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Spontaneous Elimination of Serum Hepatitis C Virus (HCV) RNA in Chronic HCV Carriers: A Population-Based Cohort Study

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The natural course of hepatitis C virus (HCV) infection has not been fully elucidated. To investigate whether HCV is spontaneously eliminated in chronic carriers, a long-term population-based cohort study was conducted on 435 chronic HCV carriers. Individual characteristics, serum HCV RNA, and liver function tests were analyzed, and ultra sonography (US) was performed in all subjects. Subjects were followed up for 7.2 ± 2.4 years (mean ± SD). Serum HCV RNA was spontaneously eliminated in 16/435 (3.7%) individuals during this period; thus, the incidence of spontaneous elimination of serum HCV RNA was 0.5%/year/person. Multivariate analysis revealed that both a low value of ZTT and no US finding of chronic liver disease were associated with spontaneous viral elimination in HCV carriers. Three of these 16 individuals had chronic hepatitis, and 13 of them had normal ALT levels. When the neutralization of binding (NOB) assay that evaluates inhibition of the HCV envelope-2 protein binding to human cells was examined using sera from these 16 individuals, the NOB antibody was detected in only 3 cases with chronic hepatitis. These results suggest that serum HCV RNA is spontaneously eliminated in chronic HCV carriers in a population, and that the development of NOB antibody is associated with a natural resolution of chronic hepatitis in the minority of them. ***J. Med. Virol. 71:56–61, 2003.*** © 2003 Wiley-Liss, Inc.

KEY WORDS: hepatitis C; epidemiology; chronic hepatitis; neutralization of binding; NOB antibody

INTRODUCTION

Hepatitis C virus (HCV) infection occurs worldwide, and it is estimated that 170 million people are infected [Cohen, 1999]. In more than 70% of cases, the HCV infection is chronic, and the disease progresses to chronic hepatitis, cirrhosis and finally to hepatocellular carcinoma (HCC) [Kiyosawa et al., 1990; Tong et al., 1995; Seeff, 1999; Di Bisceglie, 2000]. HCV infection causes progressive liver disease in most infected individuals; on the other hand, it remains unclear whether HCV viremia can be spontaneously eliminated from infected individuals. In order to elucidate the incidence of spontaneous disappearance of HCV viremia, thereby contributing to our understanding of the natural course of HCV infection, a population-based cohort study is essential. For more than 10 years since 1991, we have carried out an epidemiological study of HCV infection in an area endemic for this disease [Ishibashi et al., 1996; Yoshii et al., 1999; Kuboki et al., 1999]. This population-based cohort study enabled us to elucidate the incidence of spontaneous elimination of serum HCV RNA in chronically HCV-infected individuals. In this study, we also examined the neutralization of binding (NOB) assay [Rosa et al., 1996] to evaluate if the serum obtained from individuals with spontaneous elimination of serum HCV RNA inhibits the HCV envelope-2 protein binding to human cells. The development of NOB antibody in the host has been shown to be closely associated with natural resolution of chronic hepatitis C [Ishii et al., 1998].

The results of this study show the evidence and incidence of spontaneous elimination of serum HCV RNA in chronically HCV-infected individuals in population.

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SUBJECTS AND METHODS

Subjects

During the period from 1991 to 1995, 7,925 inhabitants in total, from whom informed consent had been obtained, underwent a mass survey health examination for HCV infection [Ishibashi et al., 1996]. Antibodies to HCV antigens (anti-HCV), HCV RNA, HCV genotype, hepatitis B surface antigen (HBsAg), and liver function tests were examined using blood samples. The survey revealed that 1,078 inhabitants were positive for anti-HCV, of whom 846 were also positive for HCV RNA. Of these subjects, 526 have subsequently been followed up every year until May, 2001. Subjects with a history of antiviral therapy using interferon or with seropositivity for HBsAg, were excluded; 435 individuals positive for serum HCV RNA were finally enrolled in this study (Fig. 1). The study protocol conformed to the ethical guidelines of the Helsinki Declaration of 1975.

Methods

The primary outcome analyzed in this cohort study was the incidence of spontaneous elimination of serum HCV RNA in chronic HCV carriers in population. The incidence of elimination of serum HCV RNA was calculated by the person-years method [Kahn and Sempos, 1989]. Analysis of factors associated with spontaneous elimination of HCV RNA was performed as follows: 14 potentially relevant associated factors, representing data obtained at the time serum HCV RNA was found positive in each subject, were assessed by univariate analysis with Fisher's exact test and Student's t-test for categorical and continuous variables. A two-tailed $P < 0.05$ was considered significant. Univariate predictors with a low P value were then included in a forward stepwise multiple logistic regression model to identify the important factors for spontaneous elimination of serum HCV RNA.

Virological assays and liver function tests. Anti-HCV was examined by an enzyme immunoassay kit (HCV EIAII Abbott; Dainabot, Tokyo, Japan). Serum HCV RNA was detected by using the Amplicor HCV RNA Detection Kit (Nippon Roche, Tokyo, Japan). When the Amplicor HCV RNA assay showed seronegativity of HCV RNA, we further tested to confirm the result by nested reverse-transcription PCR using specific primers encoding the 5′ noncoding region of HCV, as described previously [Widell et al., 1991]. HCV typing was carried out by a PCR-based genotyping assay using type-specific primers, according to a previous report [Widell et al., 1994]. HBsAg was examined by an enzyme immunoassay kit (HBsAg Dainapac; Dainabot, Tokyo, Japan). Liver function tests included alanine aminotransferase (ALT; normal value <30 IU/L), aspartate aminotransferase (AST; normal value <30 IU/L), zinc sulfate turbidity test (ZTT; normal value <11 Kunkel units) and gamma glutamyl transpeptidase (gamma-GTP; normal value <45 IU/L).

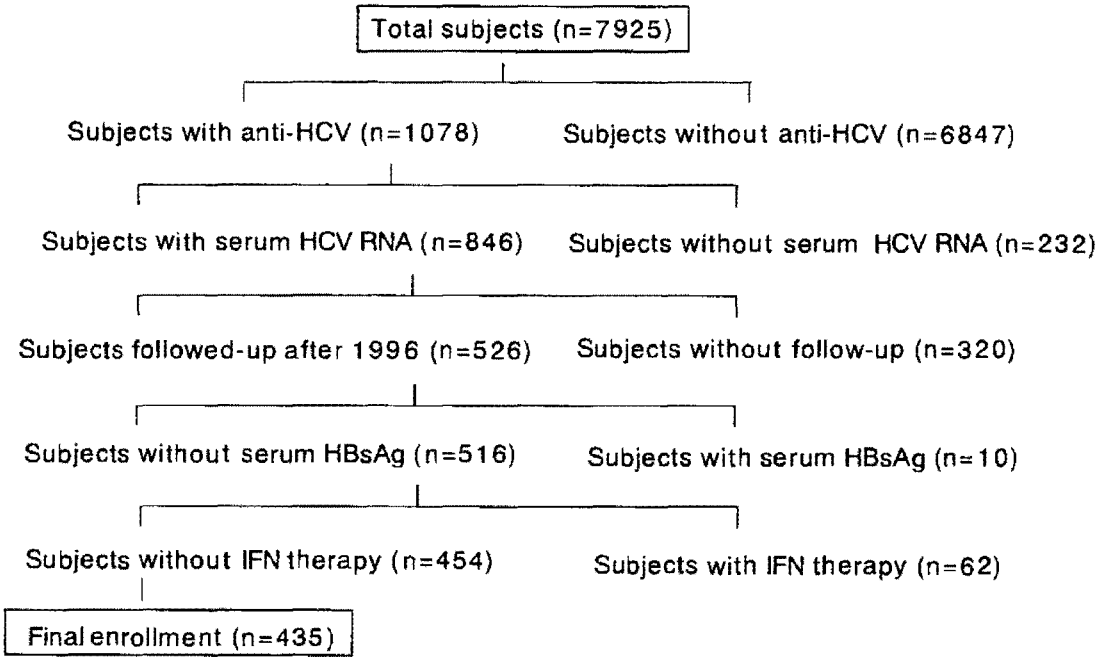

Fig. 1. Flow of subjects through the study. During the period from 1991 to 1995, 7,925 inhabitants in total underwent a mass survey health examination for HCV infection. Finally, 435 individuals positive for HCV RNA were enrolled in this study.

Neutralization of binding assay. For the NOB assay, the preserved sera at −20°C, that were collected before spontaneous elimination of HCV RNA from individuals, were used. The NOB assay was carried out as previously described [Rosa et al., 1996]. Briefly, 1 μg of the recombinant HCV-E2 protein was mixed with sera and incubated for 30 min at 37°C. Molt-4 cells (10^5 per well) were added to the mixture and incubated on ice for 1 hr. After washing, the amount of HCV-E2 bound to Molt-4 was assessed by fluorescein flow cytometry. The NOB antibody was determined as positive when the serum showed 50% neutralization of HCV-E2 binding to cells.

Ultra-sonography. Morphology of both liver and spleen was investigated by ultra-sonography (US) in all subjects. US was performed by two expert examiners. The main purpose was to detect HCC. We also checked a morphological sign of chronic liver disease to assess whether the subject had chronic HCV-related liver disease. Splenomegaly above the normal spleen index value (the index is defined as the product of the length and width of the spleen measured in centimeters; the normal value is below 35), as well as the findings of irregularity of internal echo of the liver and dullness of the liver edge, were defined as the morphological sign of chronic liver disease [Steinmaurer et al., 1984; Sherlock and Dooley, 1993].

RESULTS

Spontaneous Elimination of Serum HCV RNA in Chronically HCV-Infected Individuals

Four hundred thirty-five individuals with positivity for both anti-HCV and HCV RNA were followed up and analyzed consecutively every year. The follow-up period was 7.2 ± 2.4 years (mean ± SD, range: 1 to 10 years) from 1991 to 2001. Serum HCV RNA was spontaneously eliminated in 16/435 (3.7%) individuals during this period (Table I). Their serum HCV RNA were persistently negative to the end of follow-up, once HCV RNA clearance was achieved (Fig. 2). Based on the follow-up period, the incidence of spontaneous elimination of serum HCV RNA in chronic HCV carriers was 0.5%/year/person.

ALT Levels in Individuals with Spontaneous Elimination of Serum HCV RNA

ALT values were within the normal range in 13/16 (81.3%), but at a high level above the normal range in 3/16 (18.7%), when serum HCV RNA was found positive. Three cases (case 5, 11, 16) with ALT abnormality were diagnosed as having chronic hepatitis with ALT fluctuation with abnormally high levels before disappearance of serum HCV RNA and morphological findings by US. The ALT level of the three cases finally became normal when serum HCV RNA spontaneously became negative. The ALT levels of the other 13 cases were within the normal range over the follow-up period (Fig. 2). In 419 subjects with persistent positivity for serum HCV RNA, ALT values were within the normal range in 265/419 (63.2%), but at a high level above the normal range in 154/419 (36.8%), when serum HCV RNA was found positive. At the end-point of follow-up, ALT values were at a persistently high level in 107/154 (69.5%). The subjects with persistent positivity for HCV RNA had a higher rate for persistent ALT abnormality than those with spontaneous elimination of HCV RNA at the end of follow-up (69.5 vs. 0%; $P = 0.031$).

Factors Associated with Spontaneous Elimination of HCV RNA in Chronically HCV-Infected Individuals

Table II shows the unadjusted incidence of spontaneous elimination of HCV RNA in chronic HCV carriers with and without individual factors, and their significance by univariate analysis. Low value of ZTT under 11 Kunkel units (36.5 vs. 87.5%, $P = 0.00006$), no history of

TABLE I. Chronic HCV Carriers With Spontaneous Elimination of Serum HCV RNA*

						History	
Case no.	Age, sex	HCV genotype	ALT fluctuation	US chronicity	NOB Ab	Hepatitis	BTF
1	61 M	1b	–	–	–	–	–
2	60 M	1b	–	–	–	–	–
3	65 M	1b	–	–	–	–	–
4	71 F	1b	–	–	–	–	–
5	68 M	2b	+	+	+	+	–
6	71 M	2b	–	–	–	–	–
7	55 F	1b	–	–	–	–	–
8	77 F	1b	–	–	–	–	–
9	65 F	1b	–	–	–	–	–
10	71 F	2a	–	–	–	–	–
11	77 F	1b	+	+	+	+	–
12	78 F	1b	–	–	–	–	–
13	66 F	1b	–	–	–	–	–
14	66 M	2b	–	–	–	–	–
15	72 F	1b	–	–	–	–	–
16	38 F	2b	+	+	+	–	–

*US, ultra sonography; BTF, blood transfusion; NOB Ab, neutralization of binding antibody.

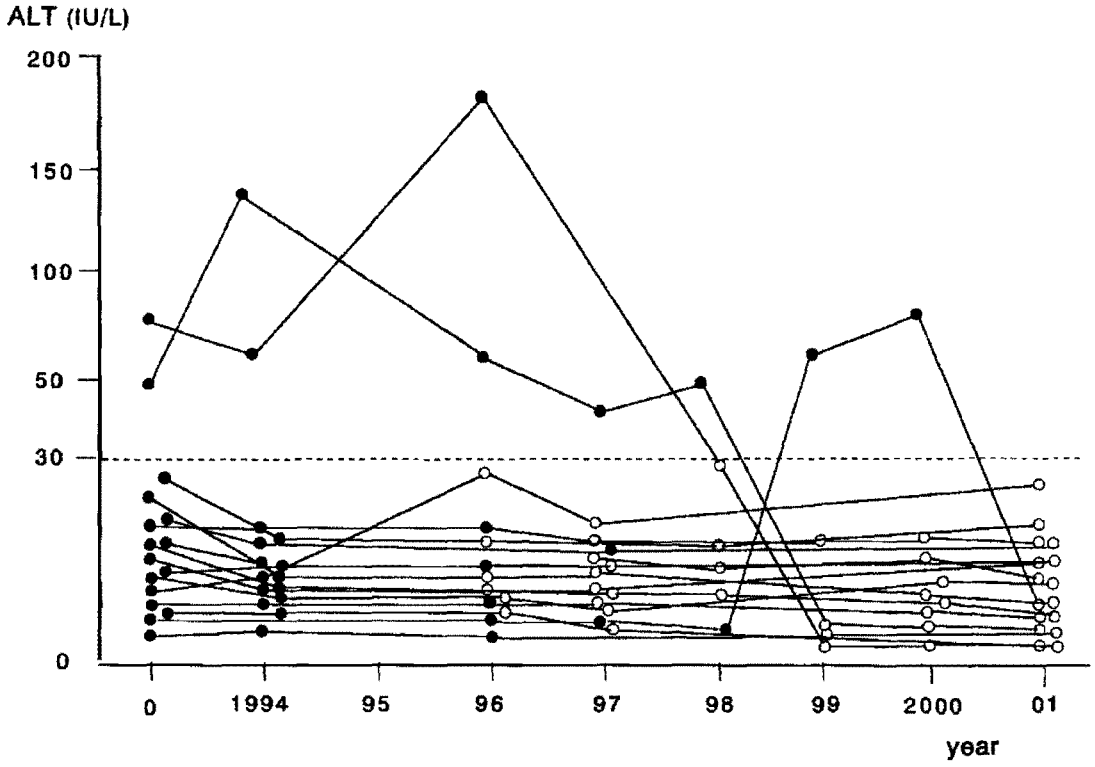

Fig. 2. Changes of ALT levels and the HCV RNA status in sera of 16 individuals showing spontaneous elimination of serum HCV RNA. Year 0 represents the first point when serum HCV RNA was detected. The solid and open circles denote serum HCV RNA-positive and serum HCV RNA-negative, respectively.

TABLE II. Univariate Analysis of Factors for Spontaneous Elimination of Serum HCV RNA in Chronic HCV Carriers*

	Serum HCV RNA		
	Persistent positivity [n = 419] (%)	Spontaneous elimination [n = 16] (%)	*P* value
Age (mean ± SD)	68.4 ± 9.3	66.6 ± 9.9	0.370
Sex (Male)	161 (38.4)	6 (37.5)	1.000
AST <30 IU/L	189 (45.1)	11 (68.7)	0.054
ALT <30 IU/L	265 (63.2)	13 (81.3)	0.111
Gamma-GTP <45 IU/L	368 (87.8)	15 (93.8)	0.706
ZTT <11 Kunkel	153 (36.5)	14 (87.5)	0.00006
History			
Hepatitis (−)	281 (67.1)	14 (87.5)	0.105
Jaundice (−)	383 (91.4)	16 (100.0)	0.383
Operation (−)	218 (52.0)	9 (56.3)	0.803
BTF (−)	336 (80.2)	16 (100.0)	0.032
Acupuncture (−)	297 (70.9)	10 (62.5)	0.576
Alcohol intake (−)	250 (59.7)	11 (68.8)	0.948
US: no finding of CLD	129 (30.8)	13 (81.3)	0.00007
HCV genotype 1b	187 (44.6)	11 (68.8)	0.108

*BTF, blood transfusion; US, ultra sonography; CLD, chronic liver disease.

blood transfusion (80.2 vs. 100%, $P = 0.032$) and no US finding of chronic liver disease (30.8 vs. 81.3%, $P = 0.00007$) were associated with spontaneous viral elimination in chronically HCV-infected individuals. Table III shows the results of stepwise multiple logistic regression modeling from the pool of 5 variables with low P values from the univariate analysis. The factor "no history of blood transfusion" was excluded from this multiple logistic regression modeling because it was present in all cases with spontaneous elimination of serum HCV RNA. Both a low value of ZTT under 11 Kunkel units (odds ratio 1:8.726, $P = 0.006$) and no US finding of chronic liver disease (odds ratio 1:4.768, $P = 0.010$) were confirmed to be significantly associated with spontaneous viral elimination in chronically HCV-infected individuals.

Detection of NOB Antibody in Individuals with Spontaneous Elimination of Serum HCV RNA

The NOB antibody was detected in 3 of 16 cases with spontaneous elimination of HCV RNA (Table I). All the three cases (case 5, 11, 16) had biochemical evidence of chronic hepatitis with morphological signs of chronic liver disease by US. The two of them had a history of acute hepatitis. The remaining 13 cases negative for the NOB antibody did not show both ALT abnormality and US finding of chronic liver disease over the follow-up period, suggesting that they were healthy seropositive HCV carriers.

DISCUSSION

The natural outcome of chronic HCV infection has not been precisely elucidated. Since most individuals infected with HCV are asymptomatic and live an ordinary life, it is difficult to clarify whether spontaneous termination of HCV viremia occurs in chronic HCV carriers in the general population. In this cohort study, we showed that serum HCV RNA is spontaneously eliminated in chronic HCV carriers with a disappearance rate of approximately 0.5%/year/person, and that the development of NOB antibody is associated with natural resolution of chronic hepatitis in some of them.

Recently, spontaneous seroreversion of anti-HCV in the general population has been reported from the research group of Italy [Kondili et al., 2002]. They showed that the seroreversion of anti-HCV with negativity for serum HCV RNA was found in 7/36 (19.4%) anti-HCV-positive individuals at a median follow up of 7 years. The frequency of spontaneous elimination of serum HCV RNA in our study (3.7%) is lower than that of Kondili et al. [2002]. This difference might be due to the different genetic backgrounds or the different age distribution of HCV-infected individuals in population.

Interestingly, all the individuals showing spontaneous elimination of serum HCV RNA had no history of blood transfusion, which may cause infection with a high amount of the virus. Lack of exposure to a large quantity of the virus through blood transfusion may be one of the important factors in spontaneous viral elimination. Multivariate analysis revealed that a low titer of ZTT within the normal level and no finding of chronic liver disease by US were the most important factors associated with spontaneous elimination of HCV RNA, suggesting that lack of liver disease in chronic HCV carriers may facilitate clearance of HCV.

Elimination of serum HCV RNA in adult patients with established chronic liver diseases due to HCV infection is rare unless antiviral therapy using interferon is employed. On the basis of patients' medical records, spontaneous elimination of HCV has been reported rarely in some cases of chronic HCV-related disease [Yousuf et al., 1992; Yokosuka et al., 1999; Yoshikawa et al., 2001]. It has been reported that such elimination was found in 6/310 (2%) patients with HCV-related chronic liver disease who were positive for serum HCV RNA; all these cases were in the terminal stage of HCC [Yokosuka et al., 1999]. Reduction in the amount of HCV may occur in the terminal stage of HCC because the optimal environment for viral growth is lost as a result of replacement of hepatocytes by tumor cells. Surgical stress may also induce immunological changes that lead to eradication of HCV from patients with chronic hepatitis C [Yoshikawa et al., 2001]. In general, spontaneous eradication of the virus from the host is a very rare event in patients with established chronic hepatitis C, although a small percentage of these patients naturally clear the virus and achieve resolution of chronic hepatitis with the development of NOB antibody [Ishii et al., 1998]. In this study, we showed that individuals with spontaneous elimination of serum HCV RNA included those with recovery from chronic hepatitis in the general population. The three cases showing spontaneous elimination of HCV RNA had chronic hepatitis with fluctuation of abnormal ALT values before the disappearance of serum HCV RNA. The NOB antibody with a high level of neutralization

TABLE III. Factors for Spontaneous Elimination of HCV RNA by Multivariate Analysis*

Factor	Adjusted OR	95% CI	P value
Normal AST (<30 IU/L)	1.35	0.40, 4.54	0.629
Normal ZTT (<11 Kunkel)	8.73	1.89, 40.38	0.006
No history of hepatitis	1.76	0.37, 8.45	0.487
No US finding of chronic liver disease	4.77	1.46, 15.61	0.010
HCV genotype, non-1b	0.46	0.15, 1.42	0.177

*US, ultra sonography.

activity exceeding 50% was detected in the sera of only these 3 individuals with chronic hepatitis. This finding suggests that the development of NOB antibody is associated with natural resolution of chronic hepatitis C. However, the results of the NOB assay should be interpreted cautiously, because the NOB assay was not performed on sera of individuals who were persistently positive for serum HCV RNA. A previous study [Rosa et al., 1996] showed that the NOB antibody with a high level of neutralization activity exceeding 50% was not detectable in any serum samples from 34 patients with chronic HCV infection with different HCV genotypes. Thus, despite the limitation of our assay, the presence of such antibodies in serum from 3 chronic hepatitis cases showing spontaneous elimination of HCV RNA is of considerable interest. Although the appearance of NOB antibody is likely related to the natural resolution of chronic hepatitis, the involvement of this antibody in viral clearance is still not understood. The mechanisms that cause spontaneous elimination of serum HCV RNA in HCV carriers without evidence of liver disease remain unknown. Antiviral cytokines induced during infection may be a key factor in inhibiting the multiplication of virus in the host. As the host immune response against HCV differs among individuals, the genetic factors specific to each individual may play a role in controlling HCV infection. Genetic factors associated with the course of HCV infection need to be further clarified at the human genome level.

In conclusion, we provide evidence of spontaneous viral elimination in population, with an estimate of its incidence, from a long-term cohort study of chronic HCV carriers. Spontaneous HCV elimination occurs in HCV carriers without evidence of liver disease, or in those with chronic hepatitis whose immune system develops NOB antibody. These findings contribute to a better understanding of the natural course of HCV infection in population.

REFERENCES

Cohen J. 1999. The scientific challenge of hepatitis C. Science 285: 26–30.

Di Bisceglie AM. 2000. Natural history of hepatitis C: Its impact on clinical management. Hepatology 31:1014–1018.

Ishibashi M, Shinzawa H, Kuboki M, Tsuchida H, Takahashi T. 1996. Prevalence of inhabitants with anti-hepatitis C virus antibody in an area following an acute hepatitis C epidemic: age-and area-related features. J Epidemiol 6:1–7.

Ishii K, Rosa D, Watanabe Y, Katayama T, Harada H, Wyatt C, Kiyosawa K, Aizaki H, Matsuura Y, Houghton M, Abrignani S, Miyamura T. 1998. High titers of antibodies inhibiting the binding of envelope to human cells correlate with natural resolution of chronic hepatitis C. Hepatology 28:1117–1120.

Kahn HA, Sempos CT. 1989. Follow-up studies: person-years. In: Kahn HA, Sempos CT, editors. Statistical methods in epidemiology. Monographs in epidemiology and biostatistics, vol. 12. New York: Oxford University Press. p 206–225.

Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, Alter HJ. 1990. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 12:671–675.

Kondili LA, Chionne P, Costantino A, Villano U, Lo Noce C, Pannozzo F, Mele A, Giampaoli S, Rapicetta M. 2002. Infection rate and spontaneous seroreversion of anti-hepatitis C virus during the natural course of hepatitis C virus infection in the general population. Gut 50:693–696.

Kuboki M, Shinzawa H, Shao L, Ishibashi M, Yoshii E, Suzuki K, Saito K, Saito T, Togashi H, Takahashi T, Yasumura S, Fukao A. 1999. A cohort study of hepatitis C virus (HCV) infection in an HCV epidemic area of Japan: age and sex-related seroprevalence of anti-HCV antibody, frequency of viremia, biochemical abnormality and histological changes. Liver 19:88–96.

Persistence of viremia in patients with type-C chronic hepatitis during long-term follow-up. Yousuf M, Nakano Y, Tanaka E, Sodeyama K, Kiyosawa K. 1992. Scand J Gastroenterol 27:812–816.

Rosa D, Campagnoli S, Moretto C, Guenzi E, Cousens L, Chin M, Dong C, Weiner AJ, Lau JY, Choo QL, Chien D, Pileri P, Houghton M, Abrignani S. 1996. A quantitative test to estimate neutralizing antibodies to the hepatitis C virus: Cytofluorimetric assessment of envelope glycoprotein 2 binding to target cells. Proc Natl Acad Sci USA 93:1759–1763.

Seeff LB. 1999. Natural history of hepatitis C. Am J Med 107:10S–15S.

Sherlock S, Dooley J. 1993. Ultrasound, computed tomography and magnetic resonance imaging. In: Sherlock S, Dooley J, editors. Diseases of the liver and biliary system, ninth edition. London: Blackwell Scientific Publications. p 63–65.

Steinmaurer HJ, Jirak P, Walchshofer J, Clodi PH. 1984. Accuracy of sonography in the diagnosis of diffuse liver parenchymal diseases, comparison of sonography and liver histology. Ultraschall Med 5:98–103.

Tong MJ, El-Farra NS, Reikes AR, Co RL. 1995. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 332:1463–1466.

Widell A, Mansson AS, Sundstrom G, Hansson BG, Nordenfelt E. 1991. Hepatitis C virus RNA in blood donor sera detected by the polymerase chain reaction: comparison with supplementary hepatitis C antibody assays. J Med Virol 35:253–258.

Widell A, Shev S, Mansson S, Zhang YY, Foberg U, Norkrans G, Flyden A, Weiland O, Kurkus J, Nordenfelt E. 1994. Genotyping of hepatitis C virus isolates by a modified polymerase chain reaction assay using type specific primers: epidemiological applications. J Med Virol 44:272–279.

Yokosuka O, Kojima H, Imazeki F, Tagawa M, Saisho H, Tamatsukuri S, Omata M. 1999. Spontaneous negativation of serum hepatitis C virus RNA is a rare event in type C chronic liver diseases: analysis of HCV RNA in 320 patients who were followed for more than 3 years. J Hepatol 31:394–399.

Yoshii E, Shinzawa H, Saito T, Shao L, Kuboki M, Saito K, Togashi H, Takahashi T, Mizokami M. 1999. Molecular epidemiology of hepatitis C virus infection in an area endemic for community-acquired acute hepatitis C. Tohoku J Exp Med 188:311–316.

Yoshikawa M, Morimoto Y, Shiroi A, Yoshiji H, Kuriyama S, Fukui H. 2001. Spontaneous elimination of serum HCV-RNA after total gastrectomy for early gastric cancer in a patient with chronic hepatitis C. Am J Gastroenterol 96:922–923.