Antibodies to Hepatitis C Virus in Prospectively Followed Patients with Posttransfusion Hepatitis

Shou-Dong Lee, Shinn-Jang Hwang, Rei-Hwa Lu, Kwok-Hung Lai, Yang-Te Tsai, and Kwang-Juei Lo

In an attempt to investigate the incidence and clinical course of type C viral hepatitis among patients with posttransfusion hepatitis, antibodies to hepatitis C virus (anti-HCV) in sera were measured from 42 prospectively followed cardiovascular surgery patients who developed hepatitis after blood transfusions. Of these, 35 (83.3%) had anti-HCV seroconversion during a 6- to 12-month follow-up period. The mean interval between blood transfusion and onset of active anti-HCV seroconversion was ∼3 months after the first elevation of serum alanine aminotransferase levels (18.1 vs. 64 weeks). There was no correlation between fluctuations in serum alanine aminotransferase levels and anti-HCV titers. Of 26 patients with type C posttransfusion hepatitis who were followed >1 year, 20 (76.9%) continued to have abnormal serum alanine aminotransferase levels. The results indicate that HCV is the major agent of posttransfusion hepatitis in Taiwan. Furthermore, it plays an important role in chronic hepatitis among transfused patients.

Non-A, non-B (NANB) hepatitis is a recognized disease caused by hepatotropic agents that are not serologically related to either hepatitis A virus or hepatitis B virus. Despite the use of hepatitis B surface antigen in screening of all blood donors after the 1970s, transfusion-associated hepatitis still occurs occasionally in blood recipients, and the NANB virus may play a major role [1-4]. According to some reports [2, 4-8], the incidence of NANB hepatitis after transfusion ranges from 6% to 17% in different countries. Thus NANB hepatitis remains the most common serious event after blood transfusion.

To reduce the incidence of transfusion-related NANB hepatitis, a specific serologic test for the NANB virus is urgently needed. Unfortunately, all attempts to develop such a test have been unsuccessful, although a specific blood test for the hepatitis C virus (HCV) was developed recently using recombinant DNA technology [9, 10]. This test showed that 80% of chronic NANB posttransfusion hepatitis in patients in Italy and Japan and 58% in patients in the United States were caused by HCV [10].

In an attempt to investigate the incidence and clinical course of NANB hepatitis after blood transfusions in Taiwan, we prospectively studied NANB hepatitis after transfusions in Taipei from 1981 to 1989 [4]. In that study, 13% of patients who had cardiovascular surgery and blood transfusions developed posttransfusion hepatitis, and 92% of such patients had NANB hepatitis. We used an ELISA to detect antibodies to HCV (anti-HCV), using stored serum samples from the prospective study of posttransfusion hepatitis [4], to determine the frequency with which HCV was responsible. The clinical course of HCV-related posttransfusion hepatitis and its correlation with anti-HCV persistence were also evaluated.

Materials and Methods

A prospective study of NANB hepatitis among patients who had cardiovascular surgery and blood transfusions at Veterans General Hospital in Taipei between 1981 and 1989 revealed 42 patients with posttransfusion hepatitis [4]. All were followed for serum liver aminotransferase levels periodically for >6 months. During the study, serum samples were obtained before and 3 days after the blood transfusion. Further serum samples were collected weekly in the first month, biweekly in the next 2 months, and monthly thereafter until 1 year. When hepatitis was diagnosed, the patient was placed on long-term clinical follow-up; the interval between samplings depended on the clinical course of disease.

Hepatitis was diagnosed when, from 2 weeks to 6 months after transfusion, two consecutive serum alanine aminotransferase (ALT) levels exceeded two times the upper normal value (>90 IU/L) in samples drawn at intervals >2 weeks. NANB hepatitis was diagnosed if there was no serologic evidence of acute hepatitis A or B, cytomegalovirus (CMV), or Epstein-Barr virus (EBV) infection. Other likely causes of hepatitis (drugs or heart failure) were also excluded. Chronic hepatitis was defined as serum ALT levels remaining high 1 year after transfusion.

Serum samples from patients were stored at −70°C until they were assayed for anti-HCV. Data on serum ALT levels and other viral hepatitis markers for each patient were obtained from the original study [4]. Serum anti-HCV (HCV antibody ELISA test system; Ortho Diagnostics, Raritan, NJ) was measured by ELISA with a microplate technique done following manufacturer’s instructions. Briefly, 20 μL of serum was added with specimen diluent to the C100-3
recombinant antigen [9]—coated microwell plate and incubated for 60 min at 37°C. After the plate was washed, 200 μl of murine monoclonal anti-IgG conjugated to horseradish peroxidase was added, and the plate was incubated for 60 min at 37°C. The microplate was again washed, 200 μl of o-phenylenediamine: 2 HCl substrate was added to each well, and the plate was incubated at room temperature for 30 min. Fifty microliters of 4 N sulfuric acid was added to each well, and the plate was read at 490 nm using a microplate reader. Specimens with absorbance values greater than the cutoff value were considered reactive. Anti-HCV seroconversion was defined as antibody reactive in two or more sequential sera or repeatedly reactive.

Results

Of 42 cardiovascular surgery patients who developed hepatitis after blood transfusions, 39 (92.8%) were considered to have NANB hepatitis, 2 (4.8%) had CMV hepatitis, and 1 (2.4%) was infected by EBV. Anti-HCV seroconversion was documented in 35 patients (83.3%) during 6 months to 1 year of follow-up, including 2 with CMV infection and 1 with EBV infection.

The mean age of the 35 patients with type C hepatitis was 50.3 years (range, 18–73); 71.4% were men. After blood transfusion, the onset of active anti-HCV seroconversion (mean, 18.1 weeks; range, 8–40) occurred ~12 weeks after the first elevation of serum ALT (mean, 6.4 weeks; range, 2–16). Most patients (83%) had elevated serum ALT 4–12 weeks after transfusion; 76% had onset of active anti-HCV seroconversion 13–28 weeks later.

Of the 35 patients with type C hepatitis, 19 (54.3%) were asymptomatic, and only 7 (20%) had total serum bilirubin levels >2 mg/dl; the highest was 6.7. Peak serum ALT levels >1000 IU/l were noted in 11 patients (31.4%), and only 2 (5.7%) had levels <300 IU/l. There was no case of fulminant hepatic failure. Of the 35 patients, 26 were followed >1 year. After 1 year, serum ALT levels remained abnormal in 20 (76.9%) but had returned to normal in 6 (23.1%). Three (50%) of the latter lost detectable anti-HCV during the follow-up period (mean, 2.5 years; greatest, 6), while 4 (20%) of the former lost their antibodies (mean follow-up, 3.6 years; greatest, 9).

Representative patterns of the serum anti-HCV response in patients with type C posttransfusion hepatitis and correlations with serum ALT levels are shown in figures 1 and 2. Passive transfer of anti-HCV was noted in six patients (17.1%). In two patients, active anti-HCV was not detected in the follow-up serum samples and their passive antibodies disappeared at weeks 1 and 3. In both, the levels of passive anti-HCV were low (peak absorbance value of anti-HCV, 0.557 and 0.676 OD). Their serum ALT levels returned to normal within 1 year (figure 1, case 1). Another four patients had active anti-HCV responses after their passive antibodies disappeared (figure 1, case 3). Levels of their passive antibodies (peak absorbance value of anti-HCV, 2.745, 1.638, 0.848, and 0.618 OD) were relatively high compared with those of cases 1 and 3. Among these four, three were followed and had abnormal serum ALT levels >1 year, and serum anti-HCV persisted >6–8 years (figure 1, case 3).

Passive anti-HCV was not detected in sera in most patients (82.9%) (figure 2). All patients had a typical delayed active anti-HCV response that usually began 8–40 weeks after transfusion. Most patients had persistent anti-HCV throughout the follow-up period, and chronic hepatitis usually developed (figure 2, case 6). Of patients followed >1 year, eight had serum anti-HCV undetectable at 8–21 months after transfusion.
Figure 2. Representative response patterns of serum antibodies to hepatitis C virus (anti-HCV) in patients with type C posttransfusion hepatitis and its correlation with serum alanine aminotransferase (ALT) levels. Case 6, Passive transfer of anti-HCV was not detected. Interval between onset of hepatitis and the anti-HCV seroconversion was ~4 months. Anti-HCV remained high through 2 years of follow-up. Chronic hepatitis also developed. Case 19, Serum ALT levels returned to normal within 12 months of transfusion. Serum anti-HCV was not persistent. Case 21, Transient detectable anti-HCV did not correlate with fluctuations in serum ALT levels. Chronic hepatitis developed. Case 24, Active anti-HCV was transiently detected between months 6 and 7 after transfusion and reappeared at month 21. Detectable anti-HCV was at low levels. O.D. = optical density of serum anti-HCV by ELISA. Horizontal dotted lines show upper normal limits for serum alanine aminotransferase (ALT); solid horizontal lines are cutoff value of positive anti-HCV.

Of these, all except one had abnormal serum ALT levels after 1 year (figure 2, cases 19, 21). No correlation was found between the fluctuations in serum ALT levels and those in anti-HCV titers. In three patients anti-HCV reappeared in serum at low levels at months 21, 24, and 38 (figure 2, case 24). None had received blood transfusion during the follow-up period.

Discussion

NANB is the most common type of transfusion-related hepatitis in the world [1-4]. Since the development of a specific antibody test for HCV [9], we studied the incidence of HCV infections in prospectively followed patients who developed the disease after blood transfusions.

Consistent with reports from Western countries [10-13], HCV appears to be a major cause of NANB posttransfusion hepatitis in Taiwan. In our study, seroconversion to anti-HCV was found not only in patients with NANB hepatitis but also in transfused patients with CMV or EBV infection.

In about half of our patients with type C hepatitis, symptoms were not clinically apparent despite fluctuating serum ALT levels. Only 20% had jaundice at the acute stage. Although the clinical symptoms of patients with acute HCV infection were relatively mild, the progression to chronicity was high: 77% had biochemical evidence of evolution toward chronic hepatitis. This is similar to reports from the United States [11] and Spain [13].

During this study, we observed that once developed, serum anti-HCV may persist ~9 years. However, antibodies in some patients disappear temporarily after a variable period, particularly in those in whom serum anti-HCV was low. This
was likely due to the insensitivity of the present anti-HCV assay. Furthermore, sequential serum samples from prospectively followed patients with type C hepatitis showed that anti-HCV seroconversion usually was delayed until 8–40 weeks after transfusion. The undetectable levels of anti-HCV and late anti-HCV seroconversion suggest that some donors capable of transmitting HCV hepatitis cannot be detected by the present assay.

The passive transfer of anti-HCV was observed in 17% of our prospectively followed patients with type C hepatitis. Two (5.7%) had no active anti-HCV response after passive anti-HCV disappeared, probably because HCV is a poor immunogen, the patient's immune response to the antigen was low, or the present assay lacks sufficient sensitivity. This suggests that some anti-HCV negative hepatitis is due to HCV. Thus, the incidence of HCV infections among patients with NANB hepatitis may be underestimated at present.

In a study by Alter et al. [11], all 3 acute type C posttransfusion hepatitis patients whose serum ALT returned to normal lost detectable anti-HCV during follow-up compared with 1 (6.7%) of 15 whose ALT levels remained elevated. This study suggested that the disappearance of anti-HCV may occur in patients in whom HCV infections resolve. Conversely, anti-HCV persistence seems to be a sign of chronicity. In our study, half of the six patients with acutely resolved hepatitis lost anti-HCV during follow-up; however, the antibody persisted in 80% of our patients who developed chronic hepatitis. In four patients, serum ALT levels continued to fluctuate after loss of anti-HCV (figure 2, case 21). Although loss of anti-HCV may occur in patients in whom hepatitis resolves, it is not known if these patients remain infectious.

Since the present HCV antibody assay cannot reliably distinguish between infectious and noninfectious blood in patients with HCV infections [13], it probably indicates an immune response to nonstructural protein of HCV. The present test, will assist clinicians and scientists in identifying and studying the epidemiology of HCV infections and will also assist significantly in screening out blood donors who might transmit HCV.

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References