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Acquisition of GB Virus Type C and Lower Mortality in Patients With Advanced HIV Disease

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(See the Editorial Commentary by Gretch, on pages 1020–1.)

Background. GB virus type C (GBV-C) is transmitted by sexual or parenteral exposure and is prevalent among patients receiving blood products. GBV-C is associated with lower human immunodeficiency virus (HIV) RNA and better survival among HIV-infected patients. Open questions are the presence and the direction of any causal relationship between GBV-C infection and HIV disease markers in the context of highly active antiretroviral therapy (HAART).

Methods. We used a limited access database obtained from the National Heart, Lung, and Blood Institute's Viral Activation Transfusion Study (VATS), a randomized controlled trial of leukoreduced vs nonleukoreduced transfusions to HIV-infected transfusion-naive patients. Blood samples from 489 subjects were tested for GBV-C markers. Cox regression models and inverse probability of treatment weights were used to examine the association between GBV-C coinfection and mortality in the VATS cohort.

Results. We found a significant reduction in mortality among GBV-C coinfected VATS subjects, after adjusting for HAART status, HIV RNA level, and CD4 cell count at baseline. Acquisition of GBV-C RNA (n = 39) was associated with lower mortality in 294 subjects who were GBV-C negative at baseline, adjusting for baseline covariates (hazard ratio = 0.22, 95% confidence interval [CI]: .08–.58) and in models in which weights were used to control for time-updated covariates (odds ratio = 0.21, 95% CI: .08–.60).

Conclusions. GBV-C viremia is associated with lower mortality, and GBV-C acquisition via transfusion is associated with a significant reduction in mortality in HIV-infected individuals, controlling for HIV disease markers. These findings provide the first evidence that incident GBV-C infection alters mortality in HIV-infected patients.

GB virus type C (GBV-C) is a nonpathogenic human virus that is transmitted parentally, sexually, and vertically and is highly prevalent among individuals infected with human immunodeficiency virus (HIV) [1–7]. Investigators have observed an association between GBV-C infection and prolonged survival among HIV-infected individuals in some, though not all, studies [8–18]. A meta-analysis of studies including 1294 HIV-infected subjects found a relative risk of mortality of 0.41 (95% confidence interval [CI]: .23–.69) for those with GBV-C coinfection [19]. These findings are consistent with reported biological effects of GBV-C, which induces an HIV-inhibitory cytokine profile, decreases T-cell activation, blocks interleukin 2–mediated CD4 T-cell proliferation, and reduces expression of the HIV entry receptors CCR5 and CXCR4 in vitro [17, 20, 21].

Recent analyses have sought to explore the relationships among HIV, GBV-C, and highly active antiretroviral therapy (HAART) [12, 22–26]. Earlier studies examined GBV-C infection that preceded or was concurrent with HIV infection, and the time sequence of

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the relationship among GBV-C viremia, HIV disease markers (RNA load, CD4 cell count), and exposure to HAART has not been fully investigated. An analysis that can account for acquisition of GBV-C infection, time-updated HAART status, and HIV disease markers could potentially clarify the relationship between HIV and GBV-C. We examined the effect of both preexisting GBV-C infection and incident GBV-C infection via transfusion on all-cause mortality. We report survival and changes in HIV RNA based on known dates of GBV-C acquisition and changes in HAART status, HIV RNA, and $CD4^+$ cell count in a cohort of transfusion-naive HIV-infected subjects receiving transfusions.

MATERIALS AND METHODS

The Viral Activation Transfusion Study (VATS) was a double-blind clinical trial initiated in July 1995 to evaluate the effects of leukoreduced (LR) vs non-LR allogeneic transfusion on patients infected with HIV and cytomegalovirus, which has been previously described [27, 28]. In brief, transfusion-naive participants with symptomatic anemia that required red cell transfusions were randomized to receive either a filtered LR or standard non-LR blood. Per enrollment criteria, study participants had advanced HIV disease; 78% had an AIDS-defining illness at baseline [28].

Blood samples from participants were collected pretransfusion, weekly posttransfusion for 1 month, and quarterly thereafter; pretransfusion and posttransfusion samples were collected for second transfusion episodes, if any, and stored at −70°C. In June 1996, about 1 year after the start of the accrual period for VATS, HAART was introduced into HIV treatment and became available to some but not all study subjects. At baseline, 24% of recruited subjects were receiving HAART (≥3 antiretroviral drugs, including at least 1 protease inhibitor or nonnucleoside reverse transcriptase inhibitor), 55% were treated with some other combination of antiretroviral therapy, and 21% were receiving no antiretroviral treatment [28].

Of the 531 enrolled subjects, 489 (92%) had paired pretransfusion and final samples available for GBV-C evaluation. We tested all available paired (baseline and final) plasma samples for GBV-C E2 antibody, using the anti-GBenv μplate enzyme immunoassay and for RNA using the quantitative GBV-C RNA reverse-transcription polymerase chain reaction assay (RT-PCR; Roche Diagnostics, Penzberg, Germany). We also tested all interim blood samples from individuals with evidence of incident GBV-C viremia or acquisition of GBV-C antibody between the pretransfusion and final samples using both antibody and RNA assays. If only the final sample tested RNA positive, it was retested to confirm viremia.

For this analysis we used the VATS limited-access public use dataset from the National Heart, Lung, and Blood Institute, combined with the GBV-C results provided by the Blood

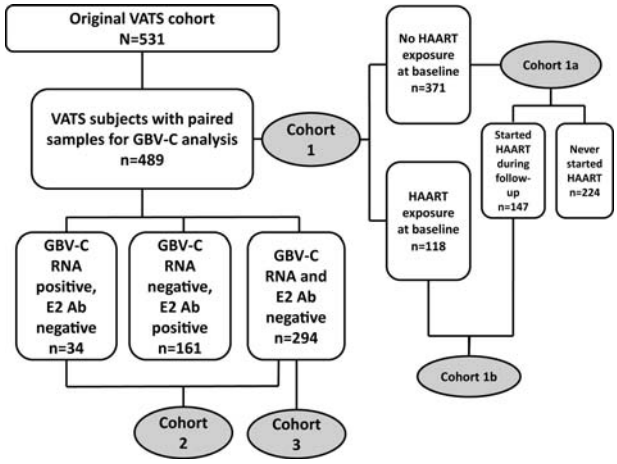

Figure 1. Flow chart of Viral Activation Transfusion Study cohort and subcohorts by GB virus type C and highly active antiretroviral therapy status. Abbreviations: GBV-C, GB virus type C; HAART, highly active antiretroviral therapy; VATS, Viral Activation Transfusion Study.

Systems Research Institute (San Francisco, California). As part of the VATS study, written informed consent was obtained from all study subjects [27], and this supplemental study protocol was approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley.

Statistical Analysis

Study subjects were assigned to groups based on GBV-C status at baseline: GBV-C RNA positive, E2 antibody positive, or both GBV-C markers negative [9]. GBV-C status at visits for which no plasma sample was tested/available was imputed as the most recent measured GBV-C status. We assumed that once a subject became GBV-C viremic, he or she remained GBV-C viremic until the end of follow-up (intention-to-treat analysis), an assumption that should yield a more conservative estimate of the effect of GBV-C viremia.

We analyzed all VATS subjects as a single cohort (cohort 1), and as 2 subcohorts according to baseline GBV-C status (Figure 1). Cohort 2 consisted of VATS subjects who were E2 antibody negative at baseline (n = 328). Cohort 3 consisted of subjects who were GBV-C RNA and E2 antibody negative at baseline (n = 294). In addition, we stratified cohort 1 by time-updated HAART status. Cohort 1a included subjects who never received HAART and subjects who started HAART during follow-up (follow-up time was censored following initiation of HAART; n = 371). Cohort 1b included subjects who were receiving HAART at baseline as well as those who initiated HAART during follow-up (n = 265).

Subjects were followed from date of VATS study entry to either death, loss to follow-up, or censoring at clinical trial conclusion (up to 3.5 years after baseline). We analyzed time

to all-cause mortality using Kaplan-Meier estimates and compared survival by baseline GBV-C RNA and E2 antibody status using the log-rank test. Cox proportional hazard regression models were used to calculate unadjusted hazard ratios (HRs) for time-updated GBV-C RNA status, as well as HR adjusted for baseline $\log_{10}$ HIV RNA, $CD4^+$ T-lymphocyte count (square root transformed) and HAART use. As outcomes we examined all-cause mortality in cohorts 1, 1a, 1b, 2, and 3; all-cause mortality or virological progress (>1 $\log_{10}$ increase in HIV RNA between t and $t+1$) [11] for cohort 1a, and all-cause mortality or virological failure (detection of 2 consecutive plasma HIV RNA levels >500 copies/mL at t and $t+1$ if at least 1 plasma HIV RNA level ≤500 copies/mL had been detected after initiation of HAART) [23] for cohort 1b.

In order to control for time-updated confounding [29, 30] by HIV disease markers in the relationship between GBV-C viremia and mortality, we applied inverse probability of treatment weights (IPTWs) and marginal structural models to estimate the effect of GBV-C acquisition on mortality for cohort 3 [31]. IPTW provides an alternative approach to adjusting for time-varying variables, such as HIV RNA, which affect mortality and subsequent GBV-C status and are thus confounders, but are also affected by prior GBV-C status and thus cannot be adjusted for in standard multivariable regression. IPTW reweights subjects to create balanced covariate distributions between exposure groups in the reweighted population. We estimated stabilized weights using pooled logistic regression models of the probability of GBV-C acquisition among subjects who remained GBV-C negative, given time-updated HAART status, HIV RNA, CD4 count, and cumulative units transfused (denominator) and baseline covariates (numerator). We examined the association of GBV-C acquisition and mortality after adjusting for baseline and time-updated covariates using weighted pooled logistic regression of mortality on current GBV-C status, time, and baseline HIV markers. Standard errors were estimated using the robust sandwich estimator [32]. All comparisons were 2-sided with a 5% significance level and were done using Stata software, version 11.2 (StataCorp, College Station, Texas).

RESULTS

Of the 489 VATS subjects tested for GBV-C RNA and E2 antibody who comprised cohort 1, 294 (60%) were RNA and E2 antibody negative, 34 (7%) were RNA positive, and 161 (33%) were E2 antibody positive at baseline; none had both RNA and E2 antibody at baseline and during follow-up, and hence these 3 groups were mutually exclusive (Figure 2). For all subjects, median follow-up time from baseline to final visit was 8.4 months (interquartile range = 2–21.8 months). Two hundred sixty-seven (55%) subjects died during study follow-up, and 46 (9%) withdrew from the study or were lost to follow-up. Six subjects lost measurable GBV-C RNA during follow-up. Table 1 provides an overview of subject characteristics at baseline and last day of follow-up by baseline GBV-C RNA and E2 antibody status.

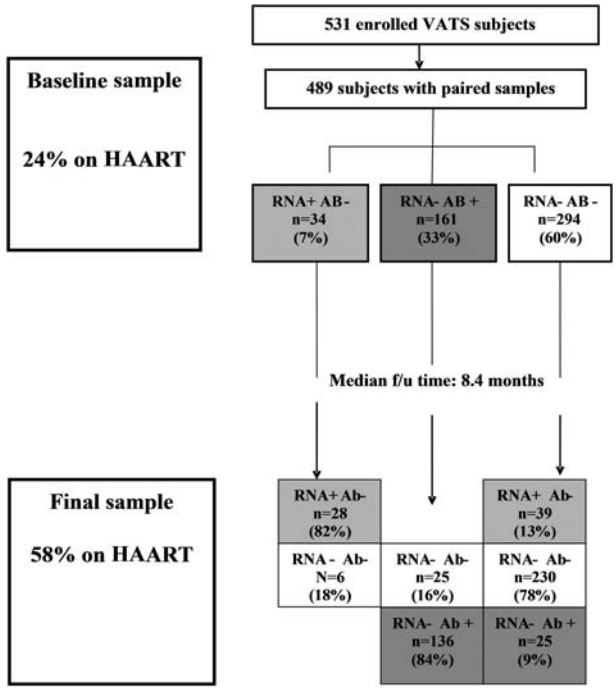

Figure 2. Patterns of GB virus type C infection status at baseline and final sample—Viral Activation Transfusion Study, 1995–1999. Abbreviations: HAART, highly active antiretroviral therapy; VATS, Viral Activation Transfusion Study.

Mortality Among VATS Subjects—Cohorts 1 and 2

Kaplan-Meier survival estimates were significantly higher for subjects with GBV-C viremia at baseline compared with those who were GBV-C RNA negative at baseline, regardless of baseline E2 antibody status ($P = .02$ for cohort 1, Figure 3; $P = .03$ for cohort 2, data not shown). Current GBV-C viremia, higher baseline CD4 cell counts, lower baseline HIV RNA, and HAART use at baseline predicted lower mortality in univariable Cox regression analyses of cohort 1 (unadjusted HR for GBV-C viremia = 0.32, 95% CI: .19–.54; Table 2). The association between mortality and time-updated GBV-C RNA status remained significant for GBV-C RNA (adjusted HR = 0.42, 95% CI: .24–.73) after adjusting for baseline CD4, HIV RNA, and HAART status. Similarly, univariable and multivariable Cox regression analysis of cohort 2 showed a significant unadjusted HR for time-updated GBV-C RNA status (unadjusted HR = 0.33, 95% CI: .20–.57) and lower mortality among subjects with current GBV-C viremia after adjusting for baseline HAART status, HIV RNA, and CD4 cell counts (adjusted HR = 0.46, 95% CI: .26–.82).

Table 1. Characteristics of 489 Participants With HIV Infection, by Baseline GB Virus Type C Status, Viral Activation Transfusion Study Cohort 1[a], 1995–1999

Characteristic	GBV-C RNA Positive (n = 34)	GBV-C E2 Antibody Positive (n = 161)	GBV-C Negative (n = 294)
Follow-up time, months			
Mean (SD)	17.0 (12.6)	11.2 (11.5)	12.9 (12.6)
Median (IQR)	17.5 (3.9–28.9)	6.6 (1.8–21.0)	9.2 (2.0–23.4)
HIV RNA, $\log_{10}$ per mL			
Baseline, mean (SD)[b]	3.6 (1.2)	4.6 (1.0)	4.6 (1.1)
Last day of follow-up, mean (SD)[b]	3.4 (1.1)	4.4 (1.2)	4.4 (1.3)
CD4 cells/μL			
Baseline, median (IQR)[c]	71.5 (20–230)	11.0 (3–61)	15.0 (3–61)
Last day of follow-up, median (IQR)[c]	129.0 (50–230)	11.0 (2–93)	19.5 (3–118)
Antiretroviral exposure (HAART)			
Baseline, No. (%)[d]	14 (41.2)	40 (24.8)	64 (21.8)
By the last day of follow-up, No. (%)	24 (70.6)	88 (54.7)	170 (57.8)
Age, years			
Mean (SD)[b]	37.3 (7.0)	40.3 (7.3)	37.6 (7.4)
Sex, No. (%)			
Male	28 (82.4)	126 (78.3)	234 (79.6)
Race, No. (%)			
White, non-Hispanic	17 (50.0)	94 (58.8)	146 (49.7)
Black, non-Hispanic	13 (38.2)	50 (31.3)	97 (33.0)
Other	4 (11.8)	16 (10.0)	51 (17.4)
HIV risk behavior, No. (%)[e]			
Heterosexual sex	9 (26.5)	46 (28.8)	99 (33.7)
Men having sex with men	22 (64.7)	97 (60.6)	171 (58.2)
Injection drug use[d]	9 (26.5)	53 (33.1)	63 (21.4)
Transfusion status			
Leukoreduced, No. (%)	19 (55.9)	85 (52.8)	135 (45.9)

Abbreviations: GBV-C, GB virus type C; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation; VATS, Viral Activation Transfusion Study.

[a] All VATS subjects with paired baseline and final samples available for GBV-C evaluation.

[b] *P* value for *F* test <.001.

[c] *P* value for Kruskal-Wallis rank test <.001.

[d] *P* value for χ^2 test <.05.

[e] HIV risk behavior groups are not mutually exclusive.

Mortality Among GBV-C–Negative Subjects Who Acquired GBV-C RNA During Follow-up—Cohort 3

VATS subjects received blood transfusions during study follow-up and thus were at risk of parenteral GBV-C transmission via transfusion [4, 33]. We restricted cohort 3 to those who were GBV-C RNA and E2 antibody negative at baseline to compare the hazard of all-cause mortality between subjects who presumably acquired GBV-C via transfusion and those who remained GBV-C negative during follow-up. We included 294 subjects in this subanalysis; 39 (13%) became GBV-C RNA positive between baseline and the last follow-up visit. In the unadjusted analysis, GBV-C acquisition was associated with a substantial reduction in mortality (HR = 0.22, 95% CI: .09–.56; Table 3). In Cox proportional hazard regression analysis adjusting for baseline HAART status, HIV RNA, and CD4 cell counts, the relative hazard of mortality in the GBV-C RNA-positive group was 0.22 (95% CI: .08–.58), compared with the GBV-C RNA-negative group (Table 3). Among 39 subjects with incident GBV-C infection, GBV-C RNA was inversely correlated with mortality (unadjusted HR = 0.25, 95% CI: .09–.72; adjusted HR = 0.17, 95% CI: .02–1.34).

IPTW was used to further adjust for possible confounding by time-updated HAART status, CD4 count, HIV RNA, and cumulative units transfused. In the treatment models to estimate IPTW, a lower time-updated HIV RNA was predictive of GBV-C acquisition (odds ratio [OR] = 0.73; *P* = .06). The marginal structural model estimates using stabilized IPTWs showed reduced mortality associated with GBV-C acquisition (OR = 0.19, 95% CI: .07–.55; Table 4). Additional weights to account for potentially informative censoring had a small

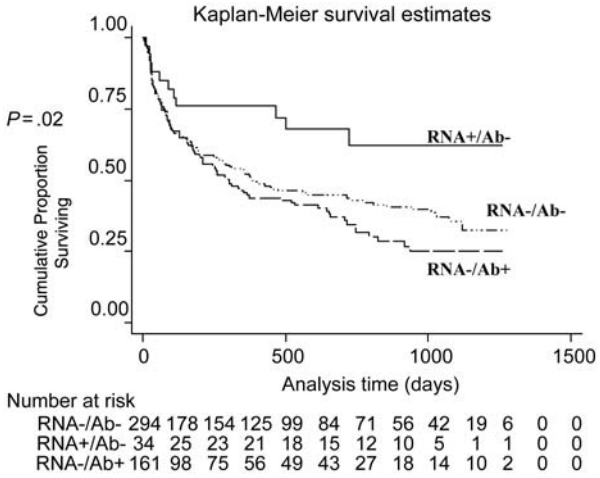

Figure 3. Kaplan-Meier estimates of survival according to GB virus type C RNA and E2 antibody status at baseline, Viral Activation Transfusion Study, cohort 1 (n = 489).

effect on the point estimate (OR = 0.21, 95% CI: .08–.60). Using alternative model specifications in the IPTW and mortality models did not substantively change findings.

Stratified Analysis for Mortality or Virological Failure

To examine mortality or virological progress/failure in cohorts 1a (n = 371) and 1b (n = 265), we stratified participants according to HAART status at baseline and during study follow-up (data not shown). In cohort 1a, the Cox proportional hazard regression analysis of subjects before initiation of HAART (follow-up started at baseline and censored by initiation of HAART) showed a trend toward lower mortality or virological progression among GBV-C RNA-positive subjects

Table 3. Unadjusted and Adjusted Hazard Ratios for Mortality Using Cox Proportional Hazard Regression Models, Viral Activation Transfusion Study Cohort 3[a] (n = 294)

Characteristic	No. of Deaths	Unadjusted HR (95% CI)	Adjusted HR[b] (95% CI)
Time-updated			
GBV-C RNA negative	153	1.00	1.00
GBV-C RNA positive	6	0.22 (.09–.56)	0.22 (.08–.58)[c]
Baseline			
HAART use		0.67 (.45–1.01)	0.99 (.66–1.48)
HIV RNA (log_{10}/mL)		1.46 (1.25–1.71)	1.21 (1.01–1.45)
CD4 cells/μL (square root)		0.87 (.82–.91)	0.89 (.84–.94)

Abbreviations: CI, confidence interval; GBV-C, GB virus type C; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HR, hazard ratio.

[a] Viral Activation Transfusion Study subjects who were GBV-C RNA and E2 antibody negative at baseline.

[b] HR is adjusted for baseline HAART status, HIV RNA, and CD4 cell count at time t_0.

[c] Using robust method for calculation of standard errors, $P = .002$.

Table 2. Unadjusted and Adjusted Hazard Ratios for Mortality Using Cox Proportional Hazard Regression Models, Viral Activation Transfusion Study Cohort 1[a] (n = 489)

Characteristic	No. of Deaths	Unadjusted HR	95% CI	Adjusted HR[b]	95% CI
Time-updated					
GBV-C RNA negative	252	1.00		1.00	
GBV-C RNA positive	15	0.32	.19–.54	0.42	.24–.73
GBV-C E2 antibody[c]		1.09	.85–1.41	…	…
Baseline					
HAART use		0.35	.26–.46	0.87	.65–1.17
HIV RNA (log_{10}/mL)		1.71	1.53–1.91	1.09	.95–1.26
CD4 cells/μL (square root)		0.80	.77–.84	0.88	.85–.92
Heterosexual sex		1.18	.91–1.52	…	…
Injection drug use		1.04	.78–1.38	…	…
Men having sex with men		0.77	.60–.98	…	…
Male		0.75	.55–1.02	…	…
Nonwhite race		0.97	.82–1.14	…	…
Leukoreduced transfusion		0.81	.63–1.02	…	…

Abbreviations: CI, confidence interval; GBV-C, GB virus type C; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HR, hazard ratio.

[a] All Viral Activation Transfusion Study subjects with paired baseline and final samples available for GBV-C evaluation.

[b] HR is adjusted for baseline HAART status, HIV RNA, and CD4 cell count at time t_0.

[c] E2 antibody values are imputed for subjects with antibody$^{+/+}$ or antibody$^{-/-}$ results at baseline/final sample.

Table 4. Estimates of the Effect of GB Virus Type C Acquisition on Mortality Using Pooled Logistic Regression Models and Weighted Marginal Structural Models, Viral Activation Transfusion Study Cohort 3[a] (n = 294)

Estimate	OR	95% CI
Unweighted estimates		
Unadjusted[b]	0.25	.10– .63
Adjusted for baseline covariates[c]	0.25	.09–.70
Weighted estimates		
Stabilized IPTW[d]	0.19	.07–.55
Stabilized IPTW and IPCW[d]	0.21	.08–.60

Odds ratio estimated in the pooled logistic regression models estimates discrete relative hazard of mortality. Inverse probability of treatment weight: range = 0.08–1.90, mean = 1.01; inverse probability of censoring weight: range = 0.08–3.64, mean = 1.02.

Abbreviations: CI, confidence interval; IPCW, inverse probability of censoring weight; IPTW, inverse probability of treatment weight; OR, odds ratio.

[a] Viral Activation Transfusion Study subjects who were GB Virus Type C RNA and E2 antibody negative at baseline,

[b] OR is adjusted for time since first transfusion.

[c] OR is adjusted for time since first transfusion, baseline highly active antiretroviral therapy (HAART) status, human immunodeficiency virus (HIV) RNA, and CD4 cell count at time t_0.

[d] OR is adjusted for time since first transfusion, baseline HAART status, HIV RNA, and CD4 cell count at time t_0, and time-updated HAART status, HIV RNA, CD4 cell count, and cumulative units at time t.

after adjusting for baseline covariates (unadjusted HR = 0.47, 95% CI: .23–.84; adjusted HR = 0.59, 95% CI: .33–1.04). Mortality or virological failure risk among those receiving HAART at baseline or starting HAART during study follow-up (cohort 1b follow-up started at study entry or at HAART initiation, respectively) was not significantly different between GBV RNA positive and negative subjects in the Cox regression models (unadjusted HR = 0.69, 95% CI: .43–1.11; adjusted HR = 0.72, 95% CI: .44–1.17).

DISCUSSION

We found that incident GBV-C infection is associated with substantially reduced mortality among patients with advanced HIV disease. Previous studies of GBV-C and survival demonstrated that persistent GBV-C infection [14, 16] or GBV-C infection late in the course of HIV infection [19] are associated with prolonged survival. No previous study has assessed the effect of incident GBV-C infection on survival. This association remained highly significant after adjustment for both baseline and time-updated HAART status, HIV RNA, and CD4 cell counts among subjects who were GBV-C RNA and E2 antibody negative at baseline. Our analysis also showed that GBV-C viremia was associated with a nonsignificant trend toward slower progression to virological failure or death among HIV-infected subjects, regardless of their use of HAART.

To our knowledge this is the first report showing a survival benefit of incident GBV-C infection among patients who were infected with HIV prior to GBV-C acquisition. All previous studies on the effect of GBV-C coinfection have described prevalent GBV-C infection, which likely preceded HIV infection [34], and have thus been unable to resolve whether acquisition of GBV-C improves survival rather than or in addition to simply acting as a marker for higher CD4 cell counts and/or lower HIV RNA [35–38]. The target cell for GBV-C infection remains unknown; however, both $CD4^+$ and $CD8^+$ lymphocytes and B cells from GBV-C–infected individuals contain and produce GBV-C particles [39], suggesting that CD4 cell levels alone do not explain the observed association between GBV and survival. Nevertheless, neither our unadjusted analyses, nor those adjusted for baseline covariates, are likely to provide unbiased estimates of the effect of GBV-C viremia on survival. Although focusing on subjects who were initially GBV-C negative and adjusting for baseline CD4 counts and HIV RNA partially addresses confounding, the resulting estimate remains subject to bias because subjects with lower HIV RNA levels after baseline are more likely to acquire GBV-C [33].

In order to address this challenge, we applied IPTW to estimate the effect of GBV-C on mortality among subjects who were initially GBV-C negative, adjusting for both baseline and time-updated confounders. The validity of IPTW depends on correct specification of our adjustment models. Although we considered several model specifications with little effect on the results, the numbers of subjects who acquired GBV-C during follow-up limited the complexity of the models we could use to control for time-updated confounders. However, our weighted results support the hypothesis that GBV-C acquisition reduces mortality, independent of time-updated HIV disease markers.

We used several approaches to evaluate HAART as an important potential confounder and an effect modifier of the effect of GBV-C on survival. First, we adjusted for baseline HAART use, assuming no effect modification between HAART and GBV-C in cohorts 1 and 3. Secondly, we stratified cohort 1 by HAART status to examine HIV RNA outcomes and mortality in each group. Finally, we adjusted for time-updated HAART use in our IPTW analysis. Although we did not find a significant effect of GBV-C RNA on the composite outcome of virological failure or mortality in subcohorts 1a or 1b when adjusted for baseline covariates, we found a consistent trend toward lower mortality among GBV-C RNA–positive subjects in all models and subcohorts. Our power to detect a protective effect of GBV-C in cohorts 1a and 1b, as well as our ability to evaluate effect modification by HAART use, was limited by study size. However, we are unaware of a larger study of HIV-infected individuals with

transfusion-related incident GBV-C infection who have not received HAART. Several previous studies have reported that GBV-C viremia during late stages of HIV infection is associated with better HIV disease outcomes [19, 36]. GBV-C viremia detected 5–6 years after HIV seroconversion was associated with significantly longer survival but not if GBV-C was measured 12–18 months after HIV seroconversion. Stage of HIV infection was suggested as an explanation for inconclusive findings of the previous studies [15, 16]. Unfortunately, we were not able to estimate the date of HIV seroconversion for VATS subjects. Given the clinical status of the VATS cohort, subjects had advanced HIV disease and presumably had seroconverted several years before enrollment [28].

Our study has a number of limitations. First, our estimate of survival benefit related to GBV-C infection may be an underestimate, as E2 antibody–positive subjects were classified with GBV-C RNA–negative ones in cohorts 1 and 3. Among 294 subjects who were GBV-C RNA and E2 antibody negative at baseline (cohort 3), 25 subjects became E2 antibody positive in the final sample, despite remaining GBV-C RNA negative. This could be due to either passive antibody transfusion or formation of E2 antibody by the subject in response to transient undetected GBV-C viremia. Misclassification of transient GBV-C–viremic subjects as GBV-C negative would be expected to bias our estimated hazard toward the null. Secondly, the VATS cohort had a relatively short follow-up time (<3.5 years). Most previous studies have examined the effect of GBV-C coinfection for a period of 4–8 years [36]. We cannot rule out that we might have seen a different effect if the VATS cohort had been followed for a longer period of time. Thirdly, we note potential misclassification of "HAART" status owing to binary categorization of the "HAART" variable. The "no HAART" group included subjects who never received any antiretroviral, as well as those who were receiving monotherapy or dual therapy that did not qualify as HAART.

In conclusion, we found that GBV-C viremia is associated with lower mortality in HIV-infected patients, after adjusting for baseline HIV RNA, CD4 counts, and HAART status, consistent with other reports. In addition, we found a significant reduction in mortality associated with incident GBV-C infection during follow-up among HIV-infected subjects receiving transfusions, even after controlling for time-updated HIV disease markers using inverse weights. Our analysis is strengthened by our ability to estimate the effect of time-updated GBV-C viremia on mortality, while appropriately controlling for time-updated HIV RNA, CD4 cell counts, and HAART status, thus, establishing a clear temporal sequence among HIV disease markers, GBV-C viremia, and mortality. There is substantial in vitro evidence demonstrating inhibition of HIV replication by GBV-C. Establishing clinical evidence for a causal relationship between GBV-C infection and HIV disease outcomes will require larger observational studies of incident GBV-C infections in HIV-infected transfusion recipients. Such studies can be designed to characterize the acute and long-term impact of GBV-C acquisition on virological, immunological, and clinical consequences of HIV infection.

Notes

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