

## ROUTES OF INFECTION, VIREMIA, AND LIVER DISEASE IN BLOOD DONORS FOUND TO HAVE HEPATITIS C VIRUS INFECTION

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**Abstract Background.** For many people infected with the hepatitis C virus (HCV), the route of exposure, risk of transmission, and severity of associated liver disease are unknown. We studied these variables in people who donated blood voluntarily.

**Methods.** Blood donors who tested positive for HCV antibodies on enzyme immunoassay were classified according to whether the results of a confirmatory second-generation recombinant immunoblot assay (RIBA) for HCV were positive, negative, or indeterminate. The evaluations also included an assessment of risk factors, a physical examination, serial determinations of alanine aminotransferase levels and HCV serologic assays, a polymerase-chain-reaction assay for HCV RNA, testing of sexual contacts and family members, and liver biopsies in some participants who were HCV-positive by RIBA.

**Results.** A total of 481 donors were studied, among whom 248 were positive for HCV by RIBA, 102 had indeterminate results, and 131 were HCV-negative. In a logistic-regression analysis, significant risk factors for HCV infection among the HCV-positive participants were a history of blood transfusion in 66 (27 percent;  $P < 0.001$  for the comparison with RIBA-negative donors), intrana-

sal cocaine use in 169 (68 percent,  $P < 0.001$ ), intravenous drug use in 103 (42 percent,  $P = 0.001$ ), sexual promiscuity in 132 (53 percent,  $P = 0.002$ ), and ear piercing among men ( $P < 0.05$ ). Nine of 85 sexual partners of HCV-positive donors were anti-HCV-positive; 8 had used intravenous drugs or received transfusions. HCV RNA was found in 213 HCV-positive donors (86 percent), 3 who had indeterminate results by RIBA (2 of these 3 tested positive with a more specific, third-generation RIBA), and none who were HCV-negative. Of the HCV-positive donors, 69 percent had biochemical evidence of chronic liver disease; among 77 donors positive for HCV by RIBA who underwent liver biopsy, 5 had severe chronic hepatitis or cirrhosis, 66 had mild-to-moderate chronic hepatitis, and 6 had no evidence of hepatitis.

**Conclusions.** Among volunteer blood donors, prior blood transfusion, intranasal cocaine use, intravenous drug use, sexual promiscuity, and ear piercing in men are risk factors for HCV infection. The high frequency of intravenous drug use was unexpected, because these donors had denied such use when questioned directly at the time of their blood donations. (N Engl J Med 1996; 334:1691-6.)

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APPROXIMATELY 36,000 of the 6 million people who donate blood each year in the United States repeatedly test positive when screened for antibody to the hepatitis C virus (HCV),<sup>1</sup> and about half are found to be carriers of HCV who require counseling and medical evaluation.<sup>2</sup> The total number of such carriers in the United States may be as high as 3.5 million.<sup>3,4</sup>

To address controversial questions about the interpretation of HCV tests, patterns of transmission, and the severity of associated disease,<sup>5-12</sup> we assessed the reliability and value of the supplemental recombinant immunoblot assay (RIBA) in diagnosing HCV infection; primary routes of HCV transmission in an asymptomatic population of blood donors; the infectivity of the persons confirmed to be anti-HCV-positive by the detection of HCV RNA in their blood and the study of their sexual partners, family members, or both; and the

relation between positivity for anti-HCV and evidence of chronic liver disease.

### METHODS

#### Serologic Studies

Initially, anti-HCV was detected in the samples of donated blood by a first-generation enzyme immunoassay (EIA 1.0, Ortho Diagnostics, Raritan, N.J.) measuring reactivity to a nonstructural HCV antigen (C100-3). In June 1992, a second-generation assay (EIA 2.0, Ortho) was substituted that had greater sensitivity because it also detected reactivity to the HCV core protein (c22) and another nonstructural antigen (c33c). All the blood samples were assayed at the National Institutes of Health (NIH) by a second-generation RIBA (RIBA 2.0, Chiron, Emeryville, Calif.). In this assay, the antigens used in the second-generation enzyme immunoassay and a component of the C100-3 protein (5-1-1) were fixed to a nitrocellulose strip, overlaid with sample, allowed to react with enzyme-labeled antibody, and observed for a color change in the added solution of substrate. Reactivity to at least two of the four HCV antigens (5-1-1, C100-3, c33c, and c22) was considered to indicate a positive result; no reactivity, a negative result; and reactivity to only one antigen, an indeterminate result.

#### Enrollment of Patients, Initial Evaluation, and Follow-up

Volunteer donors of whole blood from the Greater Chesapeake and Potomac Region of the American Red Cross were screened for anti-HCV by enzyme immunoassay and RIBA; those whose donations repeatedly tested positive were notified by letter of the results of the initial assays and invited to enroll in the study beginning in March 1991. Because of limited resources, we could enroll only 10 percent of all potentially eligible donors. We sought to enroll 200 participants in the

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group positive for HCV by RIBA and 100 participants each in the group negative for HCV by RIBA and the group with indeterminate results by RIBA. Participants were enrolled consecutively until the approximate target numbers were reached. Donors who chose to be in the study but could not be enrolled were advised to seek follow-up care through their personal physicians; these donors and those who chose not to enroll were not contacted personally for additional information. However, a subgroup of these donors was randomly selected according to the results on the RIBA, and their demographic characteristics and transfusion histories were reviewed.

After the study participants provided written informed consent to the American Red Cross, information about them was released to the Department of Transfusion Medicine at the NIH, where the study was conducted. The study protocol was reviewed and approved by the institutional review boards of the NIH and the American Red Cross. Although the NIH physicians were generally aware of each donor's HCV status as determined by RIBA at the time of interview, the subjects were all evaluated in a standardized fashion regardless of their RIBA status. Medical histories were taken by a physician, including an extensive questionnaire about demographic variables, any history of hepatitis, risk factors for HCV exposure, and a sexual history. A second questionnaire about recreational drug use was completed in private by each participant. The physical examination focused on signs of chronic liver disease.

During the first year of the study, blood samples were obtained every three months to measure liver function and for the performance of HCV serologic tests. Participants negative for HCV by RIBA were considered to be uninfected and followed for 6 to 12 months, whereas those with positive or indeterminate results were enrolled in a long-term follow-up study that is anticipated to extend for at least 5 years.

All the participants were offered an assessment of their infectivity that included the testing of their sexual partners, children, and parents for anti-HCV. Contacts and family members who consented to such testing were asked about possible parenteral exposure to HCV.

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

Serum samples from each participant were studied by the reverse-transcription polymerase-chain-reaction (PCR) assay at least once during follow-up for the detection of HCV RNA.<sup>13</sup> Nucleotide primers corresponding to sequences in the 5' noncoding region of the HCV genome were used for amplification in the nested reverse-transcription PCR. The amplified gene product was identified by staining with ethidium bromide after gel electrophoresis. All specimens were tested in duplicate. If the test results were discrepant, another duplicate PCR was performed, and a specimen was considered to be HCV-positive if at least two of the four tests were positive.

#### Histologic Evaluation of Liver-Biopsy Specimens

Liver-biopsy specimens were obtained from 77 of the 248 participants who were positive for HCV by the second-generation RIBA; of these, 60 underwent biopsy as part of an adjunctive study relating the histologic activity of liver disease to serum alanine aminotransferase levels.<sup>3</sup> All the biopsy specimens were analyzed by the NIH Anatomic Pathology Department and the Liver Section of the NIH and the National Institute of Diabetes and Digestive and Kidney Diseases. No biopsy specimens were obtained from participants with negative or indeterminate results on the RIBA.

#### Statistical Analysis

All the statistical analyses were performed with standard statistical software (SAS, Cary, N.C.; and BMDP, Los Angeles). The associations between HCV status and categorical variables (including the continuous variables, which were analyzed with cutoff values) were assessed with the unadjusted chi-square statistic, except that when the expected number in any cell of a two-by-two table was less than five, a two-tailed Fisher's exact test was used.<sup>14</sup> The associations with continuous variables were assessed by the two-tailed Wilcoxon rank-sum statistic.<sup>15</sup> A logistic-regression model<sup>16</sup> was used in the multivariate modeling of associations. All the factors for which the P value on univariate analysis was less than 0.05 were entered in the model, but the factors with the highest P values on multivariate analysis were

Table 1. HCV Status as Determined by RIBA in the Study Participants and in All Blood Donations Found to Be Positive for Anti-HCV by Enzyme Immunoassay among 954,316 Consecutive Donations.\*

VARIABLE	TOTAL No.	RESULTS OF SECOND-GENERATION RIBA		
		INDETERMINATE		
		POSITIVE	MINUTE	NEGATIVE
<i>number (percent)</i>				
Donations positive for anti-HCV by EIA	4585†	2375 (52)	743 (16)	1422 (31)
Study participants				
Enrolled	481‡	248 (52)	102 (21)	131 (27)
Followed for ≥6 mo	382	191 (50)	77 (20)	114 (30)
<i>number of months</i>				
Duration of follow-up				
Median	11	15	12	7
Range	0–45	0–45	0–29	0–23

\*Because anti-HCV-negative donors may have donated blood more than once during the study period, the number of donations exceeds the number of donors, but given the large numbers, this would only minimally affect the calculations of prevalence. The proportion with positive, negative, or indeterminate results on the RIBA would not be affected, because donors positive for anti-HCV were not allowed to donate again. EIA denotes enzyme immunoassay.

†Represents approximately 0.5 percent of all donations screened.

‡Represents approximately 10 percent of all anti-HCV-positive donors.

dropped sequentially until all the factors in the model had P values of less than 0.05.

## RESULTS

### Anti-HCV Status

From March 1991 through August 1994, 954,316 consecutive blood donations were screened for anti-HCV by enzyme immunoassay; of these, 4585 (0.5 percent) repeatedly tested positive (Table 1), and 481 of those donors (10 percent) were enrolled in the study. All the donors with positive or indeterminate results on the RIBA were invited to enroll in the study, but only 1003 of the 1422 with negative results were invited to do so, because the target number of participants was rapidly reached. Of the 131 participants who were positive on the first-generation enzyme immunoassay and negative on the RIBA, 109 (83 percent) were anti-HCV-negative on the second-generation enzyme immunoassay.

The final proportions of participants in the three study groups reflected the distribution of anti-HCV-positive donors in the overall donor population (Table 1). Of the 481 participants enrolled in the study, 382 had been followed for six months or more as of August 1994.

### Demographic Variables

#### Comparison of Participants According to RIBA Status

The characteristics of the participants that were significantly associated with a positive RIBA for HCV were young age, black race, history of liver disease, no college education, no previous blood donation, and a

history of sexually transmitted disease (Table 2). The participants with indeterminate results on the RIBA were generally similar to those with negative results, but slightly larger proportions of the former were female, had no college education, and were giving blood for the first time.

*Comparison of the Study Participants with All Donors*

Available demographic data on 1,267,295 donations of blood to the American Red Cross from January 1991 through December 1994 (a period that brackets the period of the study) were compared with the data on the study participants. The two groups were similar in terms of age, sex, and the percentage of African Americans. African Americans made up 10 percent of the overall donor population and 13 percent of the enrolled population (P=0.08). Among the study participants there was a higher percentage of persons with no college education (38 percent vs. 28 percent of all donors, P<0.001), a lower percentage of first-time donors (15 percent vs. 19 percent, P=0.01), and a higher percentage with a history of blood transfusions (18 percent vs. 9 percent, P=0.02). The participants negative for HCV by RIBA were similar to the entire group of donors with respect to age, sex, race, and transfusion history (data not shown), but a higher percentage of the former had attended college or graduated from college (28 percent vs. 16 percent, P=0.003), and a lower percentage were first-time donors (2 percent vs. 19 percent, P<0.001).

*Comparison of the Study Participants with Nonparticipants*

A sample of 181 donors distributed among the categories of HCV status as determined by RIBA was randomly selected from among those who chose not to enroll. The variables of sex, age, level of education, race, and transfusion history were similar between these donors and the study participants (data not shown). The percentage of first-time donors was higher among the donors who declined to enroll (22 percent, vs. 15 percent for the study participants; P=0.03).

**Risk Factors**

The risk factors for exposure to HCV that were significantly associated with positivity for HCV on the RIBA in the logistic-regression analysis are shown in Table 3. These factors were a history of blood transfusion in 66 participants (27 percent), intranasal cocaine use in 169 (68 percent), intravenous drug use in 103 (42 percent), and sexual promiscuity (defined as a history of sexually transmitted disease, sex with a prostitute, more than five sexual partners per year, or a combination of these) in 132 (53 percent). The high frequency of intravenous drug use was unexpected, since these participants had denied such use when they were questioned directly about it at the time of their blood donations. In the process of screening donors, intravenous drug use at any time in one's life was a reason for exclusion. Questions about blood transfusion, the exchange of sex for money or drugs, and sexually trans-

**Table 2. Characteristics of Blood Donors According to HCV Status.**

CHARACTERISTIC	RESULTS OF SECOND-GENERATION RIBA			P VALUE*
	POSITIVE (N = 248)	INDETERMINATE (N = 102)	NEGATIVE (N = 131)	
Female sex — no. (%)	109 (44)	52 (51)†	48 (37)	0.17
Mean (±SD) age — yr	37±9	42±12	44±12	<0.001
No college education — no. (%)	134 (54)	29 (28)†	21 (16)	<0.001
Black race — no. (%)‡	47 (19)	5 (5)	9 (7)	0.002
First-time donor — no. (%)	60 (24)	8 (8)§	3 (2)	<0.001
History of liver disease — no. (%)¶	77 (31)	7 (7)	7 (5)	<0.001
History of sexually transmitted disease — no. (%)	70 (28)	12 (12)	13 (10)	<0.001

\*Values shown are for the comparison of the HCV-positive group with the HCV-negative group. P>0.05 for the comparison of the indeterminate group with the negative group, except as noted.

†P=0.03 for the comparison with the negative group.

‡The comparison among study groups was not significant for any other race.

§P=0.045 for the comparison with the negative group.

¶Denotes a history of jaundice, hepatitis, or abnormal liver function.

mitted disease are limited to the year before the donation. Asking about cocaine use is not required in screening donors. Sixty-five percent of the transfusion recipients and 74 percent of the intravenous drug users reported that those events had taken place before 1980. A history of ear piercing was significantly associated with positive or indeterminate results on the RIBA among men, but there was no such association among women. Except for increased rates of intranasal cocaine

**Table 3. Potential Risk Factors for Exposure to HCV in the Study Participants.**

RISK FACTOR	RESULTS OF SECOND-GENERATION RIBA			MULTIVARIATE ANALYSIS*	
	POSITIVE (N = 248)	INDETERMINATE (N = 102)	NEGATIVE (N = 131)	ODDS RATIO (95% CI)	P VALUE
	<i>number (percent)</i>				
Transfusion	66 (27)	9 (9)	11 (8)	9.6 (4.4–20.7)	<0.001
Intranasal cocaine use	169 (68)	25 (25)†	14 (11)	8.0 (3.9–16.5)	<0.001
Intravenous drug use	103 (42)	5 (5)	2 (2)	12.5 (2.7–57.1)	0.001
Sexual promiscuity‡	132 (53)	27 (26)	31 (24)	3.0 (1.5–5.9)	0.002
Ear piercing among men§	42 (30)	7 (14)¶	0	∞	<0.05
Tattooing	52 (21)	9 (9)	5 (4)	—	—
Imprisonment	61 (25)	6 (6)	2 (2)	—	—
Needle stick**	10 (4)	1 (1)	2 (2)	—	—
Acupuncture	11 (4)	2 (2)	1 (1)	—	—

\*The positive group was compared with the negative group in a logistic-regression model. P<0.001 for all univariate comparisons between these groups, except in the case of needle stick and acupuncture (P>0.05). Dashes indicate that the risk factor shown did not meet the criteria for inclusion in the model. CI denotes confidence interval.

†P=0.003 for the univariate comparison with the negative group.

‡Defined as a history of sexually transmitted disease, sex with a prostitute, five or more sexual partners per year, or a combination of these.

§Among women, no significant differences were found between study groups. The percentages shown are based on a total of 139 men in the positive group, 50 in the group with indeterminate results, and 83 in the negative group.

¶P<0.001 for the univariate comparison with the negative group.

||Because no men who were negative for HCV by RIBA had pierced ears, the estimated relative odds is infinite. The P value shown was derived by approximation.

\*\*Data refer to needle-stick injuries in health care workers.

use and ear piercing in the men with indeterminate results, the participants with indeterminate results were similar to those with negative results in having a low frequency of potential risk factors for exposure to HCV.

A history of being tattooed or being imprisoned was associated with positive results by RIBA in the univariate analysis but not the multivariate analysis, because of the close association of these variables with intravenous drug use. Positive results by RIBA were not associated with employment in a health care profession, needle-stick injuries, or acupuncture treatments.

Follow-up questionnaires about intranasal cocaine use, completed by 137 of 169 participants positive for HCV by RIBA who acknowledged such use (81 percent), revealed that 115 (84 percent) shared straws during the cocaine use, 60 (44 percent) used cocaine intranasally three or more times per day, 40 (29 percent) had epistaxis during the cocaine use, and 37 (27 percent) observed epistaxis in others.

#### Transmission of HCV to Sexual Partners and Family Members

Sexual contacts and family members were tested for anti-HCV by the second-generation enzyme immunoassay (Table 4). The only contacts who tested positive for anti-HCV were the sexual partners of participants positive for HCV by RIBA. Of 85 such partners, 9 (11 percent) were positive for anti-HCV; 8 of these had used intravenous drugs or received transfusions. Similarly, two parents who tested positive for anti-HCV had histories of intravenous drug use or transfusion. Four of five children who tested positive were tested perinatally or as young children (under the age of 18 months). The positive results in these children may reflect the passive transfer of antibody, since none of them tested positive for HCV RNA by PCR. Follow-up testing for anti-HCV was negative in two of the children; the other two did not return for follow-up testing. The offspring with

chronic HCV infection was a 36-year-old woman who had received multiple transfusions as a child in the 1970s. Overall, of a total of 141 contacts of participants with HCV, only 1, a sexual partner, had results of anti-HCV testing that could not be explained by prior parenteral exposure or the passive transfer of antibody.

#### Detection of HCV RNA

HCV RNA was demonstrated in 213 participants with positive results by RIBA (86 percent), 3 participants with indeterminate results (3 percent), and no participants with negative results. Of 35 participants positive for HCV by RIBA but negative for HCV RNA, 34 were tested for HCV RNA at least twice (mean number of tests, 3; range, 2 to 7). PCR testing was always performed on the sample obtained at entry into the study, and subsequent tests were performed on samples obtained at intervals of three months or more. Hence, there was no evidence of intermittent viremia in the samples tested. Of the three participants with indeterminate results by RIBA who had viremia detectable by PCR, two tested positive with a more specific third-generation RIBA (RIBA 3.0, Chiron) that has not yet been licensed by the Food and Drug Administration.

#### Extent of Liver Disease

At the initial evaluation, biochemical evidence of liver disease was found in 138 of 248 participants positive for HCV by RIBA (56 percent) and in 11 of 131 participants negative for HCV (8 percent,  $P < 0.001$ ). When the follow-up period was included in the analysis, the percentage of participants with at least one elevated alanine aminotransferase measurement (upper limit of normal, 41 units per liter) increased to 69 percent among participants positive for HCV by RIBA and 15 percent among those negative for HCV ( $P < 0.001$ ). The median alanine aminotransferase level in the HCV-positive group was 48 units per liter (range, 4 to 556). During follow-up, most HCV-positive participants had either persistently normal alanine aminotransferase levels (31 percent) or peak levels no more than twice the upper limit of normal (42 percent). In 15 percent of HCV-positive participants, the alanine aminotransferase levels were more than twice the upper limit of normal, and in 12 percent they were more than three times that upper limit.

An elevated alanine aminotransferase level was strongly correlated with the presence of HCV RNA. Of 190 participants positive for HCV by RIBA who were followed for more than six months and had at least three blood samples tested, 110 of the 161 participants who had detectable HCV RNA (68 percent) had abnormal alanine aminotransferase levels, as compared with 5 of the 29 participants without detectable HCV RNA (17 percent,  $P < 0.001$ ). Conversely, 24 of 29 participants who tested negative for HCV RNA (13 percent of the 190 HCV-positive donors) had persistently normal alanine aminotransferase levels.

Of the 77 participants positive for HCV by RIBA who underwent liver biopsy, 6 (8 percent) had no histo-

Table 4. Results of a Second-Generation Enzyme Immunoassay for Anti-HCV Performed among the Sexual Contacts and Family Members of 121 Anti-HCV-Positive Study Participants, According to the HCV Status of the Participants as Determined by RIBA.

PARTICIPANT'S HCV STATUS	SEXUAL PARTNERS	CHILDREN	PARENTS
	no. positive/no. tested (%)		
Positive (n = 98)	9/85 (11)*	5/47 (11)†	2/9 (22)‡
Indeterminate (n = 15)	0/15	0/2	None tested
Negative (n = 8)	0/8	None tested	None tested

\*Of the nine anti-HCV-positive partners, eight had defined parenteral exposures to HCV.

†Of the five anti-HCV-positive children, four had probable passive transfer of antibody and the fifth had a parenteral risk factor for exposure to HCV.

‡Both anti-HCV-positive parents had defined parenteral exposures to HCV.

logic evidence of hepatitis; these 6 had normal alanine aminotransferase levels, and 5 of the 6 were negative for HCV RNA. Sixty-six (86 percent) had mild-to-moderate chronic hepatitis, and five (6 percent) had severe tissue lesions (indicating severe chronic hepatitis or cirrhosis). All the participants with severe lesions had detectable HCV RNA and peak alanine aminotransferase values more than twice the upper limit of normal.

No participant with HCV infection (who was positive for HCV by RIBA, positive for HCV RNA, or both) had characteristic symptoms of hepatitis. Fatigue was common, but its frequency was similar in infected and uninfected participants. HCV-related syndromes were detected in three participants with chronic infection, two with porphyria cutanea tarda, and one with essential mixed cryoglobulinemia.

### DISCUSSION

Although the available resources permitted the enrollment of only 10 percent of the more than 4000 donors identified as positive for anti-HCV, the demographic characteristics of the study participants and their HCV status as determined by RIBA suggested that the participants were generally similar to both the entire population of blood donors and those who did not enroll. Only the level of education and the proportion who were first-time donors differed. We used donors positive for HCV on enzyme immunoassay and negative by RIBA as a control group, rather than donors negative on enzyme immunoassay. This decision was based on cumulative data indicating that donors negative for HCV by RIBA are not infected with HCV.<sup>17</sup> In our study, no participant positive by enzyme immunoassay and negative by RIBA was positive for HCV RNA or subsequently positive by RIBA. Furthermore, 83 percent of these participants later tested negative with the more specific second-generation enzyme immunoassay. Although on repeated testing 15 percent of participants negative by RIBA had slight elevations of alanine aminotransferase, these elevations did not appear to be related to HCV infection, as was evidenced by the absence of HCV RNA and markers of active infection with the hepatitis A and B viruses.

This study validates the efficacy of the RIBA in confirming the presence or absence of HCV infection in asymptomatic people. Most study participants who were positive for HCV by RIBA had risk factors for the parenteral acquisition of HCV, biochemical indexes of chronic hepatitis, and detectable HCV RNA, whereas there were very low rates of each of these factors in the participants negative by RIBA (Fig. 1). Aside from a slightly higher frequency of risk factors for parenteral HCV infection, the group with indeterminate results by RIBA was similar to the group with negative results (Table 3 and Fig. 1). Indeterminate results are common with this assay, but only rarely do they indicate HCV infection.

Positivity for HCV by RIBA was associated with younger age, lower educational level, and black race, factors also associated with hepatitis B virus and hu-

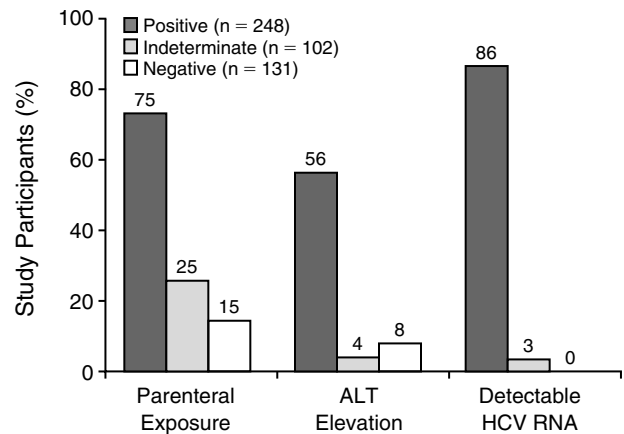


Figure 1. Frequency of Parenteral Exposure to HCV, Elevated Levels of Alanine Aminotransferase (ALT), and Detectable Serum Levels of HCV RNA in the Study Participants, According to the Results of a Second-Generation RIBA.

Numbers above the bars are percentages.  $P < 0.001$  for the comparison of the HCV-positive group with the other two groups for each variable. For parenteral exposure,  $P = 0.03$  for the comparison of the group with indeterminate results with the HCV-negative group. When serial determinations of alanine aminotransferase during follow-up were included in the analysis, the proportion of participants with at least one elevated measurement increased from 56 percent to 69 percent in the HCV-positive group and from 8 percent to 15 percent in the HCV-negative group.

man immunodeficiency virus infections.<sup>18</sup> These factors correlate with socioeconomic status and with routes of transmission, such as intravenous drug use, that are more common among the economically disadvantaged.

In the logistic-regression analysis, five factors were associated with confirmed HCV infection: intravenous drug use, blood transfusion, ear piercing in men, intranasal cocaine use, and sexual promiscuity. It is a matter of concern that a substantial number of participants — 23 percent of the cohort and 42 percent of participants positive for HCV by RIBA — acknowledged prior intravenous drug use in our study, even though they had denied such use at the time of screening. Only one study participant was currently using intravenous drugs; none considered themselves addicts or thought that their past intravenous drug use would affect the safety of their blood.<sup>19</sup>

If ear piercing in men is combined with other established sources of blood-borne HCV transmission,<sup>20-23</sup> including transfusion, intravenous drug use, tattooing, acupuncture, and occupational exposure, then 75 percent of participants positive for HCV by RIBA had risk factors for parenteral transmission. Even this high proportion may be an underestimate, because some participants may not have been aware of a transfusion in the remote past or may not have revealed a history of intravenous drug use. HCV transmission by other routes may be a less important factor than previously suggested.<sup>5,24</sup>

The strong independent association between intranasal cocaine use and HCV infection raises the questions of whether that practice is a surrogate for other behav-

ior that would foster transmission of the virus and whether the mechanics of intranasal cocaine use permit blood-borne transmission among users. If contaminated with blood, a device shared during intranasal cocaine use could convey virus to denuded nasal mucosa, allowing it to enter the bloodstream. Although this hypothesis was not proved in the present study, intranasal cocaine use could be an unrecognized route of parenteral transmission of HCV or other viruses.

The sexual transmission of HCV has been controversial.<sup>5,25-29</sup> Our study showed a significant association between HCV infection and a history of sexual promiscuity. However, when we tested the long-term sexual partners of 85 participants positive for HCV by RIBA, only 1 partner was positive for anti-HCV and (according to that partner) had no other potential parenteral exposures. Although the possibility of sexual transmission of HCV has not been ruled out, these data imply, as do other studies,<sup>30,31</sup> that such transmission is very inefficient.

The high frequency of detectable viremia in the study participants (86 percent) indicates that most people positive for HCV by RIBA are infected and infectious,<sup>32,33</sup> but 13 percent of our participants who were positive on RIBA were HCV-negative by PCR on at least two occasions. This suggests that some people recover from HCV infection and retain specific antibody in the absence of viremia.

Finally, although biochemical and histologic evidence of chronic liver disease was common, elevations of serum alanine aminotransferase were slight and tissue lesions generally mild. Only 6 percent of participants positive for HCV by RIBA had severe histologic lesions, even though most had been infected for more than a decade.

## REFERENCES

- Alter MJ. The detection, transmission, and outcome of hepatitis C virus infection. *Infect Agents Dis* 1993;2:155-66.
- McCullough J. The nation's changing blood supply system. *JAMA* 1993;269:2239-45.
- Shakil AO, Conry-Cantilena C, Alter HJ, et al. Volunteer blood donors with antibody to hepatitis C virus: clinical, biochemical, virologic, and histologic features. *Ann Intern Med* 1995;123:330-7.
- Alter MJ, Mast EE. The epidemiology of viral hepatitis in the United States. *Gastroenterol Clin North Am* 1994;23:437-55.
- Alter MJ, Hadler SC, Judson FN, et al. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. *JAMA* 1990;264:2231-5.
- Alter HJ. Descartes before the horse: I clone, therefore I am: the hepatitis C virus in current perspective. *Ann Intern Med* 1991;115:644-9.
- Alter MJ, Coleman PJ, Alexander WJ, et al. Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. *JAMA* 1989;262:1201-5.
- Alter MJ. Inapparent transmission of hepatitis C: footprints in the sand. *Hepatology* 1991;14:389-91.
- Everhart JE, Di Bisceglie AM, Murray LM, et al. Risk for non-A, non-B (type C) hepatitis through sexual or household contact with chronic carriers. *Ann Intern Med* 1990;112:544-5.
- Alter HJ. Chronic consequences of non-A, non-B hepatitis. In: Seeff LB, Lewis JH, eds. *Current perspectives in hepatology*. New York: Plenum Medical, 1989:83-97.
- Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995;332:1463-6.
- Seeff LB, Buskell-Bales Z, Wright EC, et al. Long-term mortality after transfusion-associated non-A, non-B hepatitis. *N Engl J Med* 1992;327:1906-11.
- Shindo M, Di Bisceglie AM, Cheung L, et al. Decrease in serum hepatitis C viral RNA during alpha-interferon therapy for chronic hepatitis C. *Ann Intern Med* 1991;115:700-4.
- Fleiss JL. *Statistical methods for rates and proportions*. 2nd ed. New York: John Wiley, 1981.
- Snedecor GW, Cochran WG. *Statistical methods*. 7th ed. Ames: Iowa State University Press, 1980.
- Hosmer DW Jr, Lemeshow S. *Applied logistic regression*. New York: John Wiley, 1989.
- Cuthbert JA. Hepatitis C: progress and problems. *Clin Microbiol Rev* 1994;7:505-32.
- Leitman SF, Klein HG, Melpolder JJ, et al. Clinical implications of positive tests for antibodies to human immunodeficiency virus type 1 in asymptomatic blood donors. *N Engl J Med* 1989;321:917-24.
- Conry-Cantilena C, Melpolder JC, VanRaden M, Alter HJ. Characteristics and motivation of anti-HCV positive blood donors admitting intravenous drug use. *Transfusion* 1995;35:58S. abstract.
- Mitsui T, Iwano K, Masuko K, et al. Hepatitis C virus infection in medical personnel after needlestick accident. *Hepatology* 1992;16:1109-14.
- Kiyosawa K, Sodeyama T, Tanaka E, et al. Hepatitis C in hospital employees with needlestick injuries. *Ann Intern Med* 1991;115:367-9.
- Abildgaard N, Peterslund NA. Hepatitis C virus transmitted by tattooing needle. *Lancet* 1991;338:460.
- Kiyosawa K, Tanaka E, Sodeyama T, et al. Transmission of hepatitis C in an isolated area in Japan: community-acquired infection. *Gastroenterology* 1994;106:1596-602.
- Alter MJ, Margolis HS, Krawczynski K, et al. The natural history of community-acquired hepatitis C in the United States. *N Engl J Med* 1992;327:1899-905.
- Eyster ME, Alter HJ, Aledort LA, Goedert JJ. Cotransmission of hepatitis C virus (HCV) and human immunodeficiency virus from men with hemophilia to their sexual partners. *Blood* 1990;76:398a. abstract.
- Melbye M, Biggar RJ, Wantzin P, Krogsgaard K, Ebbesen P, Becker NG. Sexual transmission of hepatitis C virus: cohort study (1981-9) among European homosexual men. *BMJ* 1990;301:210-2.
- Lissen E, Alter HJ, Abad MA, et al. Hepatitis C virus infection among sexually promiscuous groups and the heterosexual partners of hepatitis C virus infected index cases. *Eur J Clin Microbiol Infect Dis* 1993;12:827-31.
- Eyster ME, Alter HJ, Aledort LM, Quan S, Hatzakis A, Goedert JJ. Heterosexual co-transmission of hepatitis C virus (HCV) and human immunodeficiency virus (HIV). *Ann Intern Med* 1991;115:764-8.
- Esteban JI, Esteban R, Viladomiu L, et al. Hepatitis C virus antibodies among risk groups in Spain. *Lancet* 1989;2:294-7.
- Brettler DB, Mannucci PM, Gringeri A, et al. The low risk of hepatitis C virus transmission among sexual partners of hepatitis C-infected hemophilic males: an international, multicenter study. *Blood* 1992;80:540-3.
- Weinstock HS, Bolan G, Reingold AL, Polish LB. Hepatitis C virus infection among patients attending a clinic for sexually transmitted diseases. *JAMA* 1993;269:392-4.
- Aach RD, Stevens CE, Hollinger FB, et al. Hepatitis C virus infection in post-transfusion hepatitis — an analysis with first- and second-generation assays. *N Engl J Med* 1991;325:1325-9.
- Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 1989;321:1494-500.