

## Hepatitis C virus RNA detection in serum and peripheral blood mononuclear cells of patients with hepatitis C

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### Abstract

**AIM:** To investigate the existence and clinical significance of hepatitis C virus (HCV) RNA in the serum and peripheral blood mononuclear cells (PBMC) of patients with hepatitis C.

**METHODS:** HCV RNA was detected by nested polymerase chain reaction (Nested PCR) in serum and in PBMC of 46 patients with acute hepatitis C (AHC) and in 42 patients with chronic hepatitis C (CHC).

**RESULTS:** The positive rate of HCV RNA in PBMC of patients with CHC was markedly higher than that of patients with AHC ( $P < 0.01$ ). The positive rates of HCV RNA in serum of patients with AHC and CHC and in PBMC of patients with CHC were significantly higher than those of anti-HCV positive patients with normal alanine aminotransferase (ALT) levels ( $P < 0.01$ ). HCV RNA was negative in the serum of two patients, but could be detected in PBMC. In 12 patients, anti HCV was negative while HCV RNA was positive in serum.

**CONCLUSION:** (1) detection of serum HCV RNA by nested PCR might be helpful in the early diagnosis of anti-HCV negative hepatitis C; (2) liver damage in patients with hepatitis C might be correlated with HCV-viremia; (3) infection of PBMC by HCV might play an important role in chronic liver damage in patients with HCV and in the chronicity of its clinical course; and (4) PBMC might be considered as a "reservoir" for HCV.

**Key words:** Hepatitis C; RNA; Viral analysis; Monocytes; Polymerase

chain reaction

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### INTRODUCTION

In recent years, detection of hepatitis C virus (HCV) RNA by polymerase chain reaction (PCR) in peripheral blood mononuclear cells (PBMC) of patients with chronic hepatitis C has been reported abroad<sup>[1-3]</sup>. To investigate HCV RNA in PBMC of patients with hepatitis C in our country, 88 cases of acute or chronic hepatitis C have been examined by nested PCR. HCV RNA in serum, anti-HCV, and alanine aminotransferase (ALT) were assayed as well.

### MATERIALS AND METHODS

#### Patients

Eighty eight patients with hepatitis C were selected from December 1992 to June 1995, 46 of them had acute hepatitis C (AHC) (36 with post-transfusive hepatitis C (PTHC) and 10 with sporadic hepatitis C (SHC)) and 42 had chronic hepatitis C (CHC). All of the patients had elevated serum alanine ALT with antibody to HCV or positive HCV RNA in serum. Hepatitis A, B, D, and E virus infection were excluded in all. Eighteen patients had positive anti HCV only, but with normal ALT, and 10 healthy blood donors served as control.

#### Isolation of PBMC and extraction of HCV RNA

PBMC was isolated from 5 mL heparinized venous blood with Ficoll Hypaque density gradient centrifugation and washed 5 times with Hank's balanced salt solution, and then stored at  $-70^{\circ}\text{C}$ . Each specimen each from positive and negative serum HCV RNA was compared with positive and negative PCR amplification. HCV RNA in serum and PBMC was extracted as described by Chomczynski<sup>[4]</sup>.

#### Nested-PCR

Two pairs of oligonucleotide primers deduced from the 5' terminal noncoding region of HCV were synthesized on a 391 DNA synthesizer (Applied Biosystems, United States). Reverse transcription was carried out at  $42^{\circ}\text{C}$  for 45 min in  $10\ \mu\text{L}$  of Tris HCl buffer (50 mmol/L, pH8.4) containing 8 mmol/L  $\text{MgCl}_2$ , 30 mmol/L KCl, 1 mmol/L dithiothreitol, 50 pmol of an antisense primer, 10 mmol/L each of

**Table 1** Detection of serum anti-hepatitis C virus and hepatitis C virus RNA in serum and peripheral blood mononuclear cells of patients with hepatitis C

Groups	Cases	Anti-Hepatitis C virus (+)	Serum Hepatitis C virus RNA (+)	Peripheral blood mononuclear cells-Hepatitis C virus RNA (+)
		cases (%)	cases (%)	cases (%)
Acute hepatitis C	46	36 (78.3)	40 (86.9) <sup>d</sup>	15 (32.6)
Post-transfusive hepatitis C	36	31 (86.1) <sup>f</sup>	32 (88.9)	14 (38.9)
Shronic hepatitis C	10	5 (50.0)	8 (80.0)	1 (10.0)
Chronic hepatitis C	42	40 (95.7) <sup>a</sup>	34 (80.95) <sup>d</sup>	33 (78.57) <sup>bd</sup>
Anti-hepatitis C virus (+) only	18	18 (100)	5 (27.7)	2 (11.1)
Total	106	94 (88.7)	79 (74.5)	50 (47.2)

<sup>a</sup>*P* < 0.05 vs Acute hepatitis C; <sup>b</sup>*P* < 0.01 vs Acute hepatitis C; <sup>d</sup>*P* < 0.01 vs Anti-hepatitis C virus (+) only; <sup>f</sup>*P* < 0.01 vs Shronic hepatitis C.

**Table 2** Relationship of anti-hepatitis C virus and hepatitis C virus RNA in serum and peripheral blood mononuclear cells

Clinical type	Number (%)
Anti-HCV+ (S+, PBMC+)	46 (52.3)
Anti-HCV+ (S-, PBMC-)	12 (13.6)
Anti-HCV+ (S+, PBMC-)	16 (18.2)
Anti-HCV+ (S-, PBMC+)	2 (2.3)
Anti-HCV- (S+, PBMC+)	0
Anti-HCV- (S-, PBMC-)	0
Anti-HCV- (S+, PBMC-)	12 (13.6)

HCV: Hepatitis C virus S: serum; PBMC: Peripheral blood mononuclear cells

the four deoxyribonucleoside triphosphate, 10 units of RNase inhibitor, and 10 units of avian myeloblastosis virus reverse transcriptase. The first stage of PCR was performed for 35 cycles with one pair of outer primer in a DNA thermal cycler (Perkin Elmer Cetus, United States). Each reaction cycle underwent denaturation at 94 °C for 1 min, and primer annealing and extension at 60 °C for 1.5 min. Approximately 1/10 of the products were subjected to a second PCR for 35 cycles using one pair of inner primer with a reaction cycle as described above. The products of the second PCR were mixed with 3.5 µL of sample buffer, separated on 2% agarose gel, stained with ethidium bromide, and observed under ultraviolet light. The length of the second PCR amplified products was 145 bp.

#### Other reagents

Second generation anti HCV and HBsAg/anti HBs, HBeAg/anti HBe and anti HBc kits were supplied by the Beijing Si Huan Biological Engineering Products Company. Anti HAV-IgM and anti HEV-IgM kits were supplied by Beijing Ke Wei Clinical Diagnostic Regents Factory, and HDAg, anti HD and anti HD-IgM kits were supplied by Beijing Tian Yuan Biological Medical Scientific and Technological Developing Company.

## RESULTS

### Relationship between clinical classification of infection and anti HCV, HCV RNA in serum and PBMC

Of the 88 patients with acute or chronic hepatitis, 76 (86.4%) were anti-HCV positive, and 74 (84.1%) and 48 (54.6%) were HCV RNA positive in serum and PBMC, respectively (Table 1). The positive rates of anti HCV and HCV RNA in PBMC of CHC patients were much higher than those in AHC patients (*P* < 0.05 and *P* < 0.01, respectively), while there was no significant difference in the positive rate of HCV RNA in serum (*P* > 0.05) (Table 1). The positive rates of HCV RNA in serum of patients with AHC and CHC and in PBMC of patients with CHC were significantly higher than those in anti-HCV positive patients with normal ALT levels (*P* < 0.01). The positive rate of anti HCV in serum of patients infected *via* transfusion was markedly higher than that of sporadic ones (*P* < 0.01). Anti-HCV and HCV RNA in serum and PBMC of 10 adult healthy donors were all negative (Table 1).

### Relationship between anti-HCV in serum and HCV RNA in serum and PBMC

As indicated in Table 2, positive HCV RNA in PBMC occurred only in anti-HCV positive patients. None of the 12 anti-HCV negative patients were found to have HCV RNA in PBMC. Two patients were HCV RNA negative in serum but positive in PBMC.

## DISCUSSION

Some scholars abroad have found that the HCV infection rate of PBMC in patients with CHC was as high as 70%-100%<sup>[1-3]</sup> and the structure and function of PBMC also changed markedly<sup>[5,6]</sup>. These scholars suggested that HCV infection in PBMC might play an important role in the pathogenesis of hepatitis C. In this study, we used nested PCR to detect HCV RNA in PBMC of 46 patients with AHC and 42 patients with CHC. The results showed that the positive rate of HCV RNA in PBMC of patients with CHC was much higher than that of patients with AHC. This suggests that HCV infection in PBMC might be related to the chronicity of hepatitis C. The positive rates of HCV RNA in serum of patients with AHC and CHC and in PBMC of patients with CHC were significantly higher than in patients with positive anti-HCV only. But the positive rate of HCV RNA in PBMC of patients with positive anti-HCV was not significantly different compared with that of patients with AHC. This suggests that liver damage of patients with AHC was associated with HCV RNA viremia. Apart from HCV RNA viremia, liver damage of patients with CHC might also be related to HCV infection in PBMC as well. These observations are consistent with other reports<sup>[7-9]</sup>, suggesting HCV might take part directly in the process of liver damage in acute infection, while immune function disorders might be the major cause of liver lesions in chronic liver disease.

Nested PCR is highly sensitive. A positive finding of HCV RNA in PBMC may be due to contamination from HCV RNA present in serum. To investigate whether this contamination can be eliminated, the following study has been carried out. While tested by PCR, HCV RNA in the fifth time Hank's wash solution of 10 specimens from patients with positive HCV RNA were all negative, indicating that the contamination could be eliminated after washing PBMC for five times. Twenty-eight patients were HCV RNA positive in serum, but negative in PBMC while 2 patients were HCV RNA positive in PBMC, but negative in serum. These results also suggested that the influences caused by HCV RNA in serum could be excluded.

Positivity of HCV RNA in serum is a direct marker of underlying HCV infection and it is important for the diagnosis of hepatitis C. The positive rates of HCV RNA in serum of AHC and CHC were all over 80% with no significant differences between the two. The positive rates of anti-HCV in CHC and acute PTHC patients were markedly higher than those in AHC and acute SHC patients, respectively. Two patients with CHC and 10 with AHC were anti-HCV negative, but positive for HCV RNA in serum. Anti-HCV seems to be a more reliable marker for diagnosing CHC and acute PTHC as compared to AHC and acute SHC. Determination of HCV RNA in serum by nested PCR might be helpful for the early diagnosis of hepatitis C, especially acute SHC and CHC with negative anti-HCV.

In addition, we found that the positivity of HCV RNA in PBMC occurred only in anti HCV positive patients and could occur without positivity of HCV RNA in serum. This result suggests that PBMC might be an extra hepatic site for HCV storage and replication. PBMC up-take of HCV might be either by phagocytosis or direct infection. Investigations of HCV infection in PBMC might be helpful for elucidating the distribution, incubation, and replication site of HCV. It might also be useful for shedding light on, repeated infections, targets for treatment, and criteria for cure. It might also be of importance in the selection of blood donors so as to avoid transmission of HCV infection.

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