0058

DETECTION OF ANTIBODY TO HEPATITIS C VIRUS IN PROSPECTIVELY FOLLOWED TRANSFUSION RECIPIENTS WITH ACUTE AND CHRONIC NON-A, NON-B HEPATITIS

HARVEY J. ALTER, M.D., ROBERT H. PURCELL, M.D., JAMES W. SHIH, PH.D., JACQUELINE C. MELPOLDER, M.T. (A.S.C.P.), MICHAEL HOUGHTON, PH.D., QUI-LIM CHOO, PH.D., AND GEORGE KUO, PH.D.

Abstract We measured antibody (anti-HCV) to hepatitis C virus, which causes non-A, non-B hepatitis, by radioimmunoassay in prospectively followed transfusion recipients and their donors. Of 15 patients with chronic non-A, non-B hepatitis documented by liver biopsy, all seroconverted for the antibody; of 5 with acute resolving non-A, non-B hepatitis, 3 (60 percent) seroconverted. The development of anti-HCV was delayed (mean delay, 21.9 weeks after transfusion, or 15 weeks after the onset of clinical hepatitis) and took approximately one year in one patient. Antibody has persisted in 14 of the 15 patients with chronic disease (mean follow-up, ≥6.9 years; maximum, ≥12), but has disappeared in the 3 with acute resolving disease after a mean of 4.1 years. Anti-HCV was detected in samples of donor serum given to 14 (88 percent) of the 16 anti-HCV–positive patients for whom all donor samples were available. Only 33 percent of the anti-HCV–positive donors tested had an elevated serum concentration of alanine aminotransferase; 54 percent were positive for antibody to the hepatitis B core antigen (anti-HBc).

We conclude that hepatitis C virus is the predominant agent of transfusion-associated non-A, non-B hepatitis and that screening of donors for anti-HCV could prevent the majority of cases of the disease. "Surrogate" assays for anti-HBc and alanine aminotransferase would have detected approximately half the anti-HCV–positive donors involved in the transmission of hepatitis that we identified. (N Engl J Med 1989; 321:1494-500.)

NON-A, NON-B hepatitis remains the most common serious consequence of blood transfusion. Studies conducted before 1980 indicated that the risk of post-transfusion hepatitis was 7 to 12 percent.[1] Currently, the risk is presumed to be considerably lower, but it has not been measured in prospective studies. Approximately 50 percent of infected patients have biochemical evidence of chronic hepatitis, and approximately 20 percent of those with chronic hepatitis have histologic evidence of cirrhosis.[2] In addition, the non-A, non-B virus may be causally associated with hepatocellular carcinoma[3,4] and is a frequent cause of community-acquired (sporadic) hepatitis,[5] a nonpercutaneously transmitted hepatitis that is also often chronic.[6]

A system for the detection of the non-A, non-B virus has been elusive, but such a system has been reported since the recent cloning of this agent,[7] now tentatively designated the hepatitis C virus; both radioimmune and enzyme-linked assays have been developed to detect antibody (anti-HCV) to the protein expressed in the cloning experiments.[8] We have evaluated the radioimmunoassay for anti-HCV, using stored blood samples from prospective studies of transfusion-associated hepatitis,[9,10] and the frequency with which hepatitis C virus was responsible for transfusion-associated non-A, non-B hepatitis. We also determined the frequency with which an anti-HCV–positive blood donor could be identified in such cases and, by inference, the number of cases that might be prevented by the routine application of this assay in screening donors; and the relation between the specific assay for hepatitis C virus and the "surrogate" assays currently used by blood banks to reduce the transmission of non-A, non-B hepatitis.

From the Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center (H.J.A., J.W.S, J.C.M.), the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Md. (R.H.P.), and the Chiron Corp., Emeryville, Calif. (M.H., Q.-L.C., G.K.). Address reprint requests to Dr. Alter at the Department of Transfusion Medicine, Bldg. 10, Rm. 5D 56, National Institutes of Health, Bethesda, MD 20892.

In accordance with the *Journal*'s policy, the authors have stated that Drs. Houghton, Choo, and Kuo are stockholders of Chiron Corporation.

METHODS

Enrollment and Follow-up of Patients with Non-A, Non-B Hepatitis

The conduct of the prospective studies from which the samples used in the current study were derived has previously been described.[9,10] Basically, all adult patients who underwent open-heart surgery at the National Institutes of Health from 1973 to the present were enrolled if they had no evidence of preexisting liver disease, had not received any transfusions in the previous six months, were available for long-term postoperative blood sampling, and gave informed consent. Blood samples were taken from the patients weekly or biweekly for the first 3 months after transfusion, monthly for the next 3 months, and then 9 to 12 months after transfusion. Patients in whom non-A, non-B hepatitis developed were placed on long-term follow-up, during which the interval between samplings depended on their clinical course. Liver biopsies were performed in most patients in whom serum levels of alanine aminotransferase remained elevated for more than one year.

Hepatitis was diagnosed if the alanine aminotransferase level in the recipient exceeded 2½ times the upper limit of normal for our laboratory between 2 and 26 weeks after transfusion and was at least twice the upper limit of normal on repeat sampling 1 or more weeks later.[9] Non-A, non-B hepatitis was diagnosed if other hepatotropic viruses were excluded by serologic testing, if nonviral causes of hepatocellular injury were excluded by conventional clinical and laboratory studies, and if the diagnosis was agreed on by an independent review panel.[10]

Selection of Samples for Antibody Testing

For the current study, we selected from a total population of 70 patients found to have non-A, non-B hepatitis in earlier prospective studies 15 patients in whose diagnoses we had the most confidence, on the basis of the magnitude of the elevation of alanine aminotransferase levels, the presence of characteristic fluctuating patterns in alanine aminotransferase levels, the development of chronic hepatitis later confirmed by biopsy, and in 4 patients, documentation of non-A, non-B hepatitis by studies of disease transmission among chimpanzees. These 15 unequivocal cases were compared with 5 less well defined cases in which an acute episode of transfusion-associated non-A, non-B hepatitis appeared to resolve spontaneously. These self-limited cases fulfilled the diagnostic criteria for non-A, non-B hepatitis as defined in the prospective studies, but they did not involve fluctuations in alanine aminotransferase levels, biochemical evidence of chronicity, or docu-

mentation of hepatitis on biopsy. All the patients had undergone open-heart surgery and had received from 1 to 20 units of blood (mean, 12). The results from 10 of the patients (9 with chronic and 1 with acute disease) were partially described in a previous report.[8]

Other than bias due to selection on the basis of the probability of diagnosis of non-A, non-B hepatitis, there was no known selection bias that might have influenced the probability of a positive anti-HCV assay. The 20 patients selected did not differ significantly from the 50 patients not selected in age (51.7 vs. 52.1 years), sex (65 percent vs. 68 percent male), the quantity of blood transfused (11.9 vs. 13.4 units), or the frequency of chronic hepatitis (75 percent vs. 77 percent).

Testing of Donor and Recipient Samples

All serum samples from donors and recipients were retrieved from frozen storage at −20°C to −70°C. They were thawed at room temperature, and an aliquot of each sample was sent under code to Chiron Corporation, where they were tested for anti-HCV by radioimmunoassay. A sample obtained before transfusion and samples obtained 3, 6, and 9 to 12 months after transfusion were sent for initial testing. If seroconversion was documented, all available intervening samples were sent for further testing to define more precisely the time of seroconversion. Alanine aminotransferase was measured in most but not all donor samples at the time of donation, by means of a kinetic assay with a biochemical analyzer[10]; the level was considered to be elevated if it exceeded 53 IU per liter, or 2.25 SD above the normal mean log alanine aminotransferase level.[11] All donor samples were tested for antibody to hepatitis B core antigen (anti-HBc) by radioimmunoassay (CORAB, Abbott Laboratories).

Radioimmunoassay for Anti-HCV

The assay for anti-HCV was performed as previously described.[8] In essence, the cloned viral protein (C100-3) was purified from recombinant yeast by breakage with glass beads, extraction of the insoluble fraction with sodium dodecyl sulfate, removal of the sodium dodecyl sulfate by acetone precipitation, and successive chromatography on a Q-Sepharose column in 7 M urea and a Sephacryl S-300 column in 0.1 percent sodium dodecyl sulfate. The final purity of the C100-3 protein was more than 70 percent.

Microtiter plates (Immulon 2, Dynatech Laboratories) were coated with 100 ng of C100-3 and incubated with 100 μl of serum (diluted 1:100) for one hour at 37°C. Bound antibody was detected by a second incubation with ^{125}I-labeled sheep antihuman immunoglobulin (Amersham). All the assays were run in duplicate. A radioactivity count more than 3 SD above the mean for negative controls was considered a positive reaction for anti-HCV.

Results

Characteristics of Patients and Frequency of Anti-HCV Seroconversion

Table 1 shows the demographic data and clinical and histologic outcomes of the 15 patients with well-characterized chronic transfusion-associated non-A, non-B hepatitis, in relation to their anti-HCV antibody status before and after transfusion. The mean age of the patients was 51.8 years, and only 27 percent were less than 50 years old; 67 percent were men. The mean duration of the elevation of serum alanine aminotransferase concentrations was greater than or equal to 8 years (range, ≥1 to ≥13). When the alanine aminotransferase level was constantly or intermittently elevated for more than one year, chronic non-A, non-B hepatitis was considered to have developed. The presence of chronic hepatitis was confirmed by liver biopsy in each of the 14 patients who underwent this procedure. Of these 14, 3 (21 percent) had chronic persistent hepatitis, 7 (50 percent) had chronic active hepatitis, and 4 (29 percent) had cirrhosis.

Each of the 15 patients with chronic hepatitis was negative for anti-HCV before transfusion, and all became positive at varying times after transfusion (see below). Seroconversion to anti-HCV was preceded by negative results on testing of multiple samples for anti-HCV (Fig. 1 through 3) except in a few patients, in whom the earliest post-transfusion samples were positive because of passive transfer of antibody from donors with high antibody levels.

Table 2 shows similar data for the five patients with non-A, non-B hepatitis that resolved spontaneously within the first year after transfusion. When the patients with rapidly resolving disease were compared with those with chronic disease, their mean incubation periods to the onset of hepatitis were 6.4 weeks and 7.3 weeks, respectively, their mean peak acute-phase elevations of alanine aminotransferase were 1354 IU per liter (range, 287 to 2322) and 806 IU per liter (range, 421 to 2112), and the proportions of patients with icterus were 60 percent and 27 percent. The mean duration of the elevation of alanine aminotransferase in the patients with resolving hepatitis was 8.2 weeks (range, 2 to 15). Seroconversion to anti-HCV occurred in only 3 of the 5 patients with re-

Table 1. Anti-HCV Seroconversion in Prospectively Followed Patients in Whom Chronic Transfusion-Associated Non-A, Non-B Hepatitis Developed.*

Patient No.	Age/Sex	Acute Phase			Chronic Phase		Anti-HCV Seroconversion
		Onset (weeks after transfusion)	Peak ALT (IU/liter)	Peak bilirubin (μmol/liter)	Duration (yr)	Liver histologic features	
1	61/M	10	978	5.1	≥13	CAH	Yes
2	46/M	5	610	15.4	≥11	CAH	Yes
3	57/F	6	468	13.7	10½	CPH	Yes
4	54/F	9	768	10.3	8	CPH	Yes
5	71/M	9	421	17.1	>10†	CAH/cirrhosis	Yes
6	29/M	6	678	13.7	≥9	CAH	Yes
7	55/F	7	434	13.7	≥12	CAH	Yes
8	53/M	9	450	100.9	>6‡	CAH/cirrhosis	Yes
9	63/F	7	1200	153.9	4‡	CAH/cirrhosis	Yes
10	62/M	5	525	12.0	≥7	CAH/cirrhosis	Yes
11	25/M	8	505	17.1	7	CPH	Yes
12	60/M	6	2112	106.0	≥12	CAH	Yes
13	35/F	8	555	27.4	≥1	No biopsy	Yes
14	63/M	8	1600	143.6	6	CAH	Yes
15	55/M	7	785	20.5	4	CAH	Yes

*ALT denotes alanine aminotransferase, CAH chronic active hepatitis, CPH chronic persistent hepatitis, and CAH/cirrhosis CAH on initial biopsy and cirrhosis on later biopsy.

†Patient died of causes unrelated to liver disease.

‡Patient died of causes directly or indirectly related to liver disease.

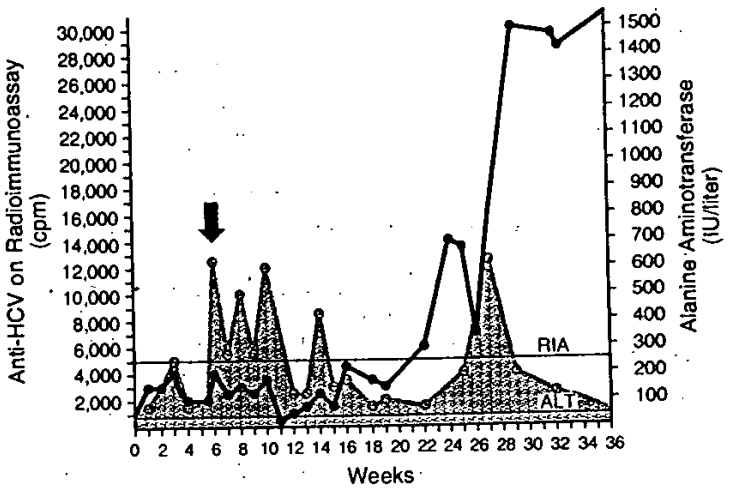

Figure 1. Serial Alanine Aminotransferase and Anti-HCV Levels in Patient 2, Demonstrating the Characteristic Long Interval between Transfusion and the First Appearance of Detectable Antibody.

The shaded area shows the level of alanine aminotransferase, and the heavy line anti-HCV. The horizontal lines show the upper limits of normal for alanine aminotransferase (ALT) and anti-HCV on radioimmunoassay (RIA). The arrow represents the onset of hepatitis. The interval between exposure and the appearance of detectable antibody was 20 to 22 weeks. There was a correspondingly long interval (14 to 16 weeks) between the onset of hepatitis and the first appearance of the antibody. Note the fluctuating alanine aminotransferase pattern characteristic of non-A, non-B hepatitis. Antibody has persisted for more than 10 years.

solving disease (60 percent), as compared with all of the 15 with chronic disease.

Interval to Anti-HCV Seroconversion and Duration of Antibody Response

The interval from the date of transfusion to the date when the first sample with a detectable level of anti-HCV was obtained is shown in Table 3. If no anti-HCV–negative sample was obtained within two weeks of seroconversion, the interval shown represents the midpoint between the last negative sample and the first positive sample. Thus calculated, the mean interval to seroconversion in patients in whom anti-HCV developed (except Patient 4) was 21.9 weeks (range, 10 to 39). The mean interval from the onset of hepatitis to anti-HCV seroconversion was 15 weeks (range, 4 to 32). In Patient 4 seroconversion occurred more than one year after transfusion, but the absence of serial samples at that time precluded the precise measurement of the interval to seroconversion. Patient 4 had no other known potential exposure to hepatitis C virus and was not sexually active; it is highly probable that the development of anti-HCV was related to her previous blood transfusion.

The antibody has persisted throughout follow-up in all the patients with chronic hepatitis except one (Table 3). The mean duration of the antibody response in those with chronic hepatitis was greater than or equal to 6.9 years (range, 14 months to ≥12 years). In one patient who appeared to have chronic disease (Patient 3), the antibody became undetectable between 13 and 15 months after it was first detected and 29 to 31 months after transfusion (Fig. 3). A diagnosis of chronic persistent hepatitis was made by histologic examination, and alanine aminotransferase concentrations were intermittently elevated at low levels (peak, 118 IU per liter) after anti-HCV was no longer detected; during subsequent follow-up, no alanine aminotransferase value exceeded 59 IU per liter, and many values were in the normal range.

In contrast to the persistence of antibody in the majority of patients with chronic disease, anti-HCV disappeared in each of the three patients with clinically resolving hepatitis who had had anti-HCV seroconversion. One of these patients (Patient 16) recovered from a severe episode of non-A, non-B hepatitis (peak alanine aminotransferase level, 1506 IU per liter; bilirubin level, 184.7 μmol per liter) within 15 weeks of onset. Although alanine aminotransferase values in multiple samples were normal thereafter, anti-HCV persisted for 9 years, only to disappear between the 9th and the 11th year. In two patients with resolving disease (Patients 18 and 19), anti-HCV disappeared in less than 1½ and 2 years, respectively (Table 3).

Patterns of Antibody Response

Representative patterns of the anti-HCV response in patients with chronic hepatitis are shown in Figures 1 through 3. Patient 2 (Fig. 1) had a typical delayed response, in which the antibody was not detected until 20 to 22 weeks after exposure and 14 to 16 weeks after the onset of hepatitis. Once the antibody was present, its level rose to a plateau, which persisted for more than 10 years. The alanine aminotransferase levels showed the fluctuating pattern typical of non-A, non-B hepatitis.

The course of Patient 10 (Fig. 2) exemplifies the pattern of passive antibody transfer. Radioactivity counts reached 13,000 cpm (2.6 times the upper limit of normal) in the first sample obtained after transfusion and then returned to base line over the next five weeks, commensurate with the half-life of IgG and with the presence of high levels of antibody (20,425 cpm, or 4 times the upper limit of normal) in one donor. Active antibody formation was first detected 14 weeks after transfusion. The anti-HCV level then fluctuated over the next 10 weeks; there was no correlation between the fluctuations in alanine aminotransferase levels and those in anti-HCV levels. The patient has remained anti-HCV–positive for 5½ years.

The course of Patient 3 (Fig. 3) illustrates the same

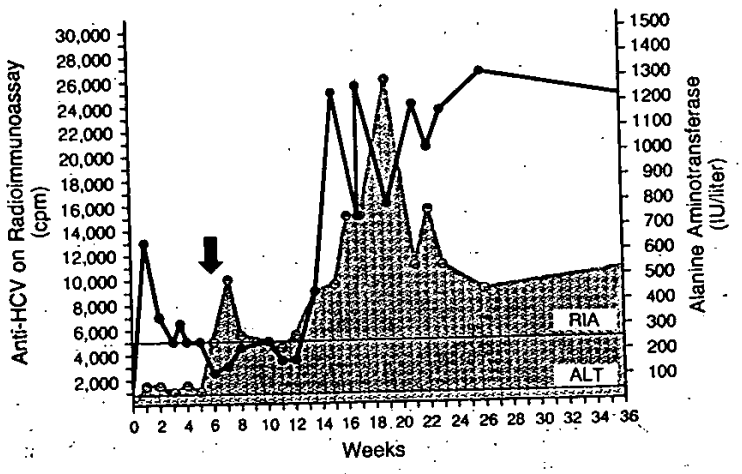

Figure 2. Passive Transfer of Anti-HCV to Patient 10 from a Donor with a High Level of Antibody.

The shaded area shows the level of alanine aminotransferase, and the heavy line anti-HCV. The horizontal lines show the upper limits of normal for alanine aminotransferase (ALT) and anti-HCV on radioimmunoassay (RIA). The arrow represents the onset of hepatitis. The inverval between exposure and the appearance of detectable antibody was 13 weeks, and between the onset of hepatitis and the appearance of detectable antibody 7 weeks. The donor tested positive for anti-HCV (20,425 cpm). The passive antibody had a typical course of decay for IgG, which was followed by the formation of active anti-HCV beginning 13 weeks after transfusion. Anti-HCV has persisted in this patient for more than 5½ years. Note that the fluctuations in radioactivity counts (performed on the same run) did not correspond to the fluctuations in alanine aminotransferase levels.

points about delayed detection of antibody (19 to 21 weeks after transfusion, or 9 to 11 weeks after the onset of hepatitis) as that of Patient 2, but also demonstrates the loss of detectable antibody after it had persisted for approximately one year. The antibody became undetectable between the 13th and 15th month after transfusion, and many samples obtained thereafter have remained negative (≥7 years).

Transient Hepatitis

In two patients with resolving hepatitis (Patients 17 and 20), anti-HCV did not develop. In Patient 17 the alanine aminotransferase level had a very sharp spike (1740 IU per liter), and he had abnormal values for only two weeks. The interval before the elevation of the alanine aminotransferase level (nine weeks) was typical of the incubation period of non-A, non-B hepatitis and was accompanied by a mild elevation in the bilirubin level (41 μmol per liter), malaise, anorexia, and nausea. There was no other obvious explanation for the symptoms and the transient rise in the alanine aminotransferase level.

Patient 20 had a peak alanine aminotransferase concentration of only 287 IU per liter with the onset at week 7 after transfusion; abnormal values persisted for only six weeks. There were no associated symptoms that suggested viral hepatitis, no hepatotoxic medications were taken, no serologic evidence of infection with other hepatotropic agents existed, and there was no obvious nonviral cause for the abnormal alanine aminotransferase values.

Except for the short period of abnormal alanine aminotransferase levels (less than six weeks), there was no consistent clinical or biochemical distinction between the two resolving cases in which anti-HCV failed to develop and the three cases in which it did (Table 2). Anti-HCV–positive donors were found in all three transient anti-HCV–positive cases (Table 4) and in neither anti-HCV–negative case.

Neither the incubation period nor the peak alanine aminotransferase or bilirubin levels reliably distinguished the individual cases in which the non-A, non-B hepatitis resolved from those in which chronic hepatitis developed. However, as a group, the patients with transient cases had a more severe acute episode, as manifested by higher mean alanine aminotrans-

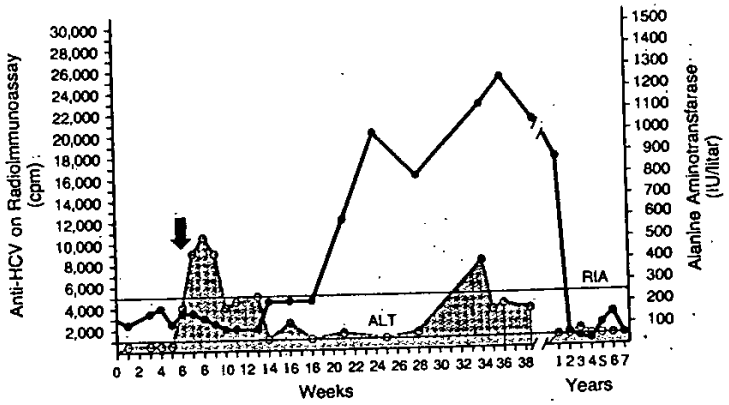

Figure 3. Serial Alanine Aminotransferase and Anti-HCV Determinations in the Only Patient (No. 3) with a Diagnosis of Chronic Hepatitis in Whom Antibody Was Not Persistent.

The shaded area shows the level of alanine aminotransferase, and the heavy line anti-HCV. The horizontal lines show the upper limits of normal for alanine aminotransferase (ALT) and anti-HCV on radioimmunoassay (RIA). The arrow represents the onset of hepatitis. The interval between exposure and the appearance of detectable antibody was 19 to 21 weeks, and between the onset of hepatitis and the appearance of detectable antibody 9 to 11 weeks. A sample taken 13 months after transfusion was anti-HCV–positive, whereas a sample taken after 15 months and all subsequent samples for 10 years were anti-HCV–negative.

Table 2. Anti-HCV Seroconversion in Prospectively Followed Patients with Acute Resolving Transfusion-Associated Non-A, Non-B Hepatitis.

Patient No.	Age/Sex	Onset (Weeks after Transfusion)	Peak ALT (IU/liter)*	Peak Bilirubin (μmol/liter)	Duration (wk)	Anti-HCV Seroconversion
16	52/M	6	1506	184.7	15	Yes
17	68/M	9	1740	41.0	2	No
18	25/F	6	915	15.4	11	Yes
19	47/F	5	2322	136.8	7	Yes
20	53/M	6	287	6.8	6	No

*ALT denotes alanine aminotransferase.

ferase levels and more frequent icterus (Tables 1 and 2).

Status of Donors and Recipients on Anti-HCV and Surrogate Assays

Table 4 shows the anti-HCV and anti-HBc status and alanine aminotransferase levels of the donors of blood to the 18 recipients who demonstrated anti-HCV seroconversion. Among the 18 recipients, an anti-HCV–positive donor was detected for 14 (78 percent); 1 patient received blood from two anti-HCV–positive donors. In two of the four recipients for whom no anti-HCV–positive donor was detected, a sample from one of their donors was unavailable for testing. Thus, in only 16 cases could we fully analyze the status of both the donors and the recipients, and an anti-HCV–positive donor was detected in 14 of them (88 percent).

Table 3. Interval between Transfusion and Anti-HCV Seroconversion and Duration of Anti-HCV Response.*

Patient No.	Transfusion–Seroconversion Interval	Onset–Seroconversion Interval	Duration of Anti-HCV Response
	wk		yr
1	15	5	≥12.0
2	21	16	≥10.5
3	16	10	>13–15 mo†
4	>52‡	>52‡	≥9.5
5	26	17	9.0§
6	13	7	≥8.5
7	23	16	≥6.5
8	26	17	4.0§
9	39	32	3.0§
10	15	10	≥5.5
11	20	12	≥8.5
12	37	31	≥10.5
13	27	19	≥1.0
14	38	30	≥7.0
15	15	8	≥7.5
16	21	15	>9–11†
18	10	4	>7–18 mo†
19	11	6	>1.0–2.0†

*The interval between blood samples was generally two weeks or less; when it was more than two weeks, the value shown represents the midpoint between the last negative sample and the first positive sample. Only in Patient 14 was the sampling interval prolonged (22 weeks).

†Anti-HCV disappeared within the specified interval.

‡The 1-year sample was negative, the 1½-year sample was weakly positive, and all later samples were positive.

§Patient died.

To investigate the relation between the specific anti-HCV assay and the two currently employed surrogate assays (measuring alanine aminotransferase levels and anti-HBc), we examined the surrogate-marker status of the anti-HCV–positive donors (Table 4). In only 12 of the 15 anti-HCV–positive donors had alanine aminotransferase levels been measured when they donated blood; 4 had elevated alanine aminotransferase levels (33 percent). Eight of the 15 anti-HCV–positive donors were positive for anti-HBc (53 percent). There was no donor with elevated alanine aminotransferase levels who was not also anti-HBc–positive, whereas there were four anti-HBc–positive donors who had normal alanine aminotransferase levels.

Overall, an anti-HCV–positive donor was detected in 88 percent of the fully evaluable cases of non-A, non-B hepatitis, and 53 percent of the anti-HCV–positive donors would have been excluded from donation by the surrogate assays, primarily that for anti-HBc.

Discussion

The cloning of the non-A, non-B hepatitis agent and the development of an assay to detect antibody against a major gene product of that agent[7,8] bring to an end more than a decade of frustration among serologists. In evaluating this assay, the first step was to test it against a coded panel of pedigreed serum samples that had been proved to transmit non-A, non-B hepatitis infection to the chimpanzee.[7] The anti-HCV assay was the first test to show reproducibly high sensitivity and 100 percent specificity against this pedigreed panel, which has become the standard for the evaluation of putative assays for the non-A, non-B hepatitis agent.[9]

In this study, we extended the epidemiologic evaluation of the anti-HCV assay using linked donor and recipient samples obtained in prospective studies of transfusion-associated hepatitis. Because the diagnosis of non-A, non-B hepatitis is often clinically imprecise, we believed it essential first to define the anti-HCV assay in a population that was itself well defined, namely those in whom histologically confirmed chronic non-A, non-B hepatitis developed; in four of these cases (Patients 1, 3, 12, and 15) the diagnosis was further confirmed by transmission experiments in the chimpanzee. In this group of 15 patients with well-documented chronic hepatitis, all demonstrated anti-HCV seroconversion. Since there was no other basis for patient selection except the certainty of the diagnosis of non-A, non-B hepatitis, its absolute concordance with a positive anti-HCV assay is quite remarkable. Hepatitis C virus appears to be the primary cause of chronic transfusion-associated non-A, non-B hepatitis.

Table 4. Anti-HCV and Surrogate Assays in Donors of Blood to Recipients in Whom Anti-HCV–Positive Post-transfusion Hepatitis Developed.*

Patient No.	No. of Donors	Anti-HCV–Positive Donor	Surrogate-Marker Status of Anti-HCV–Positive Donor	
			ALT	anti-HBc
1	18	Yes	No	No
2	18	Yes	NT	No
3	13	Yes	No	No
4	18	No	—	—
5	16	Yes	Yes	Yes
6	11	Yes	Yes	Yes
7	15	Yes	NT	No
8	20	Yes	No	Yes
9	5	Yes	Yes	Yes
10	15	Yes†	No	Yes
			No	Yes
11	5	No‡	—	—
12	19	No	—	—
13	2	Yes	No	No
14	13	Yes	Yes	Yes
15	14	No‡	—	—
16	17	Yes	NT	No
18	4	Yes	No	No
19	13	Yes	No	Yes

*ALT denotes alanine aminotransferase, and NT not tested.
†Two anti-HCV–positive donors were identified.
‡One donor sample was unavailable for testing.

We next tested five cases that were characterized by the rapid return of alanine aminotransferase values to normal. Although the rate of antibody response in these rapidly resolving cases was lower than that in the chronic cases (60 percent vs. 100 percent), it is apparent that the disease does not have to progress to chronic hepatitis for patients to manifest anti-HCV and that antibody can persist for long periods after recovery from a clinically acute episode of non-A, non-B hepatitis (nine years or more in Patient 16). Ultimately, anti-HCV disappeared in the 3 patients with resolving hepatitis, whereas it persisted in 14 of the 15 with chronic hepatitis.

There are many possible interpretations of the absence of anti-HCV in cases clinically diagnosed as non-A, non-B hepatitis, whether acute or chronic. First, the case may have been misdiagnosed, and the alanine aminotransferase elevations attributed by serologic exclusion to non-A, non-B hepatitis may have been due to nonviral hepatocellular inflammation or to a virus other than the hepatotropic viruses generally considered in the serologic-exclusion process. The likelihood of a misdiagnosis of non-A, non-B hepatitis increases when alanine aminotransferase elevations are slight, transient, or both. Second, an important potential cause of the inability to identify anti-HCV in cases that appear to be non-A, non-B hepatitis is inadequate sampling, particularly the absence of late samples. Seroconversion is uniformly delayed and may not take place for more than a year. It is critical to test samples taken over a prolonged period before concluding that a case of apparent non-A, non-B hepatitis is anti-HCV–negative. Third, an apparent case of non-A, non-B hepatitis may represent a cryptic form of hepatitis B infection in which the usual serologic markers are absent, as suggested by the finding of hepatitis B virus DNA in the liver and serum of patients who test negative for hepatitis B surface antigen.[12,13] Cryptic forms of hepatitis B infection may exist, but the high incidence of anti-HCV seroconversion in this study suggests that seronegative hepatitis B infection constitutes a very small segment of the diagnosed cases of non-A, non-B hepatitis. Fourth, cases of non-A, non-B hepatitis that are anti-HCV–negative renew speculation that there is a second non-A, non-B hepatitis agent. This has been suggested by the occurrence of multiple episodes of non-A, non-B hepatitis in a single patient,[14] by cross-challenge studies in the chimpanzee,[15] and by inactivation studies that distinguished a chloroform-sensitive agent (hepatitis C virus) and a chloroform-insensitive agent.[16] At this time, the existence of a second non-A, non-B hepatitis virus cannot be excluded, but the data in this study suggest that if another virus exists, it accounts for only a small proportion of the cases of transfusion-associated non-A, non-B hepatitis. The possibility of a second non-A, non-B hepatitis agent can now be directly explored by applying recombinant techniques similar to those that defined the hepatitis C virus. Finally, anti-HCV–negative cases may actually be due to hepatitis C virus, but they may not have an immune response detectable by the current first-generation assay. An antibody response below detectable levels may be more likely in acute, self-limited disease in which the hepatitis C virus–related antigen may be only transiently present. The more frequent finding of antibody in cases of chronic as opposed to resolving non-A, non-B hepatitis may thus reflect the persistence of sufficient hepatitis C virus antigen to boost the immune response repeatedly. The potential that anti-HCV–negative cases are nonetheless hepatitis C virus–related can now be explored with use of the polymerase chain reaction and in situ hybridization techniques.

Sequential serum samples from prospectively followed patients with non-A, non-B hepatitis indicated that the development of antibody was considerably delayed. This prolonged delay suggests that some donors capable of transmitting non-A, non-B hepatitis will not be detected by the anti-HCV assay. The adoption of this assay will thus markedly diminish the incidence of transfusion-associated non-A, non-B hepatitis, but it will not eliminate it. The prolonged window period also suggests the need to develop an assay for hepatitis C virus antigen, to increase the sensitivity of the antibody assay (perhaps with new epitopes), to explore molecular methods of viral detection, and to continue to use the surrogate assays to detect hepatitis C virus carriers who are anti-HCV–negative.

The data in this study indicate that anti-HCV persists once it develops, and we have documented per-

sistence for at least 12 years. However, we have also observed that antibody may disappear after a variable interval, particularly in patients who have rapid biochemical resolution of their hepatitis. Whether those who have lost antibody are still infectious remains open to question. The infectivity of patients who appear to have full clinical and biochemical recovery but who have persistent anti-HCV must also be explored.

Studying the anti-HCV status of both donors and recipients allowed us to predict the efficacy of this assay and evaluate the relative efficacy of the specific as compared with the surrogate assays. From our data we estimated that the routine application of this assay in donor screening would detect approximately 85 percent of those capable of transmitting non-A, non-B hepatitis. The cases that are not prevented may be due to donors who are in the window period before seroconversion, donors who carry hepatitis C virus but in whom antibody does not develop or who have lost the antibody, or donors who have other hepatitis agents.

Examination of the surrogate-marker status of the anti-HCV–positive donors suggested that in the absence of the specific anti-HCV assay, the adoption of surrogate assays by U.S. blood banks was proper; approximately 50 percent of the potential donors infected with hepatitis C virus have been excluded by the alanine aminotransferase assay, the anti-HBc assay, or both. This supports earlier predictions of a 40 to 50 percent reduction in the incidence of non-A, non-B hepatitis after the introduction of the surrogate assays.[10,11,17,18] Overall, the specific anti-HCV assay has confirmed both the usefulness and the limitations of the surrogate assays.

Measures taken to exclude donors who are at risk for exposure to the human immunodeficiency virus, the increased use of autologous blood, and the introduction of surrogate assays have all served to diminish the risk of transfusion-transmitted hepatitis. The coming introduction of the anti-HCV assay should bring a further reduction in this risk, and most important, a reduction in the long-term consequences of this common blood-borne infection.

References

1. Dienstag JL. Non-A, non-B hepatitis. I. Recognition, epidemiology, and clinical features. Gastroenterology 1983; 85:439-62.
2. 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.
3. Kiyosawa K, Akahane Y, Nagata A, Furuta S. Hepatocellular carcinoma after non-A, non-B posttransfusion hepatitis. Am J Gastroenterol 1984; 79:777-81.
4. Okuda H, Obata H, Motoike Y, Hisamitsu T. Clinicopathological features of hepatocellular carcinoma — comparison of hepatitis B seropositive and seronegative patients. Hepatogastroenterology 1984; 31:64-8.
5. 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.
6. Alter HJ, Prince AM. Transfusion-associated non-A, non-B hepatitis: an assessment of the causative agent and its clinical impact. Transfus Med Rev 1988; 2:288-93.
7. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244:359-62.
8. Kuo G, Choo Q-L, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989; 244:362-4.
9. Alter HJ, Purcell RH, Feinstone SM, Tegtmeier GE. Non-A, non-B hepatitis: its relationship to cytomegalovirus, to chronic hepatitis, and to direct and indirect test methods. In: Szmuness W, Alter HJ, Maynard JE, eds. Viral hepatitis: 1981 international symposium. Philadelphia: Franklin Institute Press, 1982:279-94.
10. Koziol DE, Holland PV, Alling DW, et al. Antibody to hepatitis B core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. Ann Intern Med 1986; 104:488-95.
11. Alter HJ, Purcell RH, Holland PV, Alling DW, Koziol DE. Donor transaminase and recipient hepatitis: impact on blood transfusion services. JAMA 1981; 246:630-4.
12. Bréchot C, Degos F, Lugassy C, et al. Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. N Engl J Med 1985; 312:270-6.
13. Alter HJ. Transfusion-associated non-A, non-B hepatitis: the first decade. In: Zuckerman AJ, ed. Viral hepatitis and liver disease. New York: Alan R. Liss, 1988:537-42.
14. Mosley JW, Redeker AG, Feinstone SM, Purcell RH. Multiple hepatitis viruses in multiple attacks of acute viral hepatitis. N Engl J Med 1977; 296:75-8.
15. Yoshizawa H, Itoh Y, Iwakiri S, et al. Demonstration of two different types of non-A, non-B hepatitis by reinjection and cross-challenge studies in chimpanzees. Gastroenterology 1981; 81:107-13.
16. Bradley DW, Maynard JE, Popper H, et al. Posttransfusion non-A, non-B hepatitis: physicochemical properties of two distinct agents. J Infect Dis 1983; 148:254-65.
17. Aach RD, Szmuness W, Mosley JW, et al. Serum alanine aminotransferase of donors in relation to the risk of non-A,non-B hepatitis in recipients: the Transfusion-Transmitted Viruses Study. N Engl J Med 1981; 304:989-94.
18. Stevens CE, Aach RD, Hollinger FB, et al. Hepatitis B virus antibody in blood donors and the occurrence of non-A, non-B hepatitis in transfusion recipients: an analysis of the Transfusion-Transmitted Viruses Study. Ann Intern Med 1984; 101:733-8.