Table 2. Multivariate logistic regression analysis of the factors associated with achievement of 80% adherence

Analysis Variable	Univariate RR (95% CI)	*P*	Multivariate RR (95% CI)	*P*
Sex (male vs. female)	2.52 (1.63–3.89)	< 0.001	2.01 (1.07–3.79)	0.030
Age (< 55 vs. ≥ 55 years)	2.24 (1.46–3.42)	< 0.001	2.38 (1.49–3.80)	< 0.001
Weight (≥ 60 vs. < 60 kg)	2.00 (1.31–3.05)	0.001	1.09 (0.63–1.89)	0.757
BMI (kg/m^2)	1.11 (0.73–1.69)	0.640		
Genotype (2 vs. 1)	1.52 (0.96–2.41)	0.072	1.84 (1.10–3.09)	0.021
HCV RNA (< 100 vs. ≥ 100 kIU/ml)	0.49 (0.18–1.34)	0.164		
White blood cell count (≥ 5000 vs. < 5000/ml)	1.16 (0.77–1.75)	0.485		
Haemoglobin (≥ 14 vs. < 14 g/dl)	2.34 (1.52–3.62)	< 0.001	1.50 (0.85–2.64)	0.161
Platelet count (≥ 15 vs. < 15 × 10^4/ml)	0.86 (0.57–1.29)	0.460		
IFN/weight (< 0.13 vs. ≥ 0.13 MU/kg)	2.24 (1.47–3.41)	< 0.001	2.42 (1.52–3.85)	< 0.001
Ribavirin/weight (< 11 vs. ≥ 11 mg/kg)	1.12 (0.75–1.81)	0.496		
Treatment experience (retreatment vs. naïve)	1.85 (1.20–2.83)	0.005	1.86 (1.15–3.01)	0.012
Treatment centre (≥ 15 cases/year centre vs. < 15 cases/year centre)	1.59 (1.05–2.41)	0.030	1.65 (1.04–2.64)	0.035
Physician's experience (≥ 19 vs. < 19 years)	1.57 (1.03–2.41)	0.038	1.54 (0.96–2.48)	0.074

BMI, body mass index; CI, confidence interval; HCV, hepatitis C virus; IFN, interferon; MU, million units; RR, relative risk.

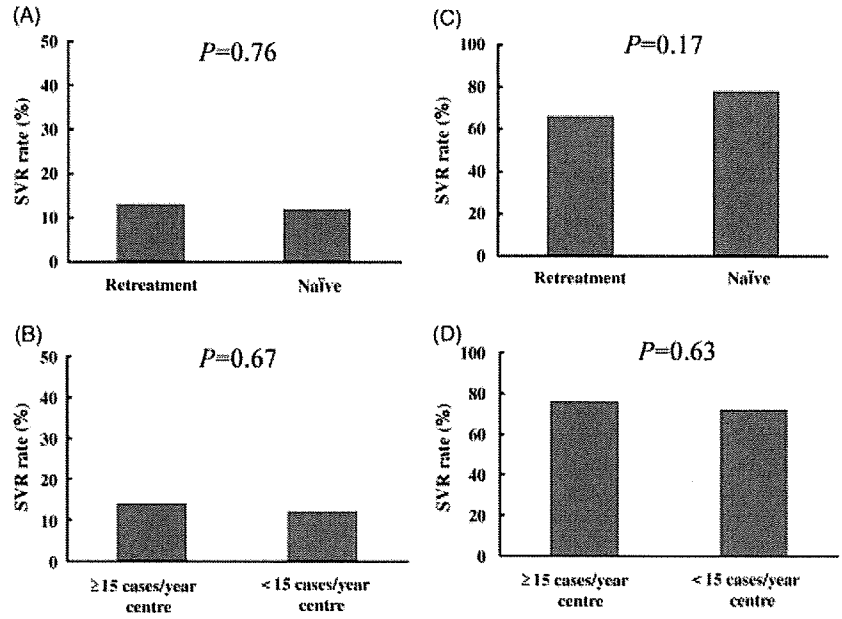

Fig. 4. Comparison of the sustained virological response (SVR) rate according to the hepatitis C virus status and patient groups. The SVR rate in the retreatment and the naïve groups (a) and in the ≥ 15 cases/year centre group and the < 15 cases/year centre group (b) among genotype 1 and high viral load (≥ 100 kIU/ml) and in the retreatment and the naïve groups (c) and in the ≥ 15 cases/year centre group and the < 15 cases/year centre group (d) among other than genotype 1 and high viral load were compared. There was no significant difference of the SVR rate between the retreatment group and the naïve group not only in genotype 1 and high viral load but also in others. There was also no significant difference of the SVR rate between the ≥ 15 cases/year centre group and the < 15 cases/year centre group.

All the SVR rates in the present study were low because IFN therapy was limited to a period of up to 24 weeks, irrespective of genotype, as described previously. The SVR rates in the retreatment and the ≥ 15 cases/year centre groups were expected to be excellent in this study because adherence to therapy was better in these groups than those in the naïve and the < 15 cases/year centre groups respectively. However, this was not the case. In the genotype 1 and high viral load group, the SVR rate of the retreatment group was almost equal to that of the naïve group. Furthermore, the SVR rate of the retreatment group tended to be lower than that of the naïve group (Fig. 4). It has been demonstrated that patients with retreatment generally respond poorly to IFN therapy and/or are difficult to treat (2, 15, 24, 25) and, thus, these patients should have backgrounds that cannot be estimated simply by parameters of clinical characteristics at baseline. Thus, the SVR rate in the retreatment group should have been essentially lower than that in the naïve group. Similarly, the SVR rate in the ≥ 15 cases/year

Table 3. Multivariate logistic regression analysis of the factors associated with a sustained virological response

Analysis Variable	Univariate RR (95% CI)	*P*	Multivariate RR (95% CI)	*P*
Sex (male vs. female)	1.52 (0.96–2.40)	0.076	1.89 (0.98–3.63)	0.057
Age (< 55 vs. ≥55 years)	1.37 (0.89–2.12)	0.158		
Weight (≥60 vs. < 60 kg)	1.10 (0.71–1.71)	0.666		
BMI (< 24 vs. ≥24 kg/m^2)	1.05 (0.68–1.63)	0.826		
Genotype (2 vs. 1)	17.49 (9.97–30.69)	< 0.001	24.23 (12.73–46.10)	< 0.001
HCV RNA (< 100 vs. ≥100 kIU/ml)	10.01 (2.82–35.56)	< 0.001	27.79 (6.92–111.56)	< 0.001
White blood cell count (≥5000 vs. < 5000/ml)	1.11 (0.72–1.72)	0.627		
Haemoglobin (≥14 vs. < 14 g/dl)	1.27 (0.80–1.99)	0.309		
Platelet count (≥15 vs. < 15 × 104/ml)	1.34 (0.87–2.07)	0.189		
IFN/weight (< 0.13 vs. ≥0.13 MU/kg)	1.40 (0.91–2.17)	0.127		
Ribavirin/weight (< 11 vs. ≥11 mg/kg)	1.42 (0.90–2.23)	0.135		
Treatment experience (naïve vs. retreatment)	1.65 (1.05–2.61)	0.032	1.40 (0.75–2.60)	0.295
Treatment centre (≥15 cases/year centre vs. < 15 cases/year centre)	1.09 (0.70–1.68)	0.712		
Physician's experience (≥19 vs. < 19 years)	1.33 (0.85–2.07)	0.210		
80% adherence (achievement vs. nonachievement)	2.29 (1.46–3.50)	0.003	2.87 (1.53–5.39)	0.001

BMI, body mass index; CI, confidence interval; HCV, hepatitis C virus; IFN, interferon; MU, million units; RR, relative risk.

centre was not significantly different from that in the < 15 cases/year centre. The patient's mean age was almost 2 years higher and the creatinine clearance was significantly lower in the ≥15 cases/year centre than in the < 15 cases/year centre. Thus, the patients in the ≥15 cases/year centre were supposed to be more difficult to treat than those in the < 15 cases/year centre and should otherwise have shown lower response rates.

The present study was not a controlled trial but a prospective study of patients treated in clinical practice at multiple institutions. Thus, patients in this study had a heterogeneous background. In controlled trials, patients are strictly selected so that compliance could be maximized. Thus, adverse events may be low in frequency. Unfortunately, the strict selection of patients as a treatment candidate is not applicable to clinical practice (2, 26–28). Also, patients are not always permitted to choose the treatment centre. Therefore, the physician's skill in selecting patients and managing adverse events as well as the patient's motivation may have an impact on the adherence to the combination therapy. In the present study, the virological response (nonresponse or relapse) to the first course in the retreated patients and the fibrosis stage, which are the major determinants of SVR, were unfortunately not available in all the patients. This information may further clarify the importance of adherence and patients' motivation. The present study suggested the importance of patients' motivation, although this had not been directly measured. Patient's motivational stage could be assessed. This could help the physician in giving appropriate advice, using motivational interviewing techniques, to improve treatment adherence. Finally, the combination therapy used in this study was based on standard IFN but not the pegylated one. Further studies with a larger number of patients treated with a combination therapy with standard IFN or pegylated IFN plus ribavirin are warranted to confirm and extend our findings.

In conclusion, the present data suggested that patient's motivation and physician's treatment experience, together with patient age, gender and dose of IFN per weight, were important for better adherence to combination therapy in patients with chronic hepatitis C. Therefore, selection of patients, who had previous treatment and were still motivated, and treatment by experienced physicians may be important for better treatment results.

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References

1. Hepatitis C–global prevalence. *Wkly Epidemiol Rec* 1999; **74**: 425–7.
2. Strader DB, Wright T, Thomas DL, *et al.* Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147–71.
3. McHutchison JG, Gordon SC, Schiff ER, *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339**: 1485–92.
4. Davis GL, Esteban-Mur R, Rustgi V, *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339**: 1493–9.
5. Poynard T, Marcellin P, Lee SS, *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352**: 1426–32.

6. National Institutes of Health Consensus Development Conference Statement: management of hepatitis C 2002 (June 10–12, 2002). *Gastroenterology* 2002; **123**: 2082–99.
7. Liang TJ, Rehermann B, Seeff LB, *et al.* Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med* 2000; **132**: 296–305.
8. Cummings KJ, Lee SM, West ES, *et al.* Interferon and ribavirin vs interferon alone in the re-treatment of chronic hepatitis C previously nonresponsive to interferon: a meta-analysis of randomized trials. *JAMA* 2001; **285**: 193–9.
9. McHutchison JG, Manns M, Patel K, *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061–9.
10. Iwasaki Y, Ikeda H, Araki Y, *et al.* Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; **43**: 54–63.
11. Tanaka Y, Hanada K, Mizokami M, *et al.* Inaugural article: a comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. *Proc Natl Acad Sci USA* 2002; **99**: 15584–9.
12. Okamoto H, Sugiyama Y, Okada S, *et al.* 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**(Part 3): 673–9.
13. Tanaka T, Tsukiyama-Kohara K, Yamaguchi K, *et al.* Significance of specific antibody assay for genotyping of hepatitis C virus. *Hepatology* 1994; **19**: 1347–53.
14. Shiratori Y, Kato N, Yokosuka O, *et al.* Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Tokyo – Chiba Hepatitis Research Group. *Gastroenterology* 1997; **113**: 558–66.
15. Shiffman ML, Di Bisceglie AM, Lindsay KL, *et al.* Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004; **126**: 1015–23.
16. Schalm SW, Weiland O, Hansen BE, *et al.* Interferon–ribavirin for chronic hepatitis C with and without cirrhosis: analysis of individual patient data of six controlled trials. Eurohep Study Group for viral hepatitis. *Gastroenterology* 1999; **117**: 408–13.
17. Davis GL, Wong JB, McHutchison JG, *et al.* Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 645–52.
18. Raptopoulou M, Tsantoulas D, Vafiadi I, *et al.* The effect of adherence to therapy on sustained response in daily or three times a week interferon alpha-2b plus ribavirin treatment of naive and nonresponder chronic hepatitis C patients. *J Viral Hepat* 2005; **12**: 91–5.
19. McHutchison JG, Ware JE Jr, Bayliss MS, *et al.* The effects of interferon alpha-2b in combination with ribavirin on health related quality of life and work productivity. *J Hepatol* 2001; **34**: 140–7.
20. Hassanein T, Cooksley G, Sulkowski M, *et al.* The impact of peginterferon alfa-2a plus ribavirin combination therapy on health-related quality of life in chronic hepatitis C. *J Hepatol* 2004; **40**: 675–81.
21. Ware JE Jr, Bayliss MS, Mannocchia M, *et al.* Health-related quality of life in chronic hepatitis C: impact of disease and treatment response. The Interventional Therapy Group. *Hepatology* 1999; **30**: 550–5.
22. Perrillo R, Rothstein KD, Rubin R, *et al.* Comparison of quality of life, work productivity and medical resource utilization of peginterferon alpha 2a vs the combination of interferon alpha 2b plus ribavirin as initial treatment in patients with chronic hepatitis C. *J Viral Hepat* 2004; **11**: 157–65.
23. Castera L, Constant A, Henry C, *et al.* Impact on adherence and sustained virological response of psychiatric side effects during peginterferon and ribavirin therapy for chronic hepatitis C. *Aliment Pharmacol Ther* 2006; **24**: 1223–30.
24. Cheng SJ, Bonis PA, Lau J, *et al.* Interferon and ribavirin for patients with chronic hepatitis C who did not respond to previous interferon therapy: a meta-analysis of controlled and uncontrolled trials. *Hepatology* 2001; **33**: 231–40.
25. Shiffman ML. Retreatment of patients with chronic hepatitis C. *Hepatology* 2002; **36**: S128–34.
26. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003; **124**: 1711–9.
27. Strader DB. Understudied populations with hepatitis C. *Hepatology* 2002; **36**: S226–36.
28. Gaeta GB, Precone DF, Felaco FM, *et al.* Premature discontinuation of interferon plus ribavirin for adverse effects: a multicentre survey in 'real world' patients with chronic hepatitis C. *Aliment Pharmacol Ther* 2002; **16**: 1633–9.

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Original Article

Clinical features of antinuclear antibodies-negative type 1 autoimmune hepatitis

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Aim: Antinuclear antibodies (ANA) are the main serologic markers of type 1 autoimmune hepatitis (AIH); however 20–30% of patients are negative for ANA. We assessed the clinical features of ANA-negative patients.

Methods: A retrospective analysis was performed of 176 patients with type 1 AIH (153 females, median age 55 years). A diagnosis of AIH was made based on the revised scoring system proposed by the International Autoimmune Hepatitis Group. ANA titers were measured using a standard indirect immunofluorescence technique.

Results: Thirty-eight patients (22%) had low titers of ANA (1:40 or 1:80), and 114 (65%) had high titers (≥ 1:160). Of 24 ANA-negative patients, 15 were positive for smooth muscle antibodies (SMA). Three of nine both ANA- and SMA-negative patients developed ANA during follow-up. The other six were diagnosed based on histological characteristics. Thirteen ANA-negative patients relapsed after the normalization of serum alanine aminotransferase (ALT) levels. ANA-negative patients more frequently showed acute presentation and, at presentation, had lower serum immunoglobulin G levels, higher serum levels of bilirubin and transaminase, and higher frequencies of histological acute hepatitis and zone 3 necrosis than those with high titers. However, the frequency of advanced stage of fibrosis was similar. The response to corticosteroids was not different among the three groups.

Conclusions: ANA-negative type 1 AIH shows acute-onset more frequently but may include not only acute autoimmune hepatitis, but also acute exacerbation of inactive chronic disease. Regarding the diagnosis of ANA-negative AIH, the determination of ANA during follow-up and the response to immunosuppressive treatment may be helpful.

Key words: acute hepatitis, antinuclear antibody, autoimmune hepatitis, immunoglobulin G

INTRODUCTION

AUTOIMMUNE HEPATITIS (AIH), first reported by Waldenström in 1950,[1] is a chronic and progressive disease of unknown cause which is characterized by histological interface hepatitis, hypergammaglobulinemia and circulating autoantibodies.[2] Antinuclear antibodies (ANA) and smooth muscle antibodies (SMA) are the main serologic markers of type 1 AIH.

The diagnosis of AIH is based on characteristic clinical, biochemical and histological findings. It is important to distinguish the disease from other forms of chronic hepatitis. Recently, the revised scoring system proposed by the International Autoimmune Hepatitis Group (IAIHG) has been applied as a useful diagnostic tool in clinical practice.[3] The sensitivity and specificity of this scoring system are more than 90%, and the diagnosis of AIH based on this system is reliable.[4–7]

However, type 1 AIH diagnosed based on this scoring system includes unusual cases that do not show typical features. Some such cases involve patients found to be negative for autoantibodies. However, ANA is negative in 20–30% of patients with type 1 AIH,[8,9] so ANA-negative patients are not rare.

A few reports have indicated the relations between ANA and clinical features in patients with type 1 AIH. Czaja *et al.*[10] reported that patients with serum ANA titers of 1:160 or higher at presentation have higher serum levels of immunoglobulin G (IgG) than those

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with ANA titers of 1:40–1:80, although these patients are similar in age, gender, serum transaminase levels and histological activity. Furthermore, ANA-positive patients have human leukocyte antigen (HLA) DR4 more frequently and have a lower frequency of liver transplantation than ANA-negative patients, while ANA patterns of indirect immunofluorescence (IIF) have no practical clinical implications.[11] However, the clinical features of ANA-negative AIH have yet to be fully elucidated. The aim of this study is to assess the clinical features of ANA-negative AIH.

METHODS

A RETROSPECTIVE ANALYSIS was performed on 176 patients with type 1 AIH (153 females, 23 males, median age 55 years) admitted to the Okayama University Hospital or six affiliated hospitals between March 1989 and April 2008. All patients were seronegative for hepatitis B surface antigen, anti-hepatitis C virus antibody, hepatitis C virus RNA (as determined via polymerase chain reaction after reverse transcription), and anti-mitochondrial antibody and underwent liver biopsy. A diagnosis of AIH was made according to the revised scoring system proposed by IAIHG.[3] A definite diagnosis of AIH based on this revised scoring system required a pretreatment score exceeding 15, while a probable diagnosis required a score of between 10 and 15. Patients with an overlapping syndrome or a coexistent disease (for example, primary biliary cirrhosis, primary sclerosing cholangitis, non-alcoholic fatty liver disease or alcohol-induced liver injury) were excluded from this analysis.

An acute presentation was defined by the presence of acute onset of symptoms (for example, jaundice and/or fatigue and/or anorexia) in conjunction with bilirubin ≥ 5 mg/dL and/or serum alanine aminotransferase (ALT) levels higher than tenfold the upper normal limit.

Liver biopsy was performed with a Vim–Silverman needle (14-G) under laparoscopy, or with a 17-G needle under ultrasonography guidance, before or just after the treatment was commenced. Liver biopsy specimens were evaluated by two pathologists and diagnosed as acute or chronic hepatitis. A diagnosis of acute hepatitis was made on the basis of the presence of histologically predominant zone 3 necrosis with minimal lymphocytic and plasma cell infiltration into portal tracts, in the absence of interface hepatitis or portal fibrosis. Liver biopsy specimens diagnosed as chronic hepatitis underwent histological staging based on the classification of Desmet *et al.*[12]

The standard initial treatment was prednisolone monotherapy (30–40 mg/day) or a combination of prednisolone (20–40 mg/day) and azathioprine (50–100 mg/day). In patients with histological low-grade inflammatory activity, the initial treatment was low-dose prednisolone (20 mg/day). Elderly patients with histological low-grade inflammatory activity and comorbidities such as osteoporosis and/or diabetes were treated with ursodeoxycholic acid (300–600 mg/day) or a combination of lower doses of prednisolone (< 20 mg/day) and ursodeoxycholic acid. An initial treatment was defined as any therapy that was started within 3 months after the diagnosis of AIH. The treatment was continued until the normalization of serum ALT levels.

Patients were divided into three groups according to serum ANA titers at presentation. The ANA titers were measured using a standard IIF technique with HEp-2 cells. The ANA titers were < 1:40 in group A, 1:40 or 1:80 in group B and ≥ 1:160 in group C. SMA was assayed by the IIF technique using rat kidney and stomach cells. A serum titer of 1:40 or greater was positive for ANA or SMA. Antibodies to liver/kidney microsome type 1 (anti-LKM-1) were measured using an enzyme-linked immunosorbent assay using recombinant cytochrome P4502D6 as the antigen, and a serum value of 50.0 index or greater was positive.

To compare the clinicopathological characteristics at presentation among the three groups, we analyzed gender, age and pretreatment score based on the revised scoring system, frequency of acute presentation, concurrent autoimmune disease, laboratory data (bilirubin, aspartate aminotransferase [AST], ALT, alkaline phosphatase [ALP], albumin, IgG, SMA titer, HLA DR4) and histological features (staging of fibrosis, rosetting of liver cells, zone 3 necrosis).

Statistics

Statistical analysis was performed using the SPSS statistical program (release 11.0.1 J; SPSS, Chicago, IL, USA).

Continuous variables were expressed as medians and ranges. The Mann–Whitney *U*-test was used to evaluate differences in the continuous variables between two groups, and the Kruskal–Wallis *U*-test was used among three groups. Dichotomous variables were compared by the χ^2-test. Correlations between two variables were calculated using Pearson correlation tests. Cumulative incidental rates were estimated using the log–rank test. *P*-values of less than 0.05 were considered significant.

RESULTS

Clinical features of 176 patients with type 1 AIH

BASED ON THE revised scoring system proposed by IAIHG,[3] the median pretreatment score of 176 patients was 18 (10–23). One hundred and thirty-six patients (77%) had definite AIH and 40 (23%) had probable AIH. Fifty-three patients (30%) showed acute presentation. Forty-two patients (26%) had concurrent autoimmune diseases: 18 had autoimmune thyroiditis, four had Sjögren's syndrome, three each had systemic lupus erythematosus, Graves' disease and ulcerative colitis, two each had autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, progressive systemic sclerosis and rheumatoid arthritis, one each had both autoimmune thyroiditis and autoimmune hemolytic anemia, both systemic lupus erythematosus and Sjögren's syndrome, and both autoimmune thyroiditis and Sjögren's syndrome.

Twenty-four patients (13%) were negative for ANA (< 1:40) (group A). Thirty-eight patients (22%) were positive for ANA titers of 1:40 or 1:80 (group B) and 114 (65%) for ANA titers of ≥ 1:160 (group C). Seventy-seven of 122 patients (63%) who were screened for SMA were positive (≥ 1:40). A correlation was shown between serum ANA titers and serum IgG levels (correlation coefficient = 0.17, $P = 0.03$).

Sixty of 87 patients (69%) screened for HLA DR status had DR4. Of 27 patients negative for DR4, nine had DR2, nine had DR9, eight had DR8 and five had DR15.

Histological acute hepatitis and cirrhosis were shown in 10 patients (6%) and 18 patients (10%), respectively. Rosetting of liver cells and zone 3 necrosis were shown in 49 patients (28%) and 53 patients (30%), respectively. Patients with acute presentation showed zone 3 necrosis more frequently than the others (53% versus 20%, $P < 0.0001$). In the 53 patients with acute presentation, zone 3 necrosis was more frequent in the 10 patients with acute hepatitis than in others (100% versus 42%; $P = 0.0009$). However, there was no difference in the frequency of rosetting of liver cells between those with acute presentation and others (36% versus 24%, $P = 0.12$).

As an initial medical treatment, 114 patients (65%) were treated with prednisolone (> 20 mg/day), 21 (12%) with low-dose prednisolone (20 mg/day) and 41 (23%) with ursodeoxycholic acid (300–600 mg/day) or a combination of lower doses of prednisolone (< 20 mg/day) and ursodeoxycholic acid. Of 135 patients treated with prednisolone ≥ 20 mg/day, 15 (11%) were transferred to other hospitals without follow-up. Of the remaining 120 patients (18 in groups A, 26 in group B, 76 in group C), 107 (89%) achieved the normalization of serum ALT levels within 6 months after the introduction of corticosteroid treatment.

ANA-negative AIH

Of 24 ANA-negative patients, 15 were positive for SMA. Three of nine SMA-negative patients developed ANA during follow-up. The other six who were negative for anti-LKM-1 were diagnosed as AIH based on histological features (interface hepatitis and portal plasma cells infiltration) and clinical course (relapse after the normalization of serum ALT levels). In terms of the histological staging of the six patients, one was classified as acute hepatitis and relapsed after the normalization of serum ALT levels. Of the remaining five patients with histological chronic hepatitis, one each was classified as F1, F3 and F4, and two as F2.

Of the 18 patients treated with prednisolone ≥ 20 mg/day as an initial medical treatment, 16 achieved normalization of serum ALT levels within 6 months after the introduction of the initial treatment, and the two who developed ANA during follow-up had a higher cumulative incidental rate of the normalization of serum ALT levels within 6 months after the introduction of the initial treatment than the remaining 16 patients (100% versus 87%; log–rank test. $P = 0.01$). However, 13 patients, of whom five were positive for neither ANA nor SMA, relapsed after the normalization of serum ALT levels.

Comparison of clinical features according to serum ANA titers

Pretreatment scores according to the revised scoring system[3] were higher in group C than in group A and group B, and definite diagnosis was more frequent in group C and group B than in group A. Acute presentation was more frequently shown in group A and group B than in group C. There were no differences in gender, age, frequency of concurrent autoimmune diseases and HLA DR status among the three groups (Table 1).

Serum bilirubin levels were higher in group A than in group C. Serum levels of AST and ALT were higher in group A and group B than in group C. Serum IgG levels were higher in group C than in group A and group B (Table 1).

Histological acute hepatitis and zone 3 necrosis were more frequently shown in group A than in group C (Table 2). Advanced stage of fibrosis (F3 + F4) was similarly shown among the three groups ($P = 0.43$).

Table 1 Clinical characteristics of three groups with type 1 autoimmune hepatitis

	Group A ($n = 24$)	Group B ($n = 38$)	Group C ($n = 114$)	P
Gender (female), n (%)	22 (92)	33 (87)	99 (87)	0.44
Age (year)	50 (16–77)	52 (18–78)	58 (16–79)	0.20
International diagnostic criteria for the diagnosis of autoimmune hepatitis				
Pre-treatment score	15 (11–21)	17 (10–21)	18 (10–23)	0.0007
Definite diagnosis, n (%)	11 (46)	29 (76)	96 (84)	0.0002
Acute presentation, n (%)	11 (46)	16 (42)	26 (23)	0.01
Concurrent autoimmune disease, n (%)	5 (21)	7 (18)	30 (26)	0.57
Bilirubin, mg/dL	1.4 (0.4–24.3)	1.1 (0.3–25.8)	0.9 (0.3–29.2)	0.05
AST, IU/L	416 (40–1690)	233 (33–1716)	139 (28–2330)	0.04
ALT, IU/L	424 (23–2162)	348 (52–2132)	161 (25–1820)	0.007
ALP, x ULN	1.0 (0.5–2.3)	1.0 (0.2–3.3)	1.1 (0.4–5.1)	0.73
Albumin, g/dL	3.8 (2.3–4.7)	4.0 (2.7–5.1)	3.8 (2.1–4.8)	0.16
IgG, mg/dL	2163 (724–3602)	2394 (1170–4200)	2625 (1085–6562)	0.009
SMA, n (%)				
≥ 1:40	15/21 (71)	19/29 (66)	43/72 (60)	0.59
≥ 1:160	6/21 (29)	8/29 (28)	24/72 (33)	0.82
HLA DR4, n (%)	9/14 (64)	17/20 (85)	34/53 (64)	0.21
Corticosteroid treatment (prednisolone ≥ 20 mg/day)				
Normalization of serum ALT levels within 6 months, n (%)	16/18 (89)	24/26 (92)	67/76 (88)	0.69
Relapse after the normalization of serum ALT levels, n (%)	11/18 (61)	15/26 (58)	34/76 (45)	0.31

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HLA, human leukocyte antigen; SMA, smooth muscle antibodies; ULN, upper limit of normal.

The cumulative incidental rate of the normalization of serum ALT levels within 6 months after the introduction of corticosteroid treatment was 89% in group A, 92% in group B and 88% in group C (log-rank test, $P = 0.69$). However, the relapse rate was 61% in group A, 58% in group B and 45% in group C, respectively ($P = 0.31$).

Table 2 Histological findings of three groups with type 1 autoimmune hepatitis

	Group A ($n = 24$)	Group B ($n = 38$)	Group C ($n = 114$)	P
Staging, n (%)				
Acute hepatitis	4 (17)	3 (8)	3 (3)	0.02
Chronic hepatitis				
F1	4 (17)	13 (34)	34 (30)	
F2	7 (29)	12 (32)	34 (30)	
F3	5 (21)	9 (24)	30 (26)	
F4	4 (17)	1 (3)	13 (11)	
Rosetting of liver cells, n (%)	6 (25)	11 (29)	32 (28)	0.94
Zone 3 necrosis, n (%)	11 (46)	14 (37)	27 (24)	0.05

DISCUSSION

IN THIS STUDY, ANA-negative patients more frequently showed acute presentation, histological acute hepatitis and zone 3 necrosis than those with high titers of ANA (≥ 1:160). Also, as described in the previous report,[13] zone 3 necrosis was more frequently shown in ANA-negative patients than in ANA-positive patients (57% versus 12%, $P < 0.0001$). Zone 3 necrosis was associated with early-stage fibrosis and 20% of patients with zone 3 necrosis showed histological acute hepatitis.[13] However, in this study, 38% of ANA-negative patients showed advanced stage of fibrosis (F3 + F4). Czaja *et al.*[10] reported that 68% of ANA-positive patients lost their antibody during corticosteroid treatment, and the loss of ANA was associated with improvements in hypergammaglobulinemia and histological necroinflammatory activity. They also reported that some patients who lost their ANA had recurrent positivity for

ANA during relapse. Thus, we consider that ANA-negative type 1 AIH may include not only acute AIH, but also acute exacerbation of inactive chronic disease.

In this study, ANA-negative patients had lower serum IgG levels at presentation than those with high titers of ANA. In the report by Abe *et al.*,[14] serum IgG levels increased as histological staging advanced. Furthermore, Montano-Loza *et al.*[15] reported that serum IgG levels had predictive value for relapse after drug withdrawal. In contrast, in some patients, ANA commonly disappear and reappear in association with their clinical events.[10] Thus, serum IgG and ANA may be associated with the pathogenesis of AIH. Although the pathogenic mechanisms of AIH have been uncertain, both cell-mediated and antibody-dependent pathways are reported to be involved.[16] In patients with ANA negativity and low serum IgG levels, a cell-mediated pathway may be predominant.

Czaja *et al.*[10] reported that ANA was detected in 60% of initially ANA-negative patients during follow-up. In this study, three ANA-negative patients developed ANA during follow-up. Of the three patients, one showed histological acute hepatitis in the absence of interface hepatitis, and the diagnosis of AIH could not be made at presentation. She was treated with 40 mg/day of prednisolone and achieved the normalization of serum ALT levels. She developed ANA 37.6 months after the introduction of the treatment. Of the other two patients, the one with histological staging F2 showed acute presentation, while the other with histological staging F4 did not. The former, who was treated with 30 mg/day of prednisolone and achieved the normalization of serum ALT levels, developed ANA 5.1 months after the introduction of the treatment. The latter, who was followed up without any treatment, developed ANA 12.7 months after the diagnosis. Thus, the determination of ANA during follow-up was useful for the diagnosis of AIH. However, in this study, five patients with neither ANA nor SMA relapsed after the normalization of serum ALT levels as a result of the initial treatment. In the IAIHG report,[3] the response to immunosuppressive treatment, especially relapse after an initial response, is affirmed to be a characteristic of AIH. The determination of ANA during follow-up and the response to immunosuppressive treatment may be helpful to extend the diagnosis of AIH in ANA-negative patients.

The diagnosis of ANA-negative AIH should be made carefully. The cause of AIH has not been fully elucidated, and the diagnosis of AIH importantly requires the exclusion of other conditions resembling AIH. Viral infections, alcohol-induced liver injury, primary biliary cirrhosis, primary sclerosing cholangitis and non-alcoholic fatty liver disease can be diagnosed almost solely using laboratory and histological examinations. In contrast, the exclusion of drug-induced liver injury based on laboratory and histological examinations may be difficult. Furthermore, several drugs (infliximab, minocycline, atorvastatin, hepatitis A vaccine) have been reported to be triggers for AIH.[17–20] It is affirmed that the recent use of known hepatotoxic drugs should not be excluded.[3] Thus, in order to exclude drug-induced liver injury, consideration of the relationship between the use of drugs and the clinical course is important.

AIH is usually classified into type 1 and type 2.[2] Type 2 patients are positive for anti-LKM-1 and are almost always young females with severe disease. In Japan, the frequency of type 2 is extremely low.[21] In this study, the six patients who were negative for both ANA and SMA during clinical course were also negative for anti-LKM-1. Furthermore, all of them were more than 40 years old at presentation and readily achieved the normalization of serum ALT levels after the introduction of treatment. Thus, we regarded them as type 1 AIH.

The detection of ANA has been important in the diagnosis of AIH, and various diagnostic criteria include ANA as an essential marker.[3,21,22] However, ANA which is directed against centromers, ribonucleoproteins, cyclin A, histones and many other antigens is unspecific for AIH and is detected in 33% of non-alcoholic fatty liver disease, 42% of primary biliary cirrhosis and 53% of primary sclerosing cholangitis.[23–26] However, the number of unusual AIH patients who do not show typical features, such as patients with acute-onset or fulminant-type AIH, autoantibody-negative patients and patients with bile duct injury, have increased, and the clinical features of AIH have diversified.[27,28] Thus, for the diagnosis to be made more easily and accurately, a new specific marker for AIH is required.

In conclusion, in our analysis, ANA-negative type 1 AIH patients had lower serum IgG levels at presentation and more frequently showed acute presentation, histological acute hepatitis and zone 3 necrosis than those with high titers of ANA. However, some ANA-negative patients also showed an advanced stage of fibrosis. ANA-negative type 1 AIH may include not only acute AIH but also acute exacerbation of inactive chronic disease. Regarding the diagnosis of AIH in ANA-negative patients, the determination of ANA during follow-up and the response to immunosuppressive treatment may be helpful. Some ANA-negative AIH patients developed ANA during follow-up and/or relapsed after the normal-

ization of serum ALT levels as a result of the initial treatment. However, ANA is not a specific marker for AIH, and the diagnosis of AIH in ANA-negative patients may not be easy. For the diagnosis to be made more easily and accurately, a new specific marker for AIH is required.

REFERENCES

1 Waldenström L. Leber Blutprotein und Narungseiweisse. *Dtsch Gesellsch Verd Stroffw* 1950; 15: 113–9.
2 Krawitt EL. Autoimmune hepatitis. *N Engl J Med* 2006; 354: 54–66.
3 Alvarez F, Berg PA, Bianchi FB *et al.* International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; 31: 929–38.
4 Papamichalis PA, Zachou K, Koukoulis GK *et al.* The revised international autoimmune hepatitis score in chronic liver diseases including autoimmune hepatitis/overlap syndromes and autoimmune hepatitis with concurrent other liver disorders. *J Autoimmune Dis* 2007; 4: 3–14.
5 Farias AQ, Gonçalves LL, Bittencourt PL *et al.* Applicability of the IAIHG scoring system to the diagnosis of antimitochondrial/anti-M2 seropositive variant form of autoimmune hepatitis. *J Gastroenterol Hepatol* 2006; 21: 887–93.
6 Kaya M, Angulo P, Lindor KD. Overlap of autoimmune hepatitis and primary sclerosing cholangitis: an evaluation of a modified scoring system. *J Hepatol* 2000; 33: 537–42.
7 Ebbeson RL, Schreiber RA. Diagnosing autoimmune hepatitis in children: is the International Autoimmune Hepatitis Group scoring system useful? *Clin Gastroenterol Hepatol* 2004; 2: 935–40.
8 Montano-Loza AJ, Carpenter HA, Czaja AJ. Features associated with treatment failure in type 1 autoimmune hepatitis and predictive value of the model of end-stage liver disease. *Hepatology* 2007; 46: 1138–45.
9 Al-Chalabi T, Boccato S, Portmann BC, McFarlane IG, Heneghan MA. Autoimmune hepatitis (AIH) in the elderly: a systematic retrospective analysis of a large group of consecutive patients with definite AIH followed at a tertiary referral centre. *J Hepatol* 2006; 45: 575–83.
10 Czaja AJ. Behavior and significance of autoantibodies in type 1 autoimmune hepatitis. *J Hepatol* 1999; 30: 394–401.
11 Czaja AJ, Cassani F, Cataleta M, Valentini P, Bianchi FB. Antinuclear antibodies and patterns of nuclear immunofluorescence in type 1 autoimmune hepatitis. *Dig Dis Sci* 1997; 42: 1688–96.
12 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; 19: 1513–20.
13 Hofer H, Oesterreicher C, Wrba F, Ferenci P, Penner E. Centrilobular necrosis in autoimmune hepatitis: a histological feature associated with acute clinical presentation. *J Clin Pathol* 2006; 59: 246–9.
14 Abe M, Hiasa Y, Masumoto T *et al.* Clinical characteristics of autoimmune hepatitis with histological features of acute hepatitis. *Hepatol Res* 2001; 21: 213–19.
15 Montano-Loza AJ, Carpenter HA, Czaja AJ. Improving the end point of corticosteroid therapy in type 1 autoimmune hepatitis to reduce the frequency of relapse. *Am J Gastroenterol* 2007; 102: 1005–12.
16 Czaja AJ. Understanding the pathogenesis of autoimmune hepatitis. *Am J Gastroenterol* 2001; 96: 1224–31.
17 Germano V, Picchianti Diamanti A, Baccano G *et al.* Autoimmune hepatitis associated with infliximab in a patient with psoriatic arthritis. *Ann Rheum Dis* 2005; 64: 1519–20.
18 Goldstein NS, Bayati N, Silverman AL, Gordon SC. Minocycline as a cause of drug-induced autoimmune hepatitis. Report of four cases and comparison with autoimmune hepatitis. *Am J Clin Pathol* 2000; 114: 591–8.
19 Pelli N, Setti M. Atorvastatin as a trigger of autoimmune hepatitis. *J Hepatol* 2004; 40: 716.
20 Berry PA, Smith-Laing G. Hepatitis A vaccine associated with autoimmune hepatitis. *World J Gastroenterol* 2007; 13: 2238–9.
21 Toda G, Zeniya M, Watanabe F *et al.* Present status of autoimmune hepatitis in Japan – correlating the characteristics with international criteria in an area with a high rate of HCV infection. Japanese National Study Group of Autoimmune Hepatitis. *J Hepatol* 1997; 26: 1207–12.
22 Hennes EM, Zeniya M, Czaja AJ *et al.*, International Autoimmune Hepatitis Group. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; 48: 169–76.
23 Manns MP, Vogel A. Autoimmune hepatitis, from mechanisms to therapy. *Hepatology* 2006; 43: S132–44.
24 Yatsuji S, Hashimoto E, Kaneda H, Taniai M, Tokushige K, Shiratori K. Diagnosing autoimmune hepatitis in nonalcoholic fatty liver disease: is the International Autoimmune Hepatitis Group scoring system useful? *J Gastroenterol* 2005; 40: 1130–8.
25 Wesierska-Gadek J, Penner E, Battezzati PM *et al.* Correlation of initial autoantibody profile and clinical outcome in primary biliary cirrhosis. *Hepatology* 2006; 43: 1135–44.
26 Angulo P, Peter JB, Gershwin ME *et al.* Serum autoantibodies in patients with primary sclerosing cholangitis. *J Hepatol* 2000; 32: 182–7.
27 Ichai P, Duclos-Vallée JC, Guettier C *et al.* Usefulness of corticosteroids for the treatment of severe and fulminant forms of autoimmune hepatitis. *Liver Transpl* 2007; 13: 996–1003.
28 Czaja AJ, Carpenter HA. Autoimmune hepatitis with incidental histologic features of bile duct injury. *Hepatology* 2001; 34: 659–65.

CLINICAL RESEARCH STUDY

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Necessities of Interferon Therapy in Elderly Patients with Chronic Hepatitis C

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ABSTRACT

BACKGROUND: The significance of antiviral therapy for elderly patients with chronic hepatitis C virus (HCV) infection has not been elucidated.

PATIENTS AND METHODS: Among 5645 patients with HCV-related chronic liver disease, the prognosis of 1917 elderly patients aged 60 years or more was analyzed. A total of 454 patients underwent interferon (IFN) therapy. By using multivariate analysis, carcinogenesis and survival were analyzed according to initial findings.

RESULTS: At 10 and 15 years, cumulative survivals in untreated elderly patients were 90.7% and 72.7% in the high platelet ($\geq$150,000/mm^3) group, 78.6% and 47.8% in the intermediate (100,000-149,000/mm^3) group, and 52.5% and 25.0% in the low platelet group (<100,000/mm^3), respectively. At 5 and 10 years, hepatocarcinogenesis rates in the intermediate and low platelet groups were 10.9% and 21.6% in the IFN group (N = 217) and 19.5% and 43.0% in the untreated group (N = 459), respectively ($P = .0005$). IFN independently decreased carcinogenesis risk with a hazard ratio of 0.56 ($P = .035$). In the high platelet group, 5- and 10-year carcinogenesis rates were 3.7% and 8.3% in the IFN-treated group (N = 228) and 5.1% and 14.0% in the untreated group (N = 585), respectively ($P = .69$). IFN treatment significantly increased cumulative survivals in the lower platelet subgroup ($P = .0001$) but did not affect the higher platelet subgroup ($P = .08$). IFN was independently associated with a longer survival in the lower platelet subgroup (hazard ratio 2.33, $P = .005$).

CONCLUSION: In elderly patients with chronic HCV, IFN for a subgroup with intermediate and low platelet counts had significant advantages in regard to hepatocarcinogenesis and survival.

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KEYWORDS: Chronic hepatitis C virus; Elderly; Hepatocellular carcinogenesis; Interferon; Survival

Hepatitis C virus (HCV) is one of the principal causes of hepatocellular carcinoma and often causes high morbidity and mortality in many countries.[1-5] Because interferon (IFN) has antiviral, antifibrotic, and anti-inflammatory actions, it is still a main arm in the treatment of chronic HCV.[6,7] Many authors have demonstrated that IFN prevents hepatocarcinogenesis and eventually prolongs the survival period of patients.[8-13] Radical eradication of HCV by IFN depends on viral load, HCV subtype, certain mutations of hepatitis virus gene, liver histology, modes of IFN administration, and various host factors, including a patient's age.[14-16] When a significant side effect occurs during IFN therapy, cessation or early withdrawal of the therapy often failed to attain a successful result. Early withdrawal and treatment failure are likely more common in elderly patients and patients with an advanced stage of liver disease.

The number and rate of elderly patients with HCV-positive chronic hepatitis are currently increasing in the United States and Japan[17-19] because of a significant decrease of new blood-borne HCV infections and an aging

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society, such as in Japan. In elderly patients with chronic hepatitis or cirrhosis type C, adverse effects of IFN are more prevalently found and hematologic disorders often disturb the completion of the therapy. As a result, IFN administration is considered less effective in elderly patients.[16,20-22] Because the fibrotic stage of liver disease is often correlated with a patient's age, an elderly patient naturally has a high risk of carcinogenesis and mortality. IFN is effective in reducing hepatocarcinogenesis and improving the survival of patients with HCV-related chronic hepatitis, but the clinical influence of IFN is considered less advantageous in elderly patients because of the short life expectancy. There has been little information on the prognosis of elderly patients with HCV-related chronic liver disease and the significance of antiviral therapy for elderly patients.

To clarify whether IFN had similar advantages between young and elderly patients, we analyzed a large cohort of HCV-positive elderly patients in regard to hepatocellular carcinogenesis and survival at a single institution. We also attempted to elucidate favorable indications and the best candidates for IFN therapy among elderly patients, if any.

CLINICAL SIGNIFICANCE

- Significant differences in hepatocarcinogenesis and survival exist among patients with HCV, according to initial platelet count.
- IFN for a subgroup with intermediate and low platelet counts had significant advantages in regard to hepatocarcinogenesis and survival of elderly patients with chronic HCV.
- Asymptomatic elderly patients with HCV should be observed carefully as to hepatocarcinogenesis by using ultrasonography when the platelet count is 150 × 1000/mm^3 or less.
- IFN therapy should be considered in elderly patients when they have intermediate and low platelet counts.
- In view of the side effects in elderly patients, treatment should be initiated as soon as possible after diagnosis of chronic HCV.

PATIENTS AND METHODS

Entire Population and Analyzed Cohorts

A total of 7235 patients were diagnosed with HCV-positive chronic liver disease with positive anti-HCV antibody and detectable HCV-RNA (nested polymerase chain reaction) and negative hepatitis B surface antigen from 1974 to 2004 at the Department of Hepatology, Toranomon Hospital, Tokyo. Anti-HCV and HCV-RNA were assayed using stored frozen sera. There were 4121 men and 3114 women, with a median age of 54 years (range, 1-92 years). We excluded 1144 patients with acute hepatitis, overt alcoholic liver disease or fatty liver, association of other types of liver disease (eg, primary biliary cirrhosis, autoimmune hepatitis), or association with hepatocellular carcinoma or other. We also excluded 446 patients with a short observation period (<6 months).

There were 3728 patients aged less than 60 years and 1917 patients aged 60 years or more. The diagnosis was established by peritoneoscopy or biopsy in 636 patients and by clinical data in 1281 patients. The ratio of women was higher (36.9% vs 54.4%, $P < .001$) and history of IFN therapy was lower (60.3% vs 23.7%, $P < .001$) in elderly patients. Median albumin value was lower (4.3 vs 4.1 g/dL, $P < .001$) and platelet count was lower (181,000 vs 155,000/mm^3, $P < .001$) in elderly patients. This study analyzed 1917 elderly patients with HCV: 454 patients (23.7%) with IFN therapy and 1463 patients (76.3%) without IFN therapy.

Interferon Treatment and Judgment of Effect

Among 454 patients with IFN therapy, 413 received IFN monotherapy and 41 received IFN plus ribavirin combination therapy as an initial antiviral therapy. Of 413 patients with IFN monotherapy, 272 patients received IFN every day for the first 2 to 8 weeks and then 2 to 3 times per week for the following 16 to 96 weeks (median, 24 weeks), 108 patients received IFN 3 times per week for 24 to 104 weeks, and 33 patients received IFN for 4 to 8 weeks. Among 346 patients without viral elimination after initial IFN therapy, 186 patients underwent repeated IFN therapy including IFN plus ribavirin combination therapy. The age at the time of initiation of therapy ranged from 60 to 84 years, with a median of 64 years.

Most patients (N = 451) with IFN therapy showed varied degrees of influenza-like symptoms, leukocytopenia, and thrombocytopenia. Forty-three patients discontinued IFN therapy because of significant adverse reactions: depression in 10 patients, marked anorexia in 9 patients; psychosis, epilepsy, or loss of consciousness in 8 patients; ophthalmic diseases in 3 patients; severe cytopenia in 3 patients; interstitial pneumonia in 2 patients; and other conditions in 8 patients. No patients had decompensated liver disease with ascites, encephalopathy, jaundice, or variceal bleeding.

Judgment of IFN effect was classified according to elimination of HCV RNA and alanine aminotransferase for 6 months after the end of treatment. Sustained virologic response was defined as persistent disappearance of HCV RNA after therapy, biochemical response was defined as normal alanine aminotransferase values without elimination of HCV RNA for at least 6 months after therapy, and no response was defined as persistently abnormal or only transient normalization of alanine aminotransferase for less than 6 months. Because 12 patients (2.6%) were lost to follow-up and 49 patients (10.8%) were still in the course of IFN therapy, the judgment was made in 393 (86.6%) of 454 patients.

Table 1 Profiles and Laboratory Data of 1917 Elderly Patients at the Initial Visit to Toranomon Hospital

	No Therapy N = 1463	IFN Therapy N = 454	P^c
Demography			
Sex (M/F)	660/803	214/240	.45
Age (y)[a]	65 (60-88)	62 (60-80)	<.001
Observation period (y)[a]	5.91 (0.5-27.6)	6.23 (0.5-17.6)	.23
Lost to follow-up (y)	165 (11.3%)	12 (2.6%)	<.001
Laboratory Data[b]			
Albumin (g/dL)	4.1 (3.8-4.3)	4.1 (3.9-4.3)	.11
Bilirubin (mg/dL)	0.6 (0.5-0.9)	0.7 (0.5-0.8)	.14
Aspartic aminotransferase (IU/L)	51 (33-83)	70 (46-106)	<.001
Alanine aminotransferase (IU/L)	56 (32-97)	90 (56-148)	<.001
Hemoglobin (g/dL)	13.8 (12.9-14.7)	14.2 (13.3-15.1)	<.001
Platelet count ($\times 1000/mm^3$)	157 (120-198)	150 (122-195)	0.12
Alpha-fetoprotein (ng/mL)	4 (3-6)	4 (3-6)	.80
HCV			
subtype 1 (1a/1b)	714 (79.2%)	154 (58.8%)	<.001
subtype 2 (2a/2b)	150 (16.6%)	102 (38.9%)	
others	38 (4.2%)	6 (2.3%)	

IFN = interferon; HCV = hepatitis C virus.
[a]Expressed by median (range).
[b]Expressed by median (25th percentile, 75th percentile).
[c]Mann–Whitney or chi-square test.

Follow-up of and Diagnosis of Hepatocellular Carcinoma

Follow-up of patients was made on a monthly to trimonthly basis after the initial visit. Imaging diagnosis was made 1 or more times per year with ultrasonography, computed tomography, or magnetic resonance imaging.

Statistical Analysis

Obtained clinical data were analyzed on an intention-to-treat basis. Nonparametric procedures were used for the analysis of background characteristics of the patients, including the Mann–Whitney *U*, Kruskal–Wallis, and chi-square tests.

Hepatocellular carcinogenesis and survival were calculated using the Kaplan–Meier test. The differences in carcinogenesis curves were tested using the log-rank test.[23] Independent factors associated with the appearance rate of hepatocellular carcinoma were studied using time-dependent Cox regression analysis.[24] The following 16 variables were analyzed for potential covariates for liver carcinogenesis at the initial hospital visit: age, sex, total alcohol intake, family history of liver disease, history of blood transfusion, association of diabetes, aspartic aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, albumin, bilirubin, hemoglobin, platelet count, serologic grouping of HCV, IFN administration, and effect of IFN treatment (time-dependent variable). A *P* value of less than .05 was considered significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 11.[25]

RESULTS

Demographics of Elderly Patients with or without Interferon Therapy

Table 1 summarizes the profiles and data of the 1917 elderly patients with or without IFN therapy during clinical course. The median age of the patients with IFN was younger by 3 years. Although aminotransferases were significantly higher in the treated group, albumin, bilirubin, and platelet count were not different between the 2 groups.

Hepatocarcinogenesis and Survival without Interferon Therapy

Liver cancer developed in 285 (19.5%) of 1463 elderly patients without IFN therapy. Hepatocarcinogenesis rates were 13.1% at the end of 5 years, 29.9% at 10 years, 45.5% at 15 years, and 55.1% at 20 years. Carcinogenesis rates were calculated in subgroups according to initial platelet count: high ($\geq$150,000/mm^3), intermediate (100,000-149,000/mm^3), and low (<100,000/mm^3). Cumulative carcinogenesis rates in the subgroups of high, intermediate, and low platelet counts were 5.1%, 14.2%, and 32.1% at 5 years, 14.0%, 34.2%, and 63.4% at 10 years, and 26.1%, 57.5%, and 74.9% at 15 years, respectively (Figure 1). The carcinogenesis rate was significantly different among the 3 subgroups ($P < .0001$).

Survival in the elderly patients without IFN therapy was 92.9% at 5 years, 76.6% at 10 years, 54.3% at 15 years, and 37.2% at 20 years. Survivals in the subgroups with high, intermediate, and low platelet counts were 97.9%, 95.9%,

and 86.8% at 5 years, 90.7%, 78.6%, and 52.5% at 10 years, and 72.7%, 47.8%, and 25.0% at 15 years, respectively (Figure 2). A significant difference was observed among the 3 subgroups ($P < .0001$).

Adverse Effects and Effect of Interferon in the Elderly

Thirty-nine patients discontinued IFN therapy because of adverse effects: severe fatigue or anorexia in 10 patients (25.6%), depression in 10 patients (25.6%), hematologic disorder in 6 patients (15.4%), ophthalmic disorders in 4 patients (10.3%), and other side effects in 9 patients (23.1%). Duration of the therapy ranged from 2 weeks to 8.1 years, with a median of 24 weeks.

Among 393 patients with available judgment of IFN effect, 140 (35.6%) had a sustained virologic response, 80 (20.4%) had a biochemical response, and 173 (44.0%) had no response.

Hepatocarcinogenesis Rates in Elderly Patients with or without Interferon

During observation, hepatocellular carcinoma developed in 334 (17.4%) of 1917 patients: 285 (19.5%) in the untreated group and 49 (10.8%) in the IFN group.

Hepatocarcinogenesis rates in the untreated and IFN groups were 13.1% and 7.0% at 5 years, 29.9% and 13.9% at 10 years, and 45.5% and 33.4% at 15 years, respectively. The carcinogenesis rate in the IFN-treated group was significantly lower than in the untreated group (log-rank test, $P < .0001$).

Carcinogenesis rates also were evaluated in the subgroups with sustained virologic response (N = 140), biochemical response (N = 80), and no response (N = 173). Cumulative carcinogenesis rates were 2.5%, 1.3%, and 9.1% at 5 years, 2.5%, 11.0%, and 18.1% at 10 years, and 2.5%, 39.6%, and 41.2% at 15 years, respectively. A significant difference was found among the 4 groups, including the untreated patient group ($P < .0001$).

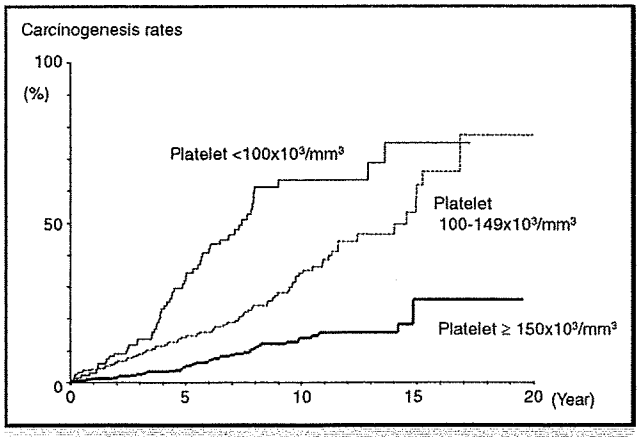

Figure 1 Hepatocarcinogenesis rates in patients without IFN therapy, according to initial platelet count. The lower the initial platelet count was, the higher the hepatocellular carcinogenesis was in the untreated cohort ($P < .0001$).

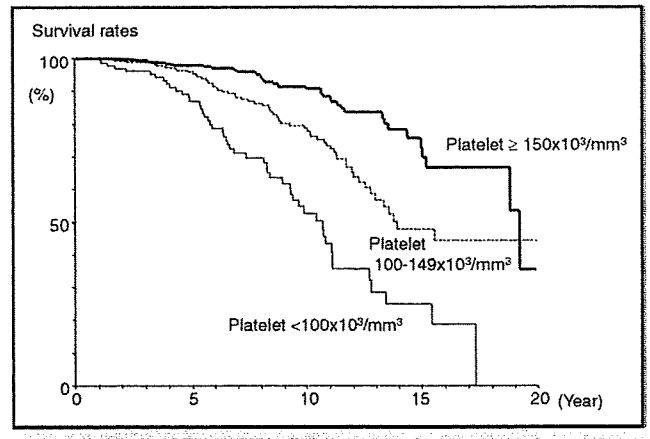

Figure 2 Cumulative survival in patients without IFN therapy, according to initial platelet count. Survival of patients with high platelet count was significantly higher than those with a low or intermediate platelet count ($P < .0001$).

Carcinogenesis rates were compared between those with or without IFN treatment in a subgroup with a high platelet count of 150,000/mm^3 or more. Cumulative carcinogenesis rates in the untreated (N = 585) and treated groups (N = 228) were 5.1% and 3.7% at 5 years, 14.0% and 13.1% at 10 years, and 26.1% and 25.9% at 15 years, respectively. The carcinogenesis rate in the IFN therapy group was slightly lower than in the untreated group, but no statistical significance was found in the high platelet subgroup ($P = .69$). Next, carcinogenesis rates were analyzed between those with or without IFN in a combined subgroup with low and intermediate platelet counts of less than 150,000 mm^3. Carcinogenesis rates in untreated (N = 459) and treated (N = 217) groups were 19.5% and 10.9% at 5 years, 43.0% and 21.6% at 10 years, and 65.3% and 39.4% at 15 years, respectively (Figure 3). The carcinogenesis rate in the group with IFN therapy was significantly lower in the untreated group ($P = .0005$).

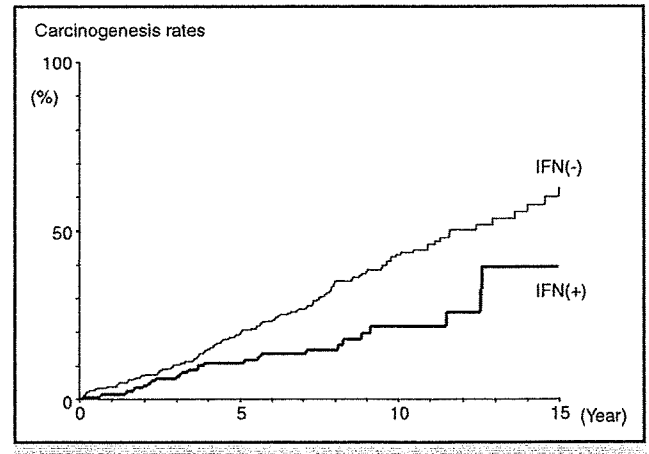

Figure 3 Hepatocarcinogenesis rates in patients with a low or intermediate platelet count. Carcinogenesis rate of patients with IFN therapy was significantly lower than those without therapy ($P = .0005$). IFN = Interferon.

Table 2 Independent Factors Associated with Hepatocellular Carcinogenesis in Elderly Patients with Hepatitis C Virus-related Chronic Liver Disease

Factors	(Category)	Hazard Ratio (95% CI)	*P*
Platelet count	1: ≥150,000/mm^3	1	
	2: 100,000-149,000/mm^3	2.42 (1.71-3.40)	<.001
	3: <100,000/mm^3	5.64 (3.88-8.22)	<.001
Alanine aminotransferase	1: <75 IU/L	1	
	2: ≥75IU/L	2.02 (1.48-2.77)	<.001
Gender	1: Female	1	
	2: Male	1.79 (1.35-2.37)	<.001
IFN	1: No therapy	1	
	2: No response	0.74 (0.44-1.25)	.26
	3: Biochemical response	0.52 (0.17-1.65)	.27
	4: Sustained virologic response	0.063 (0.009-0.449)	.006

CI = confidence interval; IFN = interferon.

Factors Affecting Hepatocellular Carcinogenesis

In the first proportional hazard analysis using IFN therapy factor as a time-dependent covariate, factors associated with carcinogenesis were explored in the entire elderly cohort. Hepatocarcinogenesis is independently associated with low platelet count ($P < .001$), high alanine aminotransferase value ($P < .001$), male sex ($P < .001$), and IFN therapy (hazard ratio = 0.67, $P = .045$).

Next, multivariate analysis was performed using factors of each IFN effect: sustained virologic response, biochemical response, no response, and no IFN therapy. Carcinogenesis was significantly associated with platelet count, male sex, alanine aminotransferase value, and sustained virologic response after IFN therapy (Table 2). Patients with low and intermediate platelet counts showed high hazard ratios and high alanine aminotransferase value; male gender showed high hazard ratios. Sustained virologic response significantly decreased the hazard ratio to 0.063 ($P = .006$).

The role of IFN treatment factor was not significant (hazard ratio 0.87, $P = .67$) in the high platelet group (≥150,000/mm^3), but it was significant (hazard ratio 0.56, $P = .035$) in the low or intermediate platelet group (<150,000/mm^3).

Survival of Elderly Patients

A total of 276 patients (14.4%) died during observation: 255 (17.4%) in the untreated group and 21 (4.6%) in the treated group. Crude survivals in the untreated and IFN groups were 92.9% and 98.7% at 5 years, 76.6% and 92.6% at 10 years, and 54.3% and 70.4% at 15 years, respectively. Survival in the IFN-treated group was significantly higher ($P < .0001$).

When a subgroup with high platelet counts (≥150,000/mm^3) was analyzed, survivals in the untreated and IFN groups were 97.9% and 99.6% at 5 years, 90.7% and 94.5% at 10 years, and 72.7% and 76.9% at 15 years, respectively. Survival was not significantly different ($P = .08$). Survival also was analyzed in a subgroup with low or intermediate platelet count (<150,000/mm^3). Cumulative survivals in the untreated and treated groups were 93.2% and 97.5% at 5 years, 70.8% and 89.9% at 10 years, and 41.2% and 64.9% at 15 years, respectively (Figure 4). Survival in the IFN therapy group was significantly higher than in the untreated group ($P = .0001$).

Factors Affecting Survival in the Elderly

Independent factors associated with survival were explored in all the elderly patients. Multivariate hazard analysis disclosed that survival is independently associated with low platelet count ($P < .001$), male sex ($P < .001$), older age ($P < .001$), and IFN therapy (hazard ratio = 0.56, $P = .041$).

In the high platelet group (≥150,000/mm^3), only gender and age were independently associated with survival. The factor of IFN therapy only showed a hazard ratio for death of 0.70 in the multivariate analysis. In the low or intermediate platelet group (<150,000/mm^3), platelet count, age,

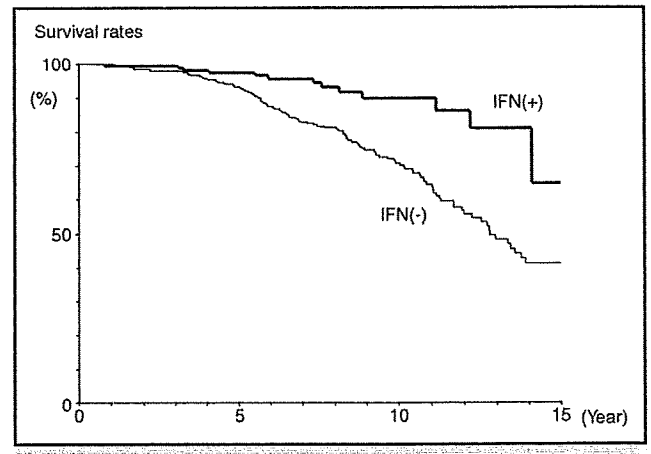

Figure 4 Cumulative survival in patients with a low or intermediate platelet count. Survival of patients with IFN therapy was significantly higher than those without therapy ($P = .0001$). IFN = Interferon.

Table 3 Independent Factors Associated with Survival Period in Elderly Patients with Hepatitis C Virus-related Chronic Liver Disease

Factors	(Category)	Hazard Ratio (95% CI)	*P*
Subgroup with High Platelet Count ($\geq$150,000/mm^3)			
Gender	1: Female	1	
	2: Male	2.81 (1.46-5.41)	.002
Age	by 1 y	1.11 (1.04-1.18)	.002
IFN	1: No	1	
	2: Yes	0.70 (0.32-1.18)	.39 (NS)
Subgroup with Low or Intermediate Platelet Count (<150,000/mm^3)			
Platelet count	1: 100,000-149,000/mm^3	1	
	2: <100,000/mm^3	3.14 (2.19-4.50)	<.001
Age	by 1 y	1.09 (1.05-1.13)	<.001
IFN	1: No	1	
	2: Yes	0.43 (0.24-0.77)	.005
Gender	1: Female	1	
	2: Male	1.56 (1.09-2.22)	.015

CI = confidence interval; IFN = interferon; NS = not significant.

IFN therapy, and sex were independently associated with hepatocellular carcinogenesis. IFN significantly decreased the hazard of death by 0.43 in the subgroup of low or intermediate platelet count ($P = .005$) (Table 3).

DISCUSSION

This retrospective study was undertaken to evaluate whether IFN therapy could decrease hepatocellular carcinogenesis and increase survival in HCV-positive elderly patients aged 60 years or more at the initial hospital visit. Because it seemed to require at least 5 years to obtain a statistical difference in carcinogenesis rates and survival between IFN-treated and untreated groups, a prospective randomized trial with untreated control patients is difficult to perform from both ethical and medical viewpoints. We therefore attempted to carry out this retrospective study to show an impact of IFN treatment with a statistical adjustment and stratification using a large number of patients under a long-term observation period.

There were significant differences in carcinogenesis and survival among patients with HCV, according to initial platelet count. Because this study dealt with all patients with HCV-related hepatitis who visited Toranomon Hospital irrespective of IFN treatment, evaluation of liver histology was performed in approximately two thirds of the patients. Platelet count has been considered a simple indicator for the progression of hepatitis, and the patients without liver biopsy were well stratified by the initial platelet count in our study. From statistics of the nationwide census for the longevity of each age group in 2003, the life expectation was 21.9 and 27.5 years for 60-year-old Japanese men and women, respectively, and 18.0 and 23.07 years for 65-year-old Japanese men and women, respectively. In view of the median age (65 years) of the untreated cohort with HCV infection, the survival of patients with high platelet counts was almost the same as that of the general population in Japan (Figure 2). Physicians should consider the longevity without IFN therapy and the cost, side effects, and risks caused by IFN for more stratified age groups of the elderly.

Although several authors have shown that effects of both IFN monotherapy[20,26,27] and IFN plus ribavirin combination therapy[28,29] were not different between elderly and younger patients with chronic HCV in regard to viral elimination and normalization of transaminase, recent reports[16,21] have shown lower virologic response rates. A possible low response rate in the elderly was closely associated with a high rate of adverse reactions,[16,20,21] and hematologic side effects seemed significant in the elderly group.[22] The low discontinuation rate (43/454, 9.5%) in the current study was partly attributable to the low rate of IFN plus ribavirin combination therapy. Horiike et al,[27] Floreani et al,[16] and Koyama et al[21] recommended IFN therapy for select patient groups with a low HCV RNA titer, non-genotype 1, or relatively young age of less than 65 years.

We previously reported a high carcinogenesis rate in elderly patients with chronic HCV who underwent IFN therapy.[30] When crude hepatocarcinogenesis rates were compared between untreated and IFN-treated groups in the current study, IFN significantly decreased the carcinogenesis rate in the elderly patients with varied severity of liver disease. As was found in the general results of patients, including the younger age group,[13] carcinogenesis in patients with sustained virologic response was significantly lower than that of patients with no response or without IFN therapy. The carcinogenesis rate was low for several years after cessation of IFN administration and increased gradually after 8 years in the group with a biochemical response (Figure 3). The cancer appearance curve of the biochemical response group implied that the normal and stable hepatitis

state in the early years contributed to suppress the process of carcinogenesis, and that reactivation of hepatitis induced the progression of hepatic oncogenesis in the later years.

Among patients with a high platelet count and mild liver disease, IFN did not decrease the rate of hepatocarcinogenesis. IFN significantly decreased the carcinogenesis rate in patients with a low or intermediate platelet count. In view of the less effective rate and high adverse reaction rate by IFN in elderly patients, IFN therapy should be considered primarily for those with a low platelet count of 150,000/mm^3 or less. Because low platelet count was closely associated with advanced disease and high risk for carcinogenesis, treatment efficacy appeared prominent in the subgroup with low and intermediate platelet counts. The best candidates for IFN therapy were those with a low platelet count, also in regard to cost-effectiveness. Because a low platelet count is closely associated with advanced stages of liver disease, IFN therapy should be avoided for elderly patients with decompensated cirrhosis or severely decreased platelet count of less than 50,000/mm^3. A sustained virologic response improves clinical symptoms in decompensated cirrhosis,[31] but IFN often induces severe complications even in young patients with decompensated cirrhosis.[32] An elderly patient with hepatitis without decompensation can be a candidate for IFN therapy if careful, close hematologic monitoring is performed. Low-dose, intermittent, long-term IFN therapy also should be considered for these patients to obtain a sustained biochemical response without creating profound and irreversible side effects. Because elderly patients generally showed some difficulties with IFN treatment, our current study demonstrated practical information about carcinogenesis and the life expectancy of elderly patients with HCV and the order of priority in management of IFN for these patients. IFN administration is preferably considered and initiated at the age of 60 years or less to reduce side effects.

CONCLUSIONS

IFN for a subgroup with low and intermediate platelet counts had significant advantages in regard to hepatocarcinogenesis and survival of elderly patients with chronic HCV.

References

1. Bruix J, Calvet X, Costa J, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet.* 1989;2:1004-1006.
2. Colombo M, Kuo G, Choo QL, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet.* 1989;2:1006-1008.
3. Ikeda K, Saitoh S, Koida I, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 cases with viral and alcoholic cirrhosis. *Hepatology.* 1993;18:47-53.
4. Williams I. Epidemiology of hepatitis C in the United States. *Am J Med.* 1999;107:2S-9S.
5. Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol.* 2007;13:2436-2441.
6. Davis GL, Balart LA, Schiff ER, et al. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter, randomized, controlled trial. *N Engl J Med.* 1989;321:1501-1506.
7. Di Bisceglie AM, Martin P, Kassianides C, et al. Recombinant interferon alfa therapy for chronic hepatitis C: a randomized, double-blind, placebo-controlled trial. *N Engl J Med.* 1989;321:1506-1510.
8. Nishiguchi S, Kuroki T, Nakatani S, et al. Randomized trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet.* 1995; 346:1051-1055.
9. Mazzella G, Accogli E, Sottili S, et al. Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. *J Hepatol.* 1996;24:141-147.
10. Kasahara A, Hayashi N, Mochizuki K, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology.* 1998;27:1394-1402.
11. Niederau C, Lange S, Heintges T, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology.* 1998;28: 1687-1695.
12. International Interferon-alpha Hepatocellular Carcinoma Study Group. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. *Lancet.* 1998;351:1535-1539.
13. Ikeda K, Saitoh S, Arase Y, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. *Hepatology.* 1999;29: 1124-1130.
14. Tsubota A, Chayama K, Ikeda K, et al. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. *Hepatology.* 1994;19:1088-1094.
15. Hagiwara H, Hayashi N, Mita E, et al. Quantitative analysis of hepatitis C virus RNA in serum during interferon alpha therapy. *Gastroenterology.* 1993;104:877-883.
16. Floreani A, Monola E, Carderi I, et al. Are elderly patients poor candidates for pegylated interferon plus ribavirin in the treatment of chronic hepatitis C? *J Am Geriatr Soc.* 2006;54:549-550.
17. Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl.* 2003;9:331-338.
18. Taura N, Hamasaki K, Nakao K, et al. Aging of patients with hepatitis C virus-associated hepatocellular carcinoma: long-term trends in Japan. *Oncol Rep.* 2006;16:837-843.
19. Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. *Intervirology.* 2006;49:7-17.
20. Alessi N, Freni MA, Spadara A, et al. Efficacy of interferon treatment (IFN) in elderly patients with chronic hepatitis C. *Infez Med.* 2003;11: 208-212.
21. Koyama R, Arase Y, Ikeda K, et al. Efficacy of interferon therapy in elderly patients with chronic hepatitis C. *Intervirology.* 2006;49:121-126.
22. Nudo CG, Wong P, Hilzenrat N, Deschenes M. Elderly patients are at greater risk of cytopenia during antiviral therapy for hepatitis C. *Can J Gastroenterol.* 2006;20:589-592.
23. Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. *J Am Stat Assoc.* 1958;53:457-481.
24. Cox DR. Regression models and life tables. *J R Stat Soc.* 1972;34: 248-275.
25. SPSS Inc. *SPSS for Windows Version 11.0 Manual.* Chicago, IL: SPSS Inc.; 2001.
26. Bresci G, Del Corso L, Romanelli AM, et al. The use of recombinant interferon alfa-2b in elderly patients with anti-HCV-positive chronic active hepatitis. *J Am Geriatr Soc.* 1993;41:857-862.

27. Horiike N, Masumoto T, Nakanishi K, et al. Interferon therapy for patients more than 60 years of age with chronic hepatitis C. *J Gastroenterol Hepatol.* 1995;10:246-249.
28. Thabut D, Le Calvez S, Thibault V, et al. Hepatitis C in 6,865 patients 65 yr or older: a severe and neglected curable disease? *Am J Gastroenterol.* 2006;101:1260-1267.
29. Honda T, Katano Y, Urano F, et al. Efficacy of ribavirin plus interferon-alpha in patients aged > or = 60 years with chronic hepatitis C. *J Gastroenterol Hepatol.* 2007;22:989-995.
30. Arase Y, Ikeda K, Suzuki F, et al. Long-term outcome after interferon therapy in elderly patients with chronic hepatitis C. *Intervirology.* 2007;50:16-23.
31. Iacobellis A, Siciliano M, Perri F, et al. Peginterferon alfa-2b and ribavirin in patients with hepatitis C virus and decompensated cirrhosis: a controlled study. *J Hepatol.* 2007;46:206-212.
32. Nevens F, Goubau P, Van Eyken P, et al. Treatment of decompensated viral hepatitis B-induced cirrhosis with low doses of interferon alpha. *Liver.* 1993;13:15-19.

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特集 肝・胆道・膵がん治療の動向―最新のエビデンス

肝がん
6)発がん予防と再発予防*

池田健次**

Key Words：hepatocellular carcinoma, interferon, prevention, recurrence, nucleoside analogue

はじめに

肝癌のほとんどは，肝炎ウイルスやアルコールなどなんらかの原因による慢性肝疾患に発生する．このうち，わが国ではB型肝炎・C型肝炎ウイルスが肝癌の原因の約90％を占めている．本稿では，これらウイルス性肝炎に対する「原因療法」である抗ウイルス療法や「対症療法」である抗炎症療法を行えば慢性肝疾患からの発癌をどれだけ抑制できるか，根治治療後の肝癌再発をどれだけ抑制できるかをまとめた．

C型慢性肝炎に対するインターフェロン療法の肝発癌抑制効果

当院で腹腔鏡肝生検により確定診断したC型慢性肝炎について，インターフェロン治療の有無およびその治療効果により発癌率がどの程度の影響を受けるかをretrospectiveコホートにより検討した[1)2)]．

1．対　象

対象は1970年より2000年までの間に診断したC型慢性肝炎2,166例．全例hepatitis B surface(HBs)抗原陰性で，診断時の初期血清にてhepatitis C virus(HCV)抗体陽性，HCV-RNA陽性が確認されている．男性は1,421例，女性745例，年齢の中央値は50歳(14～78歳)であった．インターフェロン治療を行った例は1,654例(76.4％)，行わなかったのは512例で，無治療例はインターフェロンが導入される1987年以前の症例が多かった．

2．方　法

インターフェロンの治療効果は，SVR(インターフェロン終了24週間後HCV-RNA陰性化)，BR(インターフェロン終了後6か月以上ALT正常化)，NR(上記以外の効果)に分け，発癌率の検討を行った．

経過観察からの脱落例は223例(10.3％)で，インターフェロン群164例(9.9％)，無治療群59例(11.5％)であった．全体の症例の観察期間は0.1～33.6年，中央値は10.7年であった．発癌率はKaplan-Meier法で行い，治療有無別・治療効果別の発癌率はログランクテストで行った．発癌率に寄与する要因は，Cox比例ハザードモデルで検討した．

3．成　績

(1)インターフェロンの治療効果

1,654例に行ったインターフェロンの治療効果は，SVR 606例(36.6％)，BR 266例(16.1％)，NR 782例(47.3％)であった．

(2)肝癌発癌率

中央値10.7年の間に，199例(9.2％)の肝癌発癌がみられた．このうち，96例はインターフェロ

* Prevention of hepatocellular carcinogenesis and suppression of recurrence.

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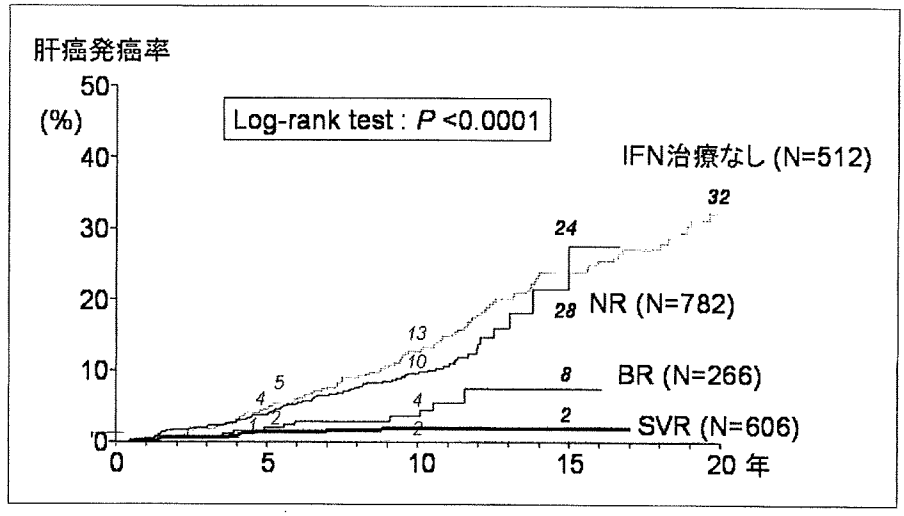

図1　インターフェロン治療効果別にみたC型慢性肝炎からの肝癌発癌率

ン治療例(96/1,654, 9.2%)，103例は無治療例(103/512, 20.1%)であった．

粗発癌率は，インターフェロン治療群・無治療群でそれぞれ，5年2.6%，4.6%，10年5.8%，12.7%，15年13.9%，23.9%で，インターフェロン治療群での発癌率は有意に低かった($P<0.0001$，ログランクテスト)．

(3)インターフェロン治療効果別にみた肝癌発癌率(図1)

インターフェロン施行例から発癌した96例のうち，SVRから発癌したのは11例(1.8%)，BRからは10例(3.8%)，NR例からは75例(9.6%)が発癌した．SVR・BR・NR別にみた粗発癌率は，5年1.4%，2.0%，3.8%，10年1.9%，3.6%，9.6%，15年1.9%，7.5%，27.6%であった．SVR群・BR群での発癌率はNR群より有意に低率であった($P<0.0001$)．

(4) C型慢性肝炎からの発癌に寄与する要因

多変量解析では，肝線維化程度(F3でのハザード比 8.68, $P<0.001$)，γ-glutamyl transpeptidase (GGTP)(50以上のハザード比 2.64, $P<0.001$)，性別(男性のハザード比 2.38, $P<0.001$)，インターフェロン治療の有無(インターフェロン治療のハザード比 0.42, $P<0.001$)，低血小板数(10万未満でのハザード比 2.22, $P<0.001$)，年齢(50歳以上のハザード比 1.90, $P=0.002$)の6要因が肝癌発癌に有意に関連する要因であった．インターフェロン治療により，発癌ハザードは0.42に低下すると計算された．

(5)インターフェロン治療効果別にみた肝癌発癌リスク

肝癌発癌に寄与する要因は，肝線維化程度(F3でのハザード比 9.90, $P<0.001$)，性別(男性のハザード比 3.44, $P<0.001$)，GGTP(50以上のハザード比 2.68, $P=0.008$)，年齢(50歳以上のハザード比 2.56, $P=0.001$)，α-fetoprotein (AFP) (20ng/ml以上のハザード比 2.34, $P=0.003$)，低血小板数(10万未満でのハザード比 2.09, $P=0.013$)があげられ，これらの共変量で補正した場合，無治療に対するSVRのハザード比は0.10($P<0.001$)，BRでは0.12($P<0.001$)，NRでは0.57($P=0.46$)であった．SVR・BR達成は有意に発癌率低下をもたらした．

4．インターフェロンの発癌抑制効果の位置づけ

2,166例と多数例のC型慢性肝炎のretrospective cohort studyからわかったことは，①インターフェロン治療を行うと(社会全体の)C型慢性肝炎からの発癌率が有意に低下すること，②インターフェロン治療でSVR・BRが得られると，無治療に比べて1/10近くまでの発癌率低下が得られることである．ことに，ウイルス排除に至らなくても，トランスアミナーゼがインターフェロン後一定期間以上の正常値を維持するだけで発癌リスクが著明に低下することの意義は大きい．

C型慢性肝炎に対するグリチルリチン製剤投与の肝発癌抑制効果

インターフェロン治療でSVR・BRが得られな

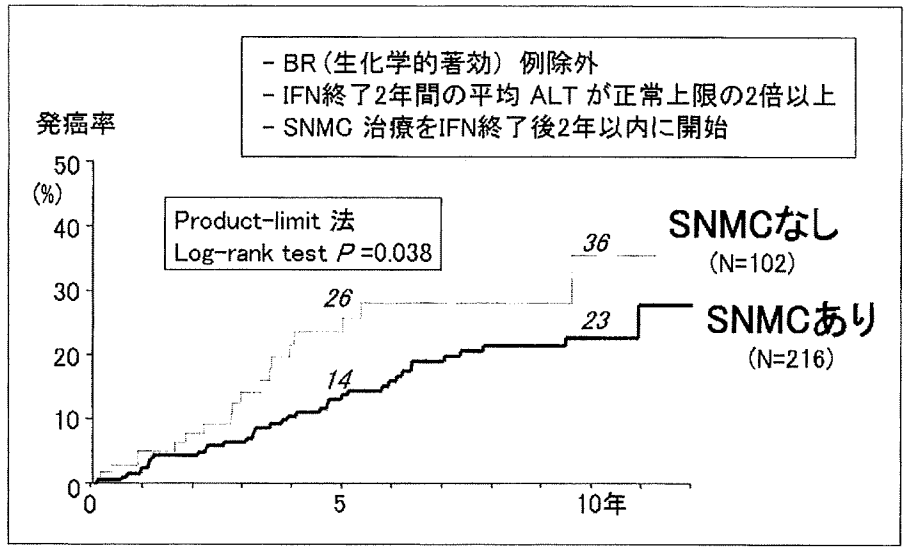

図2　SNMC投与別にみたインターフェロン無効C型慢性肝炎からの粗肝癌発癌率

かった症例に対して，グリチルリチン製剤（強力ネオミノファーゲン・シー™，以下SNMCと略）を使用し，その発癌抑制効果を1,249例のretrospective cohort studyで検討した[3)4)]．

1．対象・方法

1987年から2002年の間に当院でインターフェロン治療を行い，投与終了後6か月の時点でHCV-RNAが陽性であった1,249例を対象とした．症例の年齢は中央値53歳，男性778例・女性471例，慢性肝炎1,142例，肝硬変107例であった．SNMC治療を行ったのは453例，行わなかった例は796例で，前者ではインターフェロン無効判定時，年齢が有意に高く，AST・ALTが高く，また肝硬変の頻度が有意に高かった．

2．成　績

(1)SNMC治療群・非治療群での粗発癌率

治療群・非治療群での5年発癌率はそれぞれ11.6%，5.0%，10年発癌率は19.9%，10.6%で，SNMC治療群で有意に高率であった(P=0.0001)．

(2)インターフェロン終了1年間の平均ALT値別にみた発癌率

平均ALTを以下の6群に分けて発癌率を比較した：正常，正常値の1.5倍以内，1.5～2倍，2～3倍，3～4倍，4倍以上．それぞれの10年発癌率は，6.6%，7.2%，19.6%，15.1%，21.0%，39.3%で，平均ALT値と発癌率とは明らかな相関がみられた．

(3)インターフェロン後活動性の症例での発癌率

インターフェロン後にSNMCが使用された症例は，年齢・肝線維化・トランスアミナーゼなどすべてが発癌リスクの高い側に偏っており，インターフェロン使用例と同様のトランスアミナーゼ値の症例について，無治療例と比較して発癌率の検討を行った．

インターフェロン後に不完全著効と判定された例を除外し，かつ平均ALT値が正常値の2倍以上であった418例（SNMC群289例，非治療群129例）について発癌率を比較した．SNMCはインターフェロン治療後にトランスアミナーゼが上昇し2年以内に治療を開始した症例のみに限って検討した．このような背景の症例で，SNMC群・非治療群の肝癌発癌率を比較すると，5年発癌率は13.3%，26.0%，10年発癌率は21.5%，35.5%で，SNMC群で有意に発癌率が低かった(P=0.021)（図2）．

(4)インターフェロン無効後の発癌に寄与する独立要因

インターフェロン無効の判定後SNMC治療を開始するまでの期間を時間依存性変数として，発癌率に寄与する要因を多変量解析で検討した．肝線維化の程度（F1に比しF2～F3のハザード比2.94，F4のハザード比9.21，P<0.001）・性別（男性のハザード比2.80，P=0.006）・SNMCの有無（有のハザード比0.49，P=0.014）が独立して肝