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ORIGINAL ARTICLE

Poor Response to Pegylated Interferon and Ribavirin in Older Women Infected with Hepatitis C Virus of Genotype 1b in High Viral Loads

Hitomi Sezaki · Fumitaka Suzuki · Yusuke Kawamura · Hiromi Yatsuji · Tetsuya Hosaka · Norio Akuta · Masahiro Kobayashi · Yoshiyuki Suzuki · Satoshi Saitoh · Yasuji Arase · Kenji Ikeda · Yuzo Miyakawa · Hiromitsu Kumada

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Abstract *Background* Response to treatment in patients with chronic hepatitis C, with reference to age and gender, has not been examined fully. *Aim* The influence of gender and age on treatment with pegylated interferon (PEG-IFN) and ribavirin was evaluated in a retrospective study. *Methods* PEG-IFN and ribavirin were given for 48 weeks to 179 men and 121 women infected with hepatitis C virus (HCV) of genotype 1b in high viral loads (>100 kIU/ml). *Results* Sustained virological response at 24 weeks after treatment was poorer in women than men who were aged ≥50 years (22% vs 53%, $P < 0.001$). Among the patients aged ≥50 years who had received ≥80% of the doses of PEG-IFN, ribavirin, or both, women responded less often than men (26% vs 64%, $P < 0.001$; 33% vs 61%, $P = 0.022$; and 32% vs 63%, $P = 0.016$; respectively). In multivariate analysis, male gender, retention of indocyanine green, ribavirin dose and compliance with therapy increased sustained virological response. *Conclusions* Response to combined PEG-IFN and ribavirin is poorer in female than male patients with hepatitis C who are aged ≥50 years, irrespective of compliance with treatment. Low estrogen levels in older women could be responsible for their impaired response to PEG-IFN and ribavirin.

H. Sezaki (✉) · F. Suzuki · Y. Kawamura · H. Yatsuji · T. Hosaka · N. Akuta · M. Kobayashi · Y. Suzuki · S. Saitoh · Y. Arase · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital, Minato-ku, Tokyo 105-8470, Japan
e-mail: hitomis@mx1.harmonix.ne.jp

Y. Miyakawa
Miyakawa Memorial Research Foundation, Tokyo, Japan

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Introduction

There are an estimated 170 million people worldwide that are chronically infected with hepatitis C virus (HCV) [1]. HCV can persist in 70–80% of individuals who have been exposed to it, and it can induce chronic liver disease, through cirrhosis to hepatocellular carcinoma (HCC) in approximately 30% of them until 30–40 years after they were infected [2–4]. A number of viral and host factors influence the velocity of fibrosis progression in chronic hepatitis C. Thus, stage and grade of hepatitis are more severe in patients who are infected with HCV genotype 1 in high viral loads [5–7]. Male gender, age and intake of alcohol accelerate fibrosis, as well [8–10].

Interferon (IFN) combined with ribavirin has been the most effective and favored treatment of chronic hepatitis C to date. The combined treatment with the standard IFN can terminate HCV-1 infection with high viral loads in approximately 20% [11], and that with pegylated IFN (PEG-IFN) in >40% [12]. Owing to hemolytic side effects, however, women are less tolerant to ribavirin [13]. Although the response to combined treatment has been shown to be better in women than in men in previous studies, there remains a possibility that it could be influenced by age. Hence, there is a need for the comparison of the response between men and women in different age groups.

Virological response to PEG-IFN and ribavirin at the end of a 48-week treatment (ETR), as well as sustained virological response (SVR) 24 weeks after the completion of therapy, was compared between 179 men and 121

women who were infected with HCV-1b in high viral loads. In patients aged ≥50 years, both ETR and SVR were poorer in women than in men, irrespective of the total dose of IFN, ribavirin or both.

Methods

Study Population

From December 2001 to February 2006, 490 consecutive patients with chronic hepatitis C received combination therapy with PEG-IFN and ribavirin at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. The following inclusion criteria were met by 300 (61%) patients: they were (1) positive test results for antibodies to HCV (anti-HCV) and for HCV RNA genotype 1b by qualitative methods, and not co-infected with HCV of other genotypes; (2) negative test results for hepatitis B surface antigen or antibodies to human immunodeficiency virus type-1 (HIV-1); (3) confirmed findings of high HCV RNA levels ≥100 kIU/ml, which is the Japanese definition of high viral loads [14, 15], within the past 2 months; (4) no cirrhosis diagnosed by laparoscopy and ultrasonography, and with platelet counts $>80 \times 10^3/mm^3$; (5) body weight ≥40 kg and not pregnant or lactating; (6) total alcohol intake <500 kg in the past; (7) no HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; (8) no treatment with antivirals or immunosuppressants during the previous 3 months; and (9) with the wish to comply with the treatment protocol for 48 weeks. None of them received growth factors before or during the study period.

The 300 patients, comprising 179 men and 121 women, received PEG-IFN and ribavirin for 48 weeks and were followed for at least 24 weeks after completion of this combination therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Serum Markers of HCV Infection

Anti-HCV was determined by third-generation enzyme-linked immunosorbent assay (ELISA) by commercial kits (Ortho HCV Ab ELISA Test 3; Chiron Cooperation, Emeryville, CA, USA). HCV RNA was determined quantitatively by polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor ver. 2.0, Roche Diagnostics, Tokyo, Japan) in serum diluted tenfold at the baseline, as well as at least monthly during and after treatment; it has a dynamic range between 5 kIU/ml and 5,000 kIU/ml. Sera negative for HCV RNA (<5 kIU/ml) by quantitative assay were tested by qualitative PCR (Amplicor, Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a detection limit at 100 copies/ml.

Combined PEG-IFN and Ribavirin Therapy

Patients underwent subcutaneous administration of PEG-IFN-α2b (PEG-Intron, Schering-Plough Corp, Kenilworth, NJ, USA), weekly, at a median dose of 1.4 μg/kg (range 0.8–1.9 μg/kg), together with ribavirin orally, at a median daily dose of 11 mg/kg (range 3.7–14.2 mg/kg) for 48 weeks. The dose of ribavirin was adjusted by body weight: 600 mg for patients weighing ≤60 kg; 800 mg for those between >60 kg and <80 kg; and 1,000 mg for those ≥80 kg. It was tapered in the 99 (33%) patients in whom hemoglobin levels decreased below 10 g/dl during the combination therapy.

Statistical Analysis

Variables were compared between groups by the chi-square test, Fisher's exact probability test and the Mann–Whitney U test. Differences in the loss of HCV RNA from the serum between groups was evaluated with the Kaplan–Meier life table with use of the log rank test. The influence of various factors on the response to PEG-IFN/ribavirin was evaluated by logistic regression in univariate and multivariate analyses. Analysis of all data was performed with the computer program SPSS software (SPSS Inc., Chicago, IL, USA), and a P value less than 0.05 was considered significant.

Results

Baseline Characteristics of Male and Female Patients Infected with HCV-1b in High Loads

PEG-IFN and ribavirin were given for 48 weeks to 179 men and 121 women who had been infected with HCV-1b in high viral loads (>100 kIU/ml). Table 1 compares baseline characteristics between them. Women were older, had lower hemoglobin values and platelet counts, and lower levels of albumin, gamma-glutamyl-transpeptidase (γ-GTP) and ferritin, than men. The stage of fibrosis was lower in women than in men, although their alanine aminotransferase (ALT) levels were comparable. Three months before the start of combination therapy, IFN had been given to 75 (42%) of the 179 male patients, comparably frequently to 40 of the 121 (33%) female patients. Age distribution for men and women is shown in Fig. 1. The proportion of patients ≥60 years was higher in women than in men (39% vs 19%, $P < 0.001$).

Table 1 Baseline characteristics of 300 patients with chronic hepatitis with high-titers of HCV-1b RNA who had received PEG-IFN and ribavirin for 48 weeks and were followed for 48 weeks or longer

Characteristic[a]	Men ($n = 179$)	Women ($n = 121$)	Differences P
Age (years)	50 (19–66)	57 (30–69)	<0.001
Previous IFN treatment	75 (42%)	40 (33%)	0.146
Hemoglobin (g/dl)	15.2 (11.5–17.8)	13.5 (11.2–15.1)	<0.001
Platelets ($\times 10^3/mm^3$)	176 (88–366)	165 (91–331)	0.025
Albumin (g/dl)	3.9 (3.2–4.6)	3.8 (3.0–4.6)	0.004
ALT (IU/l)	77 (23–504)	68 (19–391)	0.078
γ-GTP (IU/l)	78 (14–409)	37 (11–171)	0.011
LDL (mg/dl)	98 (50–176)	99 (57–168)	0.920
Ferritin (mg/l)	186 (<10–1,327)	95 (<10–4 42)	<0.001
ICG_{15} (%)	14 (4–41)	13 (2–31)	0.969
Stage (F0-1/F2-3)	80/66 (50 unknown)	42/55 (57 unknown)	0.050

ALT alanine aminotransferase, *γ-GTP* gamma-glutamyl transpeptidase, *LDL* low density lipoprotein, ICG_{15}, retention of indocyanine green at 15 min

[a] The means (ranges) are given

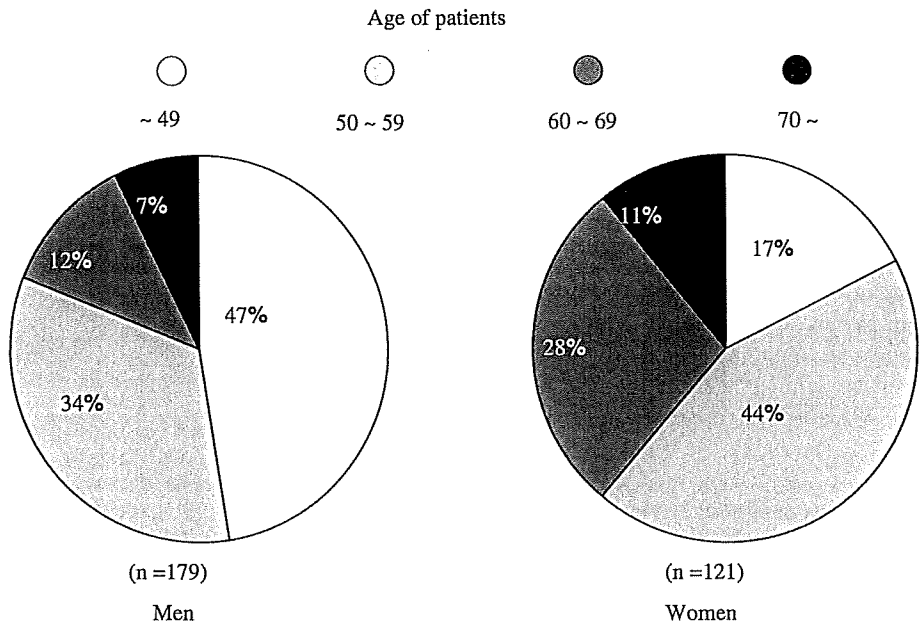

Fig. 1 Distribution of ages in the male and female patients with chronic hepatitis C who were infected with HCV-1b in high loads

Virological Response During the 48-Week Treatment with PEG-IFN and Ribavirin

On-treatment response to the combined treatment is compared between men and women in Fig. 2. Through 48 weeks on treatment, women gained a virological response less frequently than did men. ETR was achieved by 58% of women as against 83% of men ($P < 0.001$). Restricted to the patients who gained ETR, women lost HCV RNA from serum later than men did [median (range) 24.1 (2.0–36.4) vs 13.0 (2.0–48.0) weeks, $P < 0.001$]. Figure 3 depicts the on-treatment virological response in patients <50 years and those ≥50 years separately. The virological response was no different between men and women <50 years. However, it was poorer in women than

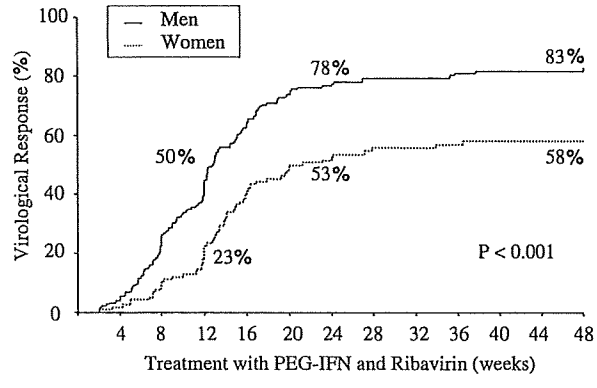

Fig. 2 On-treatment virological responses to PEG-IFN and ribavirin in male and female patients infected with HCV-1b in high viral loads

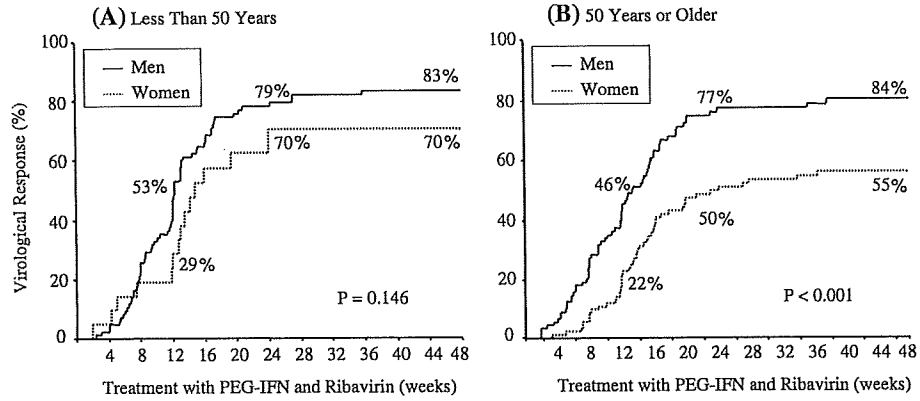

Fig. 3 On-treatment virological responses to combined IFN and ribavirin in male and female patients infected with HCV-1b in high viral loads who were less than 50 years (**a**) or 50 years or older (**b**)

in men ≥50 years. Differences between men and women in total (Fig. 2), therefore, were attributed to a poorer response of women ≥50 years to the combined treatment.

Sustained Virological Response to the 48-Week Treatment with PEG-IFN and Ribavirin

Sustained virological response 24 weeks after the completion of combined treatment was accomplished much less frequently in women than in men [33/121 (27%) vs 105/179 (59%), $P < 0.001$]. SVR was influenced by age both in men and in women (Fig. 4). It was found significantly less often in women than men who were 50 years or older.

Figure 5 illustrates the relationship between the earliest on-treatment virological response and SVR in men and women. Patients with a virological response at 4 weeks gained SVR invariably. However, in the patients with virological response in later weeks, SVR was achieved less frequently in women than in men. In the patients who had lost HCV RNA from the serum at 12 weeks, in particular, SVR was achieved significantly less often in women than in men (63% vs 88%, $P = 0.012$). The relationship

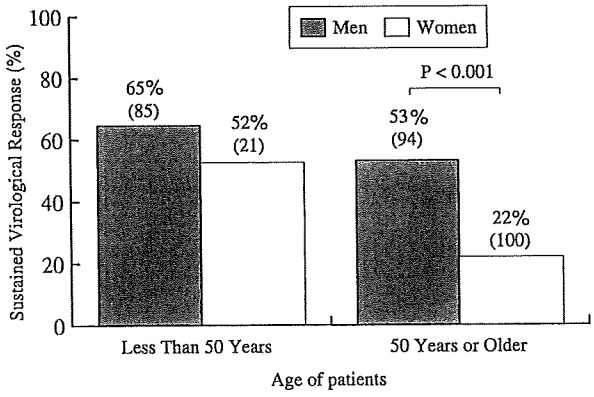

Fig. 4 Sustained virological response to PEG-IFN and ribavirin in male and female patients stratified by age. The number of patients is indicated in parentheses in each column

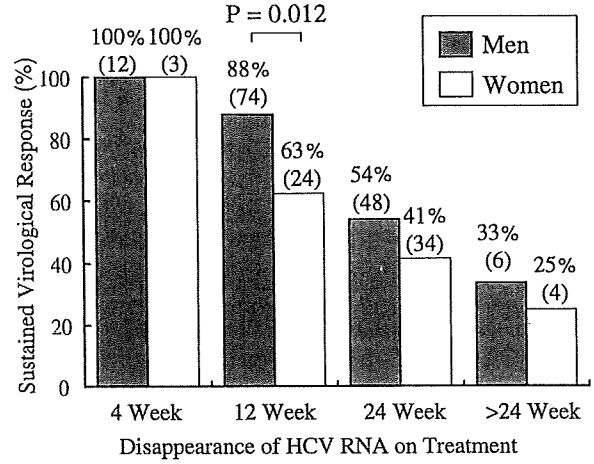

Fig. 5 Sustained virological response in male and female patients who lost HCV RNA from the serum at various weeks on treatment with PEG-IFN and ribavirin

between on-treatment virological response and SVR was compared among women in different age groups (Fig. 6). In the patients with the earliest virological response at 12 weeks and 24 weeks, SVR was achieved less frequently in women aged ≥50 years than in those <50 years, but the difference fell short of being significant due to the small numbers of patients in the comparison.

SVR and Compliance with PEG-IFN Therapy, Ribavirin Therapy, or Both

Table 2 compares compliance with the combined treatment between men and women. Either or both of PEG-IFN and ribavirin were tolerated to a lesser extent by women than by men. Thus, doses ≥80% were reached less frequently in women than in men for PEG-IFN or ribavirin, or both. The initial dose of ribavirin was no different between men and women.

SVR was achieved less frequently in women than in men who had received ≥80% of the dose of PEG-IFN

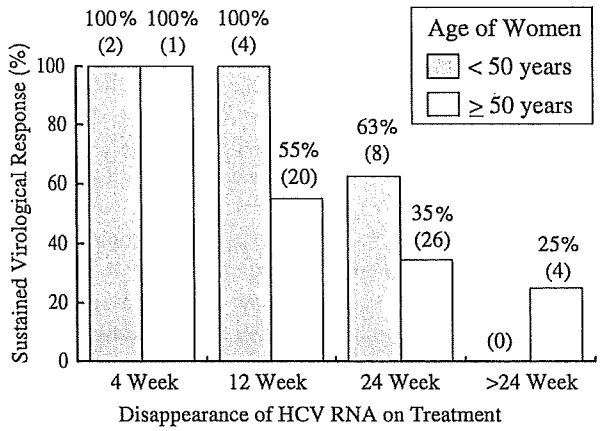

Fig. 6 Sustained virological response to PEG-IFN and ribavirin in female patients stratified by age who lost HCV RNA from serum at various weeks on treatment

Table 2 PEG-IFN and ribavirin received by patients with chronic hepatitis with high-titers of HCV-1b RNA

PEG-IFN and ribavirin	Men ($n = 179$)	Women ($n = 121$)	Differences P
Initial ribavirin dose (mg/kg body weight)	11.1 (5.0–14.1)	11.2 (3.7–14.3)	0.735
Total dose			
PEG-IFN ≥ 80%	139 (78%)	94 (53%)	<0.001
Ribavirin ≥ 80%	117 (65%)	42 (35%)	<0.001
Both ≥ 80%	110 (61%)	32 (27%)	<0.001
Withdrawn	28 (16%)	28 (23%)	0.131

[20/64 (31%) vs 92/138 (67%), $P < 0.001$], ribavirin [19/42 (45%) vs 79/120 (66%), $P = 0.027$] or both [13/32 (41%) vs 72/109 (66%), $P = 0.013$]. Again, differences were observed only in patients ≥50 years (Fig. 7). In the patients <50 years, in contrast, the rate of SVR was no different between women and men who had received ≥80% of the dose of PEG-IFN, ribavirin, or both.

The influence of age was compared between male and female patients in different age groups. SVR was achieved significantly more frequently in the men aged ≥60 years [88/145 (61%) vs 17/74 (37%), $P = 0.001$] and <60 years [17/34 (50%) vs 6/48 (13%), $P < 0.001$]. Likewise, SVR was more common in male than female patients aged 50–59 years [33/60 (55%) vs 16/53 (30%), $P = 0.013$].

Multivariate Analysis for Factors Accelerating the Response to PEG-IFN and Ribavirin Therapy

In univariate analysis, age, gender, hemoglobin, albumin, ICG_{15}, ribavirin dose and compliance with PEG-IFN therapy, ribavirin therapy, or both, influenced SVR. In multivariate analysis, only male gender, ICG_{15}, ribavirin dose and compliance with PEG-IFN, as well as both PEG-IFN and ribavirin, accelerated the chance of SVR (Table 3).

Discussion

In a retrospective study, response to PEG-IFN and ribavirin for 48 weeks was compared between 179 men and 121 women with chronic hepatitis C who had been infected with HCV-1b in high viral loads by the Japanese definition (>100 kIU/ml) [14, 15]. Loss of HCV RNA from serum occurred less often in women than in men throughout the 48 weeks of treatment. Both ETR (55% vs 83%, $P < 0.001$) and SVR (27% vs 59%, $P < 0.001$) were achieved significantly less frequently in women than in men. The observed low response to PEG-IFN and ribavirin stands at odds with the better response to antiviral treatments and slow progression of fibrosis in women than in men [9, 16, 17]. There are, however, viral and host factors other than gender that can influence the course of chronic hepatitis C and, by inference, the response to antiviral treatments.

Viral factors such as HCV genotypes and infection load affect the course of chronic hepatitis C. Thus, hepatitis is

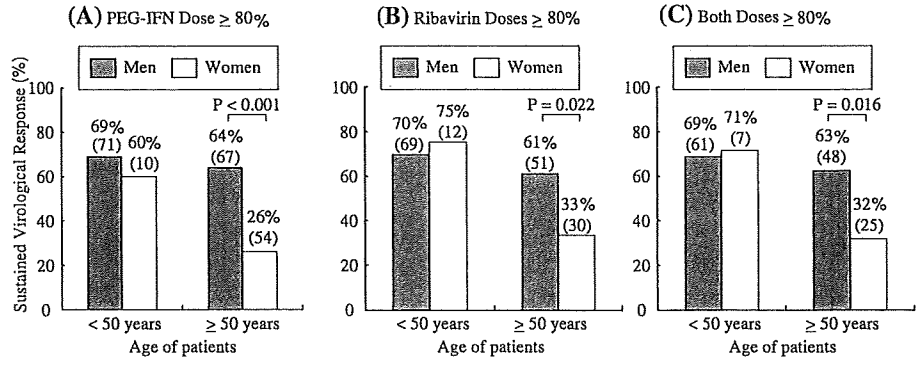

Fig. 7 Sustained virological response to PEG-IFN and ribavirin in patients who had received 80% or more of the dose of IFN (**a**), ribavirin (**b**) or both of them (**c**). Results are shown for men and women in two age groups

Table 3 Factors promoting the response to PEG-IFN and ribavirin in multivariate analysis

Factors	Odds ratio	95% Confidence interval	*P*
Male gender	3.50	1.71–7.17	0.001
$ICG_{15} \leq 13.5\%$	2.09	1.07–4.08	0.031
Ribavirin $\geq$ 11.1 mg/kg per day	2.17	1.11–4.25	0.024
Total PEG-IFN $\geq$ 80%	6.96	2.26–21.4	0.001
PEG-IFN/ribavirin $\geq$ 80%	12.66	2.32–71.4	0.003

more severe and less responsive to IFN in patients infected with HCV genotypes 1 and 4 than in those with HCV genotypes 2, 3 and 6 [18–22]. Likewise, high viral loads are associated with rapid progression of liver disease and poor response to IFN [23–25]. In our study, such viral factors were excluded in comparing the response to PEG-IFN and ribavirin between women and men. All the patients were infected with HCV genotype 1b in high viral loads (>100 kIU/ml).

Age influences the severity of chronic hepatitis C [9, 26], and disease progresses faster and response to antiviral therapy is poorer in older patients [23]. There were significant differences in age between female and male patients in our study. The women were older than the men [mean (range) 57 (30–69) years vs 50 (19–66) years, $P < 0.001$], and the proportion of patients $\geq$60 years was higher in women than in men (39% vs 19%, $P < 0.001$). Hence, the response to PEG-IFN and ribavirin was evaluated in patients aged $\geq$50 years and <50 years separately. There were no differences in the response between female and male patients <50 years, during and at the end of the 48-week treatment, as well as 24 weeks thereafter. However, ETR (55% vs 84%, $P < 0.001$) and SVR (22% vs 53%, $P < 0.001$) were gained significantly less often in women than men who were aged $\geq$50 years.

The influence of gender was observed, also, in patients aged $\geq$60 years and those aged 50–60 years. Hence, women would become less responsive than men to PEG-IFN and ribavirin after they had entered their fifties.

From a therapeutic notion, compliance with treatment can alter the response. Since ribavirin accumulates in erythrocytes and induces hemolysis, it is less tolerated in women who tend to be anemic than men without such an inclination [27]. At the baseline, women had lower levels of hemoglobin and ferritin than men. These would have been responsible for the lower tolerance to PEG-IFN and ribavirin in women than men in our study. In fact, $\geq$80% of the dose of PEG-IFN, ribavirin, or both, was tolerated less frequently in women than men ($P < 0.001$ for each). Even in the patients who had received $\geq$80% of the dose, however, the response to PEG-IFN and ribavirin was gained less frequently in women than in men. Again, the difference was due to a significantly lower response in female patients than in male patients aged $\geq$50 years, while the response was no different between those <50 years of age.

Taken altogether, the poorer response to PEG-IFN and ribavirin in women than in men was attributable to impaired response in the female patients aged $\geq$50 years. Older women with chronic hepatitis C, therefore, would be less responsive to the combined treatment with PEG-IFN and ribavirin currently in use. In support of this view, the response to human lymphoblastoid IFN for 24 weeks is dependent on gender and age [28]. The greatest physiological change precipitated in women by aging is a decreased serum concentration of bioavailable estrogen after they enter the menopause [29]. Estrogen has been shown to have an antifibrotic potential in both experimental and clinical studies. In experimental cirrhosis induced by dimethylnitrosamine in rats, administration of neutralizing antibodies to estradiol and ovariectomy enhanced fibrogenesis in female rats [30]. Hepatocytes have the receptor to estrogen [31], and myofibroblastic transformation in hepatic stellate cells of rats is inhibited in culture supplemented with this hormone [32]. Consequently, hepatic fibrosis progresses faster in menopausal women with chronic hepatitis C, and hormone replacement therapy may be able to prevent it [33]. Furthermore, in women aged $\geq$50 years, the number of estrogen receptor in hepatocytes decreases to one-half of that in those aged <50 years. This would stand in further support of the notion that the antifibrotic effects of decreased estrogen levels in patients aged $\geq$50 years with chronic hepatitis C would produce a lesser response to PEG-IFN and ribavirin.

Favorable effects of female sex hormones on hepatitis have long been suggested. Chronic hepatitis C is mild in menstruating women [34]; its activity is suppressed during pregnancy and enhanced after delivery [35]. The velocity of fibrosis progression is extremely low in young women exposed to HCV through mass-administration of immunoglobulin-D. Only two of 184 (1.2%) and four of 1,018 (0.4%) developed cirrhosis over 24 years and 20 years, respectively, in Irish and German studies [36, 37]. It does need to be pointed out, however, that the majority of women in those studies had not been followed beyond the menopause. There is a possibility that chronic hepatitis C may progress at a faster speed during their next few decades. Continued observations of them would be necessary to evaluate the validity of such an assumption.

Although decreased levels of estrogen can explain the enhanced activity of chronic hepatitis C in older women, as well as their concomitant resistance to PEG-IFN and ribavirin, it does not give an account of the better response in men than women who were aged $\geq$50 years. Feminization represented by gynecomastia is common in men

who have developed cirrhosis, and it can increase even in healthy men with age [38]. Possibly in the background of this phenomenon, circulating levels of free estrogen in men exceed those in women, after they enter their fifties, with margins widening with age [29]. It is tempting to speculate that elevated estrogen levels in men with chronic hepatitis C are responsible for their better response to the combination therapy than women who were aged ≥50 years. Whether or not such a speculation would hold would have to be evaluated by a comparison of estrogen levels between older men and women with chronic hepatitis C.

Although osteoporosis is an extrahepatic manifestation of chronic hepatitis C [39], hormone replacement therapy has been withheld for fear of potential hepatotoxicity. There is evidence, however, that oral contraceptives inhibit the progression of fibrosis in women [33]. It may lead to the possibility that the response to antiviral treatment in older women with chronic hepatitis C would be improve by substituting estrogen in them. The merit of hormone replacement therapy for them, of course, would need to be balanced against any harmful effects associated with it.

There are limitations in this study. All the patients were infected with genotype 1b in high viral loads. Hence, the results obtained may or may not be extended to patients with chronic hepatitis C who are infected with HCV of other genotypes in low viral loads. The influence of sex hormones needs to be substantiated by their determination in correlation with SVR. These limitations notwithstanding, the results obtained warrant a special caution in the treatment of women older than 50 years due to their lesser responsiveness to PEG-IFN and ribavirin.

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References

1. Cohen J. The scientific challenge of hepatitis C. *Science.* 1999;285:26–30. doi:10.1126/science.285.5424.26.
2. Alberti A, Chemello L, Benvegnu L. Natural history of hepatitis C. *J Hepatol.* 1999;31:S17–S24. doi:10.1016/S0168-8278(99)80369-9.
3. Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis.* 2000;20:17–35. doi:10.1055/s-2000-9505.
4. Seeff LB. Natural history of chronic hepatitis C. *Hepatology.* 2002;36:S35–S46. doi:10.1002/hep.1840360706.
5. Feray C, Gigou M, Samuel D, et al. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. *Gastroenterology.* 1995;108:1088–1096. doi:10.1016/0016-5085(95)90207-4.
6. Garcia-Samaniego J, Soriano V, Castilla J, et al. Influence of hepatitis C virus genotypes and HIV infection on histological severity of chronic hepatitis C. *Am J Gastroenterol.* 1997;92:1130–1134.
7. Pageaux GP, Ducos J, Mondain AM, et al. Hepatitis C virus genotypes and quantitation of serum hepatitis C virus RNA in liver transplant recipients: relationship with severity of histological recurrence and implications in the pathogenesis of HCV infection. *Liver Transpl Surg.* 1997;3:501–505. doi:10.1002/lt.500030504.
8. Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med.* 1999;340:1228–1233. doi:10.1056/NEJM199904223401602.
9. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet.* 1997;349:825–832. doi:10.1016/S0140-6736(96)07642-8.
10. Vogt M, Lang T, Frosner G, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. *N Engl J Med.* 1999;341:866–870. doi:10.1056/NEJM199909163411202.
11. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med.* 1998;339:1485–1492. doi:10.1056/NEJM199811193392101.
12. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med.* 2002;347:975–982. doi:10.1056/NEJMoa020047.
13. Hung CH, Lee CM, Lu SN, et al. Anemia associated with antiviral therapy in chronic hepatitis C: incidence, risk factors, and impact on treatment response. *Liver Int.* 2006;26:1079–1086. doi:10.1111/j.1478-3231.2006.01354.x.
14. Akuta N, Suzuki F, Kawamura Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol.* 2007;79:1686–1695. doi:10.1002/jmv.20979.
15. Sezaki H, Suzuki F, Kawamura Y, et al. Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatol Res.* 2007;37:787–792. doi:10.1111/j.1872-034X.2007.00132.x.
16. Conjeevaram HS, Fried MW, Jeffers LJ, et al. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology.* 2006;131:470–477. doi:10.1053/j.gastro.2006.06.008.
17. 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;352:1426–1432. doi:10.1016/S0140-6736(98)07124-4.
18. 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.
19. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med.* 2004;140:346–355.
20. Hui CK, Yuen MF, Sablon E, Chan AO, Wong BC, Lai CL. Interferon and ribavirin therapy for chronic hepatitis C virus genotype 6: a comparison with genotype 1. *J Infect Dis.* 2003;187:1071–1074. doi:10.1086/368217.
21. Legrand-Abravanel F, Nicot F, Boulestin A, et al. Pegylated interferon and ribavirin therapy for chronic hepatitis C virus genotype 4 infection. *J Med Virol.* 2005;77:66–69. doi:10.1002/jmv.20414.
22. Yuen MF, Lai CL. Response to combined interferon and ribavirin is better in patients infected with hepatitis C virus genotype 6

than genotype 1 in Hong Kong. *Intervirology*. 2006;49:96–98. doi:10.1159/000087270.
23. Kumada T, Toyoda H, Honda T, et al. Treatment of chronic hepatitis C with interferon alone or combined with ribavirin in Japan. *Intervirology*. 2006;49:112–118. doi:10.1159/000087273.
24. Liang TJ, Rehermann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med*. 2000;132:296–305.
25. Trepo C. Genotype and viral load as prognostic indicators in the treatment of hepatitis C. *J Viral Hepat*. 2000;7:250–257. doi: 10.1046/j.1365-2893.2000.00233.x.
26. Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. *Gut*. 2002;51:248–252. doi:10.1136/gut.51.2.248.
27. Takaki S, Tsubota A, Hosaka T, et al. Factors contributing to ribavirin dose reduction due to anemia during interferon alfa2b and ribavirin combination therapy for chronic hepatitis C. *J Gastroenterol*. 2004;39:668–673. doi:10.1007/s00535-003-1363-9.
28. Hayashi J, Kishihara Y, Ueno K, et al. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. *Arch Intern Med*. 1998;158:177–181. doi: 10.1001/archinte.158.2.177.
29. Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL. Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. *J Clin Endocrinol Metab*. 1998;83:2266–2274. doi:10.1210/jc.83.7.2266.
30. Yasuda M, Shimizu I, Shiba M, Ito S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology*. 1999;29:719–727. doi:10.1002/hep.510290307.
31. Rossini GP, Baldini GM, Villa E, Manenti F. Characterization of estrogen receptor from human liver. *Gastroenterology*. 1989;96:1102–1109.
32. Shiba M, Shimizu I, Yasuda M, Ii K, Ito S. Expression of type I and type III collagens during the course of dimethylnitrosamine-induced hepatic fibrosis in rats. *Liver*. 1998;18:196–204.
33. Di Martino V, Lebray P, Myers RP, et al. Progression of liver fibrosis in women infected with hepatitis C: long-term benefit of estrogen exposure. *Hepatology*. 2004;40:1426–1433. doi: 10.1002/hep.20463.
34. Sartori M, Andorno S, Rigamonti C, Grossini E, Nicosia G, Boldorini R. Chronic hepatitis C is mild in menstruating women. *J Gastroenterol Hepatol*. 2000;15:1411–1417. doi:10.1046/j.1440-1746.2000.02368.x.
35. Latt NC, Spencer JD, Beeby PJ, et al. Hepatitis C in injecting drug-using women during and after pregnancy. *J Gastroenterol Hepatol*. 2000;15:175–181. doi:10.1046/j.1440-1746.2000.02060.x.
36. Levine RA, Sanderson SO, Ploutz-Snyder R, et al. Assessment of fibrosis progression in untreated Irish women with chronic hepatitis C contracted from immunoglobulin anti-D. *Clin Gastroenterol Hepatol*. 2006;4:1271–1277. doi:10.1016/j.cgh.2006.05.028.
37. Wiese M, Berr F, Lafrenz M, Porst H, Oesen U. Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in Germany: a 20-year multicenter study. *Hepatology*. 2000; 32:91–96. doi:10.1053/jhep.2000.8169.
38. Braunstein GD. Gynecomastia. *N Engl J Med*. 1993;328:490–495. doi:10.1056/NEJM199302183280708.
39. Carey EJ, Balan V, Kremers WK, Hay JE. Osteopenia and osteoporosis in patients with end-stage liver disease caused by hepatitis C and alcoholic liver disease: not just a cholestatic problem. *Liver Transpl*. 2003;9:1166–1173. doi:10.1053/jlts.2003.50242.

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CLINICAL STUDIES

Predictive factors of advanced recurrence after curative resection of small hepatocellular carcinoma

Tetsuya Hosaka, Kenji Ikeda, Masahiro Kobayashi, Miharu Hirakawa, Yusuke Kawamura, Hiromi Yatsuji, Hitomi Sezaki, Norio Akuta, Fumitaka Suzuki, Yoshiyuki Suzuki, Satoshi Saitoh, Yasuji Arase and Hiromitsu Kumada

Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan

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Correspondence
Tetsuya Hosaka, MD, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan
Tel: +81 44 877 5111
Fax: +81 44 860 1623
e-mail: hosa-p@toranomon.gr.jp

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Abstract

Background: The tumour recurrence rate after resection is still high even in patients with small hepatocellular carcinoma (HCC). The advanced patterns of recurrence occasionally occur after resection. In this study, we analysed the clinical and histological characteristics of small HCC and evaluated the predictive factors of advanced tumour recurrence. *Methods:* One hundred and sixty-five patients underwent resection of small HCC measuring 3 cm or less in greatest dimension. Patterns of tumour recurrences were classified into advanced recurrence and minor recurrence based on size, number, vascular invasion and extrahepatic metastasis of recurrent tumour. We created a simple index to closely evaluate the malignant potential of small HCC, named α-foetoprotein–size ratio index (ASRI). *Results:* Overall tumour recurrence was significantly associated with tumour multiplicity ($P < 0.001$) and ASRI ($P = 0.001$). Tumour multiplicity, ASRI and tumour differentiation were independent and significant predictive factors of advanced recurrences. The overall survival rates were lower in the advanced recurrence group than the minor recurrence or the no recurrence group. *Conclusions:* Patients with advanced recurrences have a poor prognosis, although they have undergone curative resection of small HCC. On the other hand, patients with minor recurrences have a relatively good prognosis. ASRI was a useful index to predict advanced recurrence after curative resection of small HCC. The therapeutic management to prevent advanced recurrences is needed.

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in Africa and Asia, including Japan. Routine checkups are performed in patients with hepatitis or cirrhosis who constitute a significant high-risk group for HCC (1–3). Recently, technological advances in ultrasonography (US), computed tomography (CT) and magnetic resonance imaging have helped in the detection of small HCC during follow-up periods of chronic liver disease (4, 5). Moreover, resection of HCC has become safe in cirrhotic patients due to progress in surgical techniques, and perioperative management has contributed to very low operative mortality. However, the tumour recurrence rate after resection is still high even in patients with small HCCs (6–10). Recurrences in the remnant liver can occur based on two characteristics of HCC: intrahepatic metastasis from the primary tumour and de novo multicentric carcinogenicity (11–13).

Tumour status at the time of recurrence is important to improve prognosis because tumour recurrence rates after curative resection are high. The advanced patterns of recurrence occasionally occur as follows: widespread recurrence, a number of recurrent tumours, large recurrent tumour, involving vascular invasion and extrahepatic metastasis, despite curative resection (14–16). Because the therapeutic approach for recurrent tumours is limited, these cases have a poor prognosis. Therefore, it is important to pick up patients who are likely to have these advanced recurrence, and to develop effective adjuvant therapy. In the present study, we examined the clinical features of small HCC, and identified the factors associated with tumour recurrence, especially advanced recurrence and prognosis after curative resection of small HCCs using clinical data and results of histopathological examination. Furthermore, we created a simple index to closely evaluate the malignant potential of small HCC and evaluated the usefulness of this index as a predictor of recurrence of HCC after curative resection.

Patients and methods

Patients

Medical records of patients who were hospitalized at Toranomon Hospital from 1995 to 2005 were reviewed retrospectively. HCC was diagnosed by detailed imaging or histopathological examination. A total of 251 consecutive patients with tumours underwent resection as the initial therapy for HCC, and 165 of these patients were found to have HCC measuring ≤ 3 cm (greatest dimension) and were eligible for inclusion in this study. These 165 patients (127 men and 38 women; median age 61 years; range, 38–73 years) had chronic hepatitis or cirrhosis. Hepatitis B virus (HBV) surface antigen was positive in 33, anti-hepatitis C virus (HCV) was positive in 127, but neither of them was positive in eight. Table 1 lists the clinical characteristics of the 165 patients before hepatectomy. Of these, 125 patients (75.6%) were classified as grade A according to Child–Pugh classification. The median value for the indocyanine green retention rate at 15 min was 24%, and the median values for serum albumin, bilirubin, aspartic transaminase (AST), α-foetoprotein (AFP) concentration and platelet counts were 3.7 g/dl, 1.0 mg/dl, 44 IU/L, 26 ng/ml and $10.8 \times 10^4/mm^3$ respectively.

Among 165 patients, 26 patients (15.8%) had multiple tumours before resection. We conducted percutaneous ablation therapy, including ethanol injection, microwave coagulation

Table 1. Clinical characteristics of 165 patients before hepatic resection

Variables	$n = 165$
Age	62 (38–80)*
Gender (male:female)	127:38
Hepatitis B surface antigen-positive	46 (27.9%)
Anti-hepatitis C virus-positive	109 (66.1%)
Child–Pugh classification (A:B:C)	125:38:1
Serum albumin (g/dl)	3.6 (2.6–4.6)*
Serum bilirubin (mg/dl)	1.0 (0.3–2.7)*
Aspartate transaminase (IU/L)	44 (12–386)*
Prothorombin time (%)	90.8 (58.9–112.8)*
ICG R15 (%)	21 (8–68)*
Platelet count (10^4/mm^3)	12.6 (3.9–26.0)*
α-foetoprotein (ng/ml)	23 (1–7960)*
Des-γ-carboxy prothorombin (mAU/ml)	22 (< 10–1650)*
Tumour size (mm)	20 (7–30)*
Tumour number (solitary:multiple)	139:26
Vascularity positive	153 (92.7%)
ASRI	1.2 (0.03–345)*

*Values are medians (range).
ASRI, α-foetoprotein–size ratio index = AFP (ng/ml)/tumour size (mm); ICG R15, indocyanine green retention test at 15 min.

and radiofrequency ablation, for another tumour before surgery if another tumour existed in a lobe distant from the resected tumour. The term 'curative resection' indicated that no tumours were left in the remnant liver irrespective of the width of margin around the tumour; this was confirmed using (i) intra-operative US and (ii) combined US and dynamic CT conducted after 1 month of surgery.

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and its subsequent amendments, and informed consent was obtained from every patient. This study was approved by the Local Ethics Committee of Toranomon Hospital.

Follow-up and recurrence of hepatocellular carcinoma

Patients were followed up on a monthly or a bi-monthly basis after surgery by monitoring AFP and other biochemical data, and conducting US or helical dynamic CT every 3 months. The median observation period for the entire patient cohort was 6.0 years, with a range of 0.3–16.4 years. Recurrence of HCC was diagnosed by typical hypervascular characteristics on angiography and/or histological examination with fine needle biopsy specimens, in addition to certain features of CT and US.

The modes of cancer recurrence were classified into two categories: (i) advanced recurrence and (ii) minor recurrence. The patterns of recurrence were morphologically judged from the images of CT and angiography, and from histopathological findings. The pattern of recurrent tumour number > 3, tumour size > 3 cm, involving vascular invasion and/or extrahepatic metastasis was defined as advanced recurrence. The recurrent pattern, except for those described above, was defined as minor recurrence.

Imaging analysis

Ultrasonography or helical dynamic CT was carried out every 3 months for follow-up and examined for a change in imaging findings. Dynamic CT scans were performed using a single-detector helical CT scanner (Hi-Speed advantage SG; GE Yokogawa Medical Systems, Tokyo, Japan). The radiological studies included intra-arterial digital subtraction angiography (celiac and mesenteric angiography) and selective angiography of the common hepatic artery. CT arterial portography (CT-AP) and CT hepatic angiography (CT-HA) were carried out in almost all patients before surgery. HCC was diagnosed by typical hypervascular characteristics on angiography and/or CT-HA, and hypo-attenuation on CT-AP. If hepatic nodules showed iso-hypo-attenuation on CT-HA and iso-hypo-attenuation on CT-AP, histological examination was carried out with fine needle biopsy specimens before surgery.

Table 2. Pathological characteristics of small hepatocellular carcinoma

Variables	$n = 165$
Tumour differentiation (early:well:moderately:poorly)	11:32:100:22
Growth type (Eg:Ig)	138:27
Capsular formation	99 (60.0%)
Capsular infiltration	52 (31.5%)
Septum formation	42 (25.5%)
Portal vein invasion	26 (15.8%)
Intrahepatic extent of tumour	5 (3.0%)
Presence of cirrhosis	114 (69.1%)

Eg, expansive growth (well-demarcated border); Ig, infiltrative growth (poorly demarcated border).

Histopathological examination

Macroscopic and microscopic examinations were performed according to the classification of the Liver Cancer Study Group of Japan (17). All resected specimens were analysed histopathologically for tumour size, growth type, tumour differentiation, capsular formation, portal vein invasion, satellite nodules and fibrosis staging of surrounding liver. The tumour characteristics are summarized in Table 2. We categorized well-differentiated HCC that had histological features of the early stage into early HCC. Early HCC was defined as follows: macroscopically, the tumours had an indistinct margin that replaced the liver cell cords at the tumour–non-tumour boundary; microscopically, increased cell density with an increased nuclear to cytoplasm ratio and an irregular thin-trabecular pattern, and the portal tracts were involved inside the tumours together with tumour cell invasion into the portal tracts (18–20).

α-foetoprotein–size ratio index

In this study, there were patients with very high AFP levels regardless of the cohort of small HCC measuring 3 cm or less in greatest dimension. We hypothesized that HCCs with high AFP levels had more malignant potential than those with low AFP levels if each tumour size was equal. And so, we created a simple index to closely evaluate the malignant potential of small HCC, named the AFP–size ratio index (ASRI). The numerical formula of ASRI was defined as follows: ASRI = AFP levels (ng/ml)/tumour size (mm). For example, the calculated value of ASRI of HCC, with tumour size = 20 mm and AFP levels = 400 ng/ml, is 20.

Statistical analysis

Standard statistical measures and procedures were used. We used the χ^2-test to assess the significant association of risk

factors with tumour recurrence after resection. All factors found to be at least marginally associated with recurrence ($P < 0.15$) were tested by multivariate analysis. Independent factors, associated with the recurrence of HCC and prognosis, were calculated using stepwise Cox regression analysis. The χ^2-test was used to analyse differences between the clinical characteristics of HCC and the patterns of tumour recurrences. The cumulative overall survival rates after resection of small HCC were analysed using the Kaplan–Meier method, and differences in the curves were tested using the log-rank test. A P value of < 0.05 in a two-tailed test was considered significant. Data analysis was performed using the SPSS software, version 11.0 (Chicago, IL, USA).

Results

Factors associated with tumour recurrences

Univariate analysis showed that tumour recurrence was significantly associated with tumour multiplicity ($P < 0.001$), ASRI ≥ 20 ($P = 0.004$), AFP levels ≥ 1000 ng/ml ($P = 0.024$), portal vein invasion ($P = 0.035$) and serum albumin levels ≥ 3.5 g/dl ($P = 0.041$), and marginally significantly with HCV positivity ($P = 0.058$), HBV negativity ($P = 0.072$), hypervascularity of tumour ($P = 0.076$) and serum AST levels ≥ 50 IU/L ($P = 0.088$) (Table 3). Because these variables were associated, multivariate analysis was performed using the nine variables mentioned above in the model (Table 4a). The following two variables were significantly associated with overall tumour recurrence: tumour multiplicity [hazard ratio (HR) 3.06, 95% confidence interval (CI): 1.84–5.10; $P < 0.001$], ASRI ≥ 20 (HR 2.42, 95% CI: 1.41–4.18, $P = 0.001$). To evaluate risk factors except for tumour multiplicity, subgroup analysis was conducted in solitary tumour cases (Table 4b). Independent risk factors affecting the overall recurrence of HCC were the presence of portal vein invasion (HR 2.35, 95% CI: 1.31–4.20, $P = 0.004$), ASRI ≥ 20 (HR 2.23, 95% CI: 1.19–4.18, $P = 0.013$) and serum albumin < 3.5 g/dl (HR 1.74, 95% CI: 1.05–2.88, $P = 0.030$).

Predictive factors of advanced recurrences after curative resection

Tumour recurrence was diagnosed in 102 (61.8%) of the 165 patients, with a median interval of 2.77 years after curative resection. Of these, 22 (13.3%) were categorized into advanced recurrence, 80 (48.4%) were minor recurrence and the remaining 63 (38.1%) were no recurrence. The median interval to recurrence after resection was 1.82 years in the minor recurrence group and 1.01 years in the advanced recurrence group respectively. Univariate analysis showed that advanced recurrence was significantly associated with the following four factors: poorly differentiation of tumour ($P < 0.001$), ASRI ≥ 20 ($P = 0.005$), tumour multiplicity ($P = 0.017$) and AFP levels ≥ 1000 ng/ml ($P = 0.025$) (Table 5). Multivariate analysis by the Cox model was performed using the four variables mentioned above. Predictive factors of advanced recurrences after curative resection were tumour multiplicity (HR 5.65, 95% CI: 1.77–18.1, $P = 0.003$), ASRI ≥ 20 (HR 4.04, 95% CI: 1.16–14.1, $P = 0.028$) and poor differentiation of tumour (HR 2.70, 95% CI: 1.51–4.82, $P = 0.001$) (Table 6).

We compared values of ASRI by patterns of recurrences (Fig. 1). The median values of ASRI were 0.68 (minimum: 0.07–maximum: 73.0) in the no recurrence group, 1.64 (0.06–344) in the minor recurrence group and 3.28 (0.03–318) in the advanced recurrence group respectively. The values of ASRI were marginally significantly higher in the minor recurrence and the advanced recurrence group than in the no recurrence group. However, there was no significance of ASRI values stratified by tumour number.

Furthermore, we categorized the following three subgroups into the advanced recurrence group: tumour number > 3, or tumour size > 3 cm without vascular invasion and extrahepatic metastasis (multi/large nodular recurrence group), recurrent tumour with vascular invasion (vascular invasion group) and

Table 3. Factors associated with overall recurrence of small hepatocellular carcinoma by univariate analysis

Factors	Hazard ratio (95% CI)	*P*
Age (≥65 vs. < 65 years)	0.79 (0.52–1.22)	0.288
Gender (female vs. male)	0.78 (0.48–1.26)	0.316
HBV (negative vs. positive)	1.52 (0.96–2.41)	0.072
HCV (positive vs. negative)	1.53 (0.99–2.36)	0.058
Serum albumin (< 3.5 vs. ≥3.5 g/dl)	1.53 (1.02–2.31)	0.041
Serum bilirubin (≥1.5 vs. < 1.5 mg/dl)	1.11 (0.62–2.00)	0.713
AST levels (≥50 vs. ≥50 IU/L)	1.41 (0.95–2.10)	0.088
Prothorombin time (≥70 vs. < 70%)	0.67 (0.31–1.45)	0.311
ICG R 15 (≥30 vs. < 30%)	1.37 (0.89–2.12)	0.158
count (≥10^5 vs. < 10^5/mm^3)	0.81 (0.54–1.22)	0.304
AFP levels (≥1000 vs. < 1000 ng/ml)	2.01 (1.10–3.67)	0.024
ASRI (≥20 vs. < 20)	2.16 (1.28–3.64)	0.004
DCP levels (≥100 vs. < 100 mAU/ml)	1.19 (0.70–2.04)	0.517
Fibrosis stage (F4 vs. F1, 2, 3)	1.09 (0.72–1.66)	0.681
Tumour size (≥21 vs. < 21 mm)	1.088 (0.73–1.63)	0.680
Tumour number (multiple vs. solitary)	2.85 (1.74–4.65)	< 0.001
Vascularity (positive vs. negative)	2.48 (0.91–6.76)	0.076
Tumour differentiation (poorly vs. early, well, moderately)	1.15 (0.87–1.51)	0.333
Eg	1.00 (0.60–1.68)	0.987
Capsular formation	1.01 (0.68–1.52)	0.948
Infiltration to capsular	1.39 (0.92–2.10)	0.121
Septum formation	0.99 (0.63–1.56)	0.969
Portal vein invasion	1.70 (1.04–2.78)	0.035
Intrahepatic extent of tumour	1.57 (0.58–4.26)	0.380

AFP, α-foetoprotein; ASRI, α-foetoprotein–size ratio index; AST, aspartic transaminase; DCP, des-γ-carboxy prothorombin; Eg, expansive growth (well-demarcated border); HBV, hepatitis B virus; HCV, hepatitis C virus; ICG R15, indocyanine green retention test at 15 min.

Table 4a. Independent risk factors affecting the overall recurrence of hepatocellular carcinoma after curative resection of small hepatocellular carcinoma by multivariate analysis

Factors	Category	Hazard ratio (95% CI)	*P* value
Tumour number	1: solitary	1	
	2: multiple	3.06 (1.84–5.10)	< 0.001
ASRI	1: < 20	1	
	2: ≥20	2.42 (1.41–4.18)	0.001

ASRI, α-foetoprotein–size ratio index; CI, confidence interval.

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Table 4b. Independent risk factors affecting the overall recurrence of hepatocellular carcinoma after curative resection of small hepatocellular carcinoma by multivariate analysis (solitary cases only)

Factors	Category	Hazard ratio (95% CI)	*P* value
Portal vein invasion	1: −	1	
	2: +	2.35 (1.31–4.20)	0.004
ASRI	1: < 20	1	
	2: ≥20	2.23 (1.19–4.18)	0.013
Serum albumin	1: ≥3.5	1	
	2: < 3.5	1.74 (1.05–2.88)	0.030

ASRI, α-foetoprotein–size ratio index; CI, confidence interval.

Table 5. Univariate analysis for clinical factors associated with advanced recurrence

Factors	Advanced recurrence, *n* = 22 (%)	Minor recurrence, *n* = 80 (%)	No recurrence, *n* = 63 (%)
Age			
< 65 years	13 (59)	59 (73.8)	35 (55.6)
≥65 years	9 (41)	21 (26.2)	28 (44.4)
Gender			
Male	19 (86.4)	62 (77.5)	46 (73)
Female	3 (13.6)	18 (22.5)	17 (27)
HBV			
Positive	5 (22.7)	19 (23.8)	22 (34.9)
Negative	17 (77.3)	61 (76.2)	41 (65.1)
HCV			
Negative	5 (22.7)	23 (28.8)	28 (44.4)
Positive	17 (77.3)	57 (71.2)	35 (55.6)
Serum albumin			
≥3.5	11 (50)	49 (61.3)	41 (65.1)
< 3.5	11 (50)	31 (39.7)	22 (34.9)
Serum bilirubin			
< 1.5	21 (95.5)	67 (83.8)	56 (88.9)
≥1.5	1 (4.5)	13 (16.2)	7 (11.1)
AST levels			
< 50	17 (77.3)	40 (50)	42 (66.7)
≥50	5 (22.7)	40 (50)	21 (33.3)
Prothorombin time			
< 70	2 (9.1)	5 (6.3)	14 (22.2)
≥70	20 (90.9)	75 (93.7)	49 (77.8)
ICG R 15			
< 30	15 (68.2)	58 (72.5)	48 (76.2)
≥30	7 (31.8)	22 (27.5)	15 (23.8)
Platelet count			
$< 10^5$	7 (31.8)	28 (35)	30 (47.6)
$\geq 10^5$	15 (68.2)	52 (65)	33 (52.4)
AFP levels			
< 1000	17 (77.3)	73 (91.3)	61 (96.8)
≥1000	5 (22.7)*	7 (8.7)	2 (3.2)
ASRI			
< 20	15 (68.2)	70 (87.5)	60 (95.2)
≥20	7 (31.8)*	10 (12.5)	3 (4.8)
DCP levels			
< 100	18 (81.8)	68 (85)	54 (85.7)
≥100	4 (18.2)	12 (15)	9 (14.3)
Fibrosis stage			
F1, 2, 3	9 (41)	23 (28.8)	19 (31.7)
F4	13 (59)	57 (71.2)	41 (68.3)
Tumour size			
< 21	11 (50)	53 (66.3)	37 (58.7)
≥21	11 (50)	27 (33.7)	26 (41.3)
Tumour number			
Solitary	14 (63.6)	67 (83.8)	58 (92.1)
Multiple	8 (36.4)*	13 (16.2)	5 (7.9)
Vascularity			
Negative	1 (4.5)	3 (3.8)	8 (12.7)
Positive	22 (95.5)	77 (96.2)	55 (87.3)
Tumour differentiation			
Early, well, moderately	13 (59.1)	74 (92.5)	56 (88.9)
Poorly	9 (40.9)*	6 (7.5)	7 (11.1)
Eg			
Eg	21 (95.5)	63 (78.8)	54 (85.7)
Ig	1 (4.5)	17 (21.2)	9 (14.3)
Capsular formation			
Absence	6 (27.3)	33 (41.3)	22 (34.9)
Presence	16 (72.7)	47 (58.7)	41 (65.1)
Infiltration to capsular			
Absence	13 (59.1)	54 (67.9)	46 (73)
Presence	9 (40.9)	26 (32.1)	17 (27)
Septum formation			
Absence	16 (72.7)	61 (76.2)	46 (73)
Presence	6 (27.3)	19 (23.8)	17 (27)
Portal vein invasion			
Absence	17 (77.3)	65 (81.3)	54 (85.7)
Presence	5 (22.7)	15 (18.7)	6 (14.3)
Intrahepatic extent of tumour			
Absence	20 (90.9)	78 (97.5)	59 (98.3)
Presence	2 (9.1)	2 (2.5)	1 (1.7)

*Significantly higher than the other groups ($P < 0.05$).

AFP, α-foetoprotein; ASRI, α-foetoprotein–size ratio index; AST, aspartic transaminase; DCP, des-γ-carboxy prothorombin; Eg, expansive growth (well-demarcated border); HBV, hepatitis B virus; HCV, hepatitis C virus; ICG R15, indocyanine green retention test at 15 min; Ig, infiltrative growth (poorly demarcated border).

Table 6. Predictive factors of advanced recurrence after curative resection by multivariate analysis using the Cox model

Factors	Category	Hazard ratio (95% CI)	*P* value
Tumour number	1: solitary	1	
	2: multiple	5.65 (1.77–18.1)	0.003
ASRI	1: < 20	1	
	2: ≥20	4.04 (1.16–14.1)	0.028
Tumour		differentiation	1: early, well, moderately
	1		
2: poorly		(1.51–4.82)	0.001
	2.70		

ASRI, α-foetoprotein–size ratio index; CI, confidence interval.

presence of extrahepatic metastasis (extrahepatic metastasis group). The multi/large nodular recurrence group had 17 cases (77.3%), the vascular invasion group had three (13.6%) and the

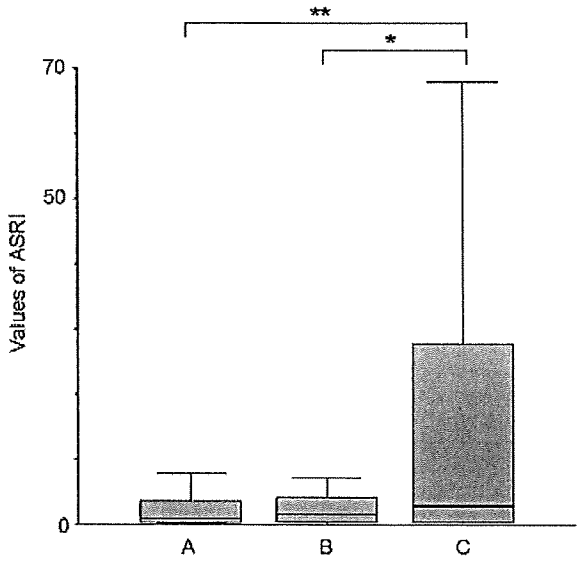

Fig. 1. Comparison with values of ASRI by patterns of recurrences. (A) No recurrence group, (B) minor recurrence group, (C) advanced recurrence group. $^{*}P=0.032$, $^{**}P=0.028$.

extrahepatic metastasis group had two (9.1%) in 22 cases of advanced recurrence. In particular, patients in the vascular invasion group had significantly higher pre-operative des-γ-carboxy prothorombin levels than those in the other two groups ($P=0.008$). Meanwhile, there was no significant difference of ASRI among the three groups.

Survival rate after curative resection by patterns of recurrences

Figure 2 shows the overall survival rates by patterns of recurrences. The overall survival rates of patients were 98.5, 93.6 and 91.8% for the first, third and fifth year in the no recurrence group; 98.8, 96.5 and 85.6% in the minor recurrence group; and 91.3, 64.5 and 35.1% in the advanced recurrence group respectively. The overall survival rates of the advanced recurrence group were significantly lower than those of the minor recurrence and the no recurrence groups (advanced recurrence vs. no recurrence: $P < 0.0001$, advanced recurrence vs. minor recurrence: $P=0.001$). Furthermore, the overall survival rates of the minor recurrence group were significantly lower than those of the no recurrence group ($P=0.009$). However, the overall survival rates of both the minor recurrence and the no recurrence groups were similar for the first 5 years after surgery.

Discussion

Our study identified the clinical, radiological and histological factors associated with advanced tumour recurrence and prognosis after curative resection of small HCC. Predictive factors of advanced recurrence were tumour number, ASRI and tumour differentiation. ASRI, which was made to reflect the malignant potential of HCC precisely, was easy to calculate and useful to predict the overall and advanced recurrence of HCC. Patients in the advanced recurrence group had a poorer prognosis than those in the minor recurrence and the no recurrence groups. On the other hand, patients in the minor recurrence group had a

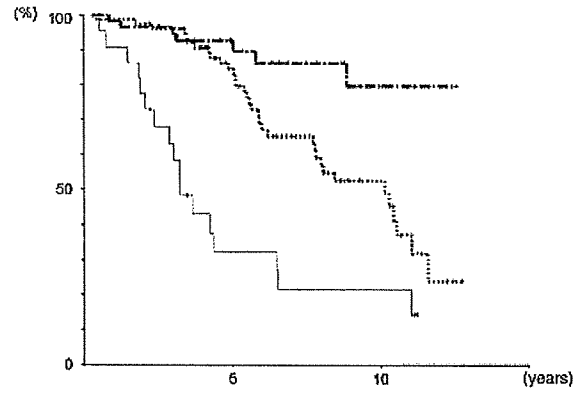

Fig. 2. Overall survival rates by patterns of recurrences; thick broken line: no recurrence group, dot line: minor recurrence group, solid line: advanced recurrence group.

prognosis similar to that of the no recurrence group for the first 5 years after resection.

Some predictors of survival and recurrence after resection were reported previously (21–24). These reports showed that the main predictors of recurrence were tumour size, tumour number, serum AFP levels, tumour differentiation, vascular invasion, etc. In the present study, we intended for patients with small HCC within 3 cm to pick up cases with high malignant potential. Therefore, tumour size was not associated with recurrence, but the other factors mentioned above were associated with recurrence as well as previous reports. However, we recently showed that ASRI was associated with both overall and advanced recurrence after resection. Small HCC with a high ASRI value may have a high malignant potential and may be likely to cause intra- or extrahepatic metastasis.

The high recurrence rate of HCC after curative resection and ablation is attributable to two principal characteristics: intrahepatic metastasis and de novo multicentric carcinogenesis. Some studies have shown that intrahepatic metastasis is an important mechanism of early recurrence after resection (13, 16, 24). In the present study, time to advanced recurrence was short: just 1 year. Furthermore, a previous study showed that tumour differentiation, which was a predictive factor of advanced recurrence in this study, was associated with intrahepatic metastasis (22). This is probably because potential metastasis depends on biological tumour factors, such as tumour differentiation. Considering these facts, a main mechanism of advanced recurrence is assumed intrahepatic metastasis. High AFP levels have been reported as a poor prognosis factor after resection of HCC (25, 26). On the other hand, it is assumed that AFP levels may increase in patients with acute or chronic active inflammation in background hepatocytes without HCC (27, 28). It is difficult to distinguish these mechanisms of AFP elevation. We created ASRI to evaluate the malignant potential of HCC by calculating AFP values per unit tumour diameter. Although it is impossible to distinguish neoplastic and inflammatory AFP elevation using this index, ASRI may mainly reflect neoplastic AFP elevation because ASRI is a predictive factor of advanced recurrence of HCC. In addition, Imamura *et al.* (24) reported that high AFP levels were associated with early recurrence within 2 years after resection, and this fact also supports our result.

α-foetoprotein levels usually tend to be higher in HBV-related HCC than those related to HCV, and this tendency has been reported by researchers in Japan, where HCV is

Liver International (2009)

predominant in HCC incidence (29). However, there was no significant difference in AFP levels between HBV- and HCV-related HCC in this study. We re-evaluated the predictive factors of recurrence after resection by stratifying this cohort into HBV- or HCV-related HCC. ASRI $\geq$ 20 was significantly associated with overall recurrence after resection in the HBV cohort, and this result was similar in HCV. Therefore, we consider ASRI as the useful index regardless of the viral aetiology, even in an HBV-endemic area.

Patients with advanced recurrence had a poor prognosis because of limitation and resistance of treatment. The overall survival rates were lower (35.1% per 5 years) in the advanced recurrence group than in the minor or the no recurrence group, in this study. On the other hand, patients with minor recurrence had a relatively good prognosis because it was possible to conduct reresection or percutaneous ablation therapy for recurrent tumour. Therefore, adjuvant therapy to prevent advanced recurrence after resection is needed. Although a number of studies of adjuvant therapy have been reported, none is effective for preventing intrahepatic metastasis after resection of HCC. Pre-/post-operative chemoembolization and chemotherapy had no benefit for tumour recurrence (30–32). Although a few authors including our hospital have reported that interferon is effective for preventing recurrence of HCC after resection, it is assumed that interferon itself suppresses de novo carcinogenesis (33–35). Recently, it was reported that sorafenib, which was a multikinase inhibitor, improved the overall survival rates in patients with advanced HCC (36). Sorafenib is expected to have the potential of effective adjuvant therapy to prevent tumour recurrence by intrahepatic metastasis, and a future report is awaited.

In conclusion, tumour number, ASRI and tumour differentiation were identified as risk factors for advanced recurrence of HCC. In particular, ASRI was easy to calculate and a useful index to predict advanced recurrence after curative resection of small HCC and to choose patients requiring adjuvant therapy after resection.

References

1. Tsukurna H, Hiyama T, Tanaka S, *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797–801.
2. Fattovich G, Giustina G, Schalm SW, *et al.* Occurrence of hepatocellular carcinoma and decompensation in Western European patients with cirrhosis type B. *Hepatology* 1995; **21**: 77–82.
3. Ikeda K, Saitoh S, Suzuki Y, *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis. A prospective observation of 2215 patients. *J Hepatol* 1998; **28**: 930–8.
4. Shinagawa T, Ohto M, Kimura K, *et al.* Diagnosis and clinical features of small hepatocellular carcinoma with emphasis on the utility of real-time ultrasonography. A study in 51 patients. *Gastroenterology* 1984; **86**: 495–502.
5. Okuda K. Early detection of hepatocellular carcinoma. *Hepatology* 1986; **6**: 729–38.
6. Lui WY, Chau GY, Loong CC, *et al.* Hepatic segmentectomy for curative resection of primary hepatocellular carcinoma. *Arch Surg* 1995; **130**: 1090–7.
7. Nagashima I, Hamada C, Naruse K, *et al.* Surgical resection for small hepatocellular carcinoma. *Surgery* 1996; **119**: 40–5.
8. Lise M, Bacchetti S, Da Pian P, *et al.* Prognostic factors affecting long-term outcome after liver resection for hepatocellular carcinoma: results in a series of 100 Italian patients. *Cancer* 1998; **82**: 1028–36.
9. Hanazaki K, Kajikawa S, Shimozawa N, *et al.* Survival and recurrence after hepatic resection of 386 consecutive patients with hepatocellular carcinoma. *J Am Coll Surg* 2000; **191**: 381–8.
10. Nagasue N, Ono T, Yarnanoi A, *et al.* Prognostic factors and survival after hepatic resection for hepatocellular carcinoma without cirrhosis. *Br J Surg* 2001; **88**: 515–22.
11. Sugimoto R, Okuda K, Tanaka M, *et al.* Metachronous multicentric occurrence of hepatocellular carcinoma after surgical treatment – clinicopathological comparison with recurrence due to metastasis. *Oncol Rep* 1999; **6**: 1303–8.
12. Kosuge T, Makuuchi M, Takayama T, *et al.* Long-term results after resection of hepatocellular carcinoma: experience of 480 cases. *Hepatogastroenterology* 1993; **40**: 328–32.
13. Poon RT, Fan ST, Ng IO, *et al.* Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. *Cancer* 2000; **89**: 500–7.
14. Matsumura T, Kanematsu T, Takenaka K, *et al.* Patterns of intrahepatic recurrence after curative resection of hepatocellular carcinoma. *Hepatology* 1989; **9**: 457–60.
15. Sonoyama T, Ochiai T, Hironaka T, *et al.* Predictors of postoperative diffuse intrahepatic recurrence of hepatocellular carcinoma. *Hepatogastroenterology* 2003; **50**: 1078–84.
16. Park JH, Koh KC, Choi MS, *et al.* Analysis of risk factors associated with early multinodular recurrences after hepatic resection for hepatocellular carcinoma. *Am J Surg* 2006; **192**: 29–33.
17. Liver Cancer Study Group of Japan. *Classification of Primary Liver Cancer*, 1st English edn. Tokyo: Kanahara & Company Ltd, 1997.
18. International Working Party. Terminology of nodular hepatocellular lesions. *Hepatology* 1995; **22**: 983–93.
19. Kojiro M, Yano H, Nakashima O. Pathology of early hepatocellular carcinoma: progression from early to advanced. *Semin Surg Oncol* 1996; **12**: 197–203.
20. Nakano M, Saito A, Yamamoto M, *et al.* Stromal and blood vessel wall invasion in well-differentiated hepatocellular carcinoma. *Liver* 1997; **17**: 41–6.
21. Belghiti J, Panis Y, Farges O, *et al.* Intrahepatic recurrence after resection of hepatocellular carcinoma complicating cirrhosis. *Ann Surg* 1991; **214**: 114–7.
22. Kumada T, Nakano S, Takeda I, *et al.* Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. *Hepatology* 1997; **25**: 87–92.
23. Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434–40.
24. Imamura H, Matsuyama Y, Tanaka E, *et al.* Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200–7.
25. The Cancer of the Liver Italian Program (CLIP) Investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* 1998; **28**: 751–5.
26. Ikai I, Arii S, Kojiro M, *et al.* Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. *Cancer* 2004; **101**: 796–802.
27. Smith JB. Occurrence of alpha-fetoprotein in acute viral hepatitis. *Int J Cancer* 1971; **8**: 421–4.
28. Silver HK, Gold P, Shuster J, *et al.* Alpha(1)-fetoprotein in chronic liver disease. *N Engl J Med* 1974; **291**: 506–8.
29. Sasaki Y, Yamada T, Tanaka H, *et al.* Risk of recurrence in a long-term follow-up after surgery in 417 patients with hepatitis B- or hepatitis C-related hepatocellular carcinoma. *Ann Surg* 2006; **244**: 771–80.

30. Wu CC, Ho YZ, Ho WL, *et al.* Preoperative transcatheter arterial chemoembolization for respectable large hepatocellular carcinoma: a reappraisal. *Br J Surg* 1995; **82**: 122–6.
31. Yamasaki S, Hasegawa H, Kinoshita H, *et al.* A prospective randomized trial of preventive effect of pre-operative transcatheter arterial embolization against recurrence of hepatocellular carcinoma. *Jpn J Cancer Res* 1996; **87**: 206–11.
32. Kohno H, Nagasue H, Hayashi T, *et al.* Postoperative adjuvant chemotherapy after radical hepatic resection for hepatocellular carcinoma (HCC). *Hepatogastroenterology* 1996; **43**: 1405–9.
33. Ikeda K, Arase Y, Saitoh S, *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor – a prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000; **32**: 228–32.
34. Kubo S, Nishiguchi S, Hirohashi K, *et al.* Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. *Ann Intern Med* 2001; **134**: 963–7.
35. Mazzaferro V, Romito R, Sciavo M, *et al.* Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44**: 1543–54.
36. Llovet J, Ricci V, Mazzaferro V, *et al.* Randomized phase III trial of sorafenib versus placebo in patients with advanced hepatocellular carcinoma (HCC). *J Clin Oncol* 2007; **25**: 1.

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Original Article

Effectiveness of combination therapy of splenectomy and long-term interferon in patients with hepatitis C virus-related cirrhosis and thrombocytopenia

Daisuke Morihara, Masahiro Kobayashi, Kenji Ikeda, Yusuke Kawamura, Hiromi Saneto, Hiromi Yatuji, Tetuya Hosaka, Hitomi Sezaki, Norio Akuta, Yoshiyuki Suzuki, Fumitaka Suzuki and Hiromitu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

Aim: To elucidate the effectiveness of combination therapy of splenectomy and long-term interferon (IFN) on survival and hepatocarcinogenesis, we retrospectively analyzed 180 patients with hepatitis C virus (HCV)-related cirrhosis and thrombocytopenia.

Methods: Group A consisted of 121 patients who received neither splenectomy nor IFN therapy. Group B consisted of 11 patients who underwent splenectomy only. Group C consisted of 32 patients who underwent IFN therapy only. Group D consisted of 16 patients who received the combination therapy splenectomy followed by IFN therapy.

Results: The viral response in group D estimated at least 6 months after IFN therapy showed sustained viral response in four patients, biochemical response in one and no response in six. Multivariate analysis using time-dependent variables showed significant improvement of survival rate in patients on the combination therapy,but no effect on the appearance rate of hepatocarcinogenesis relative to the findings in group A.

Conclusions: In this study, the splenectomy did not directly improve the prognosis, but increased the ability for patients to undergo IFN. As a result, we considered that the combination therapy of splenectomy and long-term IFN significantly improved survival rate in patients with advanced HCV-related cirrhosis and thrombocytopenia.

Key words: cirrhosis, hypersplenism, interferon, splenectomy, thrombocytopenia

Abbreviations:
AFP, Alpha-fetoprotein; ALT, Alanine aminotransferase; AST, Aspartic aminotransferase; BR, biochemical response; CT, Computed tomography; HCC, Hepatocellular carcinoma; HCV, Hepatitis C virus; ICG R15, Indocyanine green retention rate at 15 min; IFN, Interferon; MELD score, Model for End-Stage Liver Disease score; NR, No response; PLT, platelet; SVR, Sustained virological response; TTT, Thymol turbidity test; US, Ultrasonography; ZTT, Zinc sulfate turbidity test.

INTRODUCTION

THE PRESENCE OF severe thrombocytopenia in patients with cirrhosis associated with hepatitis C viral (HCV) infection limits the use of interferon (IFN) therapy. The different treatment modalities for hepatocellular carcinoma (HCC), such as hepatic resection, radiofrequency ablation, or percutaneous ethanol injection, are also limited by low platelet (PLT) counts. In patients with compensated cirrhosis and low model for end-stage liver disease (MELD) score, liver transplantation is not warranted and the use of antiviral therapy to slow down the progression to liver failure is not recommended. In other words, such patients are too healthy for transplantation and too thrombocytopenic to treat with antiviral agents. Splenectomy has been suggested for the treatment of secondary hypersplenism and thrombocytopenia as a means to improve PLT count.[1]

If patients with HCV-related cirrhosis and thrombocytopenia could receive the benefits of splenectomy[2,3] and IFN therapy,[4,5] such therapy would clinically be very useful. The combination therapy of splenectomy and long-term IFN administration may improve survival rate and reduce the incidence of hepatocarcinogenesis.

Correspondence: Dr Masahiro Kobayashi, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: mshkobayashi@toranomon.gr.jp
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However, there are only a few reports that have examined the usefulness of this combination therapy in patients with advanced HCV-related cirrhosis and low PLT count.[6] In this study, we retrospectively analyzed 180 patients with compensated cirrhosis and thrombocytopenia who had received the combination therapy of splenectomy and long-term IFN to determine the effects of such treatment on the survival rate and incidence of HCC.

PATIENT AND METHODS

Study population

A TOTAL OF 180 Japanese patients with cirrhosis, hypersplenism and low PLT count ($\leq 80 \times 10^3/\mu L$) were examined between 1990 and 2006. Their initial sera were positive for antibodies to HCV (anti-HCV; second-generation anti-HCV kit; ELISA, Dainabot, Tokyo, Japan), positive HCV-RNA (Amplicor HCV monitor assay version 2.0; Roche Diagnostics, Tokyo, Japan), and negative for hepatitis B surface antigen (HBsAg; radioimmunoassay, Dainabot). Anti-HCV was assayed using stored frozen sera at -80°C. They were diagnosed with liver cirrhosis between 1990 and 2006 at Toranomon Hospital, Tokyo, Japan. In addition to liver biopsy and/or peritoneoscopy, liver cirrhosis was also diagnosed utilizing clinical findings (e.g. presence of esophageal varices), and with computed tomographic (CT) or ultrasonographic (US) findings. The following protocol was applied in our hospital until 2000: Patients with a platelet count of less than $50 \times 10^3/\mu L$ are eligible for HCC surgery (such as hepatic resection, radiofrequency ablation, or percutaneous ethanol injection) provided they receive platelet transfusion. The decision to pursue splenectomy was individualized and based on the presence thrombocytopenia and/or intractable gastric varices, and discussed with the patients.

We retrospectively analyzed the effect of splenectomy on cirrhotic patients with low PLT count ($\leq 80 \times 10^3/\mu L$). Of the total 180 patients, 121 (67.2%) patients received neither antiviral therapy nor splenectomy (group A). Thirty-two (17.8%) patients received only IFN therapy (group C). The remaining 27 (15.0%) patients underwent splenectomy (11 patients underwent only splenectomy [group B] and 16 received IFN therapy after splenectomy [group D]). Splenectomy was performed for the following reasons; (i) low PLT count in 20 patients (six [54.5%] of group B and 14 [8.5%] of group D), (ii) low PLT count and part of treatment of gastric varices in three (one [9.0%] of group B and two [12.5%] of group D), and (iii) low PLT count and refractory esophageal varices in four (four [36.4%] of group B). None of the patients required emergency splenectomy (e.g. bleeding gastric varices or other bleeding complications related to low platelet count). Our institution does not require informed consent for retrospective analysis.

Patients background and laboratory data

Table 1 summarizes the profiles and patients of groups A, B, C and D at the time of diagnosis of liver cirrhosis. Indocyanine green test was conducted in 91.2% of the patients. Patients of group D had significantly lower PLT count ($P = 0.01$) and AST ($P = 0.01$) than patients in others groups. The proportion of group A patients who regularly consumed alcohol at ≥ 80 g/day was significantly higher than other groups. Patients of group C had significantly lower TTT ($P = 0.08$) than others.

Splenectomy

Splenectomy was performed through midline or left subcostal incision depending on body habitus and previous incisions. For group B, five patients underwent splenectomy and six underwent Hassab's operation.[7] In group D, 13 patients underwent splenectomy and three underwent Hassab's operation.

IFN treatment

Thirty-two patients received IFN therapy (group C). In group C, 21 patients received 3 million units of IFN-α (natural or recombinant) intramuscularly three times per week to maintain a low alanine aminotransferase (ALT), 11 patients received 6 million units of IFN-α to eradicate HCV. Patients of group C received IFN therapy for a median period of 0.5 years (range, 0.0–9.7 years).

Sisteen patients received the combination therapy (group D). Of these, 12 (75%) patients underwent splenectomy for the purpose of induction of antiviral therapy with IFN. The other patients (25%) had undergone splenectomy pre dating this study. In group D, 11 patients (Cases 1–4, 8, 10–13, 15–16) received 3 million units of IFN-α (natural or recombinant) intramuscularly three times per week to maintain a low ALT, 3 patients (Cases 6, 7, and 9) received 6 million units of IFN-α to eradicate HCV. For the other two patients; one (Case 5) received pegylated IFNα2b (50 μg) monotherapy and the other patient (Case 14) received pegylated IFNα2b (50 μg) plus ribavirin (400 mg) combination therapy to maintain low ALT (Fig. 1). Patients of group D received IFN therapy for a median period of 1.4 years (range, 0.2–12.4 years).

Table 1 Patient profiles and laboratory data at the time of diagnosis of cirrhosis

	Group A (Neither splenectomy nor IFN)	Group B (splenectomy)	Group C (IFN)	Group D (splenectomy + IFN)	*P**
Demography					
No. patients	121	11	32	16	
Sex (M/F)	64/57	6/5	13/19	13/3	0.07
Age (years)†	61 (32–82)	61 (42–66)	59 (36–72)	52 (36–60)	0.41
Alcohol intake of 80 g/day or more	29	0	10	0	0.03
Diabetes mellitus	12	1	4	2	0.96
Laboratory data†					
Platelet count ($\times 10^3/\mu L$)	61 (17–80)	64 (42–75)	66 (25–80)	44 (27–78)	0.01
Prothrombin activity (%)	73 (50–101)	79 (58–94)	80 (66–100)	74 (47–100)	0.88
Albumin (g/dL)	3.5 (1.7–4.8)	3.5 (2.0–4.3)	3.4 (2.5–4.1)	3.3 (2.7–4.5)	0.64
ZTT (Kunkel)	12.3 (0.7–23.3)	10.3 (3.3–18.2)	10.8 (4.4–21.0)	12.0 (6.1–17.1)	0.29
TTT (Kunkel)	14.1 (0.4–37.2)	12.0 (4.4–16.9)	7.8 (1.2–34.0)	12.7 (2.7–34.1)	0.08
Bilirubin (mg/dL)	1.5 (0.4–7.7)	1.2 (0.7–5.3)	1.1 (0.6–2.7)	1.2 (0.8–4.4)	0.03
AST (IU/L)	64 (21–652)	83 (31–157)	75 (28–216)	60 (30–154)	0.17
ALT (IU/L)	53 (11–239)	72 (24–191)	71 (18–298)	46 (14–182)	0.01
ICG R15 (%)	38 (12–96)	41 (15–64)	32 (6–62)	32 (8–53)	0.44
Alpha-fetoprotein (ng/mL)	23 (2–909)	40 (3.9–165)	29 (5–631)	11 (4–190)	0.28

ALT, alanine aminotransferase; AST, aspartic aminotransferase; ICG R15, indocyanine green retention rate at 15 min; TTT, thymol tubidity test; ZTT, zincsulfate tubidity test.
*Kruskal-Wallis test or χ^2-test. †Expressed by median (min, max).

The effect of IFN therapy was classified according to elimination of HCV-RNA and ALT value 6 months after the end of treatment. Sustained virological response (SVR) was defined as persistent disappearance of HCV RNA after therapy, biochemical response (BR) as normal ALT values without elimination of HCV RNA for at least 6 months after therapy, and no response (NR) as persistently elevated or transiently normalized ALT levels without loss of HCV RNA.

Follow up of patients

Patients were followed up on a monthly basis after the diagnosis of cirrhosis by monitoring hematologic, biochemical, and virologic data. Imaging studies were conducted three or more times per year in the majority of patients by using computerized tomography (CT) or ultrasonography (US). Angiography was performed only when HCC was highly suspected based on CT or US. When angiography detected a typical hypervascular nodule, it was considered a specific finding for HCC in these follow-up patients, and histological confirmation was usually not required in the majority of patients. If the angiographic study did not show any hypervascular staining in a small hepatic nodule, a fine needle biopsy was performed. In this cohort, 18 (12.2%) patients were lost to follow up [14 patients (11.6%) from group A, two patients (18.2%) from group B, one patient (3.1%) from group C and two patients (12.5%) from group D]. The date of the last follow-up in this study was 31 March 2007, and the median observation period of studied patients was 5.9 years (range, 0.1–19.6 years).

Statistical analysis

Non-parametric procedures were used for the analysis of background characteristics of the patients, including Kruskal–Wallis and χ^2 test. Changes in laboratory tests values after splenectomy were evaluated by using Wilcoxon signed-rank test. Survival rate was calculated from the period between diagnosis of liver cirrhosis and death in each group, by using the Kaplan–Meier method.[8] HCC appearance rate was calculated from the period between diagnosis of liver cirrhosis and appearance of HCC in each group, by again using the Kaplan–Meier method. Differences in slopes of survival and carcinogenic curves were evaluated by log-rank test. The median waiting period between diagnosis of cirrhosis and splenectomy was 1.6 months (range, 0.0–199.5 months) for groups B and C. To compensate for wait-time bias in the splenectomy groups, curves of survival and HCC appearance were also drawn from the time of diagnosis

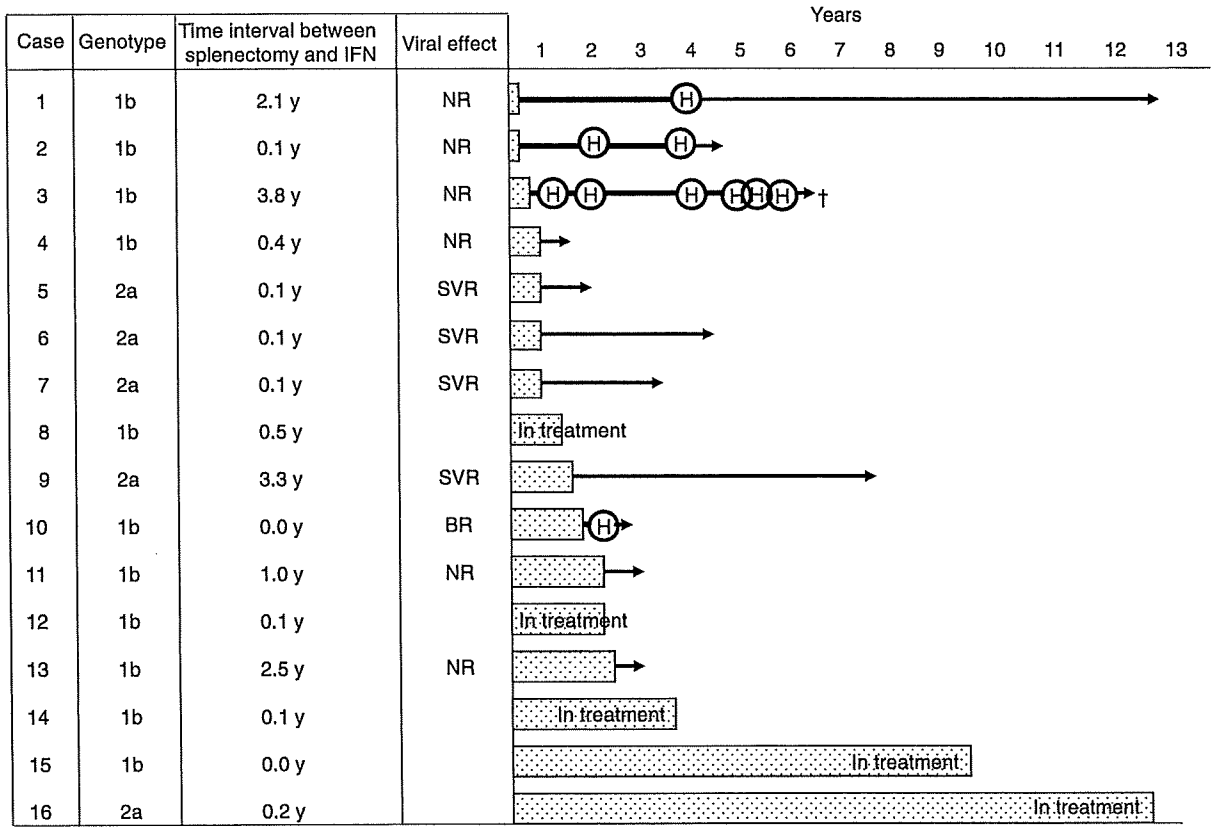

Figure 1 Individual patients who underwent splenectomy followed by long-term IFN therapy (group D). Hepatocellular carcinoma (HCC) developed in five of 16 patients. The dotted bars and arrows represent IFN therapy and follow-up period. H, appearance of HCC; SVR, sustained virological response; BR, biochemical response; NR, no response; †, death.

of cirrhosis in the groups. Independent factors associated with survival and HCC appearance were studied by using time-dependent Cox regression analysis.[9] The following 14 variables were analyzed for potential covariates for survival and liver carcinogenesis at the time of the diagnosis of cirrhosis: age, sex, habitual alcohol intake (80 g/day or more), association of diabetes, albumin, zinc sulfate turbidity test (ZTT), thymol turbidity test (TTT), bilirubin, aspartic aminotransferase (AST), ALT, PLT count, prothrombin activity, indocyanine green retention rate at 15 min (ICG R15), and alpha-fetoprotein (AFP). In addition to these variables, an interaction term of "waiting time" from the diagnosis of liver cirrhosis to splenectomy was introduced in the analysis as a time-dependent covariate. Several variables were transformed into categorical data consisting of two or three simple ordinal numbers in order to estimate the hazard ratio. All factors found to be at least marginally associated with survival and liver carcinogenesis ($P < 0.10$) were entered into multivariate Cox proportional hazard model. A P-value of less than 0.05 was considered to be significant. Statistical analyses were performed using the SPSS software (SPSS, Chicago, IL, USA).

RESULTS

Effects and complications of splenectomy

THE SPLENECTOMY GROUP consisted of 11 patients with Child–Pugh Class A (group B = 2, group D = 8), 15 with Child–Pugh Class B (group B = 8, group D = 7) and 1 with Class C (group D = 1) at operation. The median weight of the removed spleen was 430 g (range, 190–1600 g). Leukocyte count, PLT count and total bilirubin improved in most patients after sple-

nectomy. Leukocyte count increased about 1.6 times at 6 months after splenectomy [before splenectomy, median = 3200/mm^3 (range 1800–5600); after splenectomy, 5200 (3700–9000); $P < 0.001$). PLT count increased about 2.3 times at 6 months after splenectomy [before splenectomy, median = $47 \times 10^3/\mu L$ (range, $26–77 \times 10^3$); after splenectomy, 110×10^3 ($79–275 \times 10^3$); $P < 0.001$). Total bilirubin decreased about 0.6 times at 6 months after splenectomy [before splenectomy, median = 1.2 mg/dL (range, 0.6–4.4); after splenectomy, 0.7 (0.4–1.8); $P = 0.001$). Leukocyte and PLT counts reached peak levels within a month after splenectomy and were almost stabilized at six months.

Postoperative complications following splenectomy developed in three patients; hemoperitoneum ($n = 1$), portal vein thrombosis ($n = 1$) and secondary thrombocytopenia ($n = 1$). Some patients received prophylactic anticoagulation to protect against portal vein thrombosis after splenectomy. One patient with hemoperitoneum died due to multiple organ failure, while the other patients recovered with medical treatment.

Complications of splenectomy plus IFN combination therapy

Figure 1 shows patients that underwent combination therapy (group D). During the observation period, one patient (Case 3) of group D died of liver failure caused by progression of HCC. The causes of death in three other patients were not deemed to be complications related to the combination therapy. None of the patients of group D developed serious complications (e.g. portal vein thrombosis, post-operative hemorrhage, pneumonia, sepsis) from the splenectomy. Postoperatively, none of the patients showed worsening of liver biochemical test results or developed decompensated liver disease with ascites, encephalopathy, jaundice or variceal bleeding. There were also no deaths in the immediate postoperative period. Three patients (18.8%) of group D discontinued IFN therapy for the following reasons; severe thrombocytopenia (Case 1), NSAID-induced liver injury (Case 2) and peripheral neuropathy (Case 13). In contrast, eight patients (25.8%) of group C discontinued IFN therapy. Three (37.5%) of them discontinued IFN therapy due to severe thrombocytopenia. When frequency of discontinued IFN therapy was compared with group C and D, there was no significant difference ($P = 0.73$). However, there were cases, eight in group C but 0 in group D, who required a reduction in IFN dosages during treatment as compared with the beginning of treatment ($P = 0.03$). The splenectomy could have increased the ability for patients to undergo IFN.

Effect of IFN therapy after splenectomy

Eleven of 16 (68.8%) patients of group D had HCV genotype 1b and five (31.3%) had HCV genotype 2a (Fig. 1). The viral response was determined at least 6 months after IFN therapy; SVR was noted in four (36.4%) patients, BR in one (9.1%) and NR in six (54.5%). Three patients continue to receive IFN therapy at present. In this study, patients with SVR were all male and had genotype 2a. One of the patients with SVR received pegylated-IFNα–2b (Case 5, Fig. 1), while other patients received IFNα2b. Meanwhile, 18 of 32 (56.3%) patients of group C had HCV genotype 1b, 12 (37.5%) had HCV genotype 2a and two (6.3%) had HCV genotype 2b. Group C had more patients with low HCV-RNA (< 100 000 IU/mL) than group D (12 [37.5%] of group C and three [18.8%] of group D, $P = 0.09$). In group C, SVR was noted in 7(21.9%) patients, BR in six (18.8%) and NR in 17 (53.1%). Two patients continue to receive IFN therapy at present.

SVR were not significantly different between group C and D ($P = 0.43$). This result might be a reason that group D had more patients with HCV genotype 1 and higher HCV-RNA than group C.

Rate of hepatocarcinogenesis

During the follow-up period of up to 17 years (median observation period of 5.9 years), HCC developed in 65 patients (36.1%): 40 (33.1%) in group A, five (45.5%) in group B, 16 (50.0%) in group C and four (25.0%) in group D. HCC appearance rates at the end of the third year were 19.9, 20.0, 25.0 and 6.3% in group A, B, C and D, 28.5, 57.3, 34.5 and 14.1% at the end of the fifth year, and 48.2, 78.7, 43.8 and 39.8% at the end of tenth year, respectively (Fig. 2). There was no significant difference in the rate of HCC appearance among the four groups (log-rank test, $P = 0.42$). In particular, the HCC appearance rate in group D was not significantly different compared with group A (log-rank test, $P = 0.50$).

In addition, the rate of carcinogenesis correlated inversely with the duration of IFN administration (Fig. 1). For group D, 9 of 14 patients were treated with IFN for ≥ 12 months. The carcinogenic rate at the end of the 5th year in the remaining patients of the same group who were treated with IFN for < 12 months (20.0%) was higher than in those treated for ≥ 12 months (9.1%). Multivariate analysis showed that the hazard ratio of carcinogenesis for patients treated with IFN for