

ORIGINAL ARTICLE

## Persistent GB Virus C Infection and Survival in HIV-Infected Men

Carolyn F. Williams, Ph.D., Donna Klinzman, B.A., Traci E. Yamashita, M.S.,  
Jinhua Xiang, M.D., Philip M. Polgreen, M.D., Charles Rinaldo, Ph.D.,  
Chenglong Liu, Ph.D., John Phair, M.D., Joseph B. Margolick, M.D., Ph.D.,  
Dietmar Zdunek, Ph.D., Georg Hess, M.D., and Jack T. Stapleton, M.D.

### ABSTRACT

#### BACKGROUND

GB virus C (GBV-C), which is not known to be pathogenic in humans, replicates in lymphocytes, inhibits the replication of human immunodeficiency virus (HIV) *in vitro*, and has been associated with a decreased risk of death among HIV-positive persons in some, but not all, studies. Previous studies did not control for differences in the duration of HIV or GBV-C infection.

#### METHODS

We evaluated 271 men who were participants in the Multicenter Acquired Immunodeficiency Syndrome Cohort Study for GBV-C viremia (by means of a reverse-transcriptase–polymerase-chain-reaction assay) or E2 antibody (by means of an enzyme-linked immunosorbent assay) 12 to 18 months after seroconversion to positivity for HIV (the early visit); a subgroup of 138 patients was also evaluated 5 to 6 years after HIV seroconversion (the late visit).

#### RESULTS

GBV-C infection was detected in 85 percent of men with HIV seroconversion on the basis of the presence of E2 antibody (46 percent) or GBV-C RNA (39 percent). Only one man acquired GBV-C viremia between the early and the late visit, but 9 percent of men had clearance of GBV-C RNA between these visits. GBV-C status 12 to 18 months after HIV seroconversion was not significantly associated with survival; however, men without GBV-C RNA 5 to 6 years after HIV seroconversion were 2.78 times as likely to die as men with persistent GBV-C viremia (95 percent confidence interval, 1.34 to 5.76;  $P=0.006$ ). The poorest prognosis was associated with the loss of GBV-C RNA (relative hazard for death as compared with men with persistent GBV-C RNA, 5.87;  $P=0.003$ ).

#### CONCLUSIONS

GBV-C viremia was significantly associated with prolonged survival among HIV-positive men 5 to 6 years after HIV seroconversion, but not at 12 to 18 months, and the loss of GBV-C RNA by 5 to 6 years after HIV seroconversion was associated with the poorest prognosis. Understanding the mechanisms of interaction between GBV-C and HIV may provide insight into the progression of HIV disease.

From the Epidemiology Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Md. (C.F.W.); Iowa City Veterans Affairs Medical Center and University of Iowa, Iowa City (D.K., J.X., P.M.P., J.T.S.); Bloomberg School of Public Health, Johns Hopkins University, Baltimore (T.E.Y., J.B.M.); the University of Pittsburgh Graduate School of Public Health, Pittsburgh (C.R.); the University of California at Los Angeles, Los Angeles (C.L.); Northwestern University, Howard Brown Health Center, Chicago (J.P.); Roche Diagnostics, Penzberg, Germany (D.Z.); and Roche Diagnostics, Mannheim, Germany (G.H.). Address reprint requests to Dr. Stapleton at SW 34-P, GH, UIHC, Iowa City, IA 52242, or at jack-stapleton@uiowa.edu.

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**H**OST AND VIRAL FACTORS INFLUENCE the progression of human immunodeficiency virus (HIV) disease.<sup>1,2</sup> In cross-sectional studies, HIV-infected people with GB virus C (GBV-C) viremia had lower mortality rates, higher base-line CD4+ T-cell counts, a slower rate of decline in the number of CD4+ T cells, and in some studies, lower plasma HIV RNA levels than HIV-positive people who did not have GBV-C viremia.<sup>3-8</sup>

GBV-C is a flavivirus that is closely related to hepatitis C virus.<sup>9</sup> Although first detected in persons with non-A, non-B hepatitis, GBV-C has not been associated with hepatitis or any other disease in humans.<sup>10</sup> Parenteral, sexual, and vertical transmission of GBV-C have all been documented, and infection is common in many populations.<sup>7,11-17</sup> Viremia may persist for years; however, 60 to 75 percent of immunocompetent persons have spontaneous clearance of GBV-C, and this event usually coincides with the development of antibody against the GBV-C surface envelope glycoprotein E2.<sup>18-20</sup>

GBV-C replicates in CD4+ T cells,<sup>21</sup> and in vitro infection of lymphocytes with GBV-C before infection with HIV reduces the replication of HIV, suggesting a direct inhibitory effect of GBV-C on HIV.<sup>7</sup> Although six studies of HIV-positive people found a survival benefit of coinfection with GBV-C,<sup>3-8</sup> three studies did not find this effect,<sup>22-24</sup> and the nature of the relation between GBV-C and the progression of HIV disease is controversial.<sup>25</sup> To clarify the relation between GBV-C infection and the progression of HIV disease and to resolve the discrepancies in previous studies, we analyzed GBV-C status in participants in the Multicenter Acquired Immunodeficiency Syndrome (AIDS) Cohort Study whose dates of seroconversion to positivity for HIV were known.

## METHODS

### STUDY POPULATION AND DESIGN

The study population was selected from the participants in the Multicenter AIDS Cohort Study, which is ongoing in Baltimore, Chicago, Pittsburgh, and Los Angeles and consists of 5622 men who have sex with men and who were enrolled between 1984 and 1990.<sup>26-28</sup> At six-month intervals, HIV-related clinical status is assessed, an interviewer-administered questionnaire is completed, and blood is obtained for analysis, including tests for HIV seropositivity and measurement of HIV RNA levels and CD4

T-cell counts, and serum is stored as previously described.<sup>26-29</sup> The presence of antibody against hepatitis C virus and hepatitis B surface antigen was determined by immunoassay (Abbott Laboratories). Written informed consent was obtained from all participants, and this substudy was approved by the institutional review board of the University of Iowa.

To investigate the putative protective effect of GBV-C status among HIV-seropositive subjects, serum samples were tested for GBV-C RNA and E2 antibody 12 to 18 months after HIV seroconversion (the early visit) in order to determine the prevalence of GBV-C infection near the time of HIV seroconversion. Because two studies of HIV-positive people with normal CD4+ T-cell counts did not find a survival benefit associated with GBV-C infection,<sup>23,24</sup> we also studied serum samples obtained five to six years after HIV seroconversion (the late visit). To be included in this study, the date of HIV seroconversion (estimated as the midpoint between the last visit at which a subject was seronegative and the first visit at which he was seropositive) had to be known within a window of one year, and data on markers of HIV disease progression (the CD4+ T-cell count and plasma HIV RNA levels) had to be available from at least two visits after seroconversion. To avoid confounding owing to the use of highly active antiretroviral therapy, analyses included only data collected before January 1, 1996.<sup>30</sup>

### DETECTION OF GBV-C

All serum samples were assigned coded identifiers to which laboratory personnel were blinded. RNA was extracted from serum, a GBV-C-specific nested reverse-transcriptase-polymerase-chain-reaction assay was performed, and products were identified as previously described.<sup>21,31,32</sup> Positive samples were confirmed by the analysis of a second aliquot of serum obtained on the same day from the same subject. Samples with discordant results were tested a third time, and those with two positive results were defined as positive. The results for samples obtained 12 to 18 months after HIV seroconversion in 2 of 109 men were discordant (both were defined as negative), whereas none of the 50 men with positive samples 5 to 6 years after seroconversion had discordant results. For quality control, duplicate samples from 15 men were analyzed, and the same result was obtained for 14 of these duplicate samples. Antibodies against GBV-C envelope glycoprotein E2 were detected by means of the  $\mu$ Plate anti-HGenv test (Roche Diagnostics).<sup>19</sup>

**STATISTICAL ANALYSIS**

Men were defined as having viremia if GBV-C RNA was detected. Prior GBV-C infection was defined by the presence of antibody against GBV-C E2 protein in the absence of viral RNA. We used univariate descriptive statistics to investigate differences between men who were included in the analysis of samples obtained five to six years after seroconversion and those who were excluded. We compared known predictors of the risk of HIV disease progression among men with persistent GBV-C viremia, those with clearance of GBV-C, and those who were negative for GBV-C (stratified according to E2-antibody status) at both visits. We compared differences in survival rates with the use of Kaplan–Meier curves and univariate or multivariate Cox proportional-hazards models using the S-Plus SURVFIT function (MathSoft) and the SAS PHREG procedure (SAS Institute), respectively. All comparisons were two-sided, with a P value of less than 0.05 used to indicate statistical significance.

**RESULTS**

A total of 584 men in the Multicenter AIDS Cohort Study had documented HIV seroconversion. Of these, 271 men met the inclusion criteria for our study, and a subgroup of 138 men also had samples available that had been obtained five to six years after HIV seroconversion for the longitudinal analysis of GBV-C infection. The selection process is illustrated in Figure 1.

Active GBV-C infection (defined by the presence of GBV-C RNA without E2 antibody) was detected in 107 of the 271 men (39 percent), and past GBV-C infection (defined by the presence of E2 antibody without GBV-C RNA) was detected in 124 (46 percent) (Table 1). Two of the RNA-positive men were also positive for E2 antibody. Thus, a total of 231 men (85 percent) had evidence of current or previous infection with GBV-C.

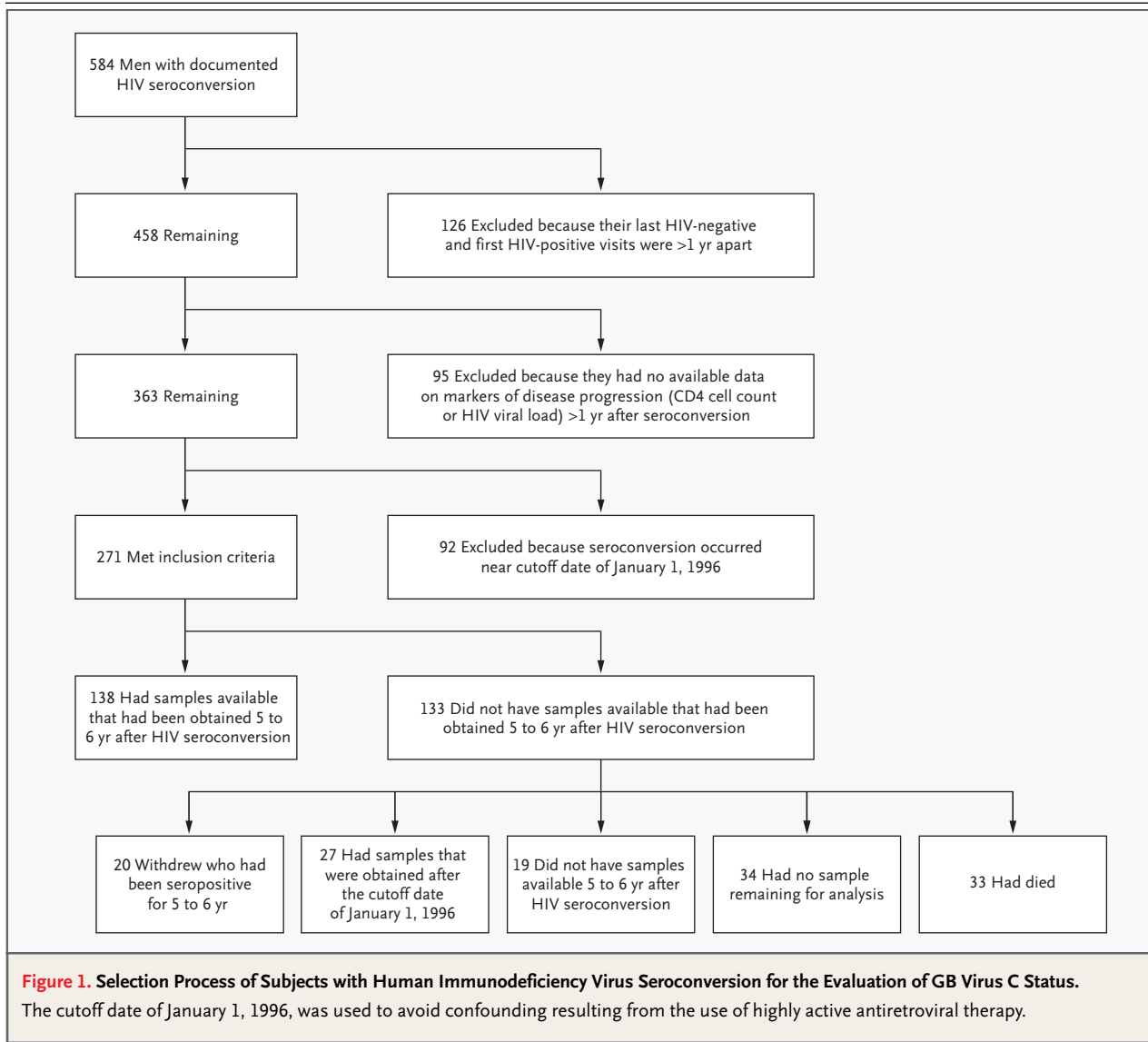
Survival rates after the early visit did not differ significantly between the men with GBV-C viremia and those without GBV-C viremia, whether or not they had evidence of previous GBV-C infection at the early visit (Fig. 2A). Similarly, when the analysis was restricted to the men evaluated both 12 to 18 months and 5 to 6 years after HIV seroconversion, rates of survival after the late visit did not differ significantly according to the GBV-C status at the early visit (Fig. 2B).

In contrast, GBV-C status five to six years after

HIV seroconversion was strongly associated with subsequent survival (Fig. 3A). Men with GBV-C viremia survived significantly longer than those without GBV-C viremia five to six years after HIV seroconversion, regardless of whether or not the latter group had E2 antibody (Fig. 3A). The risk of death among those who were negative for GBV-C RNA five to six years after HIV seroconversion (with those who were positive for E2 antibody combined with those who were negative) was 2.78 times that among men with GBV-C viremia (95 percent confidence interval, 1.34 to 5.76;  $P=0.006$ ).

To examine the effect of longitudinal changes in GBV-C status on survival, we classified the 137 men with data for both visits into four mutually exclusive categories: men with persistent GBV-C viremia; men with a prior GBV-C infection, as indicated by the absence of GBV-C viremia at both visits and the presence of E2 antibody at one or both visits; men who did not have GBV-C viremia or E2 antibody at either visit; and men who had GBV-C viremia at the early but not the late visit (i.e., those in whom GBV-C had cleared). One man acquired GBV-C RNA between the early and late visits and was excluded from the analysis. The majority of men who were negative for GBV-C RNA and positive for E2 antibody were positive for E2 antibody at both visits; however, three men lost E2 antibody between the early and late visits. The number of years since HIV seroconversion and the frequency of antiretroviral therapy did not vary significantly among the groups at either visit (Tables 1 and 2). The survival rate among those who had GBV-C viremia at the late visit was higher than that among the men in the other three groups (Fig. 3B).

Because we knew the duration of HIV infection, we did not need to control the primary survival analyses for the CD4+ T-cell count or the HIV RNA level. However, we performed multivariate analyses to determine how much of the association between GBV-C status and survival was mediated by these two variables. When the CD4+ T-cell count and HIV RNA values from the early visit were included in the analysis, the relative risks of death, as compared with the group that was positive for GBV-C RNA at both visits, were 2.57 in the group that was consistently negative for GBV-C RNA but positive for E2 antibody at either visit (95 percent confidence interval, 1.18 to 5.60;  $P=0.02$ ), 2.10 in the group that was negative for GBV-C RNA and E2 antibody at both visits (95 percent confidence interval, 0.74 to 5.97;  $P=0.16$ ), and 5.95 in the group



with clearance of GBV-C (95 percent confidence interval, 2.25 to 15.74;  $P < 0.001$ ). The effect of GBV-C status on survival was not independent of the CD4+ T-cell count and HIV RNA levels at the late visit, suggesting that GBV-C infection was associated with changes in these two variables.

To address this possibility, we examined several markers of HIV disease progression. The CD4+ T-cell count and HIV RNA levels did not differ significantly among the groups at the early visit, but did differ significantly at the late visit (Table 2). Men who were persistently positive for GBV-C RNA had a significantly slower mean rate of decline in the CD4+ T-cell count ( $-26$  cells per cubic millimeter

per year) than men with E2 antibody who were persistently negative for GBV-C RNA ( $-70$  cells per cubic millimeter per year,  $P = 0.002$ ) (Table 2) or men with clearance of GBV-C ( $-107$  cells per cubic millimeter per year,  $P < 0.001$ ); men who were persistently negative for GBV-C RNA and E2 antibody also had a greater decline in the CD4 cell count between visits, although this difference was not significant ( $-60$  cells per cubic millimeter per year,  $P = 0.09$ ) (Table 2). Early plasma HIV RNA values did not differ significantly among the groups, but the viral load increased less in the group with persistent viremia than in the other three groups.

Among the men without GBV-C RNA, the sur-

**Table 1. Characteristics of the Men with Human Immunodeficiency Virus (HIV) Seroconversion, According to Their GB Virus C (GBV-C) RNA Status.\***

Characteristic	Study Group (N=271)		Men Evaluated 5 to 6 Yr after HIV Seroconversion (N=138)		Men Not Evaluated 5 to 6 Yr after HIV Seroconversion (N=133)	
	GBV-C RNA-Positive (N=107)	GBV-C RNA-Negative (N=164)	GBV-C RNA-Positive (N=61)	GBV-C RNA-Negative (N=77)	GBV-C RNA-Positive (N=46)	GBV-C RNA-Negative (N=87)
Median year of seroconversion	1985.85	1986.13	1985.97	1985.99	1985.57	1986.39
Median time from HIV seroconversion to early visit — yr	1.25	1.27	1.25	1.27	1.24	1.27
Median age at seroconversion — yr	33.4	35.7	33.4	34.4	33.5	36.0
Positive for GBV-C glycoprotein E2 antibody at early visit — no. (%)	2 (2)	124 (76)	0	55 (71)	2 (4)	69 (79)
Median CD4+ T-cell count at early visit Cells/mm <sup>3</sup> No. evaluated	565 106	561 164	636 61	606 77	490 45	527 87
Median plasma HIV RNA level at early visit — log copies/ml	4.43	4.49	4.40	4.39	4.50	4.59
Antiretroviral therapy at early visit						
Monotherapy — %	4	6	2	4	7	8
Combination therapy — %	0	0	0	0	0	0
No. evaluated	106	163	61	77	45	86
Median follow-up after early visit — yr†	6.5	5.6	7.5	7.2	4.4	3.8
Death during follow-up — no. (%)	44 (41)	74 (45)	17 (28)	29 (38)	27 (59)	45 (52)
Rate of death/100 person-yr from early visit to study exit	6.44	7.84	3.69	5.20	12.14	11.67

\* The GBV-C RNA test results were from the sample obtained at the early visit, 12 to 18 months after HIV seroconversion.

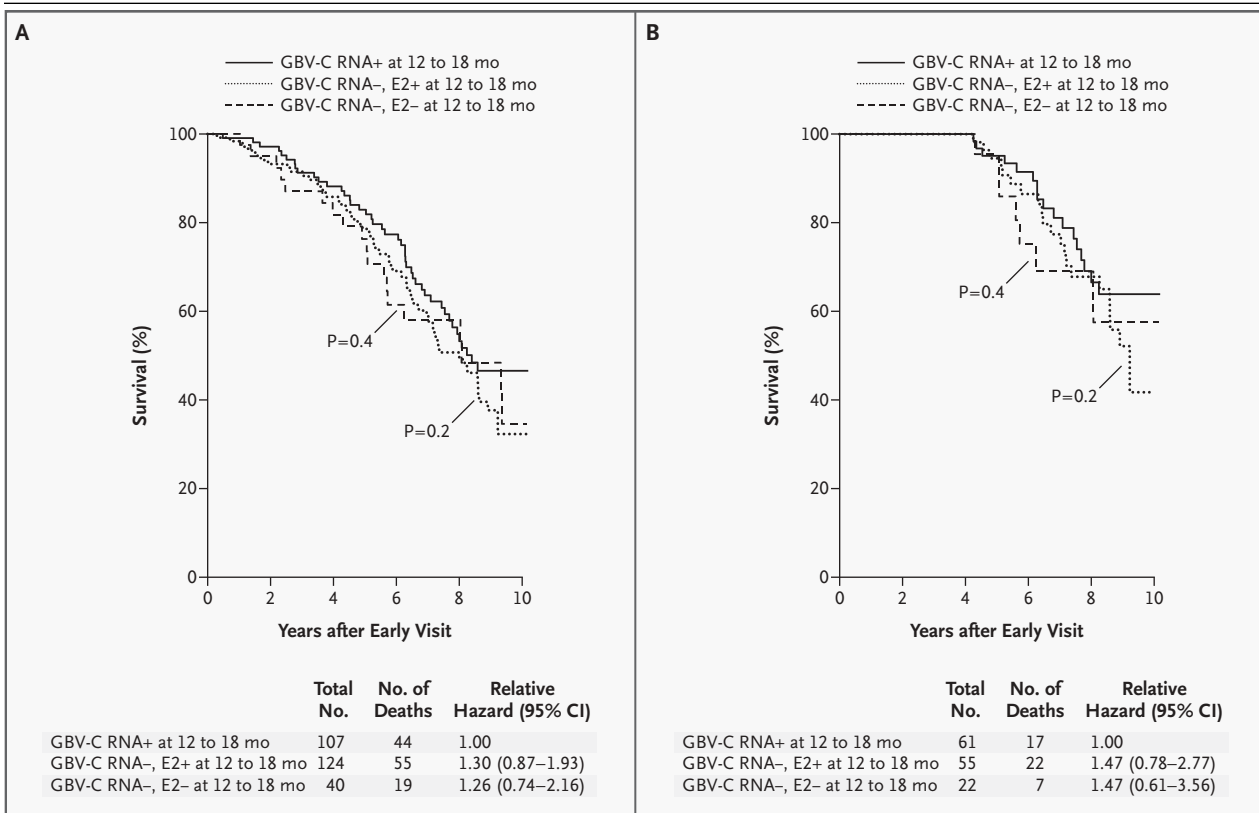
† Analyses were stopped January 1, 1996, to avoid confounding resulting from the use of highly active antiretroviral therapy.

vival rate was higher among those who were positive for E2 antibody than among those who were negative, although this difference was not significant and waned nine years after HIV seroconversion (i.e., three to four years after the late visit) (Fig. 3). E2 antibody developed in only 3 of the 12 men with clearance of GBV-C RNA between the early and late visits. The development of E2 antibody may have been predictive of a higher likelihood of survival in these men, since seven of the nine men in whom E2 antibodies did not develop died, as compared with only one of the three men in whom E2 antibody did develop; however, this comparison was not statistically significant.

We assessed the robustness of our findings in several ways. Inherent in the study design was the fact that the subgroup with data for both visits survived longer than the subgroup that did not meet this criterion. CD4+ T-cell counts were higher and the mortality rate before 1996 was lower in the former subgroup than in the latter. However, the

age at HIV seroconversion, plasma HIV RNA levels one year after seroconversion, and the use of antiretroviral therapy were similar in the two groups (Table 1). The subgroups were not biased with respect to exposure to GBV-C, since the prevalence of current and past infections with GBV-C (as defined by the presence of GBV-C RNA, E2 antibody, or both) were similar in the two subgroups (Table 1). In addition, men with persistent GBV-C viremia survived significantly longer than those with evidence of prior GBV-C infection (those who were positive for E2 antibody) and those with no evidence of prior infection (those who were negative for GBV-C RNA and E2 antibody) (Fig. 3A). Thus, GBV-C viremia was associated with a beneficial effect, and the difference in survival was not solely due to a detrimental effect associated with the loss of GBV-C RNA.

In a second analysis of this point, we considered the possibility that men who had neither GBV-C RNA nor E2 antibody at the early visit might have



**Figure 2.** Kaplan–Meier Estimates of Survival According to the GB Virus C (GBV-C) RNA and E2 Antibody Status 12 to 18 Months after Human Immunodeficiency Virus (HIV) Seroconversion (the Early Visit) among All 271 Men (Panel A) and the 138 Men Evaluated at Both 12 to 18 Months and 5 to 6 Years after HIV Seroconversion (Panel B).

Survival curves shown in Panel B start at the time of the late visit, five to six years after HIV seroconversion. P values are for the comparison with the group that was positive for GBV-C RNA, which served as the reference group in the calculations of the relative hazard of death. CI denotes confidence interval.

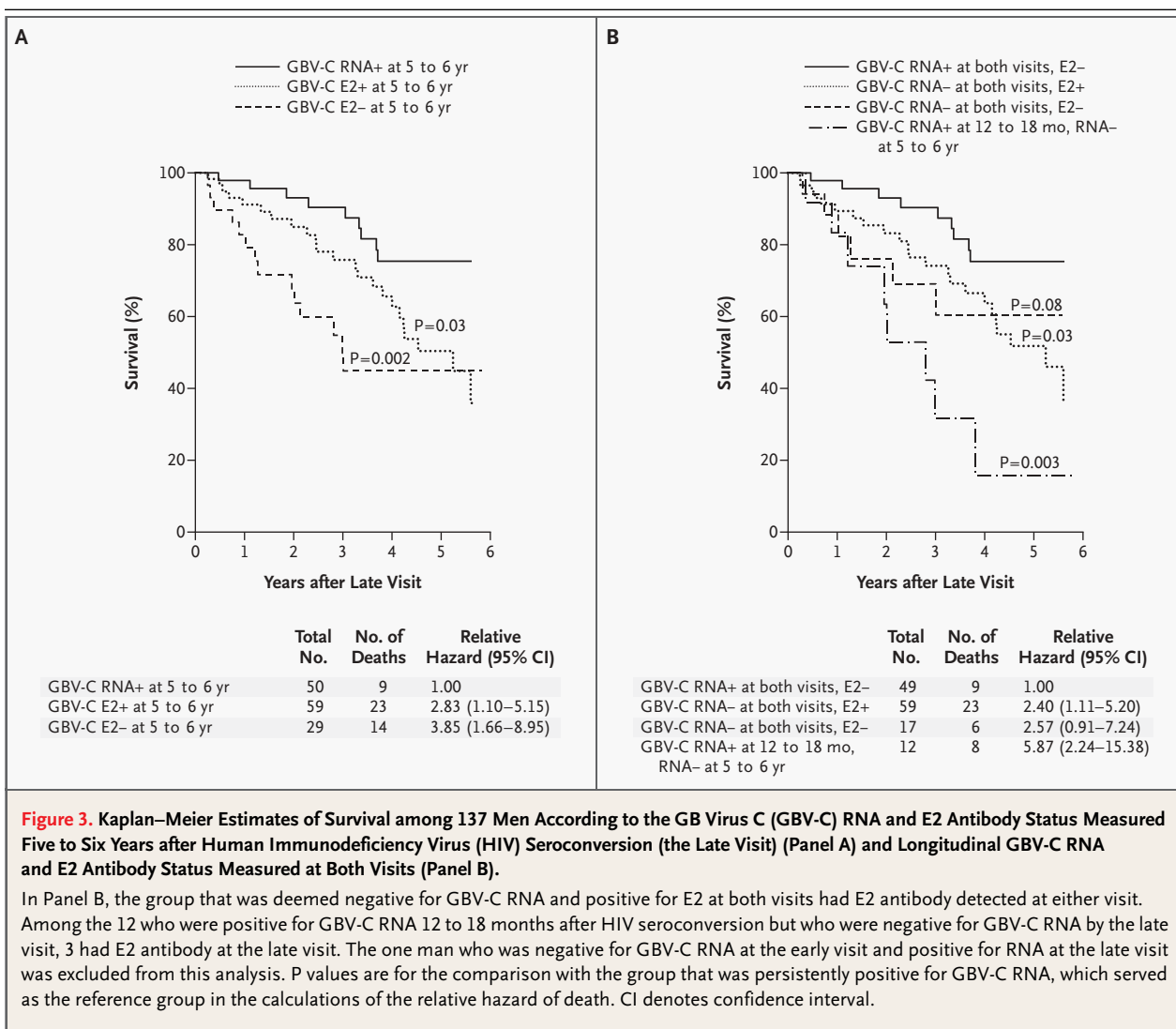
had clearance of GBV-C before the early visit without the development of GBV-C E2 antibody and thus might have been misclassified. This event might have biased our results by spuriously lowering the relative likelihood of survival among GBV-C–negative men. To assess the potential effect of a misclassification of this type, we repeated the analysis and classified the men who were negative for both GBV-C RNA and E2 antibody as positive for GBV-C RNA. This did not change the relative risk of death (data not shown).

We also explored other markers associated with the progression of HIV disease. Antibody against hepatitis C virus and hepatitis B surface antigen were infrequent in this cohort (6 percent and 4 percent, respectively) and were not associated with significant differences in mortality (data not shown). Similarly, the prevalence of heterozygosity for the

polymorphism in the gene for CC chemokine receptor 5 (*CCR5*)  $\Delta 32$ , which is associated with delayed progression of HIV disease,<sup>33</sup> was low in the study group as a whole (22 of 271) and did not differ significantly according to GBV-C status (data not shown).

#### DISCUSSION

This study provides strong evidence that GBV-C infection is associated with prolonged survival among HIV-infected people. We found that the survival advantage was large and dependent on the persistence of GBV-C viremia. Men who were positive for GBV-C RNA both 12 to 18 months and 5 to 6 years after HIV seroconversion were significantly less likely to die than men who were negative for GBV-C RNA 5 to 6 years after HIV seroconversion. Loss of



**Figure 3.** Kaplan–Meier Estimates of Survival among 137 Men According to the GB Virus C (GBV-C) RNA and E2 Antibody Status Measured Five to Six Years after Human Immunodeficiency Virus (HIV) Seroconversion (the Late Visit) (Panel A) and Longitudinal GBV-C RNA and E2 Antibody Status Measured at Both Visits (Panel B).

In Panel B, the group that was deemed negative for GBV-C RNA and positive for E2 at both visits had E2 antibody detected at either visit. Among the 12 who were positive for GBV-C RNA 12 to 18 months after HIV seroconversion but who were negative for GBV-C RNA by the late visit, 3 had E2 antibody at the late visit. The one man who was negative for GBV-C RNA at the early visit and positive for RNA at the late visit was excluded from this analysis. P values are for the comparison with the group that was persistently positive for GBV-C RNA, which served as the reference group in the calculations of the relative hazard of death. CI denotes confidence interval.

GBV-C RNA was a strong predictor of death. The survival rate 10 to 11 years after HIV seroconversion in men who were positive for GBV-C RNA at both 12 to 18 months and 5 to 6 years was 75 percent, as compared with 39 percent among those who were persistently negative for GBV-C RNA and 16 percent for those with clearance of GBV-C RNA. The survival benefit is greater than that reported for men in the Multicenter AIDS Cohort Study who are heterozygous for the *CCR5* Δ32 mutation.<sup>34</sup> Furthermore, this mutation is rare, particularly among members of minority groups in the United States and among Africans, thus reducing its attributable benefit.

Our results are strengthened by the longitudinal design of the study, which allowed us to control for

the duration of HIV infection, to test for the persistence of GBV-C infection, to take into account the presence of heterozygosity for the *CCR5* Δ32 mutation, and to analyze specimens collected before the initiation of highly active antiretroviral therapy. Previous studies of the effect of GBV-C coinfection on the progression of HIV disease that did not find a survival benefit tested only samples from patients with high CD4+ T-cell counts<sup>23,24</sup> (i.e., relatively early in their HIV infection), whereas studies that did find a survival benefit involved subjects with a broad range of CD4+ T-cell counts.<sup>3–8</sup> The GBV-C status 12 to 18 months after HIV seroconversion was not predictive of survival in our cohort, whereas the status 5 to 6 years after HIV seroconversion

**Table 2. Characteristics of the Men According to Their Status with Respect to GB Virus C (GBV-C) RNA and Glycoprotein E2 Antibody 12 to 18 Months and 5 to 6 Years after Human Immunodeficiency Virus (HIV) Seroconversion.\***

Characteristic	GBV-C RNA—Positive at Both Visits, E2 Antibody—Positive (N=49)		GBV-C RNA—Negative at Both Visits, E2 Antibody—Negative (N=59)		GBV-C RNA—Negative at Both Visits, E2 Antibody—Negative (N=17)		GBV-C RNA—Positive at 12 to 18 Mo, GBV-C RNA—Negative at 5 to 6 Yr (N=12)	
	12–18 Mo after HIV Seroconversion	5–6 Yr after HIV Seroconversion	12–18 Mo after HIV Seroconversion	5–6 Yr after HIV Seroconversion	12–18 Mo after HIV Seroconversion	5–6 Yr after HIV Seroconversion	12–18 Mo after HIV Seroconversion	5–6 Yr after HIV Seroconversion
Years after HIV seroconversion	1.3 (1.2 to 1.3)	5.6 (5.5 to 5.7)	1.3 (1.3 to 1.4)	5.6 (5.5 to 5.7)	1.4 (1.2 to 1.4)	5.6 (5.3 to 5.8)	1.3 (1.1 to 1.4)	5.5 (5.3 to 5.7)
CD4+ T-cell count — cells/mm <sup>3</sup>	641 (577 to 706)	527 (444 to 610) †‡§	685 (609 to 760)	388 (318 to 458)	593 (490 to 696)	338 (195 to 480)	744 (552 to 937)	279 (118 to 439)
No. evaluated	49	49	59	58	17	17	12	12
Annual change in CD4+ T-cell count — cells/mm <sup>3</sup> /yr ¶	-26 (-46 to -6) †§		-70 (-87 to -52)		-60 (-99 to -22)		-107 (-148 to -67)	
No. evaluated	49	49	58	58	17	17	12	12
CD8+ T-cell count — cells/mm <sup>3</sup>	792 (690 to 894)	1005 (880 to 1129)	816 (740 to 893)	964 (833 to 1095)	949 (796 to 1102)	883 (579 to 1187)	765 (508 to 1022)	766 (464 to 1078)
Mean (95% CI)	49	49	59	58	17	17	12	12
No. evaluated								
CD8+ T cells — %	40.7 (38.3 to 43.0)	50.5 (47.7 to 53.4)	40.6 (37.5 to 43.8)	53.1 (49.6 to 56.6)	44.7 (38.9 to 50.6)	53.9 (46.7 to 61.1)	37.4 (30.7 to 44.2)	56.7 (47.1 to 66.3)
Mean (95% CI)	49	49	59	58	17	16	12	12
No. evaluated								
Plasma HIV RNA — log copies/ml	4.25 (4.07 to 4.43)	4.38 (4.16 to 4.60)	4.18 (4.00 to 4.36)	4.66 (4.38 to 4.93)	4.51 (4.20 to 4.81)	4.58 (4.20 to 4.96)	4.35 (3.97 to 4.73)	4.78 (4.24 to 5.32)
Mean (95% CI)	49	31	59	39	17	14	12	10
No. evaluated								
Annual change in plasma HIV RNA — log copies/ml/yr ¶	0.02 (-0.01 to 0.05) †		0.11 (0.04 to 0.17)		0.04 (-0.06 to 0.14)		0.12 (0.01 to 0.22)	
Mean (95% CI)	31	31	39	39	14	14	10	10
No. evaluated								

\* Data for one man who was GBV-C—negative 12 to 18 months after HIV seroconversion and GBV-C—positive 5 to 6 years after HIV seroconversion were omitted. CI denotes confidence interval. P values are for pairwise comparisons.  
 † P<0.05 for the respective comparison with men who were GBV-C RNA—negative and E2 antibody—positive at both visits.  
 ‡ P<0.05 for the respective comparison with the men who were negative for both GBV-C RNA and E2 antibody at both visits.  
 § P<0.005 for the respective comparison with the men who were GBV-C RNA—positive at 12 to 18 months and GBV-C RNA—negative 5 to 6 years after HIV seroconversion.  
 ¶ The annual change in the CD4 cell count and plasma HIV RNA level describes the annual change per year for the period between the two visits.

sion was highly associated with survival, indicating that the stage of HIV infection may explain the discrepancy between the earlier studies.

Our study has also provided information on the natural history of GBV-C in HIV-positive men. First, prior or current infection with GBV-C was nearly universal in this cohort 12 to 18 months after HIV seroconversion (85 percent had viremia, E2 antibody, or both). Since E2 antibody did not always develop in men who lost GBV-C RNA, our figure probably underestimates the lifetime prevalence of GBV-C infection. Second, clearance of GBV-C was common in this cohort, occurring in 9 percent of the HIV-positive men (12 of 138) between one and six years after HIV seroconversion. E2 antibody did not develop in most of these men. The particularly poor outcome in these men may reflect the loss of a protective effect of GBV-C and suggests that losing GBV-C may also be a marker of advanced HIV infection. This study focused on men who acquired HIV by sexual transmission before effective antiretroviral therapy became available. Therefore, it may not apply to women, patients treated with antiretroviral therapy, or people infected parenterally. For instance, the rate of clearance of GBV-C may be higher among those parenterally exposed,<sup>35</sup> raising questions about the possible benefit of GBV-C in this group of HIV-positive people.

The reason for the association between GBV-C and survival in HIV-positive persons is not known. Our *in vitro* studies indicate that there is a direct viral interference,<sup>7</sup> although persistence or clearance

of GBV-C infection may represent markers of other host or HIV factors that influence the progression of HIV disease. We do not know why some of the men had clearance of GBV-C after HIV infection or why this was associated with a worse prognosis. Since our analysis was based on only two time points, we do not know when GBV-C was cleared relative to the decline in immune function. It would be of interest to know whether GBV-C clearance was associated with HIV-mediated destruction of host cells necessary for the production of GBV-C or whether clearance of GBV-C preceded the loss of CD4+ T cells.

Alternatively, persistent coinfection with GBV-C may be a marker of immunologic or other host characteristics that confer relative resistance to the progression of HIV disease. More data on the development of E2 antibody are needed for us to understand the effect of previous exposure to GBV-C and the implications of a failure to form antibodies when GBV-C is cleared. Determination of the precise *in vivo* interactions between GBV-C and HIV infections may provide new insights into the progression of HIV disease and may identify new approaches to disease-modifying treatment or vaccines.

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