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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

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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.

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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

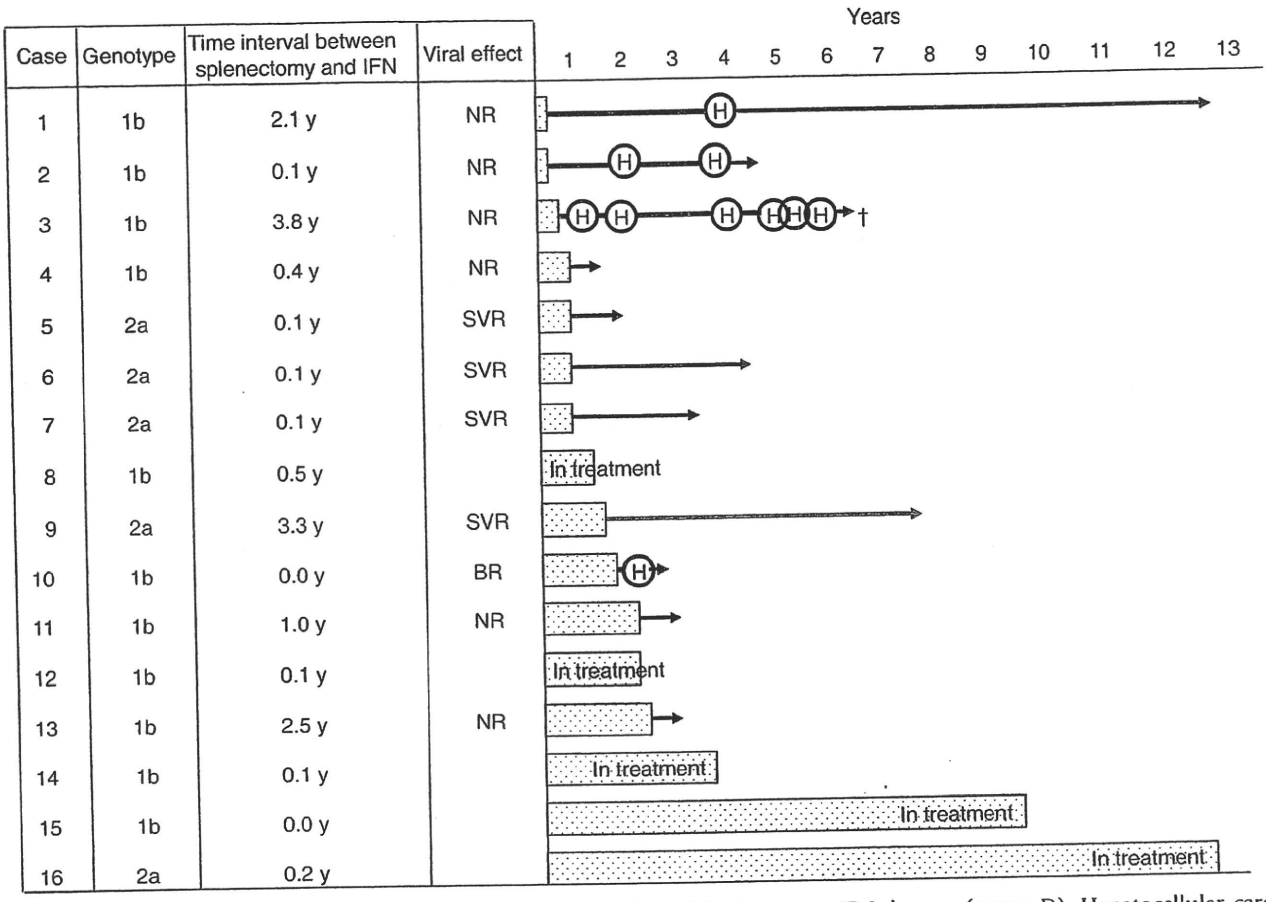

Figure 1 Individual patients who underwent splenectomy followed by long-term IFN therapy (group D). Hepatocellular carcinoma (HCC) developed in five of 16 patients. The dotted bars and arrows represent IFN therapy and follow-up period. H, appearance of HCC; SVR, sustained virological response; BR, biochemical 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

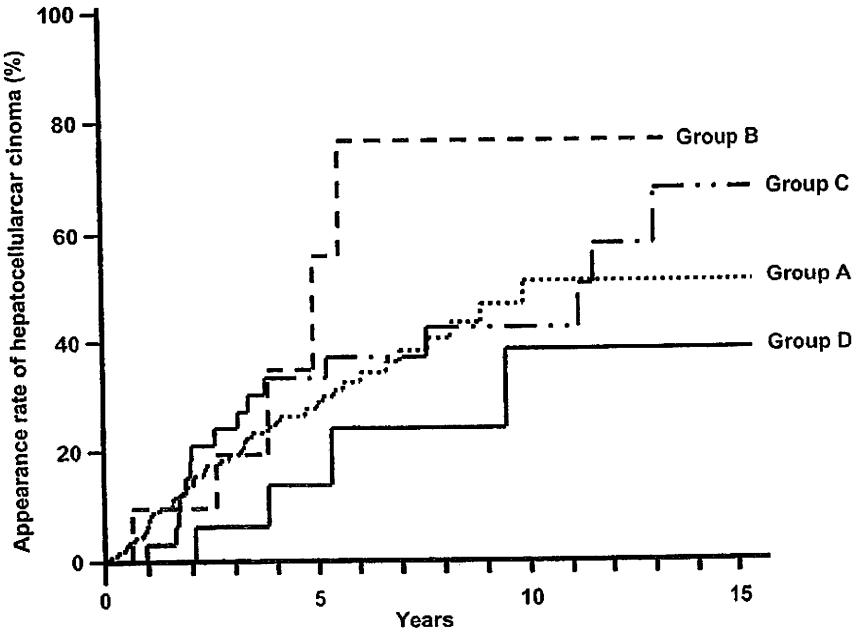

Figure 2 Crude hepatocellular carcinogenesis (HCC) curves in patients of groups A, B, C and D. There was no significant difference in the HCC appearance rate among the four groups (log-rank test, $P = 0.42$).

≥ 12 months was 0.022 after adjustments for significant covariates, but was not significantly different ($P = 0.43$).

We also assessed the effects of splenectomy and long-term IFN therapy on hepatocarcinogenesis by comparing patients of group D (splenectomy + IFN administration for ≥ 12 months) with those of group A. The combination therapy reduced the hazard ratio to 0.03 (multivariate analysis with adjustments for significant covariates), though it was significant ($P = 0.83$). We also assessed compared patients of groups C and B (splenectomy alone). Administration of IFN for ≥ 12 months reduced the hazard ratio to 0.03 (multivariate analysis after adjustments for significant covariates), but was not significant ($P = 0.83$). These results suggest that the combination of splenectomy plus long-term IFN decreased the likelihood of hepatocarcinogenesis.

Effect of splenectomy and IFN combination therapy on survival

During the observation period, one of the 16 patients of group D (Case 3) died (Fig. 1). The survival rates for groups A, B, C and D were 84.2, 90.9, 87.5 and 100% at the end of the third year, 72.0, 90.9, 87.5 and 100% at the fifth year, 41.4, 36.4, 83.3 and 83.3% at the tenth year, respectively (Fig. 3). The survival rate for patients of group D was the highest compared with the other groups (log-rank test, $P = 0.002$). We also compared the effect of combination therapy on the survival rate of patients of group A and group D. The survival rate of group D was significantly higher than of group A (log-rank test, $P = 0.004$). We also compared the effect of combination therapy on the survival rate of patients of group C and group D. The survival rate of group D was not significantly different compared with group C (log-rank test, $P = 0.29$). The combination therapy significantly improved the hazard ratio of survival to 9.69 ($P = 0.028$, multivariate analysis with adjustments for significant covariates, Table 2). These results suggest that the splenectomy simply increased the ability for patients to undergo IFN and may not directly improve patient survival.

DISCUSSION

CHRONIC HEPATITIS C virus (HCV) will continue to cause significant morbidity and mortality through to at least 2015.[10] HCV infection remains a common cause of chronic liver disease and is an increasing indication for liver transplantation. Thrombocytopenia (platelet counts $< 150 \times 10^3/\mu L$) is a common complication in patients with chronic liver disease (CLD), and is reported in as many as 76% of cirrhotic patients.[11]The ability to increase platelet levels could significantly reduce the need for platelet transfusions and facilitate the use of IFN-based antiviral therapy and other medically indicated treatments in patients with liver disease. Current treatment options for severe

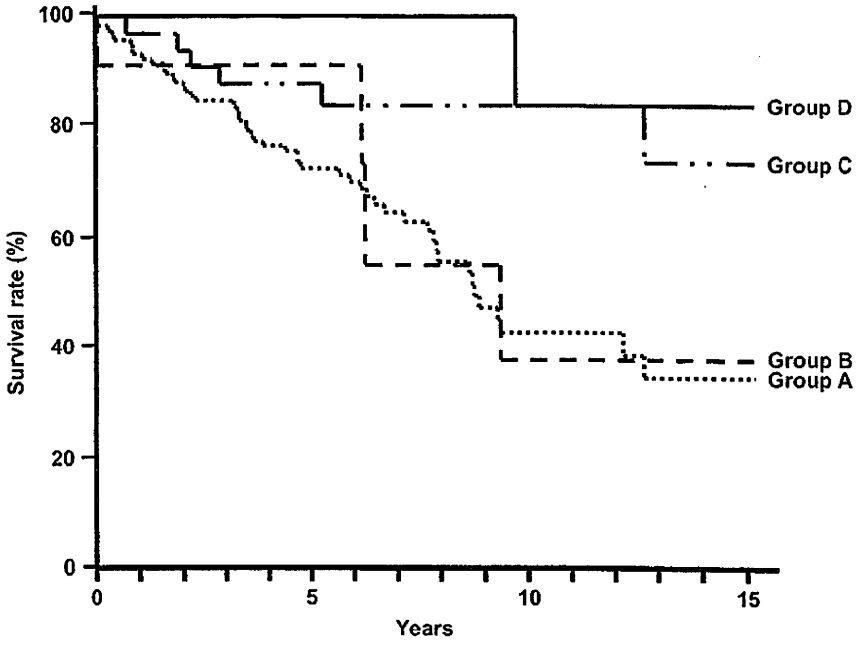

Figure 3 Survival rates for patients of groups A, B, C and D. The survival rate was significantly different for group A, B, C and D (log-rank test, $P = 0.002$). The survival rate of patients of group D was significantly higher than that of group A (log-rank test, $P = 0.004$).

thrombocytopenia include platelet transfusion, splenic artery embolization and splenectomy. We studied the usefulness of the combination therapy of splenectomy and long-term IFN in patients with advanced HCV-related cirrhosis and thrombocytopenia.

With regard to the usefulness of splenectomy, some studies reported that splenectomy improved PLT counts in cirrhotic patients with thrombocytopenia.[2,3] Furthermore, Shimada *et al.*[12] reported that splenectomy resulted in significant falls in ammonia levels and rises in serum albumin. Thus, there is evidence that splenectomy is beneficial and results in recovery of liver function by improving of blood supply to the liver.[6,13] In the present study, at 6 months after splenectomy, leukocyte count increased 1.6 times, PLT count increased 2.3 times, and total bilirubin decreased nearly 0.6 times, relative to prior the procedure. Furthermore, liver function test results also improved in most patients with splenectomy.

Table 2 Significance of combined therapy of survival rate in patients of advanced hepatitis C virus-related cirrhosis with low platelet count (time-dependent proportional hazard model)

Factors	Category	Hazard ratio (95% CI)	*P*
Combined therapy (splenectomy + IFN)	1: no 2: yes	1 9.69 (1.28–76.9)	0.028

IFN, interferon therapy.

With regard to the value of IFN therapy after splenectomy, Hayashi *et al.*[6] reported that splenectomy in patients with HCV cirrhosis can be done safely to allow application of antiviral treatment and potentially avoid transplantation.[6] In this study, only three of 16 (18.8%) patients discontinued IFN therapy after splenectomy. Among the three patients, IFN therapy was discontinued because of thrombocytopenia in only one (6.3%) patient. On the other hand, 13 (81.3%) of the 16 patients on combination therapy were able to complete the full course of IFN therapy, continue IFN therapy or stopped therapy due to NR. Thus, it may be said that IFN therapy is safe in most patients with advanced HCV-related cirrhosis and thrombocytopenia. Furthermore, the present results indicate that splenectomy is an effective method in patients with chronic HCV infection and hypersplenism to increase peripheral leukocyte and platelet counts so that subsequent IFN therapy can be better tolerated. In this study, regarding the reduction of IFN dosages during treatment when comparing group C and D, group D did not have any cases who a reduction in IFN dosages was necessitated by thrombocytopenia ($P = 0.03$). Hayashi *et al.*[6] reported that five of their seven patients underwent splenectomy and then completed a full course of pegylated IFN and ribavirin

treatment or stopped therapy due to NR, and that none of their patients required dose reductions or treatment discontinuation due to thrombocytopenia. In the present study, the viral response to IFN therapy was SVR in four (36.4%) patients, BR in one (9.1%) and NR in six (54.5%). SVR was 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. All patients with SVR of group D had genotype 2, suggesting that SVR seems to be achievable by combination therapy in patients with HCV-related cirrhosis with genotype 2 and thrombocytopenia.

We also analyzed the effect of the combination therapy on hepatocarcinogenesis in patients with advanced HCV-related cirrhosis and low PLT count. Chen *et al.*[14] reported that the 5-year tumor-free survival rate was significantly higher after hepatectomy and splenectomy than after hepatectomy alone (37 vs. 27.3%, respectively, $P = 0.003$). In contrast, Yao *et al.*[15] reported that splenectomy in early stage of tumor inoculation stimulated tumor growth and metastasis in their rat model of HCC.[15] In this study, the HCC appearance rate in patients who underwent splenectomy alone (group B) was not significantly different from that of the control (log-rank test, $P = 0.52$). In addition, the HCC appearance rate in patients who received the combination therapy was also not significantly different from the control (log-rank test, $P = 0.50$). We previously reported that long-term IFN therapy for 12 months or longer reduced the rate of hepatocarcinogenesis in patients with liver cirrhosis caused by HCV.[5] Multivariate analysis of long-term follow-up showed that the combination therapy, including IFN administration for ≥ 12 months, decreased the hazard ratio of hepatocarcinogenesis to 0.03, though this was not significant ($P = 0.83$). The reason for the lack of significance might be the small population sample of this study. Yoshida *et al.*[16] reported that IFN therapy significantly reduced the risk for HCC, especially among virologic and biochemical responders. That the combination therapy decreased the hazard ratio of hepatocarcinogenesis to 0.03 suggests the ability of long-term IFN to inhibit HCC, especially among non-responders.

We also examined the effects of the combination therapy on survival. In this study, multivariate analysis using time-dependent variables showed significant improvement of survival in patients who received the combination therapy (group D) compared with the control group (group A) (hazard ratio 3.40, $P = 0.017$; 95% CI 1.24–9.35). This may be considered the crucial finding of this study. In splenectomy, Morimasa *et al.*[17] reported no difference in survival rate between splenectomy and endoscopic injection sclerotherapy (EIS) for esophageal varices. Similarly, the survival rate in the splenectomy group in this study (group B) was not significantly different from the control ($P = 0.88$). Furthermore, the survival rate of group D was not significantly different compared with group C (log-rank test, $P = 0.29$). These results suggest that the splenectomy increased the ability for patients to undergo IFN and that the combination therapy of splenectomy and long-term IFN significantly improved survival rate in patients with advanced HCV-related cirrhosis and thrombocytopenia. The likely mechanism of action of the combination therapy is first improvement of leucopenia and thrombocytopenia following splenectomy, which allowed administration of IFN, and then IFN produced remission of liver fibrosis, control of necroinflammatory process, and induced suppression of the HCC growth process, consequently leading to improvement of survival rate. Moreno and Muriel[18] reported that IFN resulted in remission of liver fibrosis, and that control of the necroinflammatory process can therefore induce suppression of the HCC growth process. Our results also suggested that patients with NR may need to continue the combination therapy with long-term IFN therapy.

"Pegylated IFN plus ribavirin" and "eltrombopag" are promising drugs and can be potentially used in combination therapy. Recent multicenter trials have demonstrated the superiority of pegylated IFN plus ribavirin compared to pegylated IFN alone or non-pegylated combination therapy.[19,20] In addition, several promising novel agents that stimulate TPO and increase PLT count, such as the oral platelet growth factor eltrombopag, are currently in development for the prevention and/or treatment of thrombocytopenia.[21] Eltrombopag may be a substitute for splenectomy or PSE. Thus, combination therapy of pegylated IFN plus ribavirin after splenectomy or eltrombopag may improve survival rate and reduce the rate of hepatocarcinogenesis.

Our study had certain limitations. In particular, in this study, four (25%) of the patients who underwent combination therapy had a history of splenectomy. A randomized control study with a larger number of cases should be conducted to confirm the effectiveness of this therapy.

In conclusion, the combination therapy of splenectomy and long-term IFN decreased the rate of hepatocarcinogenesis and significantly improved the survival rate in patients with advanced HCV-related cirrhosis and low PLT count.

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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 ($P < .0001$).

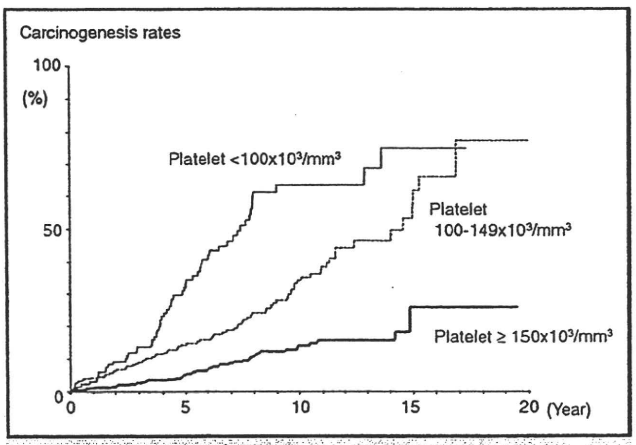

Figure 1 Hepatocarcinogenesis rates in patients without IFN therapy, according to initial platelet count. The lower the initial platelet count was, the higher the hepatocellular carcinogenesis was in the untreated cohort ($P < .0001$).

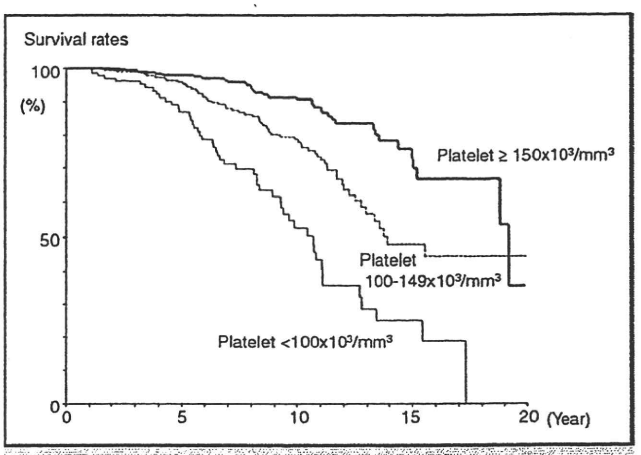

Figure 2 Cumulative survival in patients without IFN therapy, according to initial platelet count. Survival of patients with high platelet count was significantly higher than those with a low or intermediate platelet count ($P < .0001$).

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 ($P = .0005$).

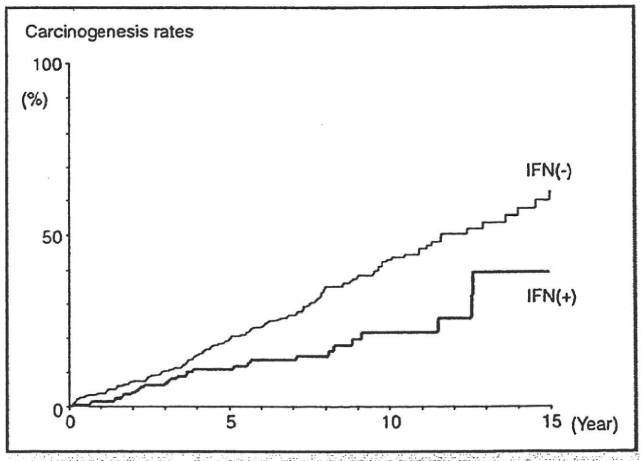

Figure 3 Hepatocarcinogenesis rates in patients with a low or intermediate platelet count. Carcinogenesis rate of patients with IFN therapy was significantly lower than those without therapy ($P = .0005$). IFN = Interferon.

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,

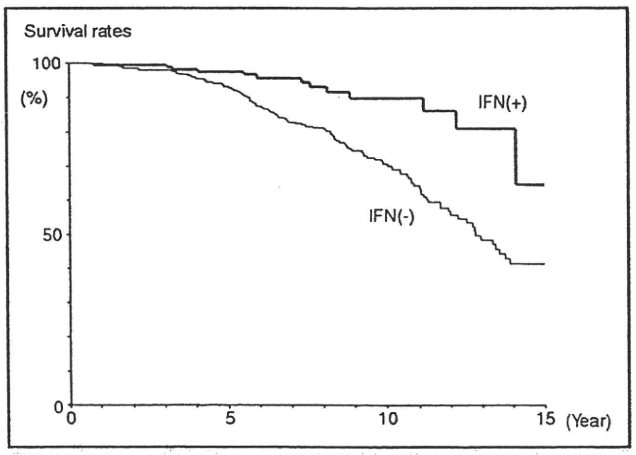

Figure 4 Cumulative survival in patients with a low or intermediate platelet count. Survival of patients with IFN therapy was significantly higher than those without therapy ($P = .0001$). IFN = Interferon.

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 (≥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.

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ORIGINAL ARTICLE

Development of hepatocellular carcinoma in elderly patients with chronic hepatitis C with or without elevated aspartate and alanine aminotransferase levels

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Abstract

Objective. Hepatocellular carcinoma (HCC) in the elderly infected with hepatitis C virus (HCV) is expected to increase globally within the next two decades. The purpose of the study was to define the natural history of elderly patients with chronic hepatitis C needs in order to prevent HCC from arising in these patients. ***Material and methods.*** Treatment-naive patients aged ≥65 years with platelet counts $>120 \times 10^3/mm^3$ were classified as 120 with aspartate and alanine aminotransferase (ASAT and ALAT) levels ≦40 IU/l (group A) and 212 with either or both levels ≥41 (group B) and followed-up for 3 years or longer without antiviral treatment. ***Results.*** Cirrhosis and HCC developed more frequently in group B than in group A ($p<0.001$ for both). In particular, of the patients aged 65–69 years at entry, cirrhosis and HCC developed more frequently in group B than in group A ($p<0.001$ and $p=0.001$, respectively). Liver-related causes of death were more common in group B than in group A (20/34 (59%) versus 1/9 (11%), $p=0.021$). HCC developed more frequently in men than in women ($p=0.033$). ***Conclusions.*** In elderly patients with chronic hepatitis C, cirrhosis and HCC develop more frequently in those with elevated transaminase levels than in those without elevated transaminase levels. Therefore, transaminase levels need to be suppressed below ≦40 IU/l, using antiviral treatments or other agents, in order to prevent cirrhosis and HCC arising in these patients. In view of rare liver-related deaths, aggressive antiviral treatment would not be necessary in the elderly with chronic hepatitis C who have normal transaminase levels.

Key Words: *Age, chronic hepatitis, cirrhosis, hepatitis C virus, hepatocellular carcinoma*

Introduction

There are an estimated 170 million people persistently infected with hepatitis C virus (HCV) worldwide, and approximately 30% of them develop serious complications during their lifetime, such as decompensated cirrhosis and hepatocellular carcinoma (HCC) [1]. The incidence of HCC in HCV carriers increases with age and is particularly high in those aged 65 years or older. Based on the shift in age-specific distribution of HCV carriers with time [2–4], HCC is expected to increase in the next 20 years, globally.

The natural history of infection with HCV is influenced by host and virological factors including age and gender [5–7], as well as viral loads and genotypes [8–10]. Thus, hepatitis proceeds slowly in HCV infections contracted by children and young women. During follow-ups carried out over 20 years, liver damage developed in a mere 3% of children who were infected with HCV during heart surgery [7], and cirrhosis emerged in only 2% of pregnant women infected with anti-D immune globulin contaminated with HCV [5].

As the average life span of human beings continues to extend, owing to improvements in sanitary

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conditions and efficient management of ailments, difficulties in the treatment of chronic hepatitis C in elderly individuals are increasingly coming to the fore. This is attributable, at least in part, to liver fibrosis accelerating in parallel with age [11], as well as less tolerability and more side effects of combined interferon (IFN) and ribavirin in these patients [6,11,12].

These constraints notwithstanding, there is a pressing need for treatment of aged individuals with antiviral agents in order to prevent the development of cirrhosis and HCC and to promote better survival with an increased quality of life. When planning antiviral treatment of the elderly, weighing its merits against untoward effects, it is essential to understand the natural history of HCV infection in these patients. However, there have been virtually no reports on the natural history of HCV infection in older adults, nor are there any solid guidelines for antiviral treatment in these patients [13].

In the 42 years from 1964 to 2005, we have followed-up 332 patients who were persistently infected with HCV and had not received any antiviral treatment. They included the 120 patients with aspartate and alanine aminotransferase (ASAT and ALAT) levels $\leqq$40 IU/l (group A) and the 212 with ASAT and/or ALAT $\geq$41 (group B), and were followed-up for 3 years or longer without receiving any antiviral treatment. It is hoped that the evolution of chronic hepatitis in these patients, with special reference to the baseline transaminase levels, will shed light on how they should be treated for the prevention of cirrhosis and HCC in the coming era of global longevity.

Material and methods

Patients

During 42 years, from 1964 through 2005, 7358 patients with HCV-RNA in the serum visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. Of these patients, 843 (11.5%) were $\geq$65 years of age at presentation, and 512 (60.7% of the elderly) had not received antiviral agents or other drugs that might suppress the replication of HCV. In order to rule out cirrhosis, 180 patients with platelet counts $<120\times10^3/mm^3$ were excluded. The remaining 332 patients were classified into the 120 with ASAT and ALAT levels $\leqq$40 IU/l (Group A) and the 212 with ASAT and/or ALAT levels $\geqq$41 IU/l (group B); they included 22 patients (10.4%) with ASAT levels $\leqq$40 IU/l and 18 (8.5%) with ALAT levels $\leqq$40 IU/l. Baseline transaminase levels were determined at least twice, 2–3 months apart, in the course of 6 months. The patients were followed-up for 3 years or longer without receiving any antiviral treatment, and tested monthly for liver function, HCV-RNA and α-fetprotein (AFP) or protein induced by the absence of vitamin K or antagonist-II (PIVKA-II). Screening for cirrhosis and HCC was carried out yearly using ultrasonography and/or computed tomography. Angiography was implemented when HCC was strongly suspected by imaging modalities. During follow-ups, herbal medicine (intravenous Stronger Neo-Minophagen C (SNMC) or oral Shousaikotou) and/or ursodeoxycholic acid was given to 51 (42.5%) patients in group A and 139 (65.6%) patients in group B. Three (2.5%) patients in group A and 24 (11.2%) patients in group B, in whom IFN was started after they had been followed-up for 3 years or longer, left the study cohorts at the initiation of treatment. Informed consent was obtained from each patient who participated in this study, and the protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Human Research Committee of the institution.

Markers of HCV infection

Qualitative assay for HCV-RNA was performed using polymerase chain reaction (PCR) with nested primers and the results were recorded as positive or negative, with the detection limit at 100 copies/ml. Quantification of HCV-RNA was carried out with the branched-DNA assay version 2.0 (Chiron Corp., Calif., USA), and the results were expressed in megaequivalents (MEq) per milliliter over a range from $<$0.5 to 120 MEq/ml.

Statistical analysis

Since certain data in the analysis were regarded to comply with non-Gaussian distribution, categorical variables at baseline were compared with the Fisher exact test and numerical values were analyzed with the Mann-Whitney U-test and the Kruskal-Wallis test. Cumulative rates of cirrhosis, HCC, and death were calculated using the Kaplan-Meier technique, and differences between curves were evaluated by the log-rank test. A p-value <0.05 with the two-tailed test was considered significant. All the analyses were carried out using the computer program SPSS ver.11.0 (SPSS Inc., Ill., USA).

Results

Treatment-naive patients older than 65 years infected with HCV

During the 42 years from 1964 through 2005, the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo admitted 332 patients aged 65 years or older with HCV who had not received any antiviral treatment, and in whom cirrhosis had not developed. In Table I we compare demographic, clinical, and virological characteristics between the 120 patients with baseline transaminase levels ≦40 IU/l and the 212 patients with levels ≧41 IU/l. ASAT and ALAT levels were higher, while platelet counts were lower in the patients with elevated transaminase levels compared with in patients without elevated transaminase levels.

When patients with baseline transaminase levels ≦40 IU/l were stratified by age, the median follow-up period was shorter in those aged 75–80 years than in those aged 65–69 or 70–74 years (4.5 versus 8.6 or 7.0 years, $p=0.011$) (Table II). Although the baseline transaminase levels were within normal limits in all of them, the median ASAT level was higher in patients aged 70–74 years than in those aged 65–70 or 75–80 years (35 versus 27 or 28 IU/l, $p=0.040$). In patients with baseline levels of both or either transaminase ≧41 IU/l, the median albumin level was lower in those aged 75–80 years than in those aged 65–69 or 70–74 years (3.9 versus 4.1 or 4.1 g /dl, $p=0.005$) (Table III).

Development of cirrhosis and HCC

Cirrhosis developed more frequently in elderly patients aged 65 years or older, with elevated transaminase levels at baseline, during follow-ups for longer than 3 years (Figure 1A). At 5 and 10 years of follow-up, cirrhosis developed in, respectively, 26% and 27% of the patients with the baseline transaminase levels ≧41 IU/l in contrast to only 4% and 13% of the patients with levels ≦40 IU/l ($p<0.001$). Likewise, HCC developed more frequently in elderly patients with elevated transaminase levels at baseline (Figure 1B). At 5 and 10 years of follow-up, HCC developed in, respectively, 22% and 26% of the patients with the baseline transaminase levels ≧41 IU/l, contrasting with only 3% and 5% of the patients with levels ≦40 IU/l ($p<0.001$).

Development of cirrhosis is compared between patients with and without elevated transaminase levels at baseline who were stratified by age (Figure 2). Cirrhosis developed more frequently in the patients with elevated transaminase levels than in those without elevated transaminase levels who were aged 65–69 years ($p<0.001$). In patients aged 70–74 years, cirrhosis tended to occur more often in those with elevated transaminase levels than in those without elevated transaminase levels during 5 years (27% versus 0%), but the difference fell short of being significant owing to the small number of patients in both groups.

Likewise, development of HCC is compared between patients with and those without elevated transaminase levels at baseline who were stratified by age (Figure 3). HCC developed more frequently in the patients with elevated transaminase levels than in those without elevated transaminase levels who were aged 65–69 years ($p=0.001$). In patients aged 70–74 and 75–80 years, HCC tended to occur more often in those with elevated transaminase levels than in those without elevated transaminase levels during 5 years (20% versus 5% and 19% versus 0%, respectively), but the difference was not significant, owing to the small number of patients in both groups.

Influence of gender on the development of cirrhosis and HCC

Figure 4 shows a comparison of the development of cirrhosis and HCC between 155 male and 177

Table I. Characteristics of patients with HCV-RNA aged 65 years or older with or without elevated transaminase (ASAT and ALAT) levels.

Features	≤40 IU/ml ($n=120$)	≥41 IU/l ($n=212$)	Differences p-value
Men	51 (42.5%)	104 (49.1%)	0.513
Follow-up (years)	7.8 (3–31.5)	8.7 (3–18.9)	0.181
ASAT (IU/l)	23 (6–40)	76 (27–496)	<0.001
ALAT (IU/l)	28 (11–40)	63 (22–411)	<0.001
Albumin (g/dl)	4.1 (2.4–4.9)	4.1 (3.2–5.3)	0.189
Platelets ($\times 10^3/mm^3$)	184 (120–343)	173 (120–313)	0.001
HCV RNA (MEq/ml)	4.5 (<0.5–120)	5.6 (<0.5–49)	0.168
HCV genotypes (1b:2a:2b:ND)	85:20:3:7	176:28:12:9	0.970

Abbreviations: HCV = hepatitis C virus; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; MEq = megaequivalents; ND = not determined. Data are expressed as the number (%) or the median with the range in parentheses.