Long-Term Mortality and Morbidity of Transfusion-Associated Non-A, Non-B, and Type C Hepatitis: A National Heart, Lung, and Blood Institute Collaborative Study

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Persons with non-A, non-B hepatitis (cases) identified in 5 transfusion studies in the early 1970s have been followed ever since and compared for outcome with matched, transfused, non-hepatitis controls from the same studies. Previously, we reported no difference in all-cause mortality but slightly increased liver-related mortality between these cohorts after 18 years follow-up. We now present mortality and morbidity data after approximately 25 years of follow-up, restricted to the 3 studies with archived original sera. All-cause mortality was 67% among 222 hepatitis C-related cases and 65% among 377 controls (P = NS). Liver-related mortality was 4.1% and 1.3%, respectively (P = .05). Of 129 living persons with previously diagnosed transfusion-associated hepatitis (TAH), 90 (70%) had proven TAH-C, and 39 (30%), non-A-G hepatitis. Follow-up of the 90 TAH-C cases revealed viremia with chronic hepatitis in 38%, viremia without chronic hepatitis in 39%, anti-HCV without viremia in 17%, and no residual HCV markers in 7%. Thirty-five percent of 20 TAH-C patients biopsied for biochemically defined chronic hepatitis displayed cirrhosis, representing 17% of all those originally HCV-infected. Clinically evident liver disease was observed in 86% with cirrhosis but in only 23% with chronic hepatitis alone. Thirty percent of non-A, non-B hepatitis cases were unrelated to hepatitis viruses A,B,C, and G, suggesting another unidentified agent. In conclusion, all-cause mortality approximately 25 years after acute TAH-C is high but is no different between cases and controls. Liver-related mortality attributable to chronic hepatitis C, though low (<3%), is significantly higher among the cases. Among living patients originally HCV-infected, 23% have spontaneously lost HCV RNA. (HEPATOLOGY 2001; 33:455-463.)

Acute non-A, non-B hepatitis, attributable predominantly to hepatitis C virus (HCV) infection, is usually a mild and asymptomatic illness.[1] Nevertheless, more than 80% of such persons develop persistent infection and most have biochemical evidence of chronic hepatitis.[1-4] Among those biopsied, about 20% display cirrhosis within 10 to 20 years, some of whom develop hepatocellular carcinoma (HCC).[5,6]

The true frequency and rate of progression of HCV–related liver disease, however, remains ill-defined. Current views on outcome derive largely from studies of persons with already established chronic hepatitis C whose dates of initial infection are frequently unknown. This approach provides information only on those who come to medical attention and fails to define the full spectrum of potential clinical outcomes. Such a narrowed focus may overestimate the frequency, rate of progression, and severity of associated liver disease. To avoid this inherent bias, it is necessary to conduct long-term, prospective studies that involve large numbers of persons evaluated from onset of well-defined acute hepatitis C.

Accordingly, in 1987, we began a follow-up study of persons involved in transfusion-associated hepatitis studies performed in the United States approximately 2 decades earlier, studies that had been designed specifically to identify the onset of acute hepatitis.[7] The goal of the follow-up study was to compare long-term mortality and morbidity between those who had developed acute non-A, non-B hepatitis, and matched transfused patients from the same studies who had not developed hepatitis.

We reported previously that all-cause mortality did not differ between these 2 groups after an average follow-up interval of 18 years; liver-related mortality, however, showed a slight but significant increase among the hepatitis cases.[7] We now present updated mortality data and outcome data among surviving hepatitis cases grouped according to their HCV status.

PATIENTS AND METHODS

Selection of Study Cohorts

The derivation of the study populations has been described.[7] Briefly, the cohort was assembled originally from 5 prospective trans-

Abbreviations: HCV, hepatits C virus; HCC, hepatocellular carcinoma; ALT, alanine transaminase; AST, aspartate transaminase; VA, Veterans' Affairs; NIH, National Institute of Health; TTV, Tranfusion Transmitted Viruses; WR, Walter Reed; PCR, polymerase chain reaction; HGV, hepatitis G virus; TAH, transfusion-associated hepatitis.

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fusion-associated hepatitis studies (2 Veterans Affairs [VA] Cooperative studies, the National Institutes of Health [NIH] Blood Bank study, the Transfusion Transmitted Viruses [TTV] study, and the Walter Reed [WR] Army Hospital study) that had been conducted between 1967 and 1980.[8-12] Serial enzyme monitoring of blood recipients had been performed in all 5 studies and, therefore, uniform criteria could be employed to diagnose hepatitis, namely the development of otherwise unexplained increases in alanine aminotransferases (ALT) between 2 and 24 weeks after transfusion.[7] The patients with acute hepatitis from the original studies, hereafter referred to as cases, were combined for current evaluation and follow-up.

Each case was matched with approximately 2 transfused individuals from the same studies who had not developed characteristic enzyme abnormalities. Matching variables included initial treatment center, sex, race, use of hepatitis immunoglobulin, a history of alcoholism, age, number of units of blood transfused, and date of transfusion. The matched individuals are hereafter referred to as controls.

The mortality data reported previously[7] were derived from all 5 original studies. Because the present report focuses on serologic and molecular changes over time, as well as long-term clinical outcomes, we confined the analysis to the 3 studies in which the original sera had been maintained in frozen storage. These included the second VA cooperative study,[9] the NIH study,[10] and the TTV study;[11] sera from the other 2 studies had either not been stored (Walter Reed study) or had been exhausted or compromised by repeated earlier serologic testing (first VA cooperative study).

Criteria to Diagnose and Categorize the Original Cases of Acute Viral Hepatitis

To assure uniformity among the 3 studies, subjects selected as hepatitis cases required the identification of raised ALT values on at least 2 occasions, occurring between 2 and 24 weeks following transfusion, at least 1 of which had to exceed twice the upper limit of normal and the second, to exceed at least the upper limit of normal. For all 3 studies, the development of hepatitis B surface antigen (HBsAg) in temporal association with biochemical evidence of hepatitis defined acute hepatitis B; the absence of HBsAg , and in 2 of the studies,[10,11] the absence of IgM antibody to hepatitis A defined non-A, non-B hepatitis. Subsequently, when HCV assays became available, repository sera of the original non-A, non-B hepatitis cases were tested for the presence of antibody to the hepatitis C virus (anti-HCV). A diagnosis of hepatitis C required the appearance and persistence of a confirmed positive test for anti-HCV temporally related to serum enzyme elevations.

Serologic and Virologic Assays

Tests for hepatitis A and B were performed using routine radioimmunoassay methods (Abbott Laboratories, North Chicago, IL). Anti-HCV testing was done by enzyme-linked immunoassay (EIA 2.0, Abbott Laboratories), and all reactive samples were confirmed by a supplementary dot-blot immunoassay (Abbott Matrix HCV) or a strip immunoblot assay (RIBA, Chiron Corp., Emeryville, CA).

Serum HCV-RNA was quantified with standards prepared from plasma from a single HCV-RNA reactive blood donor. The HCV-RNA concentration of this specimen was initially determined by the Chiron branched DNA assay and by coamplification with HIV-1 RNA standards developed in the laboratory of FBH. Preparation of standards and study specimens for polymerase chain reaction (PCR) was performed with guanidinium isothiocyanate-phenol-chloroform. PCR-based amplification was done with primers homologous to sequences in the 5′-noncoding region, and colorometric detection of PCR products was performed using a commercial kit.[13,14] HCV-RNA quality control materials were prepared from a sample of HCV (strain H) with a concentration of $10^{6.5}$ CID_{50} per mL (gift of Dr. Robert Purcell, Hepatitis Viruses Section, NIAID). Assays of samples containing 300 CID_{50} per mL (about 5400 HCV RNA copies per mL) showed a between-run coefficient of variation of 11.5%. The lower limit of detection of the assay for this study was 1,000 copies per mL. Assays were performed under code without knowledge of clinical status. Because virtually all untreated persons with chronic hepatitis C have HCV RNA levels over 5,000 copies per mL in their blood, it is extremely unlikely that there were false-negative results given the sensitivity of the assay used in this study.

Hepatitis G virus (HGV-RNA) levels in serum were measured using reverse transcription PCR in the laboratory of HJA as previously described.[15]

Original repository sera were not optimally stored for minimizing loss of nucleic acid[16] and, hence, were not examined for HCV-RNA. However, HCV-RNA testing was performed in the follow-up study because the sera were separated from the clot within 2 to 4 hours and stored at −30°C until tested.

Sequence of Evaluation of Living Cohort in the Follow-Up Study

Study subjects were contacted for an initial health-directed interview, physical examination, and phlebotomy for liver-related biochemical tests, hepatitis serologies, and baseline alpha-fetoprotein level measurement. They were then requested to return on at least 2 occasions, at 3-month intervals, for questioning regarding intervening medical problems and for repeat biochemical and serologic screening. All patients provided informed consent, and the study was approved by the relevant Institutional Review Boards.

Diagnostic Criteria for Chronic Hepatitis

An elevated ALT in at least 2 of 3 blood samples obtained during the 6-month evaluation period defined chronic hepatitis; hepatitis C was held responsible if there was accompanying and persisting RIBA-confirmed anti-HCV reactivity. Persons with chronic hepatitis were requested to undergo a liver biopsy whenever feasible. Biopsies were examined at the Armed Forces Institute of Pathology, Washington, DC by KGI and ZDG. The histologic diagnosis was reached by consensus.

Statistical Analyses

Stratified analyses were employed to adjust for differences among the 5 original studies using the SAS computer program.[17] These included analyses of variance for continuous data,[18] the Mantel-Haenszel statistic for dichotomous data,[19] and stratified Kaplan-Meier Survival curves.[20]

TABLE 1. Characteristics of Original Study Cohort by Anti-HCV Status of Cases During the Original Episode of Transfusion-Associated Hepatitis

	Anti-HCV–Positive Cases and Their Controls*		Anti-HCV–Negative Cases and Their Controls*	
Characteristic	Cases (N = 222)	Controls (N = 377)	Cases (N = 92)	Controls (N = 168)
Male sex, %	66.7	65.8	69.6	66.7
African-American race, %	15.8	13.8	17.4	17.3
Age at transfusion†	49 ± 13	49 ± 13	49 ± 14	49 ± 14
Year of transfusion†	1,975 ± 3	1,975 ± 2	1,976 ± 3	1,976 ± 2
Units of blood†	8.2 ± 7.3	7.4 ± 7.3	7.0 ± 7.3	6.4 ± 7.0
>2 units of blood, %	75.2	67.4	64.1	70.2
History of alcoholism, %	5.0	3.2	5.4	3.6

*No significant differences between cases and controls for either HCV–positive or HCV–negative cohort.

†Mean ± S.D.

TABLE 2. All-Cause and Liver-Related Mortality, by Anti-HCV Status of Cases During the Original Episode of Transfusion-Associated Hepatitis

	Anti-HCV–Positive Cases and Their Controls		Anti-HCV–Negative Cases and Their Controls	
	Cases (N = 222)	Controls (N = 377)	Cases (N = 92)	Controls (N = 168)
*All Deaths**				
At initial tracing; '89-'91, n (%)	99 (44.6)	174 (46.1)	36 (39.1)	66 (39.3)
As of 12/3/1997, n (%)	149 (67.1)	245 (65.0)	47 (51.1)	92 (54.8)
Liver-Related Deaths†				
At initial tracing; '89-'91, n (%)	5 (2.3)	5 (1.3)	2 (2.2)	1 (0.6)
As of 12/3/1997, n (%)	9 (4.1)†	5 (1.3)†	3 (3.3)	1 (0.6)

*No difference in all-cause mortality between cases and controls for either the HCV–positive or the HCV–negative cohort.

†$P = .05$ for HCV–positive cases as of 12/3/97.

RESULTS

Comparison Between Entire Cohort of Non-A, Non-B Transfusion-Associated Hepatitis Cases and Their Controls

Demographic and Historical Comparisons. In the 3 original studies, 314 persons were diagnosed as having developed acute transfusion-associated hepatitis (95, VA; 142, TTV; 77, NIH). In 222 (70.7%) of them (Table 1), the disease was attributed to HCV infection, the cause for the remaining 92 cases being undetermined. The 222 HCV–positive cases were matched with 377 controls, whereas the 92 HCV–negative cases were matched with 168 controls. There were 14 additional patients diagnosed as acute hepatitis based on characteristic enzyme abnormalities who were found to be anti-HCV–positive. However, a pretransfusion serum specimen was also found to be anti-HCV–positive, indicating that the infection had predated the transfusion. Because there was no way to determine whether the hepatitis observed in these individuals was the result of a flare in their pre-existing HCV infection or to superinfection with a non-ABC agent, they were excluded from subsequent analysis.

As shown in Table 1, comparison of the original cases and controls according to the HCV status revealed no significant differences in the proportion of males, the percent who were African-American, age at the time of transfusion, the year of transfusion, the units of blood received, or a history of alcoholism.

All-Cause and Liver-Related Mortality. At the time of the initial call-back between 1989 and 1991, approximately 18 years after the index transfusion, 99 (44.6%) of the HCV–positive group and 174 (46.1%) of their controls had expired, whereas mortality among the HCV–negative group was 36 (39.1%) for the cases and 66 (39.3%) for their controls (Table 2). Re-evaluation of all-cause mortality approximately 25 years after the index transfusion (December 31, 1997) showed a comparable increase in all groups. In the HCV–positive category, 149 (67.1%) of the cases had died as compared with 245 (65.0%) of the controls, whereas in the HCV–negative group, 47 (51.1%) of the cases and 92 (54.8%) of the controls had died (Table 2). None of the comparisons were significantly different.

Survival curves over a 20-year period, comparing cases and controls of both the HCV–positive and HCV–negative groups, are shown in Fig. 1. None of the comparison curves showed a significant difference.

Differences were found, however, in liver-related causes of death (Table 2). At initial call-back, liver disease had accounted for 5 deaths (2.3%) among the 222 HCV–positive cases and for 5 deaths (1.3%) among the 377 controls. The re-analysis 7 years later that included follow-up to December

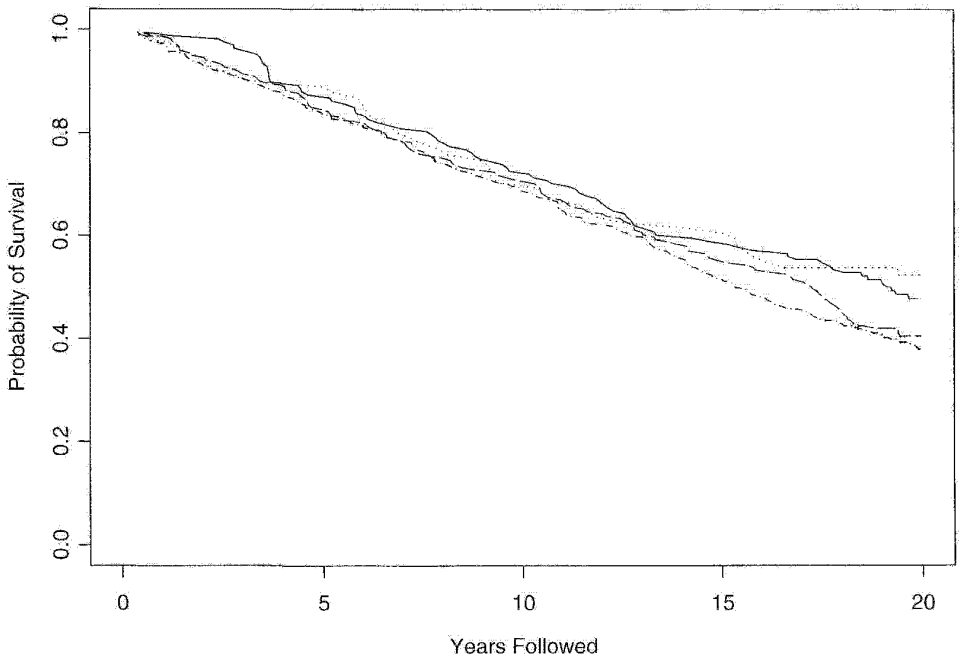

FIG. 1. Life table survival curves comparing survival rates between anti-HCV–positive hepatitis cases [n = 222, 40% (95% CI: 34%,47%) at 20 years, *dashed line*] and controls for anti-HCV–positive cases [n = 377, 38% (95% CI: 33%,43%) at 20 years, *dashed and dotted line*], as well as between anti-HCV–negative cases [n = 92, 52% (95% CI:42%,63%) at 20 years, *dotted line*] and controls for anti-HCV–negative cases [n = 168, 48% (95% CI: 40%,56%) at 20 years, *solid line*]. The lines show no significant differences.

TABLE 3. Success in Tracing and Interviewing Living Cohorts, by Anti-HCV Status of Cases During the Original Episode of Transfusion-Associated Hepatitis

	Anti-HCV–Positive Cases and Their Controls		Anti-HCV–Negative Cases and Their Controls	
	Cases (N = 123)	Controls (N = 203)	Cases (N = 56)	Controls (N = 102)
Cannot be traced, n (%)	14 (11.4)	25 (12.3)	2 (3.6)	8 (7.8)
Refused interview, n (%)	16 (13.0)	37 (18.2)	11 (19.6)	17 (16.6)
Interviewed only, n (%)	3 (2.4)	5 (2.5)	4 (7.1)	4 (3.9)
Interviewed, blood sample obtained, n (%)	90 (67.0)	136 (69.6)	39 (69.6)	73 (71.6)

1997 revealed that liver-related deaths among the HCV–positive group had increased to 9 (4.1%) for the cases but remained at 5 (1.3%) for the controls (P = .05). The causes for the 9 liver-related deaths among the HCV–positive cases, based on death certificate evaluations and medical records, where available, consisted of hepatocellular carcinoma in 3 patients, cirrhosis in 3, chronic hepatitis in 1, hepatitis unspecified in 1, and "hepatic failure" in 1. Liver-related deaths among the controls were recorded as cirrhosis in 4 and "other sequelae of liver disease" in 1.

Mortality resulting from liver disease was examined also among the patients who were HCV–negative (Table 2). Initially, mortality among the 92 cases was 2 (2.2%) as compared with 1 (0.6%) of the 168 controls; however, by the end of December 1997, mortality had risen to 3 (3.3%) among the cases but remained at 1 (0.6%) among the controls ($P = .098$). Liver-related causes of deaths among the three HCV–negative cases were HCC in 1, cirrhosis in 1, and "unspecified disorder of the liver" in 1, whereas the single liver-related death in the controls was recorded as HCC.

Follow-Up Study

Success Rate in Tracing, Interviewing, and Sampling the Living Cohort. Initial interviews began in June 1989 and ended in December 1991. Among the 314 patients diagnosed with hepatitis in the 3 original studies, 179 (57%) were still living at the time of the follow-up study, 123 among the HCV–positive and 56 among the HCV–negative groups (Table 3). One hundred twenty-nine (72.1%) of them had both original (repository) and follow-up sera available for serologic evaluation (90, HCV–positive; 39 HCV–negative), and are thus included in the morbidity analysis to follow. Those excluded consisted of 16 (8.9%) who could not be traced for follow-up evaluation, 27 (15.1%) who had refused follow-up participation, and 7 (3.9%) who permitted interview only. The success rate in tracing the controls was almost identical.

Clinical and Biochemical Characteristics. Screening of the original sera for anti-HCV revealed that 90 of the 129 hepatitis cases (70%) could be defined as definite instances of transfusion-associated hepatitis C (TAH-C) based on anti-HCV seroconversion with RIBA confirmation, and the remaining 39 (30%) were classified as transfusion-associated hepatitis cases unrelated to HCV (Table 4). Selected characteristics found at initiation of follow-up comparing the HCV–positive (90 persons) with the HCV–negative (39 persons) cases as well as all cases (129 persons) with their controls (209 persons) are shown in Table 4. HCV–positive cases were statistically significantly more likely to have hepatomegaly, as determined by physical examination, and elevations of serum ALT and AST as compared with HCV–negative cases. Comparison of cases with their controls revealed that the cases were significantly more likely to experience tiredness and anorexia, and to have hepatomegaly, tender liver, and thrombocytopenia as well as elevated levels of serum ALT and AST and bilirubin.

TABLE 4. Comparison Between Anti-HCV–Positive and Anti-HCV–Negative Non-A, Non-B Hepatitis Cases, and Between All Cases and Controls at Initiation of Follow-Up Evaluation*

Characteristic	Anti-HCV+ Cases (a) (N = 90)	Anti-HCV− Cases (b) (N = 39)	All Cases (c) (N = 129)	Controls (d) (N = 209)	P a vs. b	P c vs. d
Age at initiation of follow-up†	60 ± 12	58 ± 13	59 ± 12	59 ± 13	.28	.94
Additional transfusions, %	23	23	23	28	.88	.32
Ever consumed alcohol, %	63	74	67	70	.27	.58
Recent alcohol, >175 gm/wk, %	9	13	10	13	.34	.41
Usual alcohol, >175 gm/wk, %	23	26	24	31	.29	.17
Tiredness, %	38	36	37	26	.68	.01
Anorexia, %	17	18	17	5	.96	.0003
Jaundice, %	4	0	3	1	.31	.44
Ascites, %	1	0	0.8	0.5	1.00	1.00
Hepatomegaly, %	22	0.6	18	6	.03	.0009
Tender liver, %	12	3	10	3	.09	.01
Splenomegaly, %	7	0	5	3	.07	.38
Elevated ALT, %	44	8	34	6	<.0001	<.0001
Elevated AST, %	49	13	38	7	.0002	<.0001
Elevated total serum bilirubin, %	14	13	14	6	.69	.016
Elevated alkaline phosphatase, %	27	10	22	16	.06	.25
Alpha-fetoprotein >50 units, %	1	0	0.8	0.5	1.00	1.00
Hypoalbuminemia (<3 g/dL), %	1	0	0.8	0	1.00	.38
Thrombocytopenia (<100,000/μL), %	4	0	3	0	.31	.02

*Includes only those who were available for examination at initiation of follow-up.
†Mean ± S.D.

TABLE 5. Evaluation of Hepatitis C Virus Serologic and Biochemical Sequelae Among 129 Cases With Sera Available at Time of Original Study and at Follow-Up

Original Study Status			Follow-Up Study Status				
Anti-HCV Status	No	%	Anti-HCV	HCV-RNA	Chronic Hepatitis*	No.	%
Positive	90	69.8	+	+	+	34	37.8
			+	+	−	35	38.9
			+	−	+	1	1.1
			+	−	−	14	15.6
			−	−	+	1	1.1
			−	−	−	5	5.6
Negative	39	30.2	+	+	−	1	2.6
			−	−	+	4	10.3
			−	−	−	34	87.2

*Based on ALT criteria (see text).

Serologic Analysis and Outcome of Anti-HCV Positive Cases. Among the 90 persons with a clearly established original diagnosis of acute TAH-C, based on observed seroconversion, 69 (77%) were positive for both anti-HCV and HCV-RNA at follow-up, signifying continuing HCV infection (Table 5). The remaining 21 consisted of 15 (17%) who had anti-HCV in the absence of HCV-RNA suggesting recovery from past infection, and 6 (7%) who showed no serologic or molecular evidence of their earlier HCV infection. Thus, 21 (23%) of 90 patients infected at the time of transfusion appear to have spontaneously recovered (Fig. 2). Among the 69 patients who were HCV-RNA–positive during follow-up, 49% (38% of the total of 90 cases) had biochemical evidence of chronic hepatitis whereas the remaining 51% (39% of the total) had normal ALT values (Fig. 2). In contrast, only 1 (7%) of the 15 with anti-HCV reactivity alone and 1 (17%) of the 6 with no HCV markers had biochemically-defined chronic hepatitis. In composite, among the 90 subjects who initially showed anti-HCV seroconversion, persistent viremia was identified in 77% and biochemical evidence of chronic hepatitis in 40%, of whom 94% had accompanying HCV-RNA (Table 5 and Fig. 2).

Examination of the outcome among the 14 persons with HCV infection that preceded the transfusion and who were, therefore, not included for analysis with the legitimate new cases revealed that 6 (43%) had died before initiation of the follow-up phase, none of them from liver disease; 3 had refused further follow-up; and 5 were evaluated during callback. Four of the 5 were anti-HCV–positive, 3 with detectable HCV-RNA, 2 of whom had evidence of chronic hepatitis. The fifth person was anti-HCV–negative and had normal serum enzymes. The long-term outcome, had these 14 persons been included, would not have differed from that observed among the group clearly established to be acute transfusion-related hepatitis C.

Earlier analysis of the database of the entire cohort of these 3 studies revealed that patients with hepatitis C who were heavy alcoholics were approximately 4 times more likely to develop cirrhosis than patients with hepatitis C who were not heavy alcoholics.[21] In the present study, we examined the issue again, focusing attention on the 69 patients in follow-up who were both anti-HCV–positive and HCV-RNA–positive. Sixteen (23%) of them reported heavy alcohol use (more than 175 g of alcohol per week), of whom 9 (56%) had chronic hepatitis and/or cirrhosis. Among the remaining 53 without a history of heavy alcohol use, 25 (47%) had evidence of chronic hepatitis and/or cirrhosis. Although there is a trend toward a higher rate of chronic hepatitis and/or cirrhosis among HCV–positive persons with heavy alcoholism (56%) than among those without (47%), the data provide only marginal confidence because of the small numbers available for analysis.

Relationship Between Histologic Findings and Clinical Manifestations. At the time of planning of the follow-up study, approximately 8 years earlier, it had been agreed that liver biopsies would be requested only from those with biochemical evidence of chronic hepatitis. A total of 40 patients met this requirement during follow-up, 36 of them from the original anti-HCV seropositive group and 4 from the original anti-HCV seronegative group. Among the 34 hepatitis cases that were positive for anti-HCV and HCV-RNA (Table 6), 20 (59%) underwent biopsy; 13 (65%) of them had histologic features of mild or moderate chronic hepatitis without cirrhosis or bridging fibrosis, and seven (35%) had cirrhosis. Of the 13 patients with biopsy-proven chronic hepatitis, 10 (77%) showed no clinical evidence of liver disease, whereas 3 (23%) had mild signs or symptoms (any 1 of the following – splenomegaly, hypoalbuminemia, thrombocytopenia). In contrast, 4 (57%) of the 7 patients with cirrhosis had severe clinical manifestations (varices or ascites and/or any 2 of the following – splenomegaly, hypoalbuminemia, thrombocytopenia), whereas 2 had clinical features of mild chronic liver disease, as described earlier. The other patient with cirrhosis had no evidence of clinical liver disease although HCC was detected and resected. Thus, among the persons biopsied, clinically evident severe liver disease was confined to those with histologic evidence of cirrhosis.

The 16 TAH-C cases with biochemically defined chronic hepatitis in follow-up who were not biopsied were composed of 9 (56%) with no clinical manifestations, 3 (19%) with mild

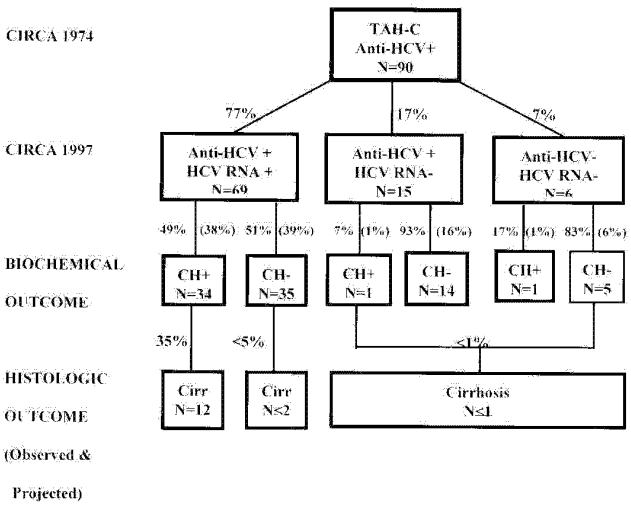

FIG. 2. Long-term serologic, biochemical, and histologic outcome among living individuals with an original diagnosis of transfusion-associated hepatitis C (TAH-C). In determining the biochemical outcome, chronic hepatitis (CH) is defined as requiring at least 2 abnormal ALT values over a 6-month period of follow-up sampling. The numbers to the left of the vertical lines represents the percent of the subset. The numbers in parentheses to the right of the vertical lines represent the percent of the entire population of transfusion-associated hepatitis cases (N = 90).

TABLE 6. Histologic Findings Versus Clinical Manifestations Among 36 Originally Anti-HCV–Positive Cases With Biochemically Defined Chronic Hepatitis as a Function of Follow-Up HCV Serologic Status

Histologic Finding vs. Clinical Manifestations	Anti-HCV–pos. HCV-RNA–pos. (n = 34)	Anti-HCV–pos. HCV-RNA–neg. (n = 1)	Anti-HCV–neg. HCV-RNA–neg. (n = 1)
Chronic Hepatitis (n = 13)	13	0	0
No signs or symptoms	10	0	0
Mild signs or symptoms*	3	0	0
Severe signs or symptoms†	0	0	0
Cirrhosis (n = 7)	7	0	0
No signs or symptoms	1‡	0	0
Mild signs or symptoms	2	0	0
Severe signs or symptoms	4	0	0
No Biopsy Performed (n = 16)	14	1	1
No signs or symptoms	8	1	0
Mild signs or symptoms	2	0	1
Severe signs or symptoms	4	0	0

*Mild Clinical Manifestations: Any 1 of the following—splenomegaly, hypoalbuminemia, thrombocytopenia.

†Severe Clinical Manifestations: Varices or ascites and/or any 2 of the following—splenomegaly, hypoalbuminemia, thrombocytopenia.

‡One patient had hepatocellular carcinoma that was successfully resected.

manifestations, and 4 (25%) with severe clinical manifestations. The findings of severe clinical manifestations in 25% of those who were not biopsied suggests that these patients also had cirrhosis. Each of these 4 patients was HCV-RNA–positive. Overall, 8 subjects (biopsied and nonbiopsied) had clinically advanced liver disease, constituting 22% of the 36 TAH-C cases with biochemically defined chronic hepatitis and 6% of the total cohort of 129 cases in the follow-up evaluation. All of the 8 cases with severe clinical manifestations were HCV-RNA–positive and had biochemical evidence of hepatitis. Conversely, none of the HCV–infected patients with normal ALT had clinical evidence of cirrhosis, supporting our estimate that cirrhosis would occur in less than 5% of this population.

Based on the frequency of cirrhosis in the biopsied population, we estimate that among the additional 14 patients with anti-HCV and HCV-RNA who had not been biopsied, 5 (35%) would likely have been identified as having cirrhosis had they actually been biopsied. Thus, the number of persons with cirrhosis (observed and estimated) among the viremic group with biochemically defined chronic hepatitis is 12. Among the 35 viremic patients with persistently normal or only a single abnormal ALT value, we estimate that fewer than 5% (2 patients) might have cirrhosis. Finally, among the remaining 21 nonviremic persons (15 with anti-HCV alone and 6 without any HCV marker), no more than 1 percent (1 person) is likely to have had cirrhosis. Accordingly, the observed and estimated frequency of cirrhosis for the entire anti-HCV–positive cohort is 15 of the original 90 seropositive cases (17%).

Serologic Analysis and Outcome of Anti-HCV Negative Cases. Follow-up of the 39 cases originally found to be hepatitis C-negative revealed that one (3%) subsequently became both anti-HCV and HCV-RNA–positive, and 4 (10%) had biochemically defined chronic hepatitis in the absence of HCV-RNA (Table 5). Thirty-four (87%) showed no biochemical or serologic markers of hepatitis or HCV infection.

To determine whether the anti-HCV–negative cases could have been the result of HGV infection, all 39 patients, as well as a random sample of the non-hepatitis controls (50 samples) and a random sample of anti-HCV–positive cases (13 samples), were tested for the presence of HGV/GBV-RNA. The rates were 7%, 8%, and 8%, respectively, suggesting that HGV/GBV-C infection was not responsible for the anti-HCV–negative cases.

Liver biopsies were performed in 2 of the 4 HCV–negative cases with abnormal aminotransferases. Moderate chronic hepatitis with incomplete cirrhosis and possibly Mallory bodies was observed in 1, and nonspecific abnormalities in the other.

DISCUSSION

Chronic hepatitis C is a pervasive worldwide problem.[22,23] Although acute hepatitis C is generally mild, HCV infection persists in the majority and may advance through incrementally severe stages of chronic hepatitis to cirrhosis, occasionally culminating in HCC. This evolution typically occurs without symptoms in the early stages of the disease.

Data from several studies suggest that the mean interval from infection to diagnosis is 10 to 18 years for chronic hepatitis, 20 to 24 years for cirrhosis, and 27 to 29 years for HCC.[5,6,24] In each of these analyses, disease onset was assumed to coincide with the reported receipt of blood transfusions. Most of the patients in these studies already had chronic liver disease by the time they came to the attention of the investigators. Infected persons without symptoms or severe chronic liver disease, possibly the majority of HCV–infected cases, would not have been referred to these tertiary care centers. Thus, such studies could underestimate the total size of the pool of infected persons, exaggerating the contribution made by those with the more severe forms of chronic liver disease. Correspondingly, the time intervals to reach a given histologic stage of liver disease also may be biased toward those who evolve more rapidly.

The advantage of the present study, designed to track both mortality and morbidity of transfusion-associated non-A, non-B hepatitis, is that the natural history evaluation began at the time of disease onset, that a concurrent control group could be evaluated, and that the study subjects have been monitored for almost 25 years. This design has permitted determination of outcome of the entire spectrum of disease severity in comparison with a well-matched control population.

Mortality data reported earlier indicated that all-cause mortality over an 18-year period was no different between the non-A, non-B hepatitis cases and the matched controls.[7] There was, however, a difference in liver-related mortality between the 2 cohorts, although the overall rate of death from liver disease was quite low. The present report, that includes data from an additional 7 years, representing a 25-year period of follow-up, indicates that despite an increasing mortality rate – hardly surprising given the ages of the study population and the underlying reasons for the transfusions – there continues to be no difference in the frequency of all-cause mortality between the cases and controls. The mortality rates of both cohorts, however, are markedly higher than that of the general population, suggesting that most deaths are the result of causes unrelated to hepatitis. In contrast, there is an increasing mortality rate from liver disease among the cases as compared with the controls, the difference being almost 3% at about 25 years after initial exposure. This suggests that liver disease as a cause of death among persons infected with HCV does begin to increase as the infected population ages and the

duration of infection increases, but that liver disease remains a relatively minor cause for patient demise.

Especially interesting in this study are the sequelae noted among the study subjects who were still alive, particularly the 129 cases that had both repository and follow-up sera available for comparative analysis, 90 of whom were HCV–positive cases. As noted earlier, an additional 14 (14%) of the originally HCV–positive cases were excluded from this analysis because they were found to be anti-HCV positive before their transfusion, attesting to the importance of having pre-exposure specimens available for examination. Had this not been recognized, the relationship of hepatitis C to the transfusion episode of record would have been overestimated. Nonetheless, the cases with pre-existing hepatitis C are of interest because they had identical outcomes to the transfusion-related cases despite their longer duration of infection. We chose not to include them in subsequent analyses because the onset of their infection was unknown, and because the study focused on transfusion-associated hepatitis. However, because their outcomes were similar to the known transfusion-associated cases and because their durations of infection had to exceed the known transfusion-related cases, the inclusion of those with pre-existent HCV would have strengthened the study conclusions that only a minority of HCV–infected individuals have severe liver disease leading to a liver-related death after 25 or more years of HCV infection.

Of the 129 acceptable cases of hepatitis, 90 (70%) were infected with HCV because of the transfusion and the remaining 39 (30%) tested negative for serologic markers of hepatitis A, B, and C. No specific cause for these latter cases could be identified. They did not appear to be related to HGV infection because the frequency of HGV-RNA detection was similar among the non-ABC hepatitis cases, the hepatitis C cases, and the non-hepatitis controls, as has been previously reported.[25,26] This suggests that these non-ABC cases were either misdiagnosed as viral hepatitis, that the levels of anti-HCV were too low to detect during the initial illness, or that yet another transfusion-transmitted virus exists, a prospect clearly warranting further study. It is noteworthy that 10% of the non-ABC cases progressed to chronic hepatitis, with cirrhosis shown in 1 of the 4 that were biopsied.

In long-term follow-up of the 90 HCV–related cases (Fig. 2), 77% remained viremic, 49% of whom had accompanying biochemical evidence of chronic hepatitis (38% of the cohort). This supports the view that HCV-RNA is an important, but not definitive, marker of HCV–related liver disease. In this regard, it is noteworthy that 51% of HCV-RNA–positive patients (39% of the cohort) did not have biochemical evidence of chronic hepatitis as measured on at least 3 occasions over a 6-month follow-up period. The existence of significant liver disease among this cohort, however, cannot be entirely excluded without a liver biopsy. Others who have performed liver biopsies among HCV–reactive persons with normal serum enzymes[27-31] have generally found mild abnormalities, although cirrhosis has been detected rarely. Based on these studies, we estimate that fewer than 5% of patients with repeatedly normal ALT values might have shown cirrhosis had they been biopsied. Continued evaluation of this group is planned because little is known of their natural history.

Seven percent of persons who were unequivocally anti-HCV– and RIBA–positive in their original sample were no longer anti-HCV– or HCV-RNA–positive in their recall sample. An additional 17% of cases retained anti-HCV in their recall sample but were HCV-RNA–negative in at least 2 samples tested by different PCR methods in 2 independent laboratories. Thus, 23% of persons clearly infected with HCV based on RIBA–confirmed antibodies subsequently cleared the virus as assessed by serial HCV-RNA and anti-HCV determinations. Whereas one cannot exclude residual virus in the liver, viral clearance also is suggested by the very low frequency of abnormal ALT and by other cohort studies.[32-34] Had it not been for the repository samples, the 7% who lost anti-HCV would not have been known to be HCV–infected. This finding has 2 major implications. First, the rate of past HCV infection in the general population may be even higher than has been estimated by serologic screenings.[35] More importantly, this finding suggests that the spontaneous clearance rate of HCV is higher than the approximate 15% that has been generally accepted,[2] and that it may indeed be as high as 20% to 25%. Indeed, in some population groups, such as young women and children, spontaneous resolution can approach 45%.[32-34] Unfortunately, the presence of HCV-RNA in the original samples of this study could not be proved because they were not preserved in a manner favorable for molecular amplification. However, it can be reasonably assumed that all patients with hepatitis C are viremic at some stage of their infection, if not throughout their full course. It seems unlikely that the absence of HCV-RNA represents a technical error because testing was performed under code in 2 laboratories, and the results were concordant. Moreover, a negative sample was confirmed by the testing of a second sample. It is, of course, conceivable that a low-level viremia existed that could not be detected in the samples tested.

Thus, these data support existing evidence that chronic hepatitis C is a slowly progressive disease in most, but not all persons, causing relatively low mortality and overt morbidity during the first 25 years after infection.[36,37] The data are even more convincing when it is recalled that 14% of the original hepatitis C cases were found to have been infected for an unknown time before the transfusion of record. The proportion of patients with severe outcomes in the pre-infected population was similarly low despite the longer duration of infection. Importantly, death from liver-related causes attributable to TAH-C was less than 3% after 25 years of follow-up.

The frequency and degree of clinical morbidity seems closely related to the extent of histologic damage. Indeed, in this study, manifest liver disease was confined almost entirely to those with cirrhosis. What remains to be determined, therefore, is the frequency and rate of progression from histologically defined chronic hepatitis alone to histologically established cirrhosis. This will require continued long-term follow-up with serial liver biopsies at defined intervals. Such studies are in progress in this cohort and others.

Although the 39 non-ABC hepatitis cases could not be distinguished on clinical or biochemical grounds from those who were anti-HCV–positive at the time of initial infection, differences were noted years later in follow-up. Compared with the seropositive cases, among whom almost one-half had evidence of chronic hepatitis, only 10% of those with seronegative hepatitis had biochemical evidence of chronic hepatitis. However, it is important to note that among the small number of non-ABC patients biopsied, one had cirrhosis in the absence of excess alcohol consumption. Thus, non-C hepatitis also may have severe outcomes. The etiology of these cases

remains unexplained. Our study shows that these cases were not related to hepatitis G.

Somewhat different outcomes have been noted among persons with "community acquired" HCV infection.[38,39] One study, from Australia, involved 98 patients with community acquired acute hepatitis C, mostly a consequence of injection drug use, who were followed up approximately 25 years after initial diagnosis.[38] Forty-six percent of them were found to have spontaneously cleared HCV, leaving 54% with persistent viremia, 69% of whom had abnormal ALT values. None of them had developed HCC, and only 8% had progressed to overt cirrhosis. Similar data were noted in a study involving 1,667 HCV–positive (33% co-infected with HIV) injection drug users in Baltimore, MD followed over a median period of 8.8 years.[39] During this time, approximately 11% underwent spontaneous seroreversion, the rate being lower in African Americans than Whites and in HIV–negative than in HIV–positive persons. End-stage liver disease was observed in 2.4%, showing a relationship to heavy alcoholism, and 2.1% died as a consequence of liver disease. However, 10 times as many individuals died from nonliver causes (22.4%), mostly from HIV infection, drug overdose, and bacterial infections. Liver biopsies among 210 patients revealed cirrhosis in only 2 subjects, the remainder showing only minimal evidence of fibrosis. These low rates of cirrhosis, coupled with the equally low rates observed in young females who had received HCV–contaminated Rh immunoglobulin 2 decades earlier,[32,34] as well as in young children followed over a 20-year period,[33] attest to the variable rates of progression to cirrhosis at the end of 2 decades of infection, ranging between 2% and 20%.

In summary, HCV was the predominant cause of transfusion-associated hepatitis, but 29% of the cases were not explained by infection with hepatitis viruses A to G. Following a mean interval ranging from 19 to 24 years after acute hepatitis C, 7% of the original cases had no residual markers of HCV infection, and 17% had antibody in the absence of viremia. Hence, 23% may have had spontaneous recovery. Of the 77% who were chronically viremic, 49% had biochemical evidence of chronic hepatitis (38 percent of the cohort). Of those with viremia and chronic hepatitis who were biopsied, 35% had cirrhosis. Not surprisingly, clinical evidence of liver disease was limited to those with cirrhosis. Because this was not a prospective study, it is not possible to determine the exact time interval when histologic cirrhosis occurred. However, these data suggest that for at least a third of the transfusion-associated HCV–infected patients with ALT abnormalities, the mean fibrosis progression rate from no fibrosis to cirrhosis can exceed a rate of 1 stage for every 5 years. For the remaining HCV–infected patients with chronic hepatitis, the disease may progress, remain the same, or even improve. Host factors such as age, gender, alcohol consumption, and genetics may exert independent effects on histologic progression, but factors responsible for the progression of fibrosis are difficult to assess for the individual patient. For the entire cohort of patients initially infected with HCV, the estimate for progression to cirrhosis is 17%. Thus, over an approximate 25-year interval, HCV infection did not lead to increased mortality and resulted in severe histologic lesions in fewer than 20%.

Whether those with histologically defined chronic hepatitis alone will progress to cirrhosis, and whether mortality and morbidity will continue to derive mainly from those with established cirrhosis, remains to be determined. Continued evaluation of mortality and morbidity in this cohort will better define the frequency and rate of liver disease progression with advancing time. The challenge now is to identify those factors responsible for promoting progression of the disease and to define markers early in the course of infection for predicting long-term outcome.

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