Prevalence, Incidence, and Clinical Characteristics of Hepatitis G Virus/GB Virus C Infection in Scottish Blood Donors

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The prevalence, incidence, clinical features, and natural history of hepatitis G virus (HGV) or GB virus C (GBV-C) were investigated in a non-remunerated blood donor population to determine its clinical significance and its impact on blood safety. Of 1020 regular blood donors, 23 (2.25%) were positive for plasma HGV/GBV-C RNA. Alanine aminotransferase levels were lower than in uninfected donors (median, 20 IU/mL; 32 IU/mL in controls; $P = .015$). Clinical examination produced no other evidence for hepatitis or for shared nonhepatic diseases. Fifteen of 17 donors excreted HGV/GBV-C in saliva (mean level, 8×10^3 copies of RNA/mL). Testing of previous donations indicated an incidence of 170–200 new infections with HGV/GBV-C per 100,000 donor-years. The absence of further clinicopathologic data and the limitations of current polymerase chain reaction–based methods for screening suggests that it is neither necessary nor practical to commence screening.

Hepatitis C virus (HCV) has been identified as the principal cause of posttransfusion hepatitis, while the development of serologic tests for blood donor screening has led to a dramatic fall in its incidence. However, in 10% of occurrences of posttransfusion hepatitis and 20% of community-acquired hepatitis, no etiologic agent has been identified. GB virus C (GBV-C) [1] or hepatitis G virus (HGV) [2] has been proposed as the etiologic agent of these residual cases. In this study, we aimed to determine the prevalence of HGV/GBV-C in a large sample of the Scottish donor population and the natural history of this population's infection with the virus and to assess the clinical significance and possible risk factors for transmission of infection in HGV/GBV-C–positive donors.

Subjects and Methods

Subjects. From October 1996 until December 1996, 1020 blood samples were collected from volunteer, consecutive, regular blood donors attending the Edinburgh and Southeast Scotland Blood Transfusion Service center. Consent to participate in the screening program was obtained from 1020 (97%) of 1052 donors interviewed. All subjects were seronegative for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and HCV. All HGV/GBV-C–positive donors were contacted and invited to attend for counseling and further investigation. At their initial consultation, repeat sampling for plasma HGV/GBV-C RNA and alanine aminotransferase (ALT) measurement was undertaken, and a history of risk behavior was sought. In addition, a sample of saliva was collected for testing for HGV/GBV-C RNA. All positive donors were referred to a single hepatologist for clinical evaluation.

Detection of HGV/GBV-C RNA and antibody in plasma. Detection and quantitation of HGV/GBV-C was done as previously described [3] on RNA extracted from 100 μL of EDTA–anticoagulated plasma or citrated plasma from blood donors that was combined into pools of 10. Positive pools were subdivided into component donations and retested. Initial screening identified 23 positive pools, which led to the identification of one positive component donation in each. RNA was also extracted from 100-μL volumes of saliva samples as described for plasma samples. IgG antibodies to GBV-C/HGV E2 antigen were detected by assay (PLATE Anti-HGenv; Boehringer Mannheim, Mannheim, Germany).

Results

Prevalence of HGV/GBV-C in blood donors. Twenty-three (2.25%) of 1020 healthy blood donors were positive for HGV/GBV-C RNA in plasma (11 men [mean age, 37.9 years] and 12 women [mean age, 33.3 years]; table 1). Nineteen returned for further investigation ~10 weeks after donation, and 18 remained HGV/GBV-C RNA–positive. HGV/GBV-C was detected in 13 of 17 saliva samples from HGV/GBV-C–viremic donors collected at that time. Levels in saliva were ~3 log lower than found in plasma (geometric means, 7.8×10^3 and 7.4×10^6 copies of HGV/GBV-C RNA/mL, respectively; table 1), but levels showed a weak correlation ($R = .442$, $P = .05$ [Spearman's rank correlation test]). The donor who had become

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Consent for HGV/GBV-C testing, recall, and examination was obtained from all blood donors who participated in the study. The study was approved by the Lothian Research Ethics Committee.

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Table 1. Epidemiologic and clinical characteristics of HGV/GBV-C–infected blood donors.

Donor[a]	Age	Sex	ALT, IU/mL	Previous donations	Plasma copies/mL[b]	Saliva copies/mL[b]	Duration of Infection,[c] years
p12	20	M	25	6	5×10^6	<100	1
p11	21	F	18	10	5×10^8	5×10^4	>2
p15	25	F	18	12	5×10^7	5×10^3	1
p16	26	F	20	17	5×10^8	5×10^4	2
p8	27	F	13	6	5×10^7	5×10^4	6
p17	27	M		14			>8
p6	28	F	18	19	<100	4×10^3	5
p9	28	F	15	9	5×10^6	5×10^4	6
p5	29	M	38	6	5×10^7	5×10^5	10
p3	30	M	47	24	5×10^5	4×10^3	2
p2	32	F		7			9
p14	32	M	33	7			>5
p4	36	F		6			>11
p18	39	M	30	28	5×10^6	5×10^3	6
p19	39	F	27	19	5×10^8	5×10^3	8
p13	40	F	15	12	5×10^7		4
p22	44	M		42			7
p7	45	F	19	11	5×10^7	5×10^3	>10
p1	47	M	20	24	5×10^6	5×10^5	7
p20	48	M	34	47	5×10^7	5×10^3	9
p10	50	M	8	8	5×10^7	5×10^4	3
p21	51	M	43	56	3×10^4	<100	9
p23	53	F	31	47	5×10^6	5×10^5	9

NOTE. ALT, alanine aminotransferase.

[a] Donors are ranked by age.

[b] HGV/GBV-C load in plasma and saliva from donor on recall.

[c] Approximate duration of HGV/GBV-C infection; time of infection is calculated as midpoint between last polymerase chain reaction–negative and first polymerase chain reaction–positive donation; in most cases these are minimum estimates, as donors have been followed prospectively for at longest 1 year (figure 1).

polymerase chain reaction (PCR)–negative in plasma produced a saliva sample that was PCR-positive (4000 copies of HGV/GBV-C RNA/mL).

One year after the original study, 11 of 14 donors who attended for follow-up remained HGV/GBV-C RNA–positive. The 3 donors who became PCR-negative showed the lowest virus loads in plasma at time of original donation (p3, 5×10^5; p18, 5×10^6; and p21, 3×10^4 copies of HGV/GBV-C RNA/mL; $P = .017$ [Kruskal-Wallis test]). The sample from p3 was reactive in the Boehringer anti-E2 ELISA (optical density [OD] of 1.6), but those from p18 and p21 were negative (ODs of 0.036 and 0.073, respectively).

Clinical assessment of HGV/GBV-C–infected blood donors. Two donors (p13, p20) of the 18 donors recalled for clinical assessment had a history of blood transfusion. Several donors had undergone ear piercing or tattoos but in establishments where virus transmission would not be considered likely. None had a history of intravenous drug use or sexual contact with a person at high risk of parenteral virus infection.

Fourteen of 23 HGV/GBV-C–positive donors were clinically examined. None had any history of jaundice or symptoms or stigmata of chronic liver disease or hepatomegaly. Five of 18 donors reported various musculoskeletal symptoms, although not of a consistent pattern and of uncertain relationship to HGV/GBV-C infection. Liver function tests were carried out for the 19 donors returning after original donation (table 1). Two donors showed a minimal elevation in ALT level (47 and 43 IU/mL [normal range, 10–40 IU/mL]). The median ALT level among HGV/GBV-C–infected donors was 20 IU/mL, lower than the median of 32 for a control group of 100 HGV/GBV-C–uninfected blood donors ($P = .015$) and a median of 61 for 91 HCV-infected donors ($P < .001$) [4]. All other liver function tests (bilirubin, alkaline phosphatase, and γ glutamyl transferase) were similarly within the normal range. All measurements in a full blood count were in the normal range for the 19 donors, apart from mild lymphopenia in 5.

Donations from 2 partners of HGV/GBV-C–infected donors who donated at the time of the original study were HGV/GBV-C PCR-negative. Three partners who attended the counseling and follow-up session were negative for HGV/GBV-C RNA in plasma and saliva and negative for antibody to HGV/GBV-C.

Incidence of HGV/GBV-C infection in blood donors. Archived samples from previous blood donations of all 23 HGV/GBV-C–infected donors were tested by PCR (figure 1). All samples from 6 donors (collected during 1986–1994) were PCR-positive, while the earliest samples from the remaining 17 (1983–1995) were PCR-negative. Two donors (p10 and p19) became PCR-positive in 1994 and 1992, respectively, but over the next 1–2 years became transiently PCR-negative and negative for antibody to E2, before becoming viremic again at the end of 1996. A total of 17 blood donors became infected with HGV/GBV-C over a mean observation period of 9.7 years. The number of susceptible blood donors investigated was 1046 (excluding the persistently viremic donors during the observation period), allowing an approximate calculation of the incidence of HGV/GBV-C infection of 0.17% per year.

One explanation for the large number of HGV/GBV-C–negative samples collected before 1990 is that HGV/GBV-C RNA sequences had degraded on long-term storage. We recalculated the incidence of HGV/GBV-C infection using shorter time intervals. When we used only the results from 1990 onwards, a total of 13 blood donors became infected over a mean interval of 6.2 years (incidence, 0.20% per year). Similarly, 8 became positive from 1992 onwards (mean observation period, 4.5 years; incidence, 0.17% per year). The similarity in these estimates suggests that degradation of HGV/GBV-C RNA during storage did not affect the calculation of incidence.

Discussion

In this study, we have demonstrated that 23 (2.25%) of 1020 healthy non-remunerated blood donors had plasma samples positive for HGV/GBV-C RNA by reverse transcription–PCR. This frequency lies within the range reported for comparable non-remunerated blood donor populations in other countries, such as Germany (1.3%–1.9%), France (4.2%), the United

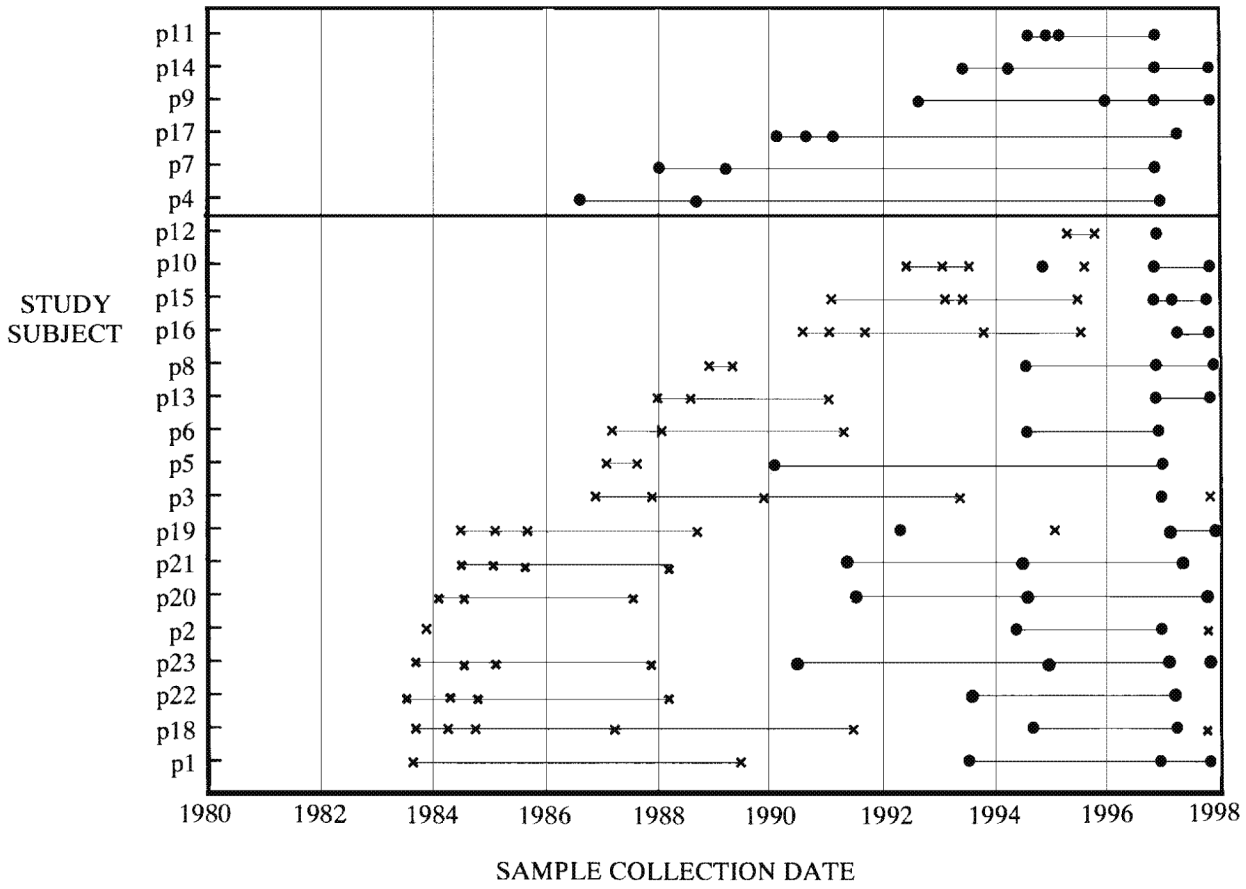

Figure 1. Retrospective testing for HGV/GBV-C RNA of archived blood donations from 23 blood donors identified as HGV/GBV-C–infected in November–December 1996. 6 donors above horizontal line are those with persistent infection throughout observation period. ●, HGV/GBV-C polymerase chain reaction–positive; ×, polymerase chain reaction–negative.

States (1.7%), Australia (4%), and Japan (0.5%–1.2%) [2, 5–8]. The male-to-female ratio of HGV/GBV-C–infected donors was 11:12, different from the 3:1 ratio observed among blood donors infected with HCV in Scotland or elsewhere. The high prevalence of HGV/GBV-C infection and the absence of significant disclosed parenteral risk factors for infection in the HGV/GBV-C–infected donors strongly suggests other routes of transmission.

Among other proposed routes of transmission of HGV/GBV-C, evidence for transmission by sexual contact includes the finding of high prevalences of infection in persons with sexually transmitted disease or with other evidence of sexual exposure with multiple partners [9]. Against this hypothesis is the absence of detectable transmission of HGV/GBV-C (either by PCR or serology) to the sex partners of 5 infected donors. Mother-to-child transmission has been documented by the frequent detection of HGV/GBV-C viremia (20%–70%) in children born of infected mothers [10]. However, the observation of de novo infection in at least 17 of the 23 HGV/GBV-C–infected blood donors in the current study indicates that the acquisition of infection occurred predominantly in adulthood.

The frequent finding of HGV/GBV-C–positive samples among archived donations from the infected donors indicates that infection may persist for several years (at least 10 years in p4). The results of this retrospective study are consistent with the finding of persistent infection of at least 2 years among 4 of 5 Australian blood donors [7] and the documented prolonged infection in varying proportions of persons infected with HGV/GBV-C through blood transfusion or treatment with blood products [11, 12].

A surprising finding that arose from the retrospective study was the relatively high incidence of HGV/GBV-C infection. The calculated rate (170–200 infections per 100,000 person-years) contrasts strongly with rates of acquisition of HCV (1.8–10 per 100,000), HIV (1.3–4.0 per 100,000), and HBV (1.7–4.0 per 100,000) in similar non-remunerated blood donor populations in Europe, the United States, Japan, and Australia.

If, in the future, donations were to be screened for HGV/GBV-C RNA by PCR, the number of new infections detected would place severe constraints on the size of the pool used. For example, even if all persistently infected donors were excluded, new infections with HGV/GBV-C over a mean donation interval of 6 months would lead to contamination of 50% of pools containing 500 component donations and 10% of pools containing 100 components.

The asymptomatic nature of HGV/GBV-C was directly demonstrated by our clinical examination of the infected donors. Two donors showed minimal elevation of ALT levels, but the mean level (20 IU/mL) and all other liver function tests were in the normal range. No subject had a previous history of acute hepatitis or jaundice. No donor had any abnormality on clinical examination, other than arthralgia in 5 persons. However, there was no consistent pattern of joint involvement, and larger studies would be required to make a more substantive link.

These findings are consistent with the absence of clinically or biochemically apparent liver disease in those infected by blood transfusion [13–15]. For example, of 79 with posttransfusion hepatitis, only 3 were infected with HGV/GBV-C alone [14], all had mild, asymptomatic hepatitis, and the absence of any correlation between ALT elevation and detection of HGV/GBV-C by PCR suggests other causes for the observed hepatitis. A difficulty encountered in investigating the HGV/GBV-C–infected donors in the current study arose from the current lack of information about the tropism and likely disease associations of HGV/GBV-C in vivo. Although HGV/GBV-C RNA can be detected in the liver, there is little evidence for its replication there. Thirteen of 17 HGV/GBV-C–positive donors showed relatively high levels of HGV/GBV-C RNA in saliva and a mean ratio to levels detected in plasma of ~1:1000. The frequency of detection and ratio to levels detected in plasma contrasts with that documented for HCV and suggests that replication of HGV/GBV-C in the respiratory or gastrointestinal tracts may occur. None of them had any history of respiratory symptoms or disease, although its secretion into saliva may represent a route of transmission, as documented for human cytomegalovirus and other herpesviruses.

In conclusion, this study demonstrates that HGV/GBV-C infection is common among our donor population but is not associated with significant hepatic disease or symptoms. We have not been able to identify a risk factor for infection in the majority of HGV-positive donors. HGV/GBV-C RNA was present in the plasma in the majority of subjects for several years and was likely to have been transfused in a large number of blood components. These findings will enable recipients of HGV/GBV-C–positive blood to be identified and the clinical sequelae investigated. In the interim, in the absence of clinicopathologic data suggesting significant morbidity in HGV/GBV-C–infected donors, blood donors should not be screened for HGV/GBV-C.

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