

## THE INCIDENCE OF TRANSFUSION-ASSOCIATED HEPATITIS G VIRUS INFECTION AND ITS RELATION TO LIVER DISEASE

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### ABSTRACT

**Background** The role of hepatitis G virus (HGV) in transfusion-associated infection and its relation to liver disease are not well understood.

**Methods** Serum samples collected between 1972 and 1995 from 357 transfusion recipients, 157 controls who did not receive transfusions, 500 randomly selected volunteer blood donors, and 230 donors of blood received by HGV-infected patients were tested for HGV RNA by qualitative and quantitative polymerase-chain-reaction assays. Samples obtained before transfusion and serially after transfusion from 79 of the 81 transfusion recipients who had transfusion-associated non-A, non-B hepatitis were available for testing.

**Results** Of the 79 patients with transfusion-associated hepatitis, 63 (80 percent) had infections related to the hepatitis C virus (HCV) and 3 had preexisting HCV and the cause of their acute hepatitis could not be determined; of the remaining 13 patients, 3 had acute HGV infection, and 10 were infected with unidentified agents. Six of the 63 patients with HCV infection who were tested (10 percent) were also infected with HGV. The three patients infected only with HGV had mild hepatitis (mean peak alanine aminotransferase level, 198 U per liter; none had jaundice); the levels of alanine aminotransferase and HGV RNA were not well correlated. The combined HCV and HGV infections were no more severe than HCV infections alone; the alanine aminotransferase values paralleled the levels of HCV RNA, but not those of HGV RNA. There were 35 HGV infections among the 357 transfusion recipients; only 3 had hepatitis with HGV as the sole viral marker. One of the 157 controls and 7 of the 500 randomly selected blood donors (1.4 percent) had detectable HGV RNA. In all eight instances in which a transfusion recipient had acute HGV infection after transfusion and samples from all donors could be tested, at least one HGV-positive donor was identified.

**Conclusions** HGV was common in a group of volunteer blood donors, and it can be transmitted by transfusion. Most HGV infections were not associated with hepatitis. HGV did not worsen the course of concurrent HCV infection. No causal relation between HGV and hepatitis has been established. (*N Engl J Med* 1997;336:747-54.)

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**C** LONING of the hepatitis C virus (HCV)<sup>1</sup> and sensitive serologic and molecular assays established HCV as the predominant cause of non-A, non-B hepatitis.<sup>2,3</sup> Conversely, it has been shown that approximately 20 percent of cases of community-acquired hepatitis<sup>4</sup> and 10 percent of cases of transfusion-transmitted hepatitis<sup>5</sup> are unrelated to HCV infection. Serum samples from patients with non-A, non-B, non-C hepatitis were used in molecular amplification and cloning that led to the discovery of a new member of the Flaviviridae family, the hepatitis G virus (HGV).<sup>6</sup> HGV is almost identical to another newly cloned agent that has been designated GB virus, type C; the two are considered to be different isolates of the same virus.<sup>7</sup>

### METHODS

#### Selection of Patients and Donor Samples

We used stored serum samples from donors and recipients that were obtained in the course of prospective studies of transfusion-associated hepatitis at the National Institutes of Health from October 1972 through December 1995. The method of enrollment, frequency and duration of follow-up, and criteria for the diagnosis of hepatitis have been described previously.<sup>3,8</sup> The study protocol was reviewed and approved by the institutional review board of the National Heart, Lung, and Blood Institute, and all the study subjects gave written, informed consent.

Between 1972 and 1995, 81 patients with transfusion-associated non-A, non-B hepatitis were identified. Serum samples obtained before transfusion and serial samples obtained after transfusion were available for HGV RNA testing from 79 of these patients (98 percent). We also tested 281 of 887 patients who received transfusions but did not contract hepatitis (32 percent) and 157 of 374 controls who did not have transfusions (42 percent). For the patients identified as having transfusion-associated HGV infection, all available samples from donors were tested for HGV RNA (a total of 230 donors); samples from 500 consecutive volunteer blood donors were also tested. The samples were stored at  $-70^{\circ}\text{C}$  before testing for HGV RNA.

#### Detection of HGV RNA and HCV RNA by the Polymerase Chain Reaction

To detect HGV RNA in a sample, nucleic acids were isolated from 100  $\mu\text{l}$  of serum by the acid guanidinium thiocyanate-phenol-chloroform extraction method. A reverse-transcription polymerase chain reaction (PCR) was carried out with an RNA PCR kit (Perkin-Elmer, Norwalk, Conn.) and a kit to prevent PCR car-

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ryover contamination (Perkin-Elmer) with the following primers (0.4 mM each) from the putative nonstructural region 5 (NS5) of the HGV genome: 5'CGAATGAGTCAGAGGACGGGGIAT3' (sense) and 5'CTCTTTGTGGTAGTAGCCGAGAGAT3' (antisense). Forty-five cycles of PCR (94°C for one minute, 55°C for one minute, and 72°C for one minute for each cycle) were performed, followed by the extension reaction at 72°C for seven minutes. PCR products were analyzed by dot blot hybridization with a <sup>32</sup>P-labeled oligonucleotide probe (5'TGCGTTACTGAGAGCA-GCTCAGATGAG3'). The sensitivity of this assay was 1 copy of HGV RNA per reaction, corresponding to approximately 20 copies per milliliter of the initial serum. PCR for HCV RNA was performed as described elsewhere.<sup>9</sup>

#### Quantitation of HGV RNA and HCV RNA

HGV RNA was quantitated with external standards containing known concentrations of HGV RNA that ranged from 1 to 10,000 copies per 50  $\mu$ l. After reverse-transcription PCR and dot blot hybridization of the samples and standards, the radioactivity of each blot was measured (Radioanalytic Imaging System, AMBIS, San Diego, Calif.). The total radioactive count of each standard was plotted against the HGV RNA copy number to establish a standard curve; the counts of radioactivity and the copy number were correlated linearly in a range from 10 to 10,000 copies per 50  $\mu$ l ( $P < 0.05$ ; correlation coefficient,  $> 0.9$ ). The copy number of each sample was determined in relation to the standard curve and was expressed as the number of copies per milliliter. HCV RNA was quantitated by a commercial assay (Monitor, Roche Molecular Systems, Branchburg, N.J.) according to the directions of the manufacturer.

#### Serologic and Biochemical Assays

In the absence of reliable serologic assays, all HGV determinations were based on the PCR findings. Antibodies to HCV were measured by a second-version enzyme immunoassay according to the directions of the manufacturer (Abbott HCV EIA 2.0, Abbott Laboratories, North Chicago, Ill.). Antibody to hepatitis E virus was measured by the method of Tsarev et al.<sup>10</sup> Alanine aminotransferase was measured by a three-point kinetic assay with an automated photometric analyzer (model 917, Hitachi-Boehringer Mannheim, Indianapolis), and the values were expressed in international units (normal range, 5 to 41 U per liter).

#### Statistical Analysis

All reported *P* values are two-sided. Standard least-squares regression analysis was used to fit a linear equation to the relation between radioactive counts and copy numbers of HGV RNA. Fisher's exact test<sup>11</sup> was used to compare proportions in two-by-two tables. All confidence intervals for binomial proportions are exact.

The chi-square test for homogeneity in R-by-C contingency tables was used to compare four groups of transfusion recipients (those with non-A, non-B, non-C hepatitis; those with hepatitis C; those with low-level elevations of alanine aminotransferase; and those with no hepatitis) with respect to the proportion of patients testing positive for HGV RNA. Because we did not have resources to ascertain the HGV RNA status of all patients, we sampled three of the four groups (the recipients with hepatitis C, those with low-level elevations of alanine aminotransferase, and those with no hepatitis), and estimated the overall number of HGV-infected patients in each group by making projections based on the sample tested. The projected counts were added to the actual count in the fully assayed group (the recipients with non-A, non-B, non-C hepatitis) to estimate the overall number of HGV infections. Exact confidence intervals for the projected counts were derived from the hypergeometric distribution<sup>12</sup> and were selected so that the confidence intervals for the grand total and for the percentages derived therefrom were at least 95 percent.<sup>13</sup> In the quantitative analysis of HGV RNA levels in the four groups, geometric means are reported because of the wide vari-

ability in values. In determining whether the four groups had similar RNA levels, we used a standard analysis of variance on the logarithms of the RNA values, again to stabilize variances and reduce the influence of extreme values.

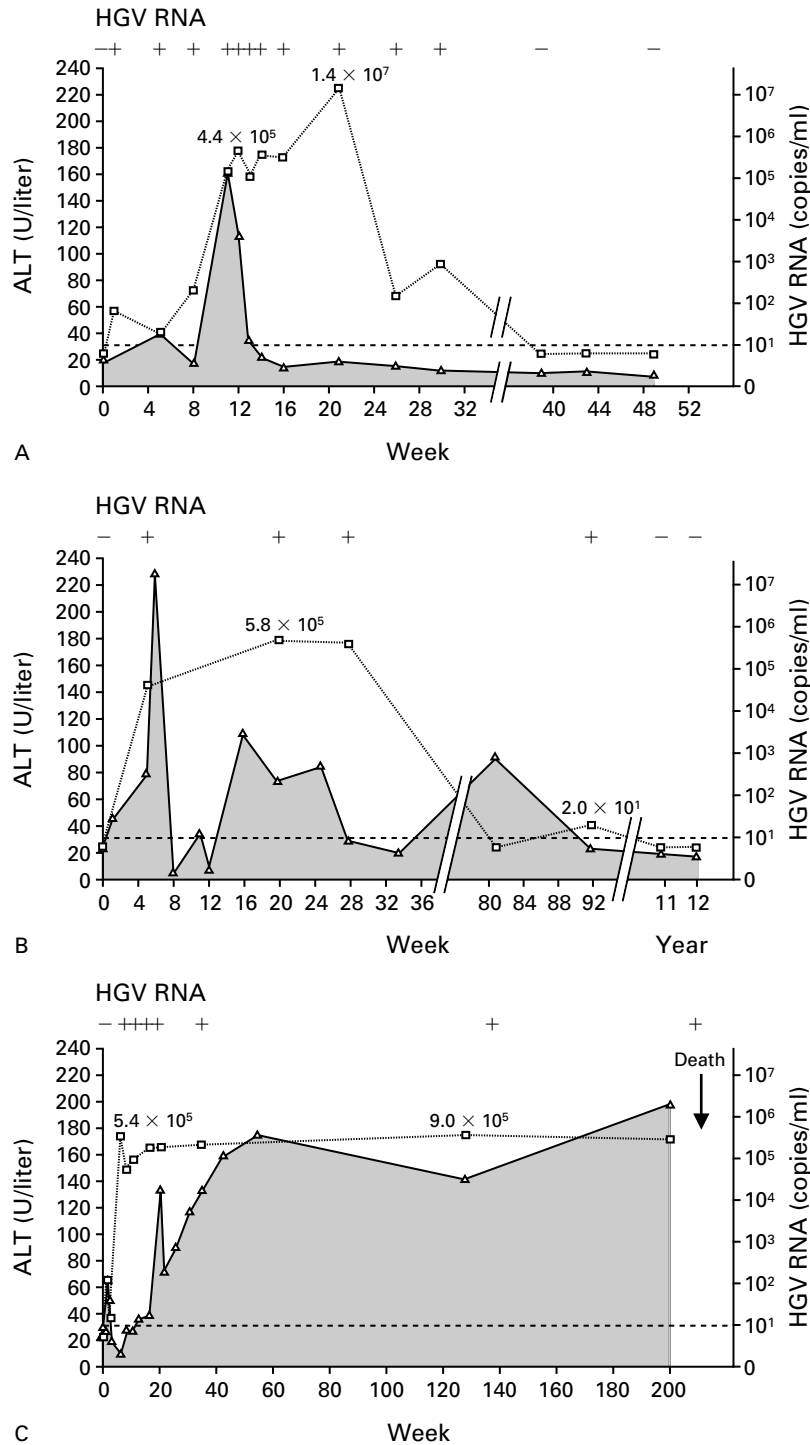
## RESULTS

### Incidence of Hepatitis G in Transfusion Recipients Followed Prospectively

Of the 79 transfusion recipients originally classified as having non-A, non-B hepatitis and for whom there were adequate blood samples obtained before and after transfusion, 63 (80 percent) had acute HCV infections that were considered to account for their hepatitis; 6 (10 percent) of these HCV-infected patients were also infected with HGV. Three patients were found to have been infected with HCV before their transfusions; the role of HCV in their post-transfusion hepatitis could not be ascertained. Only 3 of the 79 patients (4 percent) had HGV as the only viral marker. Ten of the 79 patients (13 percent) had no serologic or molecular markers for any established hepatitis virus (A through G). Thus, infection with HGV but no other hepatitis virus was present in only 4 percent of the overall group of 79 patients with non-A, non-B hepatitis and 23 percent of patients with non-A, non-B, non-C hepatitis (3 of 13). Hepatitis with no known causative agent was considerably more common.

### Clinical Course of HGV-Associated Hepatitis

Figure 1 shows the clinical course of the three patients who had acute HGV infection after transfusion but were not coinfecting with HCV. In each patient, HGV RNA was detectable within two weeks after the transfusion. All the infections were mild, with peak alanine aminotransferase levels less than 230 U per liter; no patient had jaundice, symptoms, or extrahepatic manifestations of HGV infection. The duration of the infections differed. One patient had a complete resolution of infection, with normalization of the alanine aminotransferase level within 12 weeks and clearance of HGV RNA within 40 weeks (Fig. 1A). In another patient, intermittent elevations of alanine aminotransferase and HGV RNA were noted for 80 and 92 weeks, respectively. Subsequently, the alanine aminotransferase level became normal and there was clearance of HGV RNA, but the timing of these events was imprecise because the patient was lost to follow-up for a lengthy period (Fig. 1B). In the third patient, chronic infection and chronic hepatitis (peak alanine aminotransferase level, 200 U per liter) persisted for four years, after which the patient died of unrelated causes (Fig. 1C). The interpretation of this patient's alanine aminotransferase elevations was complicated by his daily use of acetaminophen throughout follow-up, by a carcinoma of the bowel that was surgically resected, and by a primary lung cancer, which was the cause of death.



**Figure 1.** HGVRNA Infection in Three Patients with Transfusion-Associated Hepatitis.

Levels of alanine aminotransferase (ALT; shaded areas marked by triangles) and HGVRNA (squares) in the three study patients infected only with HGVRNA are shown plotted against the time since transfusion. Qualitative results of PCR for HGVRNA (positive, plus signs; negative, minus signs) are shown above each panel. The dashed lines indicate the limit of detection of HGVRNA. In each patient, the relation between the HGVRNA and ALT was inconsistent. In one patient (Panel A), HGVRNA continued to increase despite the normalization of ALT levels, and it remained detectable for at least 20 weeks after the ALT level had decreased to normal. In the second patient (Panel B), HGVRNA was elevated at week 28, when the ALT level was normal, and it was undetectable at week 80, when the ALT level was rising. In the third patient (Panel C), 20 weeks elapsed from the first appearance of HGVRNA to the first elevation of ALT. The numbers inside the panels show the values for HGVRNA in copies per milliliter.

**TABLE 1.** SEVERITY OF INFECTION WITH HGV ALONE, AS COMPARED WITH THE OTHER TYPES OF INFECTION IN THE STUDY PATIENTS.

VARIABLE	INFECTIOUS AGENT			
	HGV ALONE (N = 3)	HCV ALONE (N = 56)*	HGV AND HCV (N = 6)	NON-A-G VIRUS (N = 10)†
Alanine aminotransferase				
Mean peak value — U/liter	198	726	724	418
>10× upper limit of normal — no. (%)	0	46 (82)	5 (83)	2 (20)
>15× upper limit of normal — no. (%)	0	27 (48)	3 (50)	2 (20)
Chronic elevation — no. (%)‡	2 (67)§	40 (71)¶	3 (50)¶	3 (30)
Jaundice — no. (%)	0	18 (32)	2 (33)	0
Mean peak bilirubin — mg/dl	0.87	3.14	3.97	1.18

\*Data on one patient with fulminant hepatitis and an alanine aminotransferase level of 4493 U per liter were excluded.

†These 10 patients had no serologic or molecular markers for any established hepatitis virus (A through G).

‡An alanine aminotransferase level more than two times the upper limit of normal lasting more than one year was considered to be a chronic elevation.

§Although two of the three HGV-infected patients had prolonged elevations of alanine aminotransferase, one had an alanine aminotransferase level that became normal after 80 weeks (Fig. 1B). The other had additional illnesses, and thus there were alternative explanations for the elevations of alanine aminotransferase, as described in the text. Liver biopsies were not performed in either patient.

¶P = 0.36 by Fisher's exact test for the comparison of the group infected with HCV alone with the group infected with both HGV and HCV.

||To convert values for bilirubin to micromoles per liter, multiply by 17.1.

Quantitative levels of HGV RNA were studied in relation to the biochemical evidence of hepatocellular injury. In the first patient (Fig. 1A), the HGV RNA level correlated with the alanine aminotransferase level at first, but then continued to rise as the alanine aminotransferase level became normal. The HGV RNA level did not peak (at 14 million copies per milliliter) until 10 weeks after the alanine aminotransferase level had decreased to normal, and it remained detectable for almost 20 weeks after the normalization of alanine aminotransferase. In the second patient (Fig. 1B), the HGV RNA and alanine aminotransferase levels could not be correlated precisely because of limited sampling. Nonetheless, at week 28 the HGV RNA level was high when the alanine aminotransferase level was normal, and at week 80 HGV RNA was undetectable but the alanine aminotransferase level was rising. In the third patient (Fig. 1C), almost 20 weeks elapsed between the appearance of HGV RNA and the first elevation of alanine aminotransferase.

#### Severity of Hepatitis G and Other Hepatitis Infections

The patients infected only with HGV had less severe hepatitis than those infected only with HCV (mean peak alanine aminotransferase level, 198 vs. 726 U per liter) (Table 1). No patient with HGV had an alanine aminotransferase level more than 10 times the upper limit of normal, as compared with 82 percent of those with hepatitis C. Similarly, no

patient with HGV had jaundice, as compared with 32 percent of those with HCV.

The severity of liver disease in the 10 patients without established virologic markers for hepatitis viruses A through G (non-A-G) was intermediate between that of patients with HGV infection and that of patients with HCV infection (Table 1). Among these 10 patients, the mean peak alanine aminotransferase level was 418 U per liter, and 2 patients had alanine aminotransferase levels more than 10 times the upper limit of normal. None of the 10 patients had jaundice.

Alanine aminotransferase elevations more than two times the upper limit of normal that persisted for more than one year were found in 2 of the 3 patients with HGV alone, 40 of the 56 with HCV alone, 3 of the 6 with combined HCV and HGV infection, and 3 of the 10 with non-A-G hepatitis (Table 1).

#### Acute Infection with Both HGV and HCV

On the basis of peak alanine aminotransferase levels and the percentage of patients with jaundice, the severity of hepatitis in patients coinfecting with HGV and HCV was indistinguishable from that in patients with HCV infection alone (Table 1). This suggests that the extent of liver disease in patients with combined infection was related to infection with HCV, not HGV.

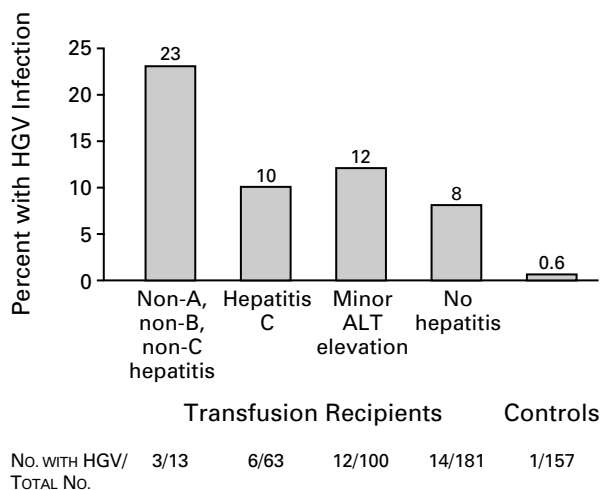
Figure 2 shows HCV and HGV viremia in relation



to alanine aminotransferase levels in two of the six patients with combined infection. In both, the hepatitis resolved rapidly. Although HGV RNA and HCV RNA became detectable two to three weeks after transfusion in each patient, the subsequent course of viremia differed markedly between the two agents. HCV RNA levels declined just before the decrease in the alanine aminotransferase level and remained undetectable throughout the period of normal alanine aminotransferase levels. In contrast, there was no correlation between HGV RNA and alanine aminotransferase. As in one of the patients infected only with HGV (Fig. 1A), HGV RNA levels in the two patients with HCV-HGV coinfection remained persistently high despite the normalization of alanine aminotransferase levels.

#### Proportion of HGV Infections Associated with Liver Disease

The frequency of HGV infection among the 357 transfusion recipients who did or did not contract hepatitis is shown in Figure 3. Although 3 of the 13 patients with non-A, non-B, non-C hepatitis had evidence of acute HGV infection (23 percent), so too did 6 of the 63 patients with hepatitis C (10 percent), 12 of the 100 patients with minor elevations of alanine aminotransferase who did not meet the



**Figure 3.** Frequency of HGV Infection in Transfusion Recipients with and without Hepatitis and in Controls Who Did Not Receive Transfusions.

The frequency of HGV infection did not differ significantly among the four groups of transfusion recipients — those with non-A, non-B, non-C hepatitis; those with hepatitis due to HCV; those with such minor elevations of alanine aminotransferase (ALT) that they did not meet the study criteria for hepatitis; and those with no biochemical evidence of hepatitis during six months of prospective follow-up. HGV RNA was significantly more prevalent among the transfusion recipients than among the controls ( $P < 0.001$ ). The numbers above the bars show the percentages with HGV infection in each group.

study criteria for hepatitis (12 percent), and 14 of the 181 patients who had persistently normal alanine aminotransferase levels after transfusion (8 percent). Among these four groups, there was no significant difference in the proportion with HGV infection ( $P = 0.26$ ). In contrast, only 1 of 157 controls who did not receive transfusions tested positive for HGV RNA ( $P < 0.001$  by Fisher's exact test when the four groups of transfusion recipients were compared with the controls). Although HGV infection appeared to be associated with transfusion, the patients who contracted hepatitis were no more likely to be infected with HGV than those without hepatitis.

There were 35 HGV infections in the four groups of transfusion recipients (Fig. 3). Each of the 13 recipients with non-A, non-B, non-C hepatitis was tested for HGV RNA, but limits on resources or the availability of samples allowed PCR testing of only 63 of 65 recipients with hepatitis C, 100 of 111 with minor alanine aminotransferase elevations, and 181 of 776 who did not contract hepatitis. A statistical extrapolation (explained in the Methods section) was used to estimate the overall number of HGV infections that would have been detected had all the transfusion recipients been tested. As a result, we projected that in the hepatitis C group there were 6 HGV infections (100 percent confidence interval, 6 to 8); in the group with minor elevations of alanine aminotransferase, 13 infections (99 percent confidence interval, 12 to 18), and in the group with no hepatitis, 60 infections (96 percent confidence interval, 35 to 96). When the group with non-A, non-B, non-C hepatitis was included in the calculation, the total projected number of HGV infections in all four groups was 82 (95 percent confidence interval, 56 to 125). Of these 82 infections, 4 percent (95 percent confidence interval, 2 to 5 percent) were projected to occur in the group with non-A, non-B, non-C hepatitis; 7 percent (95 percent confidence interval, 5 to 14 percent) in the group with hepatitis C; 16 percent (95 percent confidence interval, 10 to 29 percent) in the group with minor elevations of alanine aminotransferase; and 73 percent (95 percent confidence interval, 55 to 82 percent) in the group with no biochemical evidence of hepatitis.

#### Viremia and Clinical Outcome

To relate the severity of hepatitis to the viral burden, we performed quantitative PCR on blood samples obtained 6 to 10 weeks after exposure to HGV. The geometric-mean titer of HGV RNA did not differ significantly among the four groups when we studied the 3 patients who contracted hepatitis G, the 6 who were infected with HGV and HCV, the 12 who had minor elevations of alanine aminotransferase, and the 14 who had no biochemical evidence of liver disease ( $P = 0.48$ ). The geometric-mean titers of HGV RNA ranged from 102 to 7568 copies

of RNA per milliliter. In individual patients, the HGV RNA titers varied by as much as 5 log. The highest observed titer in the acute-phase sample (six to eight weeks) from the 35 HGV-infected patients was 2.5 million copies of RNA per milliliter.

#### Persistent HGV Infection

All 35 patients with acute HGV infection were followed for six months; 32 (91 percent) had persistent HGV RNA during that period. Most patients were not followed further because they had no evidence of hepatitis. However, eight of these patients were tested serially for up to 6 years (mean, 4.6). There was clearance of HGV within one year in one, and within three years in two others. Of the remaining five patients, two were positive for HGV RNA at the time of their deaths from causes unrelated to liver disease four to five years after their transfusions; one was positive for four years and was then lost to follow-up; and the remaining two were positive throughout follow-up. Overall, five of the eight patients had persistent viremia for four to six years, and three had viral clearance within three years.

#### Prevalence of HGV among Randomly Selected Blood Donors and among Donors to Infected Recipients

HGV RNA was detected in 7 of the 500 randomly selected blood donors (1.4 percent) who otherwise met all the standard eligibility criteria for donation. This proportion was similar to one we found previously (1.7 percent) in a separate group of 769 eligible volunteer donors, 13 of whom had HGV RNA.<sup>6</sup>

Samples were available from all donors in eight cases in which the recipient had an acute HGV infection after transfusion. At least one HGV-positive donor was identified in every case; four patients had two HGV-positive donors, and one had three. Even when samples from only some of the donors were available (that is, samples from 50 to 90 percent of the donors of blood received by a given patient), HGV-positive donors were identified in four of seven cases.

### DISCUSSION

HGV infection was highly prevalent among the volunteer blood donors we studied. The rate of HGV viremia was at least five times higher than the previously reported rate of HCV viremia among blood donors.<sup>14</sup> Although HGV infection was generally persistent, approximately one third of patients (three of eight) had clearance of the virus within three years after becoming infected. This suggests that the number of exposures to HGV considerably exceeds the number of active infections detected by PCR. Furthermore, rates of viral exposure and infection among highly selected donors may underestimate the rate in the general population.

Several of our findings give strong evidence that HGV can be transmitted by transfusion: patients neg-

ative for HGV RNA became positive shortly after transfusion; HGV RNA-positive donors were identified retrospectively for all patients with HGV infection for whom samples from all blood donors were available; and the incidence of HGV was markedly lower among patients who did not receive transfusions. Ten percent of recipients (35 of 357) were found to be infected with HGV after their transfusions. The current rate of HGV transmission is not known; transmission to at least 1 percent of recipients is probable, given the 1 to 2 percent prevalence of viremia among donors and the absence of a practical screening assay.

Despite the high rate of HGV transmission, HCV was the predominant cause of transfusion-associated hepatitis, accounting for at least 80 percent of the cases we studied. To what extent does HGV account for the residual cases? Only 3 of the 13 patients with hepatitis unrelated to HCV had acute HGV infections. We believe that the majority of cases of non-A, non-B, non-C hepatitis are due to an as yet undiscovered hepatitis agent or agents or to nonviral causes. Furthermore, even in the three patients with hepatitis who were infected only with HGV, there was a dissociation between the level of HGV RNA in serum and biochemical evidence of hepatocellular injury. This was even more striking in the patients infected with both HGV and HCV (Fig. 2), whose levels of alanine aminotransferase closely paralleled those of HCV RNA but were asynchronous with the levels of HGV RNA.

Perhaps the main reason to question the clinical effect of HGV infection is that an estimated 73 percent of such infections were unaccompanied by evidence of hepatocellular injury, and an additional 16 percent were associated with such minor elevations of alanine aminotransferase that the patients did not meet our criteria for the diagnosis of hepatitis. Given that the vast majority of HGV infections were not associated with hepatitis, that HGV had no effect on the severity of coexisting HCV infection, and that there may be unidentified agents that are more strongly associated with hepatitis than HGV, it is difficult to conclude that HGV had a causal role in the few cases of hepatitis in which it was the only identified agent. It is equally tenable to argue that these patients had clinically silent HGV infections in which the hepatitis was due to an unidentified, coexisting infectious agent or to a noninfectious cause of liver-cell injury. Indeed, one could question whether using the term "hepatitis virus" to refer to HGV is appropriate. Documentation that HGV replicates in the liver and conclusive evidence that it causes liver disease are needed before it can properly be classified as a hepatitis virus.

Supported by intramural funds at the National Institutes of Health and by Genelabs Technologies, Inc.

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*Vermont Waterfall*

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