MAJOR ARTICLE

Transmission of GB Virus Type C via Transfusion in a Cohort of HIV-Infected Patients

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Background. GB virus C (GBV-C) infection is transmitted by blood exposure and associated with lower human immunodeficiency virus (HIV) load and slower HIV disease progression. Few studies describe predictors of acute GBV-C infection following transfusion in HIV-infected patients.

Methods. We used a limited-access database from the National Heart Lung and Blood Institute's Viral Activation Transfusion Study, a randomized controlled trial of leukoreduced versus nonleukoreduced transfusions received by HIV-infected, transfusion-naive patients. Blood samples from 489 subjects were tested for GBV-C markers in pretransfusion and posttransfusion samples. We estimated the risk of acquiring GBV-C RNA and predictors of GBV-C acquisition, using pooled logistic regression.

Results. GBV-C RNA was detected ≤120 days following the first transfusion in 22 (7.5%) of 294 subjects who were GBV-C negative before transfusion. The risk of GBV-C RNA acquisition increased with each unit transfused (odds ratio, 1.09; 95% confidence interval, 1.06–1.11). Lower baseline HIV load and use of antiretroviral therapy were associated with subsequent GBV-C RNA acquisition, after control for units of blood transfused. Leukoreduced status of transfused units was not associated with GBV-C transmission.

Conclusions. Blood transfusion is associated with a significant risk of GBV-C acquisition among HIV-infected patients. Transmission of GBV-C by blood transfusion was inversely related to HIV load.

GB virus type C (GBV-C), also called hepatitis virus G, is a human flavivirus that is phylogenetically related to hepatitis C virus (HCV) [1–3]. GBV-C is primarily a lymphotropic virus that appears to replicate in T and B lymphocytes [4]. Although infection may persist, most immunocompetent individuals infected with GBV-C spontaneously clear viremia in ≤2 years [5, 6]. Anti–GBV-C envelope glycoprotein (E2) antibody usually appears after clearance of viremia and may offer some protection from reinfection [6, 7]. However, E2 antibody response to GBV-C infection is highly variable and wanes over time, making data on E2 antibody difficult to interpret. It is uncommon to detect both GBV-C RNA and E2 antibody in serum at the same time, and detection of GBV-C RNA in serum indicates active infection.

GBV-C is transmitted through sexual, parenteral, and vertical (ie, mother-to-child) routes [8–11]. Transmission of GBV-C via transfusion and transient viremia following blood transfusion have been documented by detection of 100% sequence similarity between donor and recipient posttransfusion samples [12]. The prevalence of GBV-C viremia is 1%–5% among healthy blood donors from developed countries and is much higher in developing countries [13–15]. In the United States, approximately 2% of healthy blood donors have GBV-C viremia, and 13% have E2 antibody, indicative of prior infection [15].

GBV-C was initially thought to cause hepatitis in humans, but several studies have failed to demonstrate an association between GBV-C infection and any human disease [13, 16]. Hence, blood products are not routinely screened for the presence of GBV-C RNA [13]. Because

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of the routes by which it is transmitted, GBV-C infection is more prevalent among human immunodeficiency virus (HIV)–infected individuals, with up to 43% of HIV-infected individuals exhibiting GBV-C viremia in cross-sectional studies [9, 17]. Several but not all studies have observed an association between GBV-C infection and prolonged survival among HIV-infected individuals [15], and a meta-analysis of studies including 1294 HIV-infected subjects confirmed an association between GBV-C infection and prolonged survival [18]. There are several potential mechanisms by which GBV-C infection might confer a survival advantage. GBV-C has been shown (in vivo and in vitro) to interfere with HIV replication, modulate HIV-entry receptors CCR5 and CXCR4, alter T-helper cell 1 and T-helper cell 2 cytokine profiles, decrease T-cell activation, and block interleukin 2–mediated CD4 T-cell proliferation [19–26].

In the present study, we quantified the risk of GBV-C acquisition per unit of blood transfused in 294 HIV-infected, transfusion-naive subjects documented to be negative for GBV-C RNA and E2 antibody before transfusion. To our knowledge, this is the first study to report the incidence of GBV-C viremia following transfusion in HIV-infected patients. In addition, to address the temporal ordering in the relationship between HIV and GBV-C RNA, we examined pretransfusion HIV load and CD4 cell count as predictors of GBV-C acquisition that occurred ≤120 days after transfusion.

MATERIALS AND METHODS

Plasma samples were selected for GBV-C testing from subjects previously enrolled in the prospective, multicenter Viral Activation Transfusion (VATS) clinical trial, which is sponsored by the National Heart, Lung, and Blood Institute (NHLBI). Details of subject selection and findings of the clinical trial have been previously described [27]. Briefly, VATS evaluated the effect of leukoreduction of units for transfusion into transfusion-naive HIV-infected patients between July 1995 and June 1999. In VATS, patients were randomized to receive either a filtered leukoreduced or standard nonleukoreduced blood unit [27, 28]. Study participants had advanced HIV disease and symptomatic anemia sufficient to require transfusion, with a mean HIV load (±SD) of 4.5 ± 1.1 $\log_{10}$ copies/mL, a median baseline CD4 cell count of 15 cells/μL (interquartile range [IQR], 3–73 cells/μL), and a median survival time of 8.4 months (IQR, 2–21.8 months). At last observation, the mean HIV load among those who survived was 4.3 ± 1.3 $\log_{10}$ copies/mL, and the median CD4 cell count was 22 cells/μL (IQR, 3–129.5 cells/μL).

Study samples were collected and stored at −70°C at baseline (before transfusion), weekly after transfusion for 1 month, and quarterly thereafter; pretransfusion and weekly posttransfusion samples were collected for any second transfusion episode during the study. Of the 531 subjects, 489 (92%) had paired pretransfusion and final samples available for GBV-C evaluation.

All available paired plasma samples were tested for GBV-C E2 antibody, using the anti-GBenv μPlate enzyme immunosorbant assay (Roche Diagnostics, Penzberg, Germany), and for GBV-C RNA, using quantitative GBV-C RNA reverse-transcription polymerase chain reaction (RT-PCR; Roche Diagnostics, Penzberg, Germany). 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 were tested by both antibody and RNA assays. The final sample was retested following interim testing, to confirm RNA positivity if it was the only RNA-positive sample.

To be eligible for this analysis, subjects must have had GBV-C test results and a baseline (ie, pretransfusion) sample negative for GBV-C RNA and E2 antibody (n = 294). Written informed consent was obtained from all VATS 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.

For the current analysis, we used a limited-access VATS public use dataset that provided demographic and clinical information about blood recipients in combination with GBV-C test results provided by the Blood Systems Research Institute. Personal identifiers and data elements identifying paired donor and recipients were removed from the limited-access VATS dataset provided by the NHLBI. Thus, matching of donor-recipient samples and performance of additional laboratory testing were not possible. Currently, specimens from the VATS study are housed in the NHLBI-funded Biologic Specimen and Data Repository Information Coordinating Center and can be requested for specific research projects.

Statistical Analysis

GBV-C RNA status was categorized as a binary time-varying variable (ie, positive or negative). Missing GBV-C RNA status for interim or final samples was imputed by carrying the most recent measurement forward. Cumulative units of red blood cells or platelets transfused ≤120 days after the first transfusion were described as a time-varying continuous variable. Categorical variables were compared by the χ^2 test or the Fisher exact test, as appropriate. Continuous variables were compared by the Student *t* test, if normally distributed, or the Mann–Whitney *U* test, if not normally distributed.

Risk factors for GBV-C RNA acquisition were evaluated among subjects who were negative for GBV-C antibody and RNA at study entry. Evaluation of baseline HIV load and CD4 cell counts as predictors of GBV-C RNA acquisition in subjects known to be negative for GBV-C RNA and antibody at baseline allowed us to establish a clear temporal sequence between these HIV biomarkers and GBV-C RNA acquisition. Pooled logistic regression models were used to estimate the log odds of GBV-C RNA acquisition ≤120 days after first transfusion among subjects who remained GBV-C RNA negative as

a function of cumulative units transfused and other covariates. The odds ratio (OR) estimated in such a model provides an approximation of the relative odds [29]. For each visit t following entry into study, the probability of acquiring GBV RNA by visit t was estimated among subjects who remained GBV-C RNA negative prior to visit t as a function of cumulative units transfused up until visit t. We further examined baseline $\log_{10}$ HIV load, current highly active antiretroviral therapy (HAART) status, baseline CD4 T cell count (square-root transformed), and race (nonwhite vs white) as predictors of GBV-C acquisition, after adjustment for cumulative units transfused. To ensure that HIV biomarkers were measured prior to GBV-C RNA acquisition, we used baseline HIV load and CD4 cell count in the adjusted models. HAART status was updated at each visit if the subject started HAART between a previous visit and visit t.

Repeated measures per subject were accounted for by using the robust standard errors in the form of the ''sandwich'' estimator. We did not include E2 antibody results in our analysis to define new infection, because GBV-C RNA is a measure of active infection, and E2 antibody levels may be detected transiently following transfusion because of passive antibody and are not constant over time. Data analysis was performed using Stata 10.0 (StataCorp, College Station, TX).

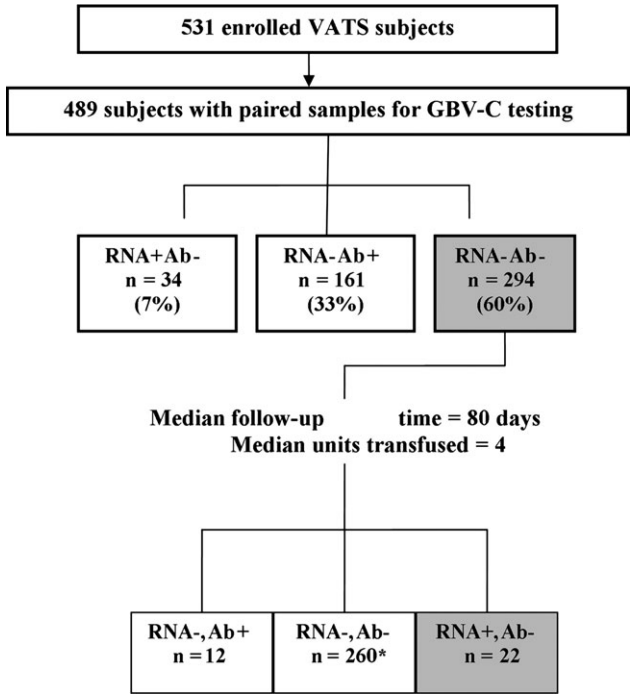

Figure 1. Patterns of GB virus type C (GBV-C) infection status at baseline and 120 days after first transfusion among members of the Viral Activation Transfusion Study (VATS) during 1995–1999. *E2 antibody (Ab) status for 171 subjects was determined >120 days after transfusion. Abbreviations: +, positive; −, negative.

RESULTS

Of the 489 subjects screened for GBV antibody and RNA, 294 (60%) were negative for both E2 antibody and RNA at baseline and thus were eligible to be included in the current analysis (Figure 1). Twenty-two study subjects (7.5%) showed evidence of having acquired GBV-C RNA ≤120 days following the first transfusion. These 22 incident GBV-C infections are presumed to have resulted from transfusion transmission of GBV-C. The incidence of GBV-C acquisition following transfusion in the study sample was estimated to be 39 cases per 100 person-years of follow-up. In no case was a subject simultaneously positive for RNA and E2 antibody, and no subject cleared viremia ≤120 days after transfusion.

The median follow-up time for the current analysis was 80 days (IQR, 33–101 days), with a slightly longer follow-up time for the group that acquired GBV-C (Table 1). This group had lower baseline pretransfusion HIV loads and a trend toward higher baseline CD4 cell counts, compared with the GBV-C–negative group ($P = .02$ and $P = .2$, respectively). Sixty-four subjects (21.8%) were receiving HAART at baseline, and an additional 39 subjects initiated HAART during follow-up. A larger percentage of patients who acquired GBV-C used antiretroviral drugs at some point during the study (59% vs 33%; $P = .01$). Those who acquired GBV-C were mostly white (82%), in contrast to the GBV-C–negative group, of whom 48% of subjects were white ($P = .01$). There was no significant difference between the 2 groups in the type of blood components transfused (platelets vs red blood cells) or leukoreduced versus nonleukoreduced components.

Mean follow-up time (±SD) from baseline to first detection of GBV-C RNA was 49 ± 36.0 days for those who acquired GBV-C RNA. Of the patients who acquired GBV-C RNA during follow-up, evidence of acquisition occurred ≤30 days following the first transfusion (early) for 12 (54.5%), while for 10 other cases acquisition occurred 31–120 days after transfusion (late). These acquisition groups were similar, except for longer follow-up time for the group with later acquisition of GBV-C RNA (data not shown). VATS subjects who acquired GBV-C viremia after transfusion had a mean GBV-C RNA load of 6.3 $\log_{10}$ copies/mL (range, 5.1–7.6 $\log_{10}$ copies/mL) at first detection. There was a significant negative correlation between GBV-C load and HIV load (correlation coefficient, –0.18; $P = .01$) among VATS subjects who acquired GBV-C during study follow-up.

Table 2 presents results of pooled logistic regression models in which the log odds of GBV-C RNA acquisition among subjects who remained GBV-C negative was estimated as

Table 1. Characteristics of Viral Activation Transfusion Study Cohort Members Initially Negative for GB Virus Type C (GBV-C) RNA and E2 Antibody, by GBV-C Acquisition Status ≤120 Days After First Transfusion

Characteristic	GBV-C RNA Acquisition (n = 22)	GBV-C RNA Negative (n = 272)	P[a]
Follow-up time, days			
Mean (SD)	85.0 (28.9)	71.2 (34.4)	.06
Median (IQR)	88.5 (80–108)	77.5 (32–100)	.08
Baseline HIV load, $\log_{10}$ copies/mL, mean (SD)	4.1 (1.4)	4.7 (1.1)	.02
Baseline CD4 T cell count, cells/μL			
Mean (SD)	70.5 (86.7)	55.5 (105.5)	.54
Median (IQR)	30.5 (7.5–95.5)	14 (3–56)	.08
HAART exposure, no. (%)			
Baseline	6 (27.3)	58 (21.3)	.51
By the last day of follow-up	13 (59.1)	90 (33.1)	.01
Cumulative units transfused			
Mean (SD)	7.2 (9.3)	5.2 (4.5)	.08
Median (IQR)	4 (2–7)	4 (2–6)	.41
Male sex, no. (%)	16 (72.7)	218 (80.2)	.41
Age at baseline, years, mean (SD)	37.2 (1.5)	37.6 (0.5)	.78
Race, no. (%)			
White, non-Hispanic	18 (81.8)	131 (48.2)	.01
Black, non-Hispanic	2 (9.1)	96 (35.3)	
Other	2 (9.1)	45 (16.5)	
HIV risk behavior, no. (%)[b]			
Heterosexual	10 (45.5)	89 (32.7)	.22
MSM	11 (50.0)	108 (58.8)	.42
Injection drug use	6 (27.3)	57 (21.0)	.49
Other	1 (4.6)	13 (4.8)	.96
Leukoreduced units transfused, no. (%)	7 (31.8)	128 (47.1)	.17
Received platelet units, no. (%)	4 (18.2)	41 (15.1)	.70

Abbreviations: HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men.

[a] Based on t test for equality of means, Wilcoxon rank-sum test for equality of medians, and χ^2 test for categorical variables.

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

a function of exposure to cumulative blood units transfused and other covariates. Each additional unit of blood transfused was associated with a 9% increase in the relative odds of GBV-C acquisition (95% confidence interval [CI], 1.06–1.11). We also investigated the odds of GBV-C acquisition as a function of baseline HIV load, baseline CD4 cell count, and current HAART exposure, after control for cumulative units transfused. There was a reduced odds of GBV-C acquisition with increased baseline HIV load (OR, 0.62 per $\log_{10}$ copies/mL; 95% CI, .40–.96) and an increased odds of GBV-C acquisition with current HAART use (OR, 4.03; 95% CI, 1.79–9.11), after control for cumulative units transfused. An increased baseline CD4 cell count was associated with a slight, nonsignificantly increased odds of GBV-C acquisition (OR, 1.05; 95% CI, .98–1.12). After adjustment for HAART use, baseline HIV load, and race, the estimated relative odds of GBV-C acquisition was 1.08 per unit transfused (95% CI, 1.05–1.11). Following adjustment for all covariates, including cumulative units transfused, current use of HAART (OR, 4.75; 95% CI, 2.02–11.18) and nonwhite race (OR, 0.15; 95% CI, .04–.64) remained significant predictors of GBV-C acquisition; however, baseline HIV load was no longer significantly associated with GBV-C acquisition (OR, 0.87; 95% CI, .54–1.41) (Table 2). Leukoreduction status and receipt of platelet units were not associated with GBV-C acquisition in regression models that controlled for cumulative units transfused.

DISCUSSION

In the present study of a transfusion-naive, HIV-infected cohort negative for GBV-C RNA and E2 antibody, we found an incidence of GBV-C infection of 39 cases per 100 person-years following transfusion. The probability of developing GBV-C viremia was associated with the number of units of blood transfused; this association remained after control for HAART use and baseline HIV load. Lower baseline HIV load, use of HAART, and race were all associated with subsequent GBV-C RNA acquisition, after controlling for units of blood transfused. Previous studies have found an inverse relationship between GBV-C load and HIV load [30, 31], and in vitro studies have demonstrated antagonism between the 2 viruses in cell culture systems [21, 22, 32]. These previous findings are consistent with our finding of an inverse relationship between GBV-C acquisition and higher HIV load. In addition, there was a trend toward higher baseline CD4 cell counts in those who acquired GBV-C, which may reflect an increase in GBV-C target cells.

We found a positive relationship between the number of units transfused and the probability of GBV-C acquisition. A positive cross-sectional relationship between the number of blood units received and GBV-C exposure (defined as detection of GBV-C RNA and/or E2 antibody) has been previously reported in immunocompetent recipients of blood ($P < .001$) [9]. However, several other studies did not find a significant association between GBV-C acquisition and number of blood transfusions [33, 34].

Blood transfusion is associated with a substantial risk of GBV-C acquisition in HIV-infected patients. Prior studies

Table 2. Odds Ratio for GB Virus Type C (GBV-C) RNA Acquisition Among 294 Human Immunodeficiency Virus (HIV)–Infected Viral Activation Transfusion Study Cohort Members

Variable	Odds Ratio (95% Confidence Interval)		
	Unadjusted	Adjusted for Cumulative Units Transfused	Adjusted for all Covariates[a]
Cumulative units of blood transfused (per unit)	1.09 (1.06–1.11)	…	1.08 (1.05–1.11)
Baseline HIV load[b]	0.61 (.40–.92)	0.62 (.40–.96)	0.87 (.54–1.41)
Current use of HAART	4.78 (2.01–11.3)	4.03 (1.79–9.11)	4.75 (2.02–11.18)
Baseline CD4 T cell count[c]	1.04 (.98–1.11)	1.05 (.98–1.12)	…
Nonwhite race (vs white)	0.21 (.06–.81)	0.15 (.04–.60)	0.15 (.04–.64)

ORs were calculated using pooled logistic regression models and estimate discrete relative hazards of GBV-C RNA acquisition.

Abbreviation: HAART, highly active antiretroviral therapy.

[a] Model is adjusted for cumulative units transfused, HIV load at baseline (before transfusion), time-varying HAART use, and race.

[b] Per $\log_{10}$ increase (in copies/mL) from baseline.

[c] Per unit increase from baseline in the square root–transformed count (in cells/μL).

using RT-PCR have reported finding GBV-C RNA in 17%–43% of HIV-infected individuals [17, 35]. Including the 7% of subjects who were GBV-C RNA positive before transfusion, the prevalence of GBV-C viremia in VATS cohort members was estimated to be 14% at 120 days after transfusion. Advanced HIV disease, high HIV load, and low CD4 cell count at baseline are among possible explanations for the low GBV-C viremia prevalence in the VATS cohort.

Previous studies have reported a higher prevalence of GBV-C RNA in immunosuppressed recipients of multiple transfusions. However, their study populations did not include HIV-infected subjects [9, 36]. Additional possible explanations for the higher prevalence of GBV-C infection found in previous studies could be geographic differences in the prevalence or differences in the laboratory methods used to detect GBV-C RNA, as many prior studies used nested RT-PCR, which has a greater sensitivity then the method used in our study [9, 17, 32, 37]. On the other hand, our estimate of the incidence of new GBV-C infection may be an underestimate, because of classification of E2 antibody–positive subjects as GBV-C RNA negative. There were 12 E2 antibody–positive subjects in the final sample at 120 days (Figure 1), and transient antibody positivity (defined as the presence of ≥1 posttransfusion E2-positive sample) was observed in 9 subjects at some point during follow-up and not necessarily in the final sample. This finding could be due to either undetected transient GBV-C viremia with subsequent E2 antibody formation or to passive transfusion of donor E2 antibody to recipients. We cannot distinguish between the 2 possibilities.

Our findings show a significantly higher incidence rate of GBV-C infection among white subjects, compared with nonwhite subjects, after adjustment for cumulative units transfused, baseline HIV load, and current use of HAART. In the original VATS cohort (n = 489), the pretransfusion prevalence of GBV-C viremia was not significantly different between whites and nonwhites (data not shown). Studies have reported a higher prevalence of GBV-C viremia among healthy blood donors in Africa [38]. In addition, HIV-infected African American women participating in the Women's Interagency HIV Study had a slightly higher prevalence of GBV-C viremia than HIV-infected women from other racial groups [39]. These findings may suggest that there are different predisposing factors for GBV-C infection among various populations.

Previous studies of transfusion-associated GBV-C infection analyzed cross-sectional samples of prevalent GBV-C infection among various groups at high risk for parenteral transmission, such as hemophiliacs, recipients of multiple transfusions, injection drug users, and transplant recipients [9, 40, 41] or patients infected with HIV [42, 43]. Acquisition of GBV-C RNA following transfusion has been studied longitudinally in recipients of multiple transfusions [6, 14, 33, 34, 44]. GBV-C infection has also been studied longitudinally in populations with known dates of HIV infection to examine the impact of prevalent GBV-C viremia on the risk of HIV acquisition [11, 45] or HIV disease progression [37, 45, 46]. Bisson et al documented a 22% higher prevalence of GBV-C viremia among those with recent HIV infection [45]. Also, Supapol et al showed that acquisition of GBV-C by infants was associated with decreased mother-to-child transmission of HIV [11]. To our knowledge, our study is the first to quantify the risk of GBV-C acquisition as a function of units transfused in HIV-infected patients receiving a transfusion and to examine pretransfusion HIV status as a predictor of subsequent GBV-C acquisition in this setting.

Our study has a number of limitations. First, while GBV-C RNA measurements were performed on pretransfusion and final samples for all subjects, interim samples were tested only for subjects with evidence of incident viremia or acquisition of

antibody. Imputation of negative results for the remaining samples could have resulted in the underestimation of GBV-C incidence if transient viremia occurred and was missed. Further, we acknowledge that our reported GBV-C incidence is a conservative estimate since testing occurred only at fixed intervals and, thus, the first detection of GBV-C RNA systematically overestimated the time of seroconversion. Second, our sample size was small, and only 22 incident GBV-C RNA infections were observed; however, this study contains the largest number of documented posttransfusion incident GBV-C infections reported to date. Third, the VATS public use dataset did not include data on hepatitis B virus or HCV infection or liver enzyme values. Previous reports have documented a higher prevalence of GBV-C RNA among patients with active or chronic HCV infection [47, 48]. There is very limited evidence concerning interactions among HIV, HCV, and GBV-C [49]. Fourth, our binary classification of HAART use included subjects who were enrolled into the study before the HAART era, as well as those who were enrolled or followed after June 1996. Thus, the ''no HAART'' group included subjects who never received any antiretroviral therapy, as well as those who were receiving some combination of drugs that did not qualify as HAART. Fifth, we cannot exclude GBV-C acquisition by routes other than transfusion (ie, sexual or nosocomial parenteral exposures) in our study population. However, given the health status of the VATS cohort, the temporal relationship, and lack of previous GBV-C E2 antibody among recipients before transfusion, it is reasonable to assume that transfusion was the most likely route of acquisition in this cohort. To confirm transmission of GBV-C via transfusion, detailed RNA sequence analysis of the donor and recipient posttransfusion samples would be required. Testing of recipient and donor specimens for GBV-C RNA and E2 antibody was not conducted as part of the VATS study. The testing of recipient specimens for GBV-C RNA and E2 antibody was conducted as part of a secondary study conducted after VATS was completed, using existing recipient specimens. Furthermore, the Roche assays used in this secondary study are no longer available, so it is not possible to test donor specimens for GBV-C RNA and E2 antibody by use of the same reagents as those for tested recipients.

Our study provides evidence of high rates of GBV-C transmission by transfusion in HIV-infected subjects, as well as an increased odds of GBV-C acquisition with lower pretransfusion HIV load and current use of HAART, after control for the cumulative numbers of units of blood transfused. Our findings thus confirm previous reports showing an inverse relationship between HIV load and GBV-C viremia [30] and in vitro interference between HIV and GBV-C [21, 22]. The present study provides support for the theory that having a lower HIV load facilitates GBV-C acquisition and could explain the lower HIV loads among GBV-C–positive patients reported in previous studies. Further investigation to clarify the mechanisms by which GBV-C and HIV interact is warranted. Establishing evidence for transfusion transmission of GBV-C will allow additional studies of the impact of acute GBV-C acquisition on the course of HIV infection in coinfected patients.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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