIV patients and in 9% of blood donors. GBV-C viremia was not associated with hepatitis. Despite approximately equal duration of HIV infection in all subgroups, the CD4 cell counts were significantly higher in GBV-C–viremic patients (344 cells/µL) compared with exposed (259 cells/µL) and unexposed (170 cells/µL) patients (P = .017 and P < .001). Furthermore, Kaplan-Meier analysis demonstrated significantly better cumulative survival in GBV-C RNA–positive HIV-infected patients, suggesting that GBV-C might be a favorable prognostic factor in HIV disease.

Coinfection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is a common complication in human immunodeficiency virus (HIV) infection, having implications on the outcome of affected patients [1, 2]. Recently, 2 new virus isolates related to the flaviviridae family, GB virus C (GBV-C) and hepatitis G virus (HGV), were identified in human sera, some from patients with cryptogenic or posttransfusion hepatitis [3, 4]. GBV-C and HGV are closely related isolates of the same virus, with >95% sequence homology [5]. Although the new virus was detected in patients with various forms of liver disease, its wide distribution among healthy persons made its clinical significance as a hepatitis agent uncertain [4, 6–8]. However, no data are available, to our knowledge, concerning the implication of GBV-C in HIV infection. Therefore, we retrospectively studied the seroprevalences of GBV-C RNA and anti–E2 antibodies in HIV-infected patients and correlated the results with the clinical follow-up data of the patients. The anti–E2 antibody is directed against the envelope 2 protein of GBV-C and was recently demonstrated to indicate exposure to the virus. Its appearance is mostly associated with viral clearance (GBV-C RNA–negative by reverse transcriptase–polymerase chain reaction [RT-PCR]) [9, 10].

Patients and Methods

We enrolled 197 HIV-infected patients who regularly attended our outpatient clinic between January 1993 and December 1994.

The stage of disease was classified according to the revised Centers for Disease Control and Prevention (CDC) staging system with respect to the European modification of this classification. One hundred twenty randomly selected blood donors from our blood bank served as controls.

Seroologic studies for the detection of HIV, HBV, and HCV. HIV antibodies were detected using an ELISA (Abbott Laboratories, Abbott Park, IL). Positive ELISA results were confirmed by Western blot. All participants were tested for anti–HCV antibodies (Abbott 2nd generation test), and positive sera were also tested for HCV-RNA by nested PCR of the 5′ untranslated region. Virus markers for HBV infection were evidence of hepatitis B surface antigen and hepatitis B core antibodies (Abbott Laboratories).

CD4 lymphocytes were measured by fluorescence-activated cell analysis (FACScan; Becton Dickinson, Heidelberg, Germany).

Detection of GBV-C RNA. RNA extracted from the equivalent of 5 µL of serum was amplified in a single-tube RT-PCR as previously described [11]. Briefly, cDNA synthesis was performed at 60°C for 30 min using rTth polymerase (Roche Molecular Systems, Branchburg, NJ) and a gene-specific antisense primer, followed by 35 cycles of amplification in a two-temperature PCR protocol (94°C for 40 s, 63°C for 60 s). PCR products were hybridized at 15°C for 10 min to an internal GBV-C–specific oligonucleotide probe. Sequences of sense and antisense primers and the hybridization probe were deduced from the 5′ untranslated region of GBV-C [11]. PCR amplicon-probe complexes were specifically detected by a microparticle capture EIA (Abbott Laboratories). Two dilutions of a GBV-C–positive serum served as a positive control, and a GBV-C–negative serum served as a negative control. The specificity of the method was demonstrated by a reference PCR and dot-blot hybridization using different primers and a different hybridization probe [11]. The detection limit of the assay was 100 molecules of an in vitro transcript per reaction.

EIA for the detection of anti–E2 antibodies. All sera were investigated by an EIA (Abbott Laboratories) for the presence of the anti–E2 antibody, which is directed against the E2 part of the presumed envelope protein of GBV-C [10].
Statistical analysis. Categorical variables were analyzed by the \( \chi^2 \) test. Group means were compared by the Student’s \( t \) test or the Mann-Whitney \( U \) test, if appropriate. Cumulative patient survival was assessed by Kaplan-Meier analysis. Equality of survival distribution was calculated by log-rank test. \( P < .05 \) was considered statistically significant. The Cox proportional-hazard regression model was performed to calculate relative risks in a multivariable model including categories for sex, age (<36 years or \( \geq 36 \) years), detection of GBV-C RNA (positive or negative), anti-E2 antibody status (positive or negative), HBV status (hepatitis B surface antigen-positive or -negative), and HCV status (anti-HCV antibody-positive or -negative) [12].

Results

Prevalences of hepatitis B and C and GBV-C. Among 197 HIV-infected patients, 20 (10.2%) were hepatitis B carriers (HBsAg-positive), and 51 (25.9%) were HCV antibody-positive. HCV RNA was detected in 39 of these 51 HCV antibody-positive patients. Three patients (1.5%) were coinfected with HBV and HCV. GBV-C RNA was detectable in 33 of 197 HIV-infected patients (16.8%) and in 1 of 120 reference group blood donors (0.8%; \( P < .001 \)). Analysis of all sera for the presence of the anti-E2 antibody (by EIA) revealed a seroprevalence of 56.8% (112/197). In comparison, only 11 of 120 (9%) blood donors were positive for the anti-E2 antibody (\( P < .001 \)). None of the antibody-positive patients had detectable GBV-C RNA levels. Only 52 of 197 HIV patients (26.4%) had no markers of GBV-C infection. With respect to GBV-C status, we differentiated viremic (GBV-C RNA-positive), exposed (anti-E2 antibody-positive/GBV-C RNA-negative), and unexposed (anti-E2 antibody-negative/GBV-C RNA-negative) HIV-infected patients.

Transmission risk factors for an infection with GBV-C were deduced from the viral prevalence in the HIV risk groups. Intravenous drug use (51.5%) and homosexuality (39.4%) were the main risk factors in our patients (table 1).

Implication of GBV-C viremia and anti-E2 antibody prevalence. No significant differences were found in means of aminotransferases (aspartate aminotransferase, alanine aminotransferase), albumin, or cholinesterase levels between virus-positive, exposed, and unexposed patients. On average, viremic patients were younger than exposed patients (33.9 ± 8.8 years vs. 40 ± 10.3 years; \( P = .006 \)), but they did not significantly differ from unexposed patients. However, GBV-C RNA-positive patients had significantly higher CD4 cell counts than patients without any exposure to GBV-C (344 cells/µL ± 189 vs. 170 ± 211; \( P < .001 \)) or to anti-E2 antibody-positive patients who had already cleared the virus (259 cells/µL ± 270; \( P = .017 \)). In analyzing subgroups in those GBV-C RNA-positive patients without HBV or HCV coinfection, higher CD4 cell counts were also evident compared with GBV-C RNA-negative patients of the same subpopulation.

Of 197 HIV-infected patients, 38 (19.3%) were classified as CDC stage I, 81 (41.1%) as CDC stage II, and 78 (39.6%) as CDC stage III. CDC stage analysis of the 33 GBV-C RNA-positive patients revealed the following distribution: 13 CDC stage I (39.4%), 19 CDC stage II (57.6%), and 1 CDC stage III (3%). In comparison, 40.1% of the exposed patients and 61.6% of the patients without exposure to GBV-C were diagnosed as CDC stage III. Significantly fewer patients had progressed to CDC stage III in the GBV-C RNA-positive group (\( P < .001 \)).

Total RNA of peripheral blood mononuclear cells (PBMC) from 3 viremic patients in the CDC stage I subgroup was investigated by strand-specific reverse transcription followed by RNase H digestion and nested PCR of the 5' untranslated region. Only positive- (not negative-) strand GBV-C RNA was detected in all 3 samples.

Survival analysis. For this analysis, we calculated the period from the first positive HIV test, available for 179 of 197 patients, to a cutoff date or death, because the exact date of HIV seroconversion was not known. Follow-up data for 28 GBV-C RNA-positive patients, 104 anti-E2 antibody-positive patients, and 47 patients without exposure to GBV-C revealed that HIV infection was of approximately equal duration in all groups before serum for the GBV-C test was taken (1530 ± 1403 days, 1490 ± 1224 days, and 1302 ± 1132 days, respectively; difference not significant). Mean survival time in the GBV-C RNA-positive group of HIV patients was 4590 ±

### Table 1. Prevalence of GBV-C RNA and anti-E2 antibody in HIV-infected patients in relation to risk factors for virus transmission.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>All HIV-infected patients</th>
<th>GBV-C RNA-positive patients</th>
<th>Anti-E2 antibody-positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homosexual promiscuity</td>
<td>104 (52.8)</td>
<td>13 (39.4)</td>
<td>59 (52.7)</td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>60 (30.5)</td>
<td>17 (51.5)</td>
<td>31 (27.7)</td>
</tr>
<tr>
<td>Heterosexual promiscuity</td>
<td>26 (13.2)</td>
<td>1 (3.0)</td>
<td>18 (16.1)</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>7 (3.5)</td>
<td>2 (6.1)</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td>Total</td>
<td>197 (100)</td>
<td>33 (100)</td>
<td>112 (100)</td>
</tr>
</tbody>
</table>
Figure 1. Kaplan-Meier survival analysis of HIV-infected patients relative to GBV-C status: GBV-C viremia (GBV-C RNA-positive), exposure to virus (anti-E2 antibody-positive, GBV-C RNA-negative), and no evidence of GBV-C infection (anti-E2 antibody-negative, GBV-C RNA-negative). Nos. of surviving patients were as follows: 25 (89%) of 28 in the GBV-C-viremic patients, 71 (68%) of 104 in exposed patients, and 24 (51%) of 47 in unexposed patients. Survival time was calculated from date of first positive HIV test (available for 179 patients). HIV infection was of equal duration in all groups before serum for GBV-C serology was taken. Survival difference is statistically significant; \( P < .001 \) (log-rank test).

279 days (95% confidence interval [CI], 4043–5137) compared with 3239 ± 143 days (95% CI, 2959–3519) in the anti-E2 antibody-positive group (\( P = .031 \), log-rank test) and with 2591 ± 217 days (95% CI, 2165–3017) in the unexposed patients (\( P < .001 \), log-rank test). The Kaplan-Meier survival analysis is illustrated in figure 1.

In the absence of detailed information about acquisition of GBV-C infection, we additionally analyzed the mean survival beginning from the date when serum was taken for analysis of the GBV-C status. For these calculations, all 197 HIV-positive patients could be considered. Twenty-nine (87.9%) of 33 GBV-C RNA-positive patients had survived beyond the cutoff date compared with 77 (68.8%) of 112 anti-E2 antibody-positive patients and with 29 (55.8%) of 52 patients without exposure to GBV-C. Mean survival of the GBV-C RNA-positive patients was 1052 ± 42 days (95% CI, 970–1134) versus 930 ± 42 days (95% CI, 847–1013) in the antibody-positive group (\( P = .041 \), log-rank test) and versus 777 ± 67 days (95% CI, 646–909) in the unexposed group of patients (\( P = .002 \), log-rank test).

A Cox proportional-hazard regression model indicated that GBV-C RNA (hazard ratio, 0.22; \( P = .007 \)) is an independent factor associated with a relative benefit on survival more distinctly than the presence of the anti-E2 antibody (hazard ratio, 0.52; \( P = .017 \)). Age of >36 years as well as HBV infection were associated with relative risks of death (hazard ratio, 2.68; \( P = .006 \) and hazard ratio, 2.26; \( P = .029 \), respectively). Gender and HCV antibody status had no prognostic value for the patients studied.

Discussion

We found a significantly increased GBV-C RNA prevalence in the HIV-infected patients compared with the control group blood donors (16.8% vs. 0.8%). The prevalence in our control group corresponds to that of volunteer blood donors reported by others \([4, 7, 8]\).

Major transmission risk factors for infection with GBV-C were intravenous drug use and homosexuality. In view of the report by Persico et al. \([13]\), who demonstrated the presence of GBV-C RNA in cell-free seminal plasma, the high prevalence of GBV-C RNA in homosexuals in our study is understandable.

In some studies, HBV and HCV infection were demonstrated to worsen the clinical course and the outcome of HIV-infected patients \([1, 2]\). We found no hepatitis (as measured by increased transaminases) associated with GBV-C infection in the immunocompromised patients. Mean levels of transaminases, slightly above the upper normal range, did not differ between patients with CDC stage I, II, or III of HIV infection, indicating the same degree of liver damage in all three CDC stages.

Of interest, GBV-C-viremic patients had distinctly higher CD4 cell counts and progressed less frequently to CDC stage III compared with exposed and unexposed patients. However, HIV infections were of approximately equal duration in all subgroups up to the time when samples were taken for GBV-C serology. Kaplan-Meier analysis indicated significantly better cumulative survival after the first positive HIV test in the GBV-C-viremic patients (figure 1). Although GBV-C RNA-positive patients were significantly younger than antibody-positive patients, they were about the same age as the unexposed patients. A multivariable Cox regression analysis confirmed that GBV-C viremia was associated with a relative benefit on survival in the HIV-infected patients independent of other variables such as age. However, the number of GBV-C RNA-positive HIV-infected patients in our study (\( n = 33 \)) is relatively small, and these results need confirmation in larger studies. Analyses of sequential samples might help to elucidate whether GBV-C and HIV are simultaneously acquired by...
shared transmission risk factors or whether one of the viruses is contracted first.

An important question for the future is whether CD4 cells are a replication site of GBV-C because decreased CD4 cell counts could explain the significantly fewer GBV-C–positive patients in CDC stage III of HIV infection. We attempted to detect viral negative-strand RNA as replication intermediate in lymphocytes of GBV-C–positive patients. However, in agreement with data from others [14], we could detect genomic but not antigenomic viral RNA in 3 PBMC samples of viremic HIV-infected patients from the CDC stage I subgroup. Further studies are necessary to investigate a possible tropism of GBV-C in PBMC subsets in detail.

Recently, relative resistance of CD4 lymphocytes to HIV infection was described in persons with high-risk sexual exposure [15]. The high prevalence of GBV-C in the homosexual risk group of our study together with the frequent detection of GBV-C in seminal plasma [13] are interesting observations in this context.

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References