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Hepatitis C virus infection: an overview

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Hepatitis C virus is an RNA virus belonging to the *Flaviviridae* family. The diagnosis of hepatitis C virus infection was based on the detection of serum antibody to hepatitis C virus (anti-HCV) using immunoassay or recombinant immunoblot assay, or the direct detection of serum hepatitis C virus RNA using polymerase chain reaction. The anti-HCV positive rate in the general population or healthy blood donors is 0.5% to 4% worldwide. Parenteral transmission was the major route of hepatitis C virus infection. High-risk groups for hepatitis C virus infection included recipients of blood transfusion of which the blood donors were not screened for anti-HCV, intravenous drug abusers, hemophiliacs, and patients who have received hemodialysis. More than 80% of patients with hepatitis C virus infection progressed into chronicity, 20% to 30% of patients with chronic hepatitis C progressed to cirrhosis after 10 to 20 years of follow-up, and some developed hepatocellular carcinoma. Hepatitis C virus was the most common cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma in Western countries and in Japan, where hepatitis B virus is not endemic. Interferon therapy at a dosage of 3 MU and subcutaneous injection 3 times per week for 6 months normalized serum transaminase in 50% of patients with chronic hepatitis C at the end of treatment. However, the relapse rate was high and only 20% to 25% of patients sustained response 1 year after discontinuing therapy. Prolonged interferon therapy up to 12 to 18 months has been suggested to improve interferon efficacy by decreasing the relapse rate and thus increasing the sustained response rate. New interferon preparation such as consensus interferon and long-acting pegylated-interferon has recently shown better treatment response than the traditional interferon regimen. The combined regimen of interferon and ribavirin was shown to have better efficacy than interferon alone in treating patients with chronic hepatitis C.

Key words: Chronic hepatitis, hepatitis C virus, interferon, ribavirin

Hepatitis C virus (HCV) was cloned in 1989 and accounted for most posttransfusion or sporadic non-A, non-B hepatitis [1-5]. Hepatitis C virus is an RNA virus belonging to the *Flaviviridae* virus family. The HCV genome consists of about 9400 nucleotides with one large open-reading frame encoding for a polypeptide (about 3010 amino acids) consisting of structural and non-structural domains (Fig. 1) [6].

The current clinical diagnosis of HCV infection is based on the detection of serum antibody to HCV (anti-HCV) using enzyme-linked immunosorbent assay (ELISA) or recombinant immunoblot assay. The first-generation anti-HCV ELISA using non-structural antigen (c100-3) has been reported to have low sensitivity and to cause false positives especially in patients with positive rheumatoid factor and hypergammaglobulinemia. The recombinant immunoblot assay has been used as a confirmation test, but its clinical use is limited because of high cost. Second- and third-generation anti-HCV ELISA, with the addition of structural antigen (c22-3) and antigens from non-structural region (c33c and NS5), greatly improved the sensitivity and specificity of the test, and had been widely used [7,8].

In the early phase of HCV infection, detection of serum anti-HCV is less sensitive, and current active infection cannot be differentiated from past resolved infection. In addition, loss of antibody in persistently infected patients has been documented in some immunocompromised hosts, such as patients receiving hemodialysis and those with human immunodeficiency virus infection. Methods that permit a direct detection of virus can augment the diagnosis from the antibody testing. Moreover, the detection of HCV viremia is a gold standard in evaluating the virological response to interferon (IFN) or other antiviral therapy. Amplification of HCV RNA by reverse transcription and polymerase chain reaction (RT-PCR) was shown

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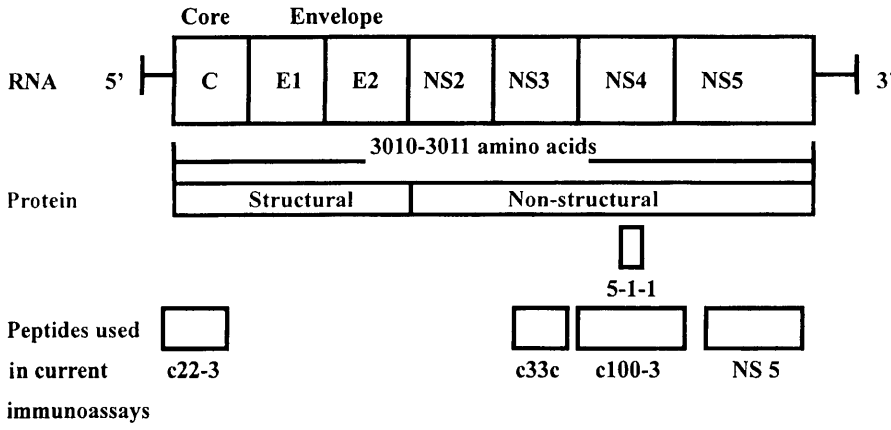

Fig. 1. Hepatitis C virus genome and peptides used in antibody to HCV immunoassays.

to be an effective means for the direct detection of HCV [9]. Qualitative measurement of HCV RNA using RT-PCR assay requires steps of RT, subsequent amplification of complementary DNA, and visualization of the amplified products using gel electrophoresis. These procedures are cumbersome and the likelihood of contamination is high. Wide range of interlaboratory variation in the results has been reported [10]. Polymerase chain reaction with standardized procedures and colorimetric detection of products (Amplicor, Roche Corp., Branchbury, NJ, US) are commercially available [11]. Quantitative measurement of HCV RNA is an important clinical tool that allows monitoring of the antiviral effect of IFN as well as predicting the treatment response. Branched chain DNA signal amplification assay (Quantiplex, Chiron Corp., Emeryvillo, CA, US) and Amplicor HCV monitor system are now commonly used in clinical practice. Quantiplex has been used widely in the quantitative measurement of serum HCV RNA [12,13]; its detection sensitivity, however, is 2×10^5 genome equivalent/mL and is not as sensitive as RT-nested PCR (10-100 copies/mL). Recently developed quantitative Amplicor HCV monitor system has shown better sensitivity in HCV RNA measurement (2000 copies/mL) compared with branched DNA signal amplification assay [14].

Hepatitis C virus genome is characterized by significant genetic heterogeneity. Quasispecies diversity in individual HCV isolates and at least 6 major genotypes were found in different geographic areas. Methods for HCV genotyping include PCR with direct sequencing, nested PCR with type-specific primers, restriction fragment length polymorphism of PCR products, and line-probe hybridization assay [15]. The major genotypes of HCV differ in their worldwide distribution [16]. The most common genotypes in the United States and Western Europe are 1a and 1b; genotypes 1b, 2a, and 2b were common in Japan and Taiwan; genotype 3 has been found in Thailand, Northern Europe, and Australia; genotype 4 is predominant in the Middle East; genotype 5 is prevalent in South Africa; and genotype 6 has been reported only in Hong Kong. Genotype 1b has been reported to be associated with high serum HCV RNA titer, more advanced disease, and suboptimal response to IFN therapy [17].

Epidemiology of Hepatitis C Virus Infection

Seroepidemiological studies have revealed an anti-HCV prevalence of 0.5% to 4% in blood donors worldwide [1,2,18-20]. However, the geographical distribution of HCV infection varied within the same country between towns, provinces, and ethnic groups. High anti-HCV prevalence rate of more than 20% has been found in specific regions of Taiwan, whereas the general HCV carrier rate in Taiwan was only 1% to 2% [21]. Identification of specific risk factors such as the use of non-disposable needle injection has been documented in high endemic area of HCV. Hepatitis C virus is the most common cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma in Western countries and in Japan, where hepatitis B virus is not endemic. Parenteral transmission was the major route of HCV infection. Sexual, intrafamiliar, and perinatal transmissions of HCV are possible but not common. High-risk groups for HCV infection included recipients of blood transfusion of which the blood donors were not screened with anti-HCV, intravenous drug abusers, hemophiliacs, and patients who received hemodialysis [19,20,22]. Other high-risk groups included patients with thalasemia requiring multiple transfusion, and organ transplantation recipients.

In patients with chronic hepatitis C, only 20% to 50% had past history of blood transfusion, whereas the others (sporadic cases) had no apparent risk factors. Tattooing, acupuncture, occupational needle stick injuries, dental procedure, operation, and use of non-disposable needle injection were possible risk factors of HCV transmission. Injection of non-disposable needles has been reported as an important route of transmission of acute community-acquired (sporadic) HCV infection in Taiwan [23]. Risk factor analysis of patients with chronic hepatitis C in Taiwan using a multivariate logistic regression analysis also revealed that blood transfusion, frequent injection with non-disposable needles, and low education level were the significant risk factors of contracting HCV infection [24]. Use of disposable needles and promotion of public education to avoid unnecessary operation, blood transfusion, or immunoglobulin injection are necessary to reduce sporadic HCV infection. Prospective studies showed that the incidence of posttransfusion hepatitis of which the blood donors were not screened for anti-HCV was around 12% [5,25], with HCV infection accounting for more than 90% of the cases. With anti-HCV screening in blood donors, the incidence of post-transfusion hepatitis C reduced dramatically [26,27].

Natural Course of Hepatitis C Virus Infection

The most remarkable and alarming aspects of HCV infection are its high rate of persistence and its ability to induce chronic liver disease. More than 80% of patients with acute HCV infection progresses into chronic hepatitis [28,29]. Persistence appears to have resulted from the ability of the virus to replicate with a high rate of mutation, resulting in a series of immunologically distinct variants or quasispecies that allow the virus to escape immunological control [30-32]. Acute HCV genotype 1b infection has been shown to have more chance to develop chronicity than other HCV genotypes, possibly because of the existence of more quasispecies in HCV genotype 1b [29]. After 10 to 20 years of follow-up, 20% to 30% of patients with chronic hepatitis C progress into cirrhosis [33].

The incidence of chronic hepatitis C progressing to irreversible cirrhosis has been reported to be as low as 20% at 10 years and as high as 8% per year [34]. Chronic infection of HCV is associated with the development of hepatocellular carcinoma. According to retrospective studies, the mean intervals between HCV infection and the detection of sequelae was 10 to 14 years for chronic hepatitis, 21 to 25 years for cirrhosis, and 28 to 29 years for hepatocellular carcinoma [35,36]. Hepatitis C virus infection at age older than 40 years, daily alcohol consumption of 50 g or more, and male gender were reported to be associated with an increased rate of fibrosis progression in patients with chronic hepatitis C [37].

Clinical and Pathological Characteristics

Clinical symptoms in patients with acute hepatitis C tended to be milder than those seen in patients infected with other hepatitis virus [38]. Most cases were asymptomatic, and only 25% of patients with post-transfusion hepatitis C developed jaundice [5,39]. The risk for fulminant or subacute liver failure is low. The incubation period of the virus ranged from 15 to 150 days (usually 6-8 weeks). Infection may not be detectable by the first-generation ELISA in detecting anti-HCV for up to 6 months, but can be detected by the second- or third-generation ELISA as early as 6 to 8 weeks after exposure. Detection of serum HCV RNA by RT-PCR can diagnose acute HCV infection as early as 1 to 2 weeks after exposure. Unlike antibodies to hepatitis A and B viruses, anti-HCV are not protective and, in most cases, are a marker for disease.

The clinical presentation of chronic hepatitis C may vary depending on the host immune system and the source and duration of infection. During long-term follow-up, patients with chronic hepatitis C may have a fluctuation of serum transaminase, which may even be normal on occasion. Most of these patients are asymptomatic or have mild fatigue. Most previous reports showed no correlation between serum levels of aminotransferase and traditional histological diagnosis of chronic lobular hepatitis, chronic persistent hepatitis, chronic active hepatitis, and cirrhosis [40-41]. Pathological evaluation using histological activity index, which contains inflammation grade of portal, periportal and lobular component, and fibrotic stage, has recently been widely used in patients with chronic hepatitis C [42-44]. Serum level of transaminase may reflect the severity of intralobular and periportal necroinflammation in liver histology, but the correlation was not strong enough for prediction [45]. In addition, the severity of hepatic fibrosis or cirrhosis is difficult to identify by clinical laboratory parameters [46]. Liver biopsy remains the gold standard in evaluating the degree of hepatic necroinflammation and fibrosis. Hepatic steatosis, bile duct damage, and portal lymphoid aggregation or follicles were histological characteristics often seen in patients with chronic HCV infection [47-50]. Portal lymphoid aggregation or follicles and bile duct damage were frequently associated with more severe periportal and portal necroinflammation, and

were frequently seen in patients with genotype 1b infection [51,52]. Hepatic steatosis was associated with obesity and was correlated with hepatic fibrosis [53].

Chronic HCV infection may be associated with various immunological disorders of the host. These disorders included (1) serum autoantibodies formation, frequently and mainly presented as antinuclear antibody and anti-smooth muscle antibody; (2) immune-complex mediated diseases, mainly presented as mixed cryoglobulinemia, glomerulonephritis, and vasculitis; and (3) other immunological disorders, such as lymphocytic sialadenitis, thyroiditis, lichen planus, and porphyria cutanea tarda [54-58]. All these findings suggested that HCV may have an important role in the pathogenesis of the immunological disorders of the host. Among Chinese patients with chronic hepatitis C, 48% and 44% of patients were associated with positive serum autoantibodies and cryoglobulinemia, respectively. Hepatitis C virus genotype and serum HCV RNA titer were not associated with the presence of these immune disorders [59,60]. Host genetic factors, such as human leukocyte antigen expression, may have a role in the manifestation of these immune disorders [61-63].

Treatment of Patients with Acute and Chronic Hepatitis C

Recombinant IFN α-2b has been used in the treatment of Chinese patients with acute posttransfusion hepatitis C. One year after a 3-month IFN treatment (3 MU, thrice a week), 44% of patients cleared the serum HCV RNA, compared with 13% in untreated patients [64]. Reports from Western countries showed similar results and indicated that IFN treatment in acute hepatitis C may prevent the disease from progressing to chronicity [65].

Patients with chronic HCV infection often progress to cirrhosis insidiously and are associated with the development of hepatocellular carcinoma. Up to date, the development of HCV vaccine was hampered by the high mutation rate of HCV genome, especially in the envelope region [66]. The treatment need of patients with chronic hepatitis C is thus urgent. Interferon was the first drug approved by most countries for the treatment of patients with chronic hepatitis C. The short-term goals of IFN therapy are (1) normalization of serum alanine transaminase (ALT) and improvement of liver necroinflammatory processes; and (2) suppression of HCV replication and sustained clearance of serum HCV RNA detected by PCR assay.

A 6-month course of alpha IFN treatment (3 MU, 3 times per week) has been shown to normalize serum ALT levels at the end of the treatment in approximately 50% of patients, but only 15% to 20% of treated patients maintained normal ALT levels during post-treatment follow-up [67,68]. The initial rate of biochemical response was not significantly improved by initiating treatment at higher doses of 5 or 6 MU, nor by dose escalation of up to10 MU in patients who failed to respond to 3 MU doses. The relapse rate can be significantly reduced by prolonging the duration of treatment from 6 to 12 or 18 months, resulting in sustained remission rates of 30% to 40% [69]. The United States National Institute of Health Consensus in 1997 stated that the optimal therapeutic regimen for hepatitis C was IFN-α given subcutaneously in a dose of 3 MU 3 times weekly for 12 months, with assessment of aminotransferase levels and HCV RNA at 3 months to allow early discontinuation in patients with no response [70]. However, a long-term IFN treatment increases the side-effects and cost of treatment, and decreases compliance of patients.

The common side-effects of IFN treatment were fever, rigor, myalgia, and headache (flu-like symptoms). These symptoms can be alleviated by premedication with acetaminophen, and usually become tolerable by patients after several weeks of injection. Other side-effects included anorexia, hair loss, body weight loss, and mental instability (Table 1). During IFN therapy, 20% to 30% of patients have experienced leukopenia and thrombocytopenia, thus requiring regular monitoring of complete blood count. However, they returned to the pretreatment value after discontinuance of the IFN treatment [71].

In most patients, there was a parallel response in serum ALT and HCV RNA levels during IFN treatment. However, it has now been shown that serum HCV RNA remains detectable in some patients who have complete biochemical response (normal ALT levels at the end of treatment). These patients were more likely to relapse

Table 1. Side-effects of interferon injection

Early side-effects (1-2 weeks after initiation)	Late side-effects (>2 weeks after initiation)
Fever/rigor	Weight loss
Myalgia	Hair loss
Fatigue	Leukopenia and thrombocytopenia
Headache	Mental instability (agitation, depression, emotional liability)
Anorexia	Increased autoimmunity (thyroid function abnormality)

after cessation of treatment, but post-treatment relapse may occur even in patients who become negative for serum HCV RNA at the end of treatment. This is probably related to the persistence of HCV RNA in liver.

Several predictive factors have been identified for a favorable response to IFN therapy (Table 2). Among the host factors, young age (<40 years) and the absence of cirrhosis were found to predict IFN response. Viral factors are currently considered the most important in predicting IFN response. Patients with low pretreatment serum HCV RNA level and HCV infection other than genotype 1b were associated with better IFN treatment results. Other factors reported to be associated with poor response were increased hepatic iron and the association with immunosuppressive status [72].

Consensus IFN is a newly developed recombinant type 1 IFN that has greater *in vitro* biological activity than other IFN-α. Clinical trials from the United States and Taiwan have shown that consensus IFN 9 μg, subcutaneous injection, 3 times per week for 6 to 12 months is a safe and effective treatment for patients with chronic hepatitis C [73,74]. Covalent attachment of a polyethylene glycol moiety to IFN-α results in a new compound (peginterferon-α) that has sustained absorption, a slower rate of clearance, and a longer half-life than unmodified IFN-α. A regimen of peginterferon α-2a 180 μg given once weekly was found to be more effective than a regimen of IFN-α-2a given 3 times weekly in treating patients with chronic hepatitis C [75, 76]

Interferon has been widely used for the treatment of chronic hepatitis C in the past 10 years. Recent data of long-term follow-up study proved that successful antiviral response prevents chronic hepatitis C patients from progressing to cirrhosis and hepatocellular carcinoma, and possibly improves survival rate [77,78].

Efficacy of other drugs in the treatment of chronic hepatitis C, such as ribavirin and ursodeoxycholic acid, was not satisfactory [79,80]. However, the combined use of IFN and ribavirin has been shown to have better efficacy than using IFN alone in treating patients with chronic hepatitis C [81-83]. Sustained clearance of serum HCV RNA can be obtained in 40% to 60% of patients receiving a combination of IFN and ribavirin. Side-effects of ribavirin were dose-related hemolysis and anemia, indirect type hyperbilirubinemia, hyperuricemia, skin rash, pruritus, and gastrointestinal upset. The current recommendations for the treatment of chronic hepatitis patients with elevated serum ALT are a 24- to 48-week regimen of IFN-α and ribavirin. Interferon alfa, 3 MU 3 times weekly, and ribavirin 1000 mg/d for patients who weighed less than 75 kg, or ribavirin 1200 mg/d for those who weighed 75 kg or more should be initiated for 24 weeks. Therapy should be discontinued at 24 weeks for patients with genotype 2 or 3. In patients with genotype 1, therapy should be stopped if serum HCV RNA test results are positive, and should be continued for a full 48-week treatment if HCV RNA test results are negative at 24 weeks [84].

Table 2. Factors of favorable response to interferon treatment in patients with chronic hepatitis C

Young age
Short duration of disease
Low body weight
Absence of cirrhosis
Low serum HCV RNA titer
HCV genotype other than genotype 1b
Negative for human immunodeficiency virus
Mutation in the NS5 region of viral genome (ISDR)

Abbreviations: HCV = hepatitis C virus; ISDR = interferon sensitivity determining region

References

1. Choo QL, 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.
2. Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW, Houghton M. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989;244:362-4.
3. Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo QL, Kuo G. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989;321:1494-500.
4. Lee SD, Tsai YT, Hwang SJ, Wu JC, Yung CH, Cheng KK, Lo KJ. A prospective study of posttransfusion non-A, non-B (type C) hepatitis following cardiovascular surgery in Taiwan. J Med Virol 1991;33:188-92.
5. Lee SD, Hwang SJ, Lu RH, Lai KH, Tsai YT, Lo KJ. Antibody to hepatitis C virus in prospectively followed patients with posttransfusion hepatitis. J Infect Dis 1991;163:1354-7.
6. Houghton M, Weiner A, Han J, Kuo G, Choo QL. Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatology 1991; 14:381-8.
7. Nakagiri I, Ichihara K, Ohmoto K, Hirokawa M, Matsuda N. Analysis of discordant test results among five second-generation assays for anti-hepatitis C virus antibodies also tested by polymerase chain reaction-RNA assay and other laboratory and clinical tests for hepatitis. J Clin Microbiol 1993;31:2974-80.
8. Lee SD, Hwang SJ, Tsai YT, Lu RH, Lo KJ. Acute post-transfusion hepatitis C in Taiwan: evaluation of two generations of hepatitis C virus diagnostic enzyme immunoassay. J

Gastroenterol Hepatol 1993;8 (Suppl):S88-91.
9. Weiner AJ, Kuo G, Bradley DW, Bonino F, Saracco G, Lee C, Rosenblatt J, Choo QL, Houghton M. Detection of hepatitis C viral sequences in non-A, non-B hepatitis. Lancet 1990;335:1-3.
10. Zaaijer HL, Cuypers HT, Reesink HW, Winkel IN, Gerken G, Lelie PN. Reliability of polymerase chain reaction for detection of hepatitis C virus. Lancet 1993;341:722-4.
11. Prati D, Capelli C, Bosoni P, Mozzi F, Zanella A, Sirchia G. Determination of hepatitis C virus RNA in the serum by the Amplicor HCV PCR kit. Vox Sang 1994;67:112-4.
12. Lau JY, Davis GL, Kniffen J,Qian KP, Urdea MS, Chan CS, Mizokami M, Neuwald PD, Wilber JC. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet 1993; 341:1501-4.
13. Chan CY, Lee SD, Hwang SJ, Lu RH, Lu CL, Lo KJ. Quantitative branched DNA assay and genotyping for hepatitis C virus RNA in Chinese patients with acute and chronic hepatitis C. J Infect Dis 1995;171:443-6.
14. Lu RH, Hwang SJ, Chan CY, Chang FY, Lee SD. Quantitative measurement of serum HCV RNA in patients with chronic hepatitis C: comparison between Amplicor HCV Monitor System and branched DNA signal amplification assay. J Clin Lab Anal 1998;12:121-5.
15. Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. J Gen Virol 1992;73:673-9.
16. Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C, Brouwer JT, Chan SW, Chayama K, Chen DS, Choo QL, Colombo M, Cuypers HTM, Date T, Dusheiko GM, Esteban JI, Fay O, Hadziyannis SJ, Han J, Hatzakis A, Holmes EC, Hotta H, Houghton M, Irvine B, Kohara M, Kolberg JA, Kuo G, Lau JYN, Lelie PN, Maertens G, McOmish F, Miyamura T, Mizokami M, Nomoto A, Prince AM, Reesink HW, Rice C, Roggendorf M, Schalm SW, Shikata T, Shimotohno K, Stuyver L, Trepo C, Weiner A, Yap PL, Urdea MS. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 1994;19:1321-4.
17. Nousbaum JB, Pol S, Nalpas B, Landais P, Berthelot P, Brechot C. Hepatitis C virus type 1b (II) infection in France and Italy. Collaborative study group. Ann Intern Med 1995;122:161-8.
18. Mansell CJ, Locarnini SA. Epidemiology of hepatitis C in the East. Semin Liver Dis 1995;15:15-32.
19. Lee SD, Chan CY, Wang YJ, Wu JC, Lai KH, Tsai YT, Lo KJ. Seroepidermiology of hepatitis C virus infection in Taiwan. Hepatology 1991;13:830-3.
20. Chen DS, Kuo GC, Sung JL, Lai MY, Sheu JC, Chen PJ, Yang PM, Hsu HM, Chang MH, Chen CJ, Hahn LC, Choo QL, Wang TH, Houghton M. Hepatitis C virus infection in an area hyperendemic for hepatitis B and chronic liver disease: the Taiwan experience. J Infect Dis 1990;162:817-22.
21. Lu SN, Chue PY, Chen IL, Wang JH, Huang JF, Peng CF, Shih CH, Chang WY. Incidence of hepatitis C infection in a hepatitis C endemic township in southern Taiwan. Kaohsiung J Med Sci 1997;13:605-8.
22. Alter MJ, Hadler SC, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Moyer LA, Fields HA, Bradley DW, Margolis HS. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. JAMA 1990;264:2231-5.
23. Chen TZ, Wu JC, Yen FS, Sheng WY, Hwang SJ, Huo TI, Lee SD. Injection with nondisposable needles as an important route for transmission of acute community-acquired hepatitis C virus infection in Taiwan. J Med Virol 1995;46:247-51.
24. Li CP, Hwang SJ, Lu CL, Chan CY, Wu JC, Lee FY, Lee SD. Risk factor analysis of patients with chronic hepatitis C in Taiwan. Chin Med J (Taipei) 1996;58:275-80.
25. Van der Poel CL, Reesink HW, Lelie PN, Leentvaar-Kuypers A, Choo QI, Kuo G, Houghton M. Anti-hepatitis C antibodies and non-A, non-B post-transfusion hepatitis in the Netherlands. Lancet 1989;2:297-8.
26. Donahue JG, Munoz A, Ness PM, Brown DE Jr, Tawn DH, McAllister HA Jr, Reitz BA, Nelson KE. The declining risk of post-transfusion hepatitis C virus infection. N Engl J Med 1992; 327:369-73.
27. Wang YJ, Lee SD, Hwang SJ, Chan CY, Chow MP, Lai ST, Lo KJ. Incidence of post-transfusion hepatitis before and after screening for hepatitis C virus antibody. Vox Sang 1994;67: 187-90.
28. Seeff LB. Natural history of hepatitis C. Hepatology 1997;6 (Suppl 1):S21-8.
29. Hwang SJ, Lee SD, Lu RH, Chu CW, Wu JC, Lai ST, Chang FY. Hepatitis C viral genotype influences the clinical outcome of patients with acute posttransfusion hepatitis C. J Med Virol (in press).
30. Tsai SL, Chen YM, Chen MH, Huang CY, Sheen IS, Yeh CT, Huang JH, Kuo GC, Liaw YF. Hepatitis C virus variants circumventing cytotoxic T lymphocyte activity as a mechanism of chronicity. Gastroenterology 1998;115:954-65.
31. Cerny A, Chisari FV. Pathogenesis of chronic hepatitis C: immunological features of hepatic injury and viral persistence. Hepatology 1999;30:595-601.
32. Farci P, Shimoda A, Coiana A, Diaz G, Peddis G, Melpolder JC, Strazzera A, Chien DY, Munoz SJ, Balestrieri A, Purcell RH, Alter HJ. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science 2000;288:339-44.
33. Di Bisceglie AM, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ. Long-term clinical and histopathological follow-up of chronic post-transfusion hepatitis. Hepatology 1991;14:969-74.
34. Tremolada F, Casarin C, Alberti A, Drago C, Tagger A, Ribero ML, Realdi G. Long-term follow-up of non-A, non-B (type C) post-transfusion hepatitis. J Hepatol 1992;16:273-81.
35. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, Alter HJ. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 1990;12:671-5.
36. Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995; 332:1463-6.
37. Poynard T, Bedossa P, Opolon P. Nature history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997; 349:825-32.
38. Chu CW, Hwang SJ, Luo JC, Wang YJ, Lu RH, Lai CR, Tsay SH, Wu JC, Chang FY, Lee SD. Comparison of clinical, virological and pathological features in patients with acute hepatitis C and acute hepatitis B. J Gastroenterol Hepatol 2001; 16:209-14.
39. Dienstag JL. Non-A, non-B hepatitis. I. Recognition, epidemiology, and clinical features. Gastroenterology 1983;85:

439-62.
40. Merican I, Sherlock S, McIntyre N, Dusheiko GM. Clinical, biochemical and histological features in 102 patients with chronic hepatitis C virus infection. Quart J Med 1993;86:119-25.
41. Healey CJ, Chapman RW, Fleming KA. Liver histology in hepatitis C infection: a comparison between patients with persistently normal or abnormal transaminases. Gut 1995;37: 274-8.
42. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431-5.
43. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991;13:372-4.
44. Scheuer PJ, Ashrafzadeh P, Sherlock S, Brown D, Dusheiko GM. The pathology of hepatitis C. Hepatology 1992;15:567-71.
45. Luo JC, Hwang SJ, Lai CR, Lu CL, Li CP, Tsay SH, Wu JC, Chang FY, Lee SD. Relationships between serum aminotransferase levels, liver histology and virological status in Chinese patients with chronic hepatitis C in Taiwan. J Gastroenterol Hepatol 1998;13:685-90.
46. Luo JC, Hwang SJ, Chang FY, Chu CW, Lu RH, Lai CR, Wang YJ, Lee PC, Tsay SH, Lee SD. Simple blood tests can predict compensated liver cirrhosis in patients with chronic hepatitis C. Hepato-Gastroenterol (in press).
47. Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992;15:572-7.
48. Gerber MA, Krawczynski K, Alter MJ, Sampliner RE, Margolis HS. Histopathology of community acquired chronic hepatitis C. Modern Pathol 1992;5:483-6.
49. Lefkowitch JH, Schiff ER, Davis GL, Perrillo RP, Lindsay K, Bodenheimer HC Jr, Balart LA, Ortego TJ, Payne J, Dienstag JL, Gibas A, Jacobson IM, Tamburro CH, Carey W, Obrien C, Sampliner R, Van Thiel DH, Feit D, Albrecht J, Meschievitz C, Sanghvi B, Vaughan RD. Pathological diagnosis of chronic hepatitis C: a multicenter comparative study with chronic hepatitis B. Gastroenterology 1993;104:595-603.
50. Czaja AJ, Carpenter HA. Sensitivity, specificity, and predictability of biopsy interpretation in chronic hepatitis. Gastroenterology 1993;105:1824-32.
51. Luo JC, Hwang SJ, Lai CR, Lu CL, Li CP, Tsay SH, Wu JC, Chang FY, Lee SD. Clinical significance of portal lymphoid aggregates/follicles in Chinese patients with chronic hepatitis C. Am J Gastroenterol 1999;94:1006-11.
52. Hwang SJ, Luo JC, Chu CW, Lai CR, Tsay SH, Chang FY, Lee SD. Clinical, virological, and pathological significance of hepatic bile duct injuries in Chinese patients with chronic hepatitis C. J Gastroenterol 2001;36:392-8.
53. Hwang SJ, Luo JC, Chu CW, Lai CR, Lu CL, Tsay SH, Wu JC, Chang FY, Lee SD. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlates. J Gastroenterol Hepatol 2001;16:190-5.
54. Angello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. N Eng J Med 1992;327: 1490-5.
55. Pawlotsky JM, Ben Yahia M, Andre C, Voisin MC, Intrator C, Roudot-Thoraval F, Deforges L, Duvoux C, Zafrani ES, Duval J. Immunological disorders in C virus chronic active hepatitis: a prospective case-control study. Hepatology 1994;19:841-8.
56. Lunel F. Hepatitis C virus and autoimmunity: fortuitous association or reality? Gastroenterology 1994;107:1550-5.
57. Strassburg CP, Manns MP. Autoimmune hepatitis versus viral hepatitis C. Liver 1995;15:225-32.
58. Gumber SC, Chopra S. Hepatitis C: a multifaceted disease. Review of extrahepatic manifestations. Ann Intern Med 1995; 123:615-20.
59. Hwang SJ, Lee SD, Li CP, Lu RH, Chan CY, Wu JC. Clinical study of cryoglobulinemia in Chinese patients with chronic hepatitis C. J Gastroenterol Hepatol 1997;12:513-7.
60. Luo JC, Hwang SJ, Li CP, Lu RH, Chan CY, Wu JC, Chang FY, Lee SD. Clinical significance of serum autoantibodies in Chinese patients with chronic hepatitis C: negative role of serum viral titier and genotype. J Gastroenterol Hepatol 1998;13:475-9.
61. Congia M, Clemente MG, Dessi C, Cucca F, Mazzoleni AP, Frau F, Lampis R, Cao A, Lai ME, De Virgiliis S. HLA Class II genes in chronic hepatitis C virus-infection and associated immunological disorders. Hepatology 1996;24:1338-41.
62. Lenzi M, Frisoni M, Mantovani V, Ricci P, Muratori L, Francesconi R, Cucca M, Ferri S, Bianchi FB. Haplotype HLA-B8-DR3 confers susceptibility to hepatitis C-related mixed cryoglobulinemia. Blood 1998;91:2062-6.
63. Thio CL, Thomas DL, Carrington M. Chronic viral hepatitis and the human genome. Hepatology 2000;31:819-26.
64. Hwang SJ, Lee SD, Chan CY, Lu RH, Lo KJ. A randomized controlled trial of recombinant interferon α-2b in the treatment of Chinese patients with acute post-transfusion hepatitis C. J Hepatol 1994;21:831-6.
65. Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, Zarski JP. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effect of dose and duration. Hepatology 1996;24:778-89.
66. Choo QL, Kuo G, Ralston R, Weiner A, Chien D, Nest GV, Han J, Berger K, Thudium K, Kuo C, Kansopon J, McFarland J, Tabrizi A, Ching K, Moss B, Cummins LB. Houghton M, Muchmore E. Vaccination of chimpanzees against infection by the hepatitis C virus. Proc Natl Acad Sci USA 1994;91:1294-8.
67. Hwang SJ, Chan CY, Lu RH, Wu JC, Lee SD. Randomized controlled trial of recombinant interferon α-2b in the treatment of Chinese patients with chronic hepatitis C. J Interferon Cytokine Res 1995;15:611-6.
68. Carithers RL Jr, Emerson SS. Therapy of hepatitis C:meta-analysis of interferon α-2b trials. Hepatology 1997;26 (Suppl 1):S83-8.
69. Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, Zarski JP. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. Hepatology 1996;24:778-89.
70. National Institutes of Health Consensus Development Conference Panel Statement: management of hepatitis C. Hepatology 1997;26 (Suppl 1):S2-10.
71. Dusheiko G. Side effects of alpha interferon in chronic hepatitis C. Hepatology 1997;26(Suppl 1):S112-21.
72. Davis GL, Lau JY. Factors predictive of a beneficial response to therapy of hepatitis C. Hepatology 1997;26(Suppl 1):S122-7.
73. Tong MJ, Reddy KR, Lee WM, Pockros PJ, Hoefs JC, Keeffe EB, Hollinger FB, Hathcote EJ, White H, Foust RT, Jensen DM, Krawitt EL, Fromm H, Black M, Blatt LM, Klein M,

Lubina J, and the Consensus Interferon Study Groups. Treatment of chronic hepatitis C with consensus interferon: a multicenter, randomized, controlled trial. Hepatology 1997;26: 747-54.
74. Hwang SJ, Lee SD, Chan CY, Lu RH, Chang FY. A randomized, double-blind, controlled trial of consensus interferon in the treatment of Chinese patients with chronic hepatitis C. Am J Gastroenterol 1999;94:2496-500.
75. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A, Hoffman J, Brunda MJ. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000;343:1666-72.
76. Heathcote EJ, Shiffman ML, Cooksley WGE, Dusheiko GM, Lee SS, Balart L, Reindollar R, Reddy RK, Wright TL, Lin A, Hoffman J, De Pamphilis J. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000; 343:1673-80.
77. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Otani S. Randomized trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051-5.
78. Mazzella G, Accogli E, Sottili S, Festi D, Orsini M, Salzetta A, Novelli V, Cipolla A, Fabbri C, Pezzoli A, Roda E. Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. J Hepatol 1996;24:141-7.
79. Lu CL, Chan CY, Hwang SJ, Lu RH, Lee SD. The efficacy of ursodeoxycholic acid in the treatment of patients with chronic hepatitis C. J Gastroenterol Hepatol 1995;10:432-7.
80. Tong MJ, Hwang SJ, Lefkowitz M, Lee SD, Co RL, Cornad A, Schmid P, Lo KJ. Correlation of serum HCV RNA and alanine aminotransferase levels in chronic hepatitis C patients during treatment with ribavirin. J Gastroenterol Hepatol 1994:9:587-91.
81. Chemello L, Cavaletto L, Bernardinello E, Guido M, Pontisso P, Alberti A. The effect of interferon alfa and ribavirin combination therapy in naive patients with chronic hepatitis C. J Hepatol 1995;23(Suppl 2):S8-12.
82. Lai MY, Kao JH, Yang PM, Wang JT, Chen PJ, Chan KW, Chu JS, Chen DS. Long-term efficacy of ribavirin plus interferon alfa in the treatment of chronic hepatitis C. Gastroenterology 1996;111:1307-11.
83. Reichard L, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomized, double-blind, placebo-controlled trial of interferon alfa-2b with and without rabavirin for chronic hepatitis C. Lancet 1998;351:83-7.
84. Liang TJ, Rehermann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment and prevention of hepatitis C. Ann Intern Med 2000;132:296-305.