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# Serological and Histological Features of Anti-HCV-Positive Individuals without Serum HCV RNA

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*Department of Internal Medicine and [1]Department of Pathology, Tohoku Koseinenkin Hospital, and [2]Department of Microbiology, Sendai Municipal Institute of Public Health, Sendai 983-8512*

KANNO, A., YAMADA, M., MURAKAMI, K. and NUMATA, N. *Serological and Histological Features of Anti-HCV-Positive Individuals without Serum HCV RNA.* Tohoku J. Exp. Med., 1998, **186** (4), 303-311 —— Antibody to hepatitis C virus (anti-HCV) in patients who are negative for HCV RNA in serum may indicate a memory of past infection of HCV. However, their clinical features have not been well understood. Fourteen anti-HCV-positive but HCV RNA-negative individuals were examined for serological and histological features. As a result, it was found that they had only antibody to HCV core antigen C22-3 with or without antibody to nonstructural viral antigen C33c on a recombinant immunoblot assay (RIBA), and that an concentration of anti-C22 was low. Liver biopsy showed two having no evidence of an obvious hepatic injury, two having a minimal change, and two having portal fibrosis. HCV RNA was not found in the liver. These results corroborate an idea that the anti-HCV in HCV RNA-negative individuals implies a past infection of HCV. Furthermore, it is suggested that a combination of an appearance pattern of antibody to HCV antigens on RIBA and anti-C22 titer are an useful marker to distinguish anti-HCV-positive individuals without viremia. —— anti-HCV; HCV RNA; RIBA; anti-C22


Recently, a specific complementary DNA clone for hepatitis C virus (HCV) was isolated (Choo et al. 1989). Development of an assay for antibody to HCV (anti-HCV) has made it possible to understand clinical features and epidemiological aspects of HCV infection (Kuo et al. 1989). Although most anti-HCV-positive patients with chronic hepatitis are viremic (Weiner et al. 1990), HCV RNA are not always detected in blood donors' sera positive for anti-HCV (Garson et al. 1990). It is supposed that anti-HCV in HCV RNA-negative patients indicates a memory of past infection of HCV (Shakil et al. 1995), but the

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clinical features have not been well understood. Further elucidation of clinical significance of anti-HCV in HCV RNA-negative individuals is required for counseling them properly. In the present study, fourteen anti-HCV-positive individuals without serum HCV RNA were examined for serological and histological features.

## Materials and Methods

Of one hundred and eighty-five anti-HCV-positive individuals who had been referred to our hospital between 1990 and 1995, one hundred and fifty-eight were positive for HCV RNA in serum. Of twenty-seven anti-HCV-positive but HCV RNA-negative individuals, fourteen were examined in this study (8 males and 6 female; mean age 52.5 years, range 28–68 years). Thirteen anti-HCV-positive individuals who were negative for HCV RNA were excluded, because sufficient clinical data were not available. Patients with acute hepatitis C were excluded. Anti-HCV was incidentally pointed out at a medical health checkup in eight, at blood donation in one, and at routine screening during follow-up of non-hepatic disease in five. Three had a history of posttransfusion hepatitis. Five had a history of a blood transfusion without hepatitis. None had a history of intravenous drug abuse, alcoholism or having hepatotoxic drugs. As comparative controls, thirteen patients with chronic hepatitis positive for both anti-HCV and HCV RNA in serum were randomly selected (8 males and 5 females; mean age 46.5 years, range 25–61 years). Three had a history of a blood transfusion. None had a history of intravenous drug abuse or having hepatotoxic drugs. More than 80 $g$ of daily alcohol consumption was recorded for two. All subjects were negative for hepatitis B surface antigen.

Anti-HCV was generally examined by a second generation enzyme immunoassay (EIA) (Ortho Diagnostic Systems, Raritan, NJ, USA), and in some instances by second generation passive agglutination (Dinabot, Tokyo). Seropositive sera were further examined by a second generation recombinant immunoblot assay (RIBA) (Ortho Diagnostic Systems), which is capable of detecting antibodies to a recombinant HCV core antigen C22-3 and nonstructural antigens C33c, C100-3 and 5-1-1. The intensity of each antibody's band on the nitrocellulose membrane was graded as 1+ to 4+. Antibody to C22-3 (anti-C22) was examined by a radioimmunoassay (Ortho Diagnostic Systems). Serum HCV RNA was examined by reverse transcription-polymerase chain reaction (PCR) using primers for amplifying the 5' untranslated region of the genome (Roche Molecular Diagnostic Systems, Branchburg, NJ, USA). The sensitivity was 10 copies of HCV genome/100 $\mu$l of serum.

Under the informed consent, liver biopsy was performed in thirteen anti-HCV-positive patients with serum HCV RNA, and also in six anti-HCV-positive individuals without serum HCV RNA even if the biochemical test showed normal results, because healthy individuals positive for anti-HCV could have abnormal

histological changes of the liver (Healey et al. 1995). Sections of the liver specimen were stained by conventional methods. A part of the liver sample was stored at −80 until used for HCV RNA examination. Histological classification of chronic hepatitis was based on the international standard criteria (Desmet et al. 1994). Histological activity index (HAI) score was used in order to numerically evaluate histological activities (Knodel et al. 1981). Periportal necrosis was graded on a 0 to 10 scale. Intralobular degeneration and focal necrosis, and portal inflammation were graded on a 0 to 4 scale. Degree of fibrosis was also graded on a 0 to 4 scale.

Approximately 2 mg of the frozen liver sample was used for extraction of total RNA by the acid-guanidinium-phenol-chloroform method. In detail, the liver was homogenized in 0.4 ml of GTC solution (4 M guanide isochiocyanate, 10 mM sodium citrate, 98 mM 2-mercaptoethanol, 0.5% sodium N-lauroyl sarcosine). Total RNA was extracted from the homogenized liver solution twice with an equal volume of phenol/chloroform solution, and then precipitated with 2 volumes of ethanol. Complementary DNA was constructed by reverse transcription with the obtained RNA solution, and then nested PCR was carried out using primers of 5′ non-coding region of the HCV genome (Numata et al. 1993). The sensitivity of the nested PCR was 5 copies of HCV genome/100 $\mu$l of serum, and the specificity was also confirmed by testing sera of patients with miscellaneous liver disease and a healthy control (data not shown).

Quantitative variables were expressed as mean ± S.D., and Mann-Whitney's U test was used for statistical analysis.

## Results

Biochemical and hematological findings of anti-HCV-positive individuals with or without serum HCV RNA are shown in Table 1. Mean levels of transaminase, zink sulfate turbidity test, total protein and $\gamma$-globulin were statistically lower in HCV RNA-negative individuals than in HCV RNA-positive patients. The number of platelet was larger in the former than in the latter. Serological and histological findings of anti-HCV-positive individuals without serum HCV RNA are shown in Table 2. On RIBA, antibody to C22-3 was found in all, with or without antibody to C33c. Antibody to 5-1-1 and C100-3 was not found in any cases. As shown in Table 2 and 3, anti-C22 titer was significantly low in HCV RNA-negative individuals as compared with HCV RNA-positive patients (9.9 ± 8.8 U vs. 165.5 ± 83.1 U, $p < 0.01$). Histopathological findings of six HCV RNA-negative individuals were as follows: Case 1. Normal liver. Case 2. Minimal change (Fig. 1). Small amount of lymphocytes infiltrated in some portal tracts, but focal necrosis was not found. Case 3. Normal liver. There were no remarkable changes except a mild fat deposition. Case 4. Minimal change. There were variously sized hepatocytes. Focal necrosis or portal inflammation was not found. Case 5. Portal fibrosis (Fig. 2). A fibrous

TABLE 1. *Biochemical and hematological findings of anti-HCV-positive individuals with or without serum HCV RNA*

| | HCV RNA (−) ($n=14$) | HCV RNA (+) ($n=13$) |
|---|---|---|
| Age (years) | 52.5±12.7 | 46.5±11.1 |
| Gender (M : F) | 8 : 6 | 8 : 5 |
| AST (U/liter) | 26.4±11.1 | 161.4±114.5** |
| ALT (U/liter) | 32.8±21.7 | 281.7±216.1** |
| ALP (U/liter) | 154.9±52.0 | 162.5±44.4 |
| γ-GTP (U/liter) | 37.1±24.2 | 85.2±87.3 |
| ZTT (U) | 7.44±3.00 | 16.06±6.32** |
| TP (g/100 ml) | 7.28±0.62 | 7.83±0.50* |
| γ-gl (g/100 ml) | 1.20±0.29 | 1.65±0.53* |
| Plt ($\times 10^3/\mu$l) | 242.9±49.1 | 172.9±48.4** |

M, male; F, female; AST, aspartate aminotransferase (normal range, <35 U/liter); ALT, alanine aminotransferase (<34 U/liter); ALP, alkaline phosphatase (105–262 U/liter); γ-GTP, gamma-glutamyltranspeptidase (5–42 U/liter); ZTT, zink sulfate turbidity test (2–12 U); TP, total protein (6.5–8.5 g/100 ml); γ-gl, gamma-globulin (0.6–1.7 g/100 ml); Plt, platelet (130–400$\times 10^3/\mu$l). *$p<0.05$; **$p<0.01$.

TABLE 2. *Serological and histological findings of anti-HCV-positive individuals without serum HCV RNA*

| Case (No.) | Anti-C22 (U) | RIBA | | | | Liver | |
|---|---|---|---|---|---|---|---|
| | | 5-1-1 | C100-3 | C33c | C22-3 | HCV RNA | Histology |
| 1 | 16 | — | — | 2+ | 4+ | — | Normal |
| 2 | 32 | — | — | 1+ | 4+ | — | Minimal change |
| 3 | 8 | — | — | — | 4+ | — | Normal |
| 4 | 6 | — | — | — | 3+ | — | Minimal change |
| 5 | 17 | — | — | 2+ | 4+ | — | Portal fibrosis |
| 6 | 8 | — | — | — | 4+ | — | Portal fibrosis |
| 7 | 5 | — | — | — | 2+ | NT | NT |
| 8 | 20 | — | — | — | 4+ | NT | NT |
| 9 | 3 | — | — | — | 2+ | NT | NT |
| 10 | 5 | — | — | — | 3+ | NT | NT |
| 11 | 1 | — | — | — | 1+ | NT | NT |
| 12 | 13 | — | — | — | 4+ | NT | NT |
| 13 | 2 | — | — | — | 2+ | NT | NT |
| 14 | 2 | — | — | — | 2+ | NT | NT |

Anti-C22: negative, <0.9 U. RIBA, recombinant immunoblot assay; NT, not tested.

TABLE 3. *Serological and histological findings of patients with chronic hepatitis positive for both anti-HCV and HCV RNA in serum*

| Case (No.) | Anti-C22 (U) | RIBA | | | | Liver | |
|---|---|---|---|---|---|---|---|
| | | 5-1-1 | C100-3 | C33c | C22-3 | HCV RNA | Histology[a] |
| 1 | 240 | 4+ | 4+ | 4+ | 4+ | + | CH mild activity mild fibrosis |
| 2 | 140 | — | — | 4+ | 4+ | + | CH mild activity no fibrosis |
| 3 | 140 | 3+ | 2+ | 4+ | 4+ | + | CH mild activity severe fibrosis |
| 4 | 110 | 2+ | 2+ | 4+ | 4+ | + | CH mild activity no fibrosis |
| 5 | 110 | 4+ | 4+ | 4+ | 4+ | + | CH mild activity no fibrosis |
| 6 | 110 | — | 2+ | 4+ | 4+ | + | CH mild activity mild fibrosis |
| 7 | 88 | — | — | 4+ | 4+ | + | CH moderate activity mild fibrosis |
| 8 | 170 | 3+ | — | 4+ | 4+ | + | CH mild activity no fibrosis |
| 9 | 340 | — | — | 4+ | 4+ | + | CH mild activity no fibrosis |
| 10 | 73 | 4+ | 4+ | 4+ | 4+ | + | CH moderate activity mild fibrosis |
| 11 | 180 | 4+ | 4+ | 4+ | 4+ | + | CH mild activity mild fibrosis |
| 12 | 140 | 4+ | 4+ | 4+ | 4+ | + | CH mild activity no fibrosis |
| 13 | 310 | — | 4+ | 4+ | 4+ | + | CH mild activity severe fibrosis |

Anti-C22 : negative, $<0.9$ U. RIBA, recombinant immunoblot assay; CH, chronic hepatitis. [a]The histological diagnosis of chronic hepatitis was based on the international standard criteria described in Materials and Methods.

enlargement with only a few inflammatory cell infiltration was found in portal tracts. There was few focal necrosis. Case 6. Portal fibrosis. A fibrotic change was found in portal tracts with a few inflammatory cell infiltration. There was no focal necrosis. HCV RNA was not detected in any liver samples. Serological and histological features of thirteen HCV RNA-positive patients with chronic hepatitis are shown in Table 3. Seven had antibody to four antigens including 5-1-1, C100-3, C33c and C22-3, and six had antibody to two antigens including C33c and C22-3 with or without antibody to 5-1-1 or C100-3. Liver biopsy showed chronic hepatitis with various activity and fibrosis. HCV RNA was found in all liver samples. As shown in Table 4, histological activities of the liver were significantly low in HCV RNA-negative individuals as compared with HCV RNA-positive patients with regard to periportal necrosis, intralobular

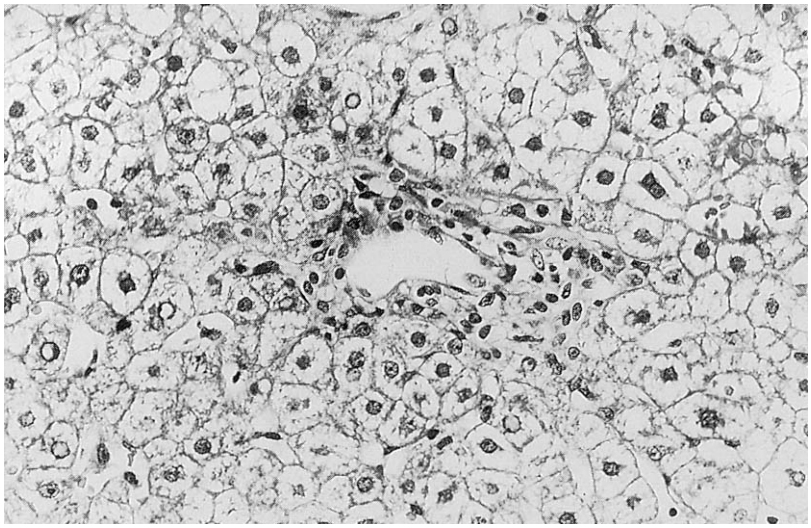

Fig. 1. Microscopic examination of the liver biopsy specimen obtained from an anti-HCV-positive individual without serum HCV RNA (Case No. 2 in Table 2). A few lymphocytes infiltrate in a portal tract. Hematoxylin and eosin, ×400.

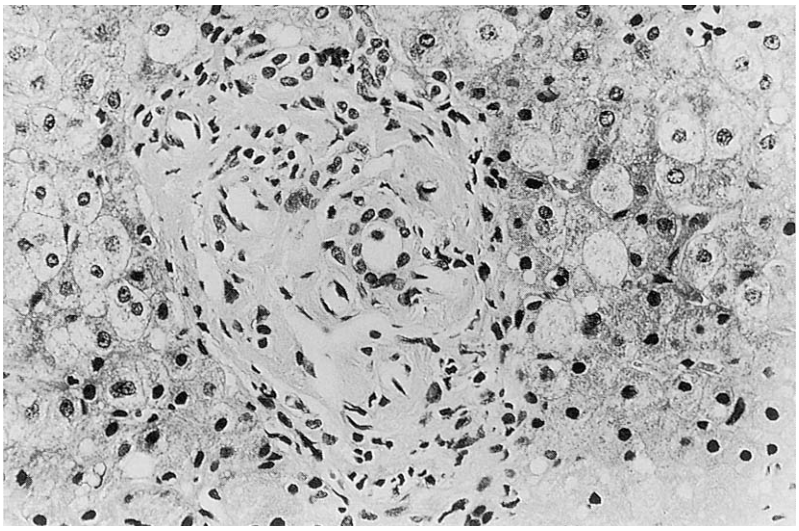

Fig. 2. Microscopic examination of the liver biopsy specimen obtained from an anti-HCV-positive individual without serum HCV RNA (Case No. 5 in Table 2). A fibrous enlargement of the portal tract is seen. Hematoxylin and eosin, ×400.

degeneration and focal necrosis, and portal inflammation. The degree of fibrosis was also lower in HCV RNA-negative individuals than in HCV RNA-positive patients, but the difference was not statistical.

## Discussion

Anti-HCV-positive patients with chronic liver disease usually have HCV RNA in serum (McGuinness et al. 1993). However, HCV RNA is not always detected in anti-HCV-positive blood donors' sera (Garson et al. 1990). Fourteen

individuals who were positive for anti-HCV but negative for serum HCV RNA were presented in this study. There are several explanations for the reason why they were negative for HCV RNA in serum in spite of presence of anti-HCV. One of the explanations is that the positive result of anti-HCV was given by nonspecific reaction, which is frequently observed in patients with hyperglobulinemia (McFarlane et al. 1990), or by presence of antibody to superoxide dismutase (Ikeda et al. 1990). However, this is not likely, because neither $\gamma$-globulin with a high level nor antibody to superoxide dismutase was found in any cases (data not shown). Another explanation is that very small amount of HCV circulated in blood. However, this is unlikely in view of the fact that HCV RNA sequences were detected neither in the serum nor in the liver. Taking these into consideration, it is suspected that the existence of anti-HCV in individuals without serum HCV RNA implies a memory of remote infection of HCV (Shibata et al. 1991). This idea is supported by the fact that two thirds of liver biopsy in six HCV RNA-negative individuals showed a normal finding or a minimal change, and that their histological activities were low (Table 4). However, the reason why portal fibrosis was found in two HCV RNA-negative individuals remains unknown. One of the explanations is that they were on the way to recover from chronic hepatitis after a natural clearance of HCV, because portal inflammation and fibrosis seem to remain for a long time after complete eradication of HCV from chronic hepatitis patients (Tsubota et al. 1997). Another explanation is that unknown agents may be incriminated. However, at least infection with hepatitis B virus (HBV) or hepatitis G virus (HGV) is not likely to be ongoing, because neither HBV DNA nor HGV RNA was not detected in sera of HCV RNA-negative individuals having portal inflammation or fibrosis (data not shown). It was demonstrated that anti-HCV-positive but HCV RNA-negative individuals had only antibody to C22-3 with or without antibody to C33c, and that the concentration of anti-C22 was lower in HCV RNA-negative individuals than in HCV RNA-positive patients. These findings are compatible with another reports (McGuinness et al. 1993; Trowbridge et al. 1996). Finally, it is suggested that a combination of an appearance pattern of antibody to HCV antigens on RIBA and

TABLE 4. *Histological activity index (HAI) score of the liver in anti-HCV-positive individuals with or without serum HCV RNA*

| | HCV RNA (−) ($n=6$) | HCV RNA (+) ($n=13$) |
|---|---|---|
| Periportal necrosis | 0 | $1.2\pm1.1$* |
| Intralobular degeneration and focal necrosis | $0.7\pm0.5$ | $1.3\pm0.8$* |
| Portal inflammation | $0.2\pm0.4$ | $2.2\pm1.0$** |
| Fibrosis | $0.3\pm0.5$ | $0.8\pm1.1$ |
| Total | $1.2\pm1.0$ | $5.6\pm3.0$** |

*$p<0.05$; **$p<0.01$

anti-C22 titer may be an useful marker to distinguish anti-HCV-positive individuals without viremia from those with viremia.

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