

GBV-C Infection and Risk of NHL among U.S. Adults

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Abstract

Some retrospective studies suggest an association between infection with GB virus-C (GBV-C) and non-Hodgkin lymphoma (NHL). We evaluated this association prospectively in a nested case-control study within the U.S. Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Cases ($N = 658$) and controls ($N = 1,316$) were individually matched by age, sex, race/ethnicity, timing of study entry, and sample selection. Prediagnostic PLCO serum samples were tested for GBV-C RNA (as a measure of active infection) and E2 antibody (active or resolved infection). Logistic regression was used to estimate odds ratios (OR) for the association between GBV-C and NHL overall and NHL subtypes. Twelve cases (1.8%) and seven controls (0.5%) were GBV-C RNA-positive. GBV-C RNA positivity was associated with NHL overall [OR, 3.43; 95% confidence interval (CI), 1.35–8.71] and, based on small numbers, diffuse large B-cell lymphoma (OR, 5.31; 95% CI, 1.54–18.36). The association with NHL persisted when the interval between testing and selection was greater than 4 years (OR, 6.00; 95% CI, 1.21–29.73). In contrast, E2 antibody positivity was not associated with NHL risk (OR, 1.08; 95% CI, 0.74–1.58). Our study demonstrates that GBV-C infection precedes development of NHL. GBV-C infection may play an etiologic role in a small proportion of NHL cases, perhaps by causing chronic immune stimulation or impaired immunosurveillance. *Cancer Res*; 74(19); 5553–60. ©2014 AACR.

Introduction

Discovered in the mid-1990s, GB virus-C (GBV-C; also known as hepatitis G virus) is a single-stranded RNA virus belonging to the *Pegivirus* genus within the family *Flaviviridae* (1–3). GBV-C is the most closely related human virus to hepatitis C virus (HCV; refs. 2–4). GBV-C is transmissible through blood and sexual exposure, particularly through blood transfusions and injection drug use (4). Following initial infection, most people clear GBV-C within a few years, but the virus can cause chronic infection in an estimated 20% to 30% of people (5, 6). The prevalence of detectable GBV-C viremia is 1% to 2% among U.S. blood donors (4).

Non-Hodgkin lymphoma (NHL) is the sixth most common cancer in U.S. men and women (7). Severe immune dysregulation due to AIDS, organ transplantation, or primary immune deficiencies strongly increases NHL risk, but it only accounts for a fraction of NHL cases (8). Direct transformation by infectious agents, including Epstein-Barr virus and human herpesvirus 8, or chronic immune stimulation by HCV and

Helicobacter pylori, are thought to play a role in some NHL subtypes (9).

Several considerations support the fact that GBV-C could also contribute to development of NHL. GBV-C RNA is commonly detected in bone marrow and spleen tissue (10, 11), and GBV-C has been demonstrated to replicate *in vitro* in peripheral blood mononuclear cells, particularly B and T lymphocytes (12, 13). Chronic GBV-C infection has been associated with decreased apoptosis of T cells, impaired T-cell activation and proliferation, and changes in immune responsiveness that may contribute to lymphomagenesis (14–16).

A recent Canadian case-control study of 553 cases and 438 controls evaluated the association of GBV-C and NHL, reporting a significantly higher prevalence of GBV-C viremia in cases than in controls (4.5% vs. 1.8%; adjusted odds ratio, 2.7; 95% CI, 1.2–6.7; ref. 17). Prior case-control studies had fewer cases and widely varying results (overall ORs, 1.1–14.4), although most studies suggested an association (18–24). Notably, these previous studies tested for GBV-C infection retrospectively, after development of NHL, which could have led to bias. Along these lines, blood transfusion is a possible route of transmission for GBV-C infection, so assessment of exposure in NHL cases who previously received blood transfusions (e.g., as therapy for anemia arising during chemotherapy) could have been a major source of confounding. It is also possible that as people develop NHL, weakened immunity could lead to GBV-C reactivation, causing an increase of detectable viremia. If such a disease effect was present, it could lead to an artifactual association in a retrospective study design. In addition, controls were often drawn from nonrepresentative convenience groups (i.e., blood donors and patients with cancer).

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We evaluated NHL risk associated with GBV-C infection in a case-control study nested in a large, U.S. general population cohort. Our study is the first to prospectively assess GBV-C status using prediagnostic serum specimens for the NHL cases. We tested subjects both for active infection as indicated by viremia (i.e., the presence of GBV-C RNA in serum) and for either active or resolved infection (as indicated by the presence of antibodies to the E2 viral capsid protein).

Patients and Methods

Study design, study population, and subject selection

The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial cohort consists of approximately 150,000 men and women between the ages of 55 and 74 years, who were randomized to enter either a screening program for several cancers (~75,000 subjects) or usual care, with the goal of determining the effects of screening on cancer-related mortality (25). Subjects were enrolled in 10 U.S. sites between 1993 and 2001. Screening arm participants received annual examinations for up to 6 years, including blood draws at baseline and 5 subsequent years (T0–T5), and were then followed for up to 12 additional years. All participants provided informed consent. For the present study, we excluded participants having a prior history of cancer, no follow-up time contributed to PLCO, a rare non-NHL cancer (as defined by the PLCO), no baseline questionnaire, no consent for etiology research, or inadequately stored serum.

Cases were defined as participants diagnosed with pathologically confirmed NHL occurring after enrollment and through April 30, 2011. Controls were eligible if they had not developed NHL at the time the case was diagnosed (measured from baseline) and were individually matched to cases 2:1 by age at baseline (5-year age groups), sex, race/ethnicity, calendar year of entry, time on study, and study year of blood draw.

Biologic specimen testing

PLCO serum samples selected for testing for GBV-C RNA (to detect viremia, or active infection) and E2 antibody (to detect active or resolved infection) were closest in time to, but preceding, NHL diagnosis or control selection (i.e., proximal samples). Testing was performed at one site (University of Iowa, Iowa City, IA) and laboratory personnel were blinded to case-control status. The testing of PLCO samples was approved by the Institutional Review Boards of the National Cancer Institute (Rockville, MD) and the University of Iowa.

Because of the lack of approved or commercial tests, the University of Iowa laboratory developed and optimized methods for detecting GBV-C RNA and antibodies (26). RNA was extracted from 20 μ L of serum using the QIAamp Viral RNA Mini Kit for amplification by a one-step reverse transcription real-time polymerase chain reaction (RT-PCR) as described previously (16, 26). RNA was amplified using 5'-NTR primers and a 6-FAM/TAMRA-labeled probe using the SuperScript II Platinum One-step Quantitative RT-PCR System (Invitrogen). Oligonucleotide primers were: sense 5'-GGCGACCGGC-CAAAA-3' [nucleotide (nt) 96-110]; antisense 5'CTTAAGACC-CACCTATAGTGGCTACC (nt 163-188); and probe 5'-FAM-

TGACCGGGATTACGACCTACCAACCCT-TAMRA (nt 131-158). Samples were amplified in an ABI Prism 7700 sequence detector at 50°C \times 50 minutes, 95°C \times 2 minutes, followed by 40 cycles (58°C \times 15 seconds, 72°C \times 1 minutes). Four negative control wells (two blanks and two water controls) were included with each set of samples, and a standard curve was generated using synthetically transcribed GBV-C RNA (26–28). If the calculated RNA concentration was 50,000 copies/mL or greater, then the result was considered positive.

Additional quality control measures were taken to ensure validity and reproducibility of GBV-C RNA testing. On all experiments, the standard curve for eight positive controls using the synthetic GBV-C RNA was reviewed; the cycle threshold (C_T) for the highest concentration was required to be within 1.0 C_T of the mean value (17.5), and the linear regression analysis of the standard curve values was required to have an $R^2 \geq 0.98$ before the experiment was accepted. In addition, the four negative control samples were required to be negative. If these criteria were not met, the RNA samples were retested. In addition to these known laboratory controls, a blinded set of positive and negative controls, provided by the testing laboratory, were aliquoted by a different laboratory and incorporated randomly into the tested batches (one set for each of 25 batches). To account for possible batch effects, cases and their matched controls were tested together, in a random order within the matching group.

Positive and negative results were initially based on an algorithm that incorporated both RT-PCR (as described above) and four nested PCR assays (using 5'-NTR and three E2 primer sets) in efforts to detect additional GBV-C infections. We assessed the reproducibility of this approach by retesting a set of PLCO samples (10 control subjects GBV-C RNA-positive according to this algorithm, and 10 control subjects GBV-C RNA-negative according to the algorithm) in a subsequent run. This duplicate testing revealed that this algorithm using the additional nested PCR primers was unreliable (data not shown), so we defined GBV-C positivity based on RT-PCR only. Positivity based on RT-PCR was reliable: all three positive control samples that were selected for retesting tested positive again, and 16 of 17 negative control samples retested negative. Thus, all of the GBV-C results reported in this article are based on the RT-PCR testing only.

Samples testing GBV-C RNA-positive at the proximal time point (indicating active infection) were further tested using another PLCO sample at the earliest available (i.e., most distal prediagnostic) time point to determine whether these infections were persistent. Because the selection was based on positivity as defined by the nested PCR algorithm, five samples (all NHL cases) that were positive based only on RT-PCR, but negative based on the algorithm, were not selected for testing at the distal time point.

To measure GBV-C antibodies, a solid-phase enzyme-linked immunosorbent assay (ELISA) based on a recombinant GBV-C E2 protein expression system was developed. Briefly, recombinant E2-Fc fusion protein (2 μ g/mL in carbonate buffer; ref. 29) was used to coat ELISA plates, which were stored at 4°C until use. Following washing, patient samples (diluted 1:200) were added to the wells for

1 hour at 37°C. Plates were washed, alkaline phosphatase-conjugated, and then anti-human λ/κ immunoglobulin light chain was added for 1 hour at 37°C. The reaction was developed with addition of para-nitrophenylphosphate substrate (incubated at 37°C), and absorbance was determined at A405 nm. All samples were tested in duplicate, and wells coated with E2-Fc protein were compared with wells coated with bovine serum albumin (BSA). A positive result was defined as absorbance values for the E2 well greater than 1.00 ELISA unit and 2.5 times that of the control BSA well.

Because GBV-C shares at least one transmission route with HCV and HIV (i.e., injection drug use) and both other viruses are associated with NHL (8, 9), we also tested for HCV and HIV infection in the proximal samples using clinically licensed antibody tests. Positive results were confirmed or evaluated for active infection by measuring viral RNA. HCV and HIV testing was performed on anonymized samples, limited to GBV-C RNA-positive cases and controls.

Statistical analysis

OR and 95% confidence intervals (CI) for the association between GBV-C and NHL overall were calculated using conditional logistic regression to adjust for the matching factors using PROC PHREG (SAS version 9.3). Using the measurements from the proximal time point, we first examined NHL risk related to GBV-C RNA positivity (i.e., active infection) as well as E2 antibody positivity (i.e., active or resolved infection). We also examined risk in relation to combinations of viremia and antibody positivity relative to negative status for both markers.

The risk of specific NHL subtypes was examined, since the Canadian study reported a particularly strong association for diffuse large B-cell lymphoma (DLBCL; ref. 17). We broke the individual matching of cases and controls and used polytomous regression (PROC LOGISTIC) to maximize the power by comparing each subgroup with all of the controls. In an additional analysis, we examined whether associations differed by latency, that is, the time between the proximal time point assessed for GBV-C infection and NHL diagnosis/selection. We also compared the mean viral RNA levels (\log_{10} copies/mL) between viremic cases and controls using PROC TTEST.

To evaluate the potential confounding effect of HIV and HCV on the association between GBV-C viremia and NHL, we excluded coinfecting people in a sensitivity analysis. Specifically, given the anonymized nature of the HCV and HIV testing, only grouped data were used in the sensitivity analysis. A 2×2 table showing the relationship between GBV-C viremia and NHL was created, and the number of GBV-C viremic cases and GBV-C viremic controls who tested positive for HCV or HIV was subtracted from the corresponding cells. Finally, the crude OR corresponding to this revised table was calculated.

To estimate the proportion of NHL cases that might be linked to GBV-C infection, the population attributable risk (PAR) was calculated on the basis of the formula: $PAR = P_e (RR - 1) / [P_e (RR - 1) + 1]$, where P_e is the prevalence of the exposure (GBV-C viremia) in controls, and RR is the relative risk (OR) associating GBV-C viremia and NHL (30).

Results

The characteristics of the cases ($N = 658$) and individually matched controls ($N = 1,316$) are presented in Table 1. The cases and controls were largely male (60.6%) of ages 60 to 69 years at randomization into the PLCO trial (57.6%), and white (94.1%). NHL developed 7.0 years on average after randomization, and the mean number of years from the proximal serum sample to NHL diagnosis was 3.7 years.

Overall, there were a total of 19 GBV-C RNA-positive cases and controls. Among the blinded positive and negative control samples incorporated into each batch, 92% (23 of 25 samples) of positive controls and 92% (23 of 25) of negative controls were correctly classified on the basis of RT-PCR testing. Twelve cases (1.8%) and seven controls (0.5%) were GBV-C RNA-positive. GBV-C RNA positivity was thus associated with NHL overall (OR, 3.43; 95% CI, 1.35–8.71; $P = 0.010$; Table 2). Risks of each NHL subtype were all elevated, but the association was statistically significant only for DLBCL (OR, 5.31; 95% CI, 1.54–18.36).

An association was apparent when the interval between GBV-C RNA measurement and case-control selection was greater than 4 years (mean interval = 7.3 years; OR, 6.0; 95% CI, 1.21–29.73; Table 3). Among the GBV-C RNA-positive subjects who were also tested at a distal time point (i.e., earlier in time), there was a suggestion that cases were more frequently positive at both time points than controls [4 of 7 (57%) vs. 2 of 7 (29%)], although this difference was not significant ($P = 0.280$). As mentioned in the Materials and Methods, 5 positive cases were not selected for testing at the distal time point.

On the basis of the 7 positive controls and 12 positive cases, viral RNA levels were higher in controls than cases (\log_{10} copies/mL = 7.8 vs. 5.9, $P = 0.006$). Furthermore, based on small numbers, the viral load varied across NHL subtypes (mean \log_{10} copies/mL ranging from 5.2 to 8.1), with the lowest levels observed among DLBCL cases (mean \log_{10} copies/mL = 5.2).

There were no differences in the prevalence of E2 antibody between cases and controls, with 45 (6.8%) and 84 (6.4%) testing positive, respectively ($P = 0.694$). Among E2 antibody-positive subjects, 1 (0.8%) was also GBV-C RNA-positive; among E2 antibody-negative subjects, 18 (1.0%) were also GBV-C RNA-positive. E2 antibody positivity was not associated with NHL overall (OR, 1.08; 95% CI, 0.74–1.58), but was significantly associated with "other" NHL subtypes (OR, 1.96; 95% CI, 1.07–3.56; Table 2). NHL was not associated with E2 antibody status in any of the follow-up periods (Table 3).

Using GBV-C uninfected subjects (i.e., negative for both GBV-C RNA and E2 antibody) as the reference group, resolved infection (i.e., GBV-C RNA-negative but E2 antibody-positive) was not associated with NHL (OR, 1.07; 95% CI, 0.73–1.58). In contrast, active infection (GBV-C RNA-positive) was significantly associated with NHL (OR, 3.45; 95% CI, 1.36–8.76; Table 4).

None of the GBV-C RNA-positive cases or controls was HIV antibody-positive. One GBV-C RNA-positive case was HCV antibody-positive; however, this sample did not have HCV RNA detected. Therefore, the antibody test represented either

Table 1. Comparison of NHL cases and controls

Characteristic	Cases (N = 658) N (%)	Controls (N = 1,316) N (%)
Sex		
Male	399 (60.6)	798 (60.6)
Female	259 (39.4)	518 (39.4)
Age at randomization, y		
55–59	166 (25.2)	332 (25.2)
60–64	197 (29.9)	394 (29.9)
65–69	182 (27.7)	364 (27.7)
70–74	113 (17.2)	226 (17.2)
Race/ethnicity		
White, non-Hispanic	619 (94.1)	1,238 (94.1)
Black, non-Hispanic	13 (2.0)	26 (2.0)
Hispanic	7 (1.1)	14 (1.1)
Asian/Pacific Islander	18 (2.7)	36 (2.7)
American Indian	1 (0.2)	2 (0.2)
Year of randomization ^a		
1994–1995	205 (31.2)	410 (31.2)
1996–1998	294 (44.7)	588 (44.7)
1999–2001	159 (24.2)	318 (24.2)
Study year of serum sample ^b		
0	46 (7.0)	92 (7.0)
1	62 (9.4)	124 (9.4)
2	208 (31.6)	416 (31.6)
4	74 (11.3)	148 (11.3)
5	268 (40.7)	536 (40.7)
Randomization to serum sample, mean years (SD)	3.3 (1.8)	3.3 (1.8)
Serum sample to NHL diagnosis, mean years (SD)	3.7 (3.3)	N/A
Randomization to NHL diagnosis, mean years (SD)	7.0 (3.8)	N/A

^aFollowing prior PLCO methods, year of randomization was defined according to fiscal year, defined as the 1-year period starting October 1 of the previous calendar year.

^bNo serum was collected in study year 3.

resolved HCV infection or a false-positive test. After subtracting the HCV-positive NHL case from the exposed group, the association between GBV-C infection and NHL remained (crude OR, 3.18; 95% CI, 1.23–8.25).

On the basis of the prevalence of GBV-C infection and the magnitude of association with NHL in our study (where $P_e = 0.005$ and $RR = 3.43$ from our results), we estimate that 1.2% of NHL cases might be linked to GBV-C infection, that is, $0.005(3.43-1)/[0.005(3.43-1) + 1] = 0.012$.

Discussion

In the present U.S. case-control study, active GBV-C infection, as indicated by GBV-C RNA positivity, was associated with an increased risk of NHL overall (OR, 3.43). Risk was elevated across all subtypes, but estimates were imprecise due to small numbers and statistically significant only for DLBCL (OR, 5.31). Of note, we show for the first time that GBV-C infection precedes the development of NHL by several years, and that the association with NHL risk appeared strongest at the longest latency (4 years or more

after testing; OR, 6.00). In contrast, resolved infection (antibody positivity in the absence of viremia) was not associated with NHL risk.

Our findings are consistent with the findings of the large, population-based Canadian case-control study in which associations of GBV-C viremia with NHL overall (OR, 2.7) and with DLBCL (OR, 5.2) were also reported (17). The consistency of the findings is remarkable considering the differences between the two studies. First, although both were matched case-control studies, the Canadian study obtained samples from NHL cases after they were diagnosed, whereas our samples were obtained before NHL diagnosis. Second, the prevalence of GBV-C viremia was higher among the Canadian controls (1.8%) compared with ours (0.5%), which may, in part, be explained by the differences in age distribution. Canadian controls were of ages 20 to 79 years, compared with our controls of ages 55 years and older, and previous studies found an inverse relationship between GBV-C viremia prevalence and age among adults (31, 32). Finally, the RT-PCR assays used to detect the GBV-C infection used different primers and were performed in

Table 2. Associations of GBV-C infection with NHL overall and NHL subtypes

	Total N	GBV-C RNA positivity ^a		E2 antibody positivity ^b	
		N (%)	OR (95% CI)	N (%)	OR (95% CI)
Controls	1,316	7 (0.53)	1.00 (reference)	84 (6.38)	1.00 (reference)
All NHL cases	658	12 (1.82)	3.43 (1.35–8.71)	45 (6.84)	1.08 (0.74–1.58)
NHL cases, by subtype					
DLBCL	145	4 (2.76)	5.31 (1.54–18.36)	8 (5.52)	0.86 (0.41–1.81)
Follicular lymphoma	88	2 (2.27)	4.35 (0.89–21.26)	1 (1.14)	0.17 (0.02–1.23)
CLL/SLL	249	4 (1.61)	3.05 (0.89–10.51)	19 (7.63)	1.21 (0.72–2.03)
Other NHL	119	1 (0.84)	1.59 (0.19–13.00)	14 (11.76)	1.96 (1.07–3.56)
Unknown NHL subtype	52	1 (1.92)	3.67 (0.44–30.37)	3 (5.77)	0.90 (0.27–2.94)

Abbreviation: CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma.

^aReferent group is RNA-negative subjects.

^bReferent group is E2 antibody-negative subjects.

different laboratories. Thus, the differences in the prevalence of GBV-C viremia may reflect the different methodologies (in addition to differences in study populations), and it is possible that our PCR assay was less sensitive than that of the Canadian study. The positive associations observed in the Canadian study and ours are consistent with results from previous but less definitive case-control studies (18–24). Some of these studies included NHL cases coinfecting with HCV (18, 21, 24), and the controls in some studies were drawn from convenience groups (blood donors and patients with cancer) that were not representative of the source population giving rise to the NHL cases (18, 20, 21, 23).

We found that among the GBV-C RNA-positive subjects who were also tested at a distal time point (i.e., earlier in time), there was a suggestion that cases were more frequently positive at both time points than controls [4 of 7 (57%) vs. 2 of 7 (29%)], although this difference was not significant ($P = 0.280$). Following transfusion acquired infection, the majority of infected people clear viremia within two years, although viremia lasting for decades has been described previously (33, 34). GBV-C viremia has been documented to persist for more than 5 years in both HIV-uninfected people and HIV-coinfecting people (5, 6). We therefore expected that some people with viremia would have had persistence at two

time points separated by up to several years, whereas others with viremia would have had a new infection.

Of further interest, among the 19 subjects with viremia, viral RNA levels were higher in controls than cases (mean \log_{10} copies/mL = 7.8 vs. 5.9; $P = 0.006$). Also, based on small numbers, the mean \log_{10} viral load across NHL subtypes varied, with lower levels among DLBCL cases. We speculate that the higher viral loads among controls may indicate acute infection, whereas the lower viral loads in cases may indicate a chronic, persistent infection that would increase risk of NHL. HIV serves as a well-documented example of high viral load during acute infection with a reduction over time (35).

Although serum GBV-C RNA is a marker of active viremia, serum E2 antibody is generally thought to serve as a marker of a resolved infection. Unfortunately, the lack of a standardized E2 antibody assay, and the fact that E2 antibody may fade to levels below the limit of detection over time, limit the usefulness of E2 antibody as an accurate marker of resolved infection. The E2 antibody detection method used in the current study has been shown to detect antibody elicited by recombinant E2 protein in mice and rabbits (36). However, it has not been directly compared with previously available assays that found an inverse relationship between

Table 3. Latency analysis of GBV-C infection and NHL

Latency	GBV-C RNA positivity ^a			E2 antibody positivity ^b		
	Controls	Cases	OR (95% CI)	Controls	Cases	OR (95% CI)
	N (%)	N (%)		N (%)	N (%)	
0–0.99 y	2 (0.58)	3 (1.73)	3.00 (0.50–17.93)	20 (5.78)	8 (4.62)	0.79 (0.35–1.83)
1–3.99 y	3 (0.64)	3 (1.28)	2.00 (0.40–9.91)	31 (6.62)	16 (6.84)	1.04 (0.55–1.95)
4.00+ y ^c	2 (0.40)	6 (2.39)	6.00 (1.21–29.73)	33 (6.57)	21 (8.37)	1.33 (0.73–2.42)

^aReferent group is RNA-negative subjects.

^bReferent group is E2 antibody-negative subjects.

^cMean, 7.3 years (range, 4.0–14.6 years).

Table 4. Association of GBV-C infection with NHL based on combined RNA and E2 antibody testing

GBV-C infection status	Cases N (%)	Controls N (%)	OR (95% CI)
Uninfected: RNA-negative, E2-negative	602 (91.49)	1,225 (93.09)	1.00 (reference)
Resolved: RNA-negative, E2-positive	44 (6.69)	84 (6.38)	1.07 (0.73–1.58)
Active: RNA-positive	12 (1.82)	7 (0.53)	3.45 (1.36–8.76)

viremia and E2 antibodies in humans (37, 38). Our finding of a high rate of concurrent GBV-C viremia and E2 antibody differs from those prior reports. Although further studies are needed to better characterize the humoral response to GBV-C, the lack of association between E2 antibody positivity and risk of NHL may not be surprising. One possibility is that resolved infection may indicate an ability to normalize lymphoma risk, whereas persistently active infection indicates chronic immune disturbances and elevated NHL risk.

Our study has several important strengths. Cases and controls were drawn from a prospective cohort, therefore minimizing potential for selection bias. We had a relatively large sample size (658 cases and 1,316 controls), necessary to examine with good precision the association of a rare exposure such as GBV-C with NHL. Because NHL subtypes are increasingly recognized as distinct entities, our data regarding NHL subtypes were a further strength, and we identified a significant association between GBV-C infection and DLBCL.

In addition, we evaluated GBV-C infection status using prediagnostic samples in cases, obtained on average 3.7 years before NHL. This aspect of our study design is unique among studies that have assessed the association between GBV-C and NHL, and it was critical for minimizing potential disease or treatment effects on GBV-C status. Also, the availability of serial samples in the PLCO cohort enabled us to assess GBV-C persistence. The findings here were based on small numbers, but they suggested an association between persistent viremia and NHL. Finally, we assessed the possibility of confounding by HIV and HCV by testing for these infections in the GBV-C-infected cases and controls.

The main limitation of our study was the small number of people with active GBV-C infection, which limited the precision of our estimates. In particular, although we observed a significantly elevated risk of DLBCL associated with GBV-C viremia, the CIs were quite wide. We also observed elevated, but not statistically significant, ORs across the other NHL subtypes. It is notable that the OR for DLBCL was still statistically significant among samples collected 4+ years before NHL diagnosis (OR, 13.6; 95% CI, 2.2–83.4), though this was based on only 3 exposed DLBCL cases (data not shown). Larger studies are needed to determine whether GBV-C viremia is more strongly associated with DLBCL than other NHL subtypes. Another limitation, noted above, is the lack of standard, commercially available RNA and E2 antibody assays. In particular, E2 antibody positivity was not associated with NHL overall but was

associated with "other" (miscellaneous or unspecified) NHL subtypes. Because the sensitivity and specificity of the antibody test are unknown, it is difficult to interpret this result, and we put greater emphasis on the associations with viremia. Indeed, the results for the RNA testing were strengthened because we incorporated a number of quality control measures to confirm the reliability and reproducibility of the RNA assay (i.e., known positive and negative controls, masked PLCO duplicates).

The association between HCV and NHL can serve as a model for understanding a possible etiologic relationship between GBV-C and NHL. HCV is an established NHL risk factor (39). Although HCV has been reported to infect lymphocytes, the virus neither integrates into the host genome nor contains a known oncogene, and HCV RNA is not detectable in NHL tumors (40) HCV is thought to increase risk of NHL through chronic host infection and long-term immune stimulation (40, 41), although direct effects on B cells are possible (42–44). GBV-C is structurally similar to HCV, and the mechanism by which it might cause NHL could be similar. Furthermore, GBV-C infection has been associated with decreased apoptosis of T cells that may contribute to the prolonged survival of HIV-infected patients coinfecting with GBV-C (14). Recent data showing that GBV-C infection reduces T-cell activation and proliferation in HIV-infected individuals suggest that GBV-C infection may impair immune surveillance and immune responsiveness, which may lead to lymphomagenesis (15, 45, 46).

In conclusion, our prospective study adds support that GBV-C infection is a risk factor for NHL. Further research is needed to confirm the GBV-C association with NHL in diverse populations. In addition, future studies should be conducted to understand immune-related mechanisms related to persistent GBV-C infection, which would inform a mechanistic understanding and assessment of whether the association is causal. The GBV-C association with NHL may have public health implications because the blood supply is not screened for GBV-C, and an estimated 1,000 units of GBV-C RNA-containing blood products are transfused daily in the United States (47). On the basis of our calculations, we estimate that about 1.2% of NHL cases might be linked to GBV-C infection. Ultimately, demonstration of a causal association with cancer would provide the rationale for a formal evaluation of the costs and benefits of screening donors for GBV-C and eliminating contaminated units of blood.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors' Contributions

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