SVR obtained via antiviral therapy for HCV cannot only prevent progression to liver cirrhosis or HCC but also prevent the development of diabetes, the potential impact of IFN therapy is quite significant.

In conclusion, this retrospective study suggests that the annual incidence of T2DM among patients with HCV is 0.8% to 1.0%. Our results indicate that SVR causes a two-thirds reduction of T2DM development in HCV-positive patients treated with antiviral drugs.

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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 (×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 (≥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 ($P < .0001$).

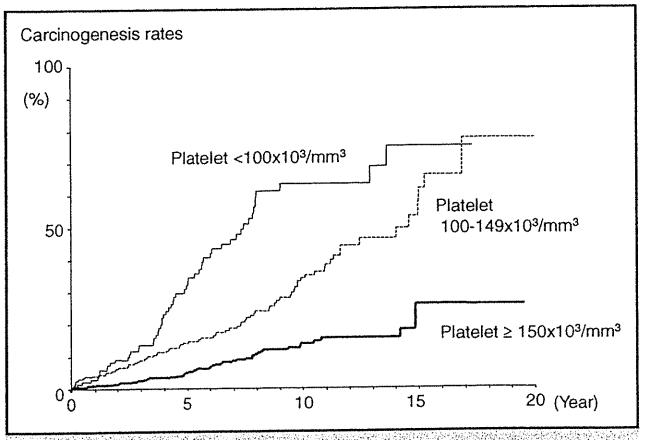

Figure 1 Hepatocarcinogenesis rates in patients without IFN therapy, according to initial platelet count. The lower the initial platelet count was, the higher the hepatocellular carcinogenesis was in the untreated cohort ($P < .0001$).

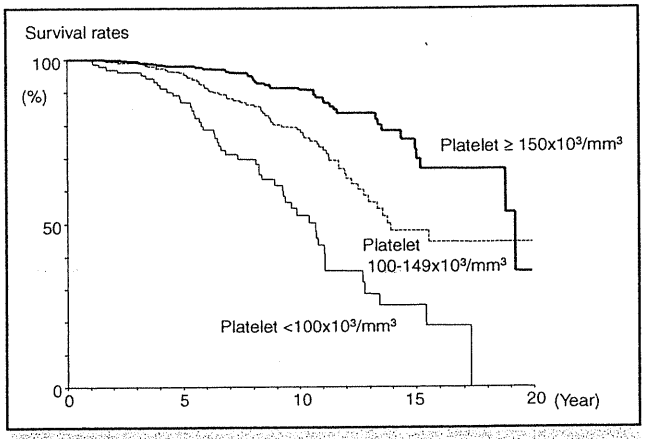

Figure 2 Cumulative survival in patients without IFN therapy, according to initial platelet count. Survival of patients with high platelet count was significantly higher than those with a low or intermediate platelet count ($P < .0001$).

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 ($P = .0005$).

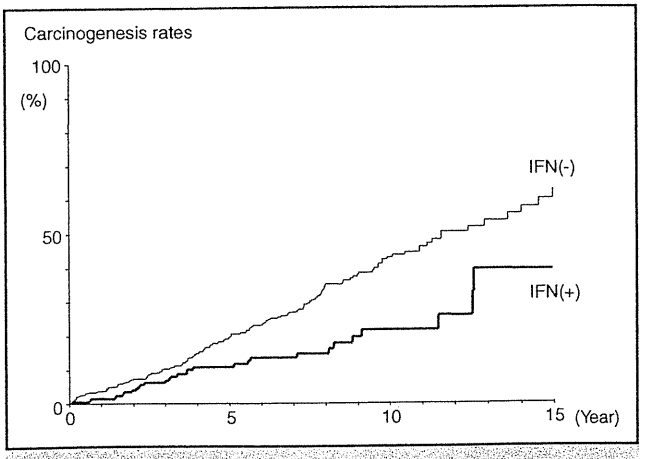

Figure 3 Hepatocarcinogenesis rates in patients with a low or intermediate platelet count. Carcinogenesis rate of patients with IFN therapy was significantly lower than those without therapy ($P = .0005$). IFN = Interferon.

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 interme-

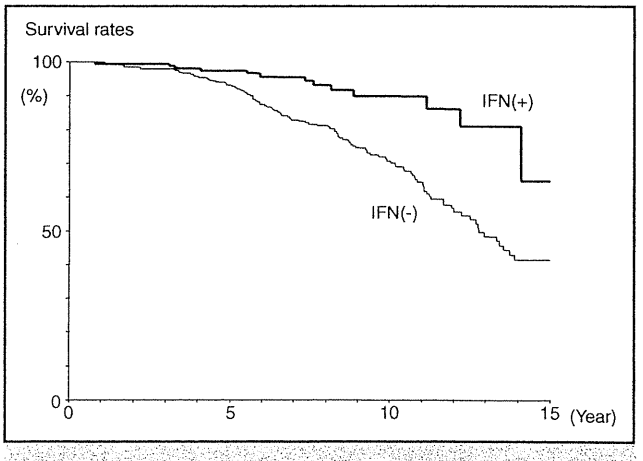

Figure 4 Cumulative survival in patients with a low or intermediate platelet count. Survival of patients with IFN therapy was significantly higher than those without therapy ($P = .0001$). IFN = Interferon.

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.

diate platelet group (<150,000/mm^3), platelet count, age, 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—LIVER, PANCREAS, AND BILIARY TRACT

Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study

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Abstract
Background Chronic hepatitis C (CHC) genotype 1b patients with high viral load are resistant to peginterferon (PEG-IFN) and ribavirin (RBV) combination therapy, especially older and female patients.
Methods To elucidate the factors affecting early and sustained viral responses (EVR and SVR), 409 genotype 1b patients CHC with high viral loads who had received 48 weeks of PEG-IFN/RBV therapy were enrolled. The amino acid (aa) sequences of the HCV core at positions 70 and 91 and of the interferon sensitivity determining region (ISDR) were analyzed. Host factors, viral factors, and treatment-related factors were subjected to multivariate analysis.
Results Male gender, low HCV RNA load, high platelet count, two or more aa mutations of ISDR, and wild type of core aa 70 were independent predictive factors for SVR. In patients with over 80% adherences to both PEG-IFN and RBV, male gender, mild fibrosis stage, and wild type of core aa 70 were independent predictors for SVR.
Conclusions Independent predictive factors for SVR were: no aa substitution at core aa 70, two or more aa mutations in the ISDR, low viral load, high values of platelet count, mild liver fibrosis and male gender.

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Keywords Chronic hepatitis C · Peginterferon and ribavirin · Core amino acid · Interferon sensitivity determining region

Abbreviations

CHC	Chronic hepatitis C
PEG-IFN	Peginterferon
RBV	Ribavirin
RVR	Rapid viral response
cEVR	Complete early viral response
LVR	Late viral response
ETR	End of treatment response
NR	Non response
SVR	Sustained viral response
ISDR	Interferon sensitivity determining region
Aa	Amino acid
ALT	Alanine aminotransferase
PLT	Platelet
HCC	Hepatocellular carcinoma

Introduction

A combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy for 48 weeks achieves a sustained viral response (SVR) rate of 40–50% in chronic hepatitis C (CHC) patients with a high viral load of genotype 1 [1–4]. The dose-reduction rate and the frequency of discontinuation of this treatment are high in aged patients [5]. The SVR rate of the therapy is lower in females than males, especially in older patients in Japan [6].

Around 30% of HCV carriers have serum alanine aminotransferase (ALT) levels within the upper limit of normal ranges [7, 8] and HCV carriers with persistently normal serum ALT (PNALT) and serum platelet (PLT) counts of over $15 \times 10^4/mm^3$ show low grade hepatic fibrosis and good prognosis [9]. Before treating HCV carriers, it is very important to predict non-response to PEG-IFN plus RBV therapy because of its medical cost, adverse effects, and its impact on the long term quality of life.

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There are many factors affecting response to IFN monotherapy and PEG-IFN/RBV therapy, including body mass index (BMI) [10, 11], steatosis [12, 13], insulin resistance [14], stage of liver fibrosis [15, 16], total cholesterol (T. Chol), triglyceride (TG), adherence to both PEG-IFN and RBV [17], race [18, 19], age [1, 2, 20], and viral factors including serum quantity of HCV RNA, HCV genotype and substitution of amino acids (aa) in the interferon sensitivity determining region (ISDR, 2209–2248) of the nonstructural protein 5A (NS5A) [21] and in the core protein [22, 23]. Early viral response is an important predictive factor in PEG-IFN/RBV therapy for CHC patients with genotype 1 and high viral loads [24–27].

The aim of this study was to elucidate the valuable predictive factors of SVR in Japanese patients with HCV genotype 1b high viral loads following 48 weeks of PEG-IFN/RBV therapy, focusing on the relationship between aa substitutions in the ISDR and at core aa 70 and 91 and early viral kinetics.

Patients and methods

Selection of patients

This retrospective study was conducted at 15 clinical sites in Japan which are part of the Study Group of Optimal Treatment of Viral Hepatitis supported by the Ministry of Health, Labor and Welfare, Japan. Eligible subjects were CHC patients, who (1) had received liver biopsy; (2) were genotype 1b with high viral load ($\geq$100 KIU/ml by Cobas Amplicor Hepatitis C Virus Test, version 2.0) at the start of PEG-IFN/RBV therapy; (3) received weekly injections of PEG-IFN-α-2b (PEG-INTRON; Shering-Plough, Kenilworth, NJ) of 1.5 μg/kg bw and oral administration of RBV (Rebetol; Shering-Plough) for 48 weeks. The amount of RBV was adjusted based on the subject's body weight; (600 mg for $\leq$60 kg bw, 800 mg for 60–80 kg bw, 1,000 mg for > 80 kg bw); (4) were examined serially for quantitative and qualitative HCV RNA; and (5) the aa sequences at positions 70 and 91 in the core region and of the ISDR in the NS5A had been determined in pretreatment sera.

Hepatitis B virus (HBV) infection, human immunodeficiency virus (HIV) infection, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease were excluded. Histopathological diagnosis was based on the scoring system of Desmet et al. [28]. The definition of alcohol abuse included patients having a history of more than 100 kg of total ethanol intake. Complete blood counts, liver function tests, serum lipids, serum ferritin, serum fibrosis markers, fasting plasma glucose (FPG), and immune reactive insulin (IRI) were examined in most cases. Written informed consent was obtained from all

patients before treatment, and the protocol was approved by the ethics committees in each site.

Study design

Four hundred and nine patients who completed 48 weeks of treatment and were followed for more than 24 weeks after treatment were enrolled in the first study (*Study design 1*).

To elucidate the effect of aa substitution of HCV core and in the ISDR on HCV dynamics, including a rapid viral response (RVR), complete early viral response (cEVR), a late viral response (LVR) and SVR, according to gender and age (<60 years ≥ 60 years), 201 of the 409 patients maintaining over 80% adherences to both PEG-IFN and RBV were enrolled in the second study (*Study design 2*).

Nucleotide sequencing of the core and NA5A gene

The nucleotide sequences encoding aa 1–191 (HCV core) and aa 2209–2248 (ISDR) were analyzed by direct sequencing as described by Akuta et al. [22, 27] and Enomoto et al. [21]. In brief, RNA was extracted from the sera and converted to cDNA and two nested rounds of polymerase chain reaction (PCR) were performed. Primers used in the PCR were as follows; (a) Nucleotide sequences of the core region: the first-round PCR was performed with CC11 (sense) and e14 (antisense) primers [22, 27], and the second-round PCR with CC9 (sense) and e14 (antisense) primers [22, 27]. (b) Nucleotide sequences of the ISDR in NS5A: the first-round PCR was performed with ISDR1 (sense) and ISDR2 (antisense) primers [21], and the second-round PCR with ISDR3 (sense) and ISDR4 (antisense) primers [21]. These sequences were compared with the consensus sequence of genotype 1b (HCV-J) [29]. Wild types virus encoded arginine and leucine at aa 70 and 91, respectively, and the aa substitutions were glutamine or histidine at aa 70 and methionine at aa 91.

Viral kinetic study

Serum HCV RNA levels were measured by PCR (Amplicor HCV RNA kit, version 2.0, Roche Diagnostics) using samples taken before treatment and at 4, 12, 24, and 48 weeks after the therapy. SVR was defined as HCV RNA negativity by qualitative analysis by PCR at 24 weeks after the treatment. RVR was defined as HCV RNA negativity at 4 weeks, cEVR as HCV RNA negativity at 12 weeks, LVR as HCV RNA negativity during 13–24 weeks and an end of treatment response (ETR) as HCV RNA negativity at the end of treatment. Patients who remained positive for HCV RNA at the end of the treatment and at 24 weeks after the therapy were defined as non-responders (NR).

Adherences to PEG-IFN and RBV

Adherences to PEG-IFN and RBV were assessed by separately calculating the actual doses of PEG-IFN and RBV received as percentages of the intended dosages. Adherences to PEG-IFN and RBV were divided into two groups; 80%≤ and <80%.

Statistical analysis

All data analyses were conducted using the SAS version 9.1.3 statistical analysis packages (SAS Institute, Cary, NC, USA). Individual characteristics between groups were evaluated by Mann–Whitney U test for numerical variables or Fisher's exact test for categorical variables. Variables exhibiting values of $p < 0.1$ in the univariate analysis were subjected to stepwise multivariate logistic regression analysis. The grade of steatosis and iron deposition in liver tissue, BMI, albumin (Alb), low density lipoprotein-cholesterol (LDL-C), homeostasis model assesment-insulin resistance (HOMA-IR), ferritin, and hyaluronic acid were excluded from multivariate logistic regression analysis because of the absence of those data in more than 10% of the patients. All p values of $p < 0.05$ by the two-tailed test were considered statistically significant.

Results

Study design 1

Baseline backgrounds, characteristics and adherences of peginterferon and ribavirin in males and females

The treatment outcome of PEG-IFN and RBV combination therapy depends on gender in Japanese patients, so in addition to aa substitutions in the ISDR in NS5A [21] or at HCV core 70 and 91 [22, 27], we compared the baseline characteristics according to gender (Table 1). Males were younger and the grade of hepatic inflammation was milder in males. The serum levels of LDL-C, PLT count, and aa substitutions of ISDR and at core 70 and 91 did not differ significantly different between males and females. The frequency of no alcohol abuse was significantly ($p < 0.0001$) higher in females than males (Some of them are not described in Table 1).

The rates of over 80% adherences to PEG-IFN and RBV were significantly lower ($p = 0.0066$, $p < 0.00001$, respectively) in females than males. Only in those above 60 years did the rate of over 80% adherence to PEG-IFN not differ significantly between males and females, but the rate of over 80% adherence to RBV was significantly lower ($p = 0.035$) in females than males (Table 1).

Table 1 Backgrounds and characteristics of male and female patients

Factors	Gender		*p* value
	Male	Female	
No. of patients	256 (62.6%)	153 (37.4%)	
Age			
Median (range)	53 (18–73)	59 (23–75)	0.00001
F stage			
F0–2	206 (80.5%)	119 (77.8%)	0.592
F3–4	50 (19.5%)	34 (22.2%)	
Grade (A factor)			
A0–1	163 (63. 7%)	79 (51.6%)	0.026
A2–3	93 (36.3%)	74 (48.4%)	
HCV RNA load 0 week (KIU/mL)			
Median (range)	1500 (100–5000 <)	1280 (100–5000<)	0.384
ALT 0 week (IU/L)			
Median (range)	74.5 (16–504)	59 (19–391)	0.001
BMI			
Median (range)	23.6 (17.5–31.2)	22.1 (16.1–33.9)	0.00033
Alb (g/dL)			
Median (range)	4.0 (3.0–5.2)	3.8 (3.0–4.8)	0.011
LDL-C (mg/dL)			
Median (range)	97 (30–185)	90 (34–174)	0.612
T-Chol (mg/dL)			
Median (range)	167 (85–273)	176 (114–261)	0.0016
PLT count ($\times 10^4/mm^3$)			
Median (range)	17.0 (8.0–31.9)	16.4 (8.1–39.9)	0.350
Amino acid mutation of ISDR			
0–1	200 (78.1%)	121 (79.1%)	0.608
2≤	56 (21.9%)	32 (20.9%)	
Amino acid substitution of core 70			
Wild	177 (69.1%)	114 (74.5%)	0.261
Mutant	79 (30.9%)	39 (25.5%)	
Amino acid substitution of core 91			
Wild	153 (59.8%)	98 (64.1%)	0.403
Mutant	103 (40.2%)	55 (35.9%)	
PEG-IFN adherence			
<80%	41 (17.7%)	42 (30.4%)	0.0066
80%≤	190 (82.3%)	96 (69.6%)	
Ribavirin adherence			
<80%	54 (23.6%)	73 (52.1%)	<0.00001
80%≤	175 (76.4%)	67 (47.9%)	
Age: <60 years			
PEG adherence			
<80%	30 (17.8%)	23 (31.5%)	0.027
80%≤	139 (82.2%)	50 (68.5%)	
Ribavirin adherence			
<80%	27 (16.2%)	31 (42.5%)	0.000029
80%≤	140 (83.8%)	42 (57.5%)	
Age: 60 years≤			
PEG adherence			
<80%	11 (17.7%)	19 (29.2%)	0.147
80%≤	51 (82.3%)	46 (70.8%)	
Ribavirin adherence			
<80%	27 (43.5%)	42 (62.7%)	0.035
80%≤	35 (56.5%)	25 (37.3%)	

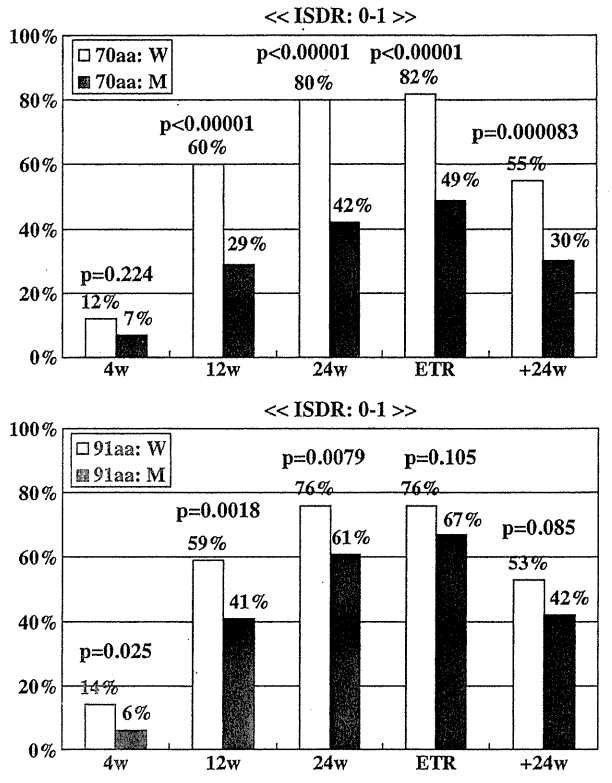

Fig. 1 Relationship between time course of serum HCV RNA negativity and amino acid substitutions in the ISDR and core amino acids 70 and 91. For cases with no or only one amino acid (aa) change in the ISDR, the rates of cEVR, LVR, ETR and SVR were significantly higher in patients with wild type core aa 70 but only the rates of RVR, cEVR, and LVR were significantly higher in patients with wild type core aa 91

Amino acid substitutions

There were no significant differences in the frequency of aa substitutions in the ISDR between males and females. Core aa substitutions at positions 70 and 91 were as follows; 291 (71.1%) were wild type and 118 (28.9%) were mutant at core aa 70, and 251 (61.4%) were wild type and 158 (38.6%) were mutant at core aa 91. There were no significant differences between males and females and between patients below and above 60 years of age.

Virological responses and aa substitutions

The rate of RVR did not differ significantly between males and females. However, more male patients showed HCV RNA negativity at 12 weeks (males vs. females; 60.7 vs. 48.4%, $p = 0.018$), 24 weeks (76.8 vs. 64.2%, $p = 0.0078$) and 48 weeks (78.2 vs. 68.6%, $p = 0.049$), and the proportion of male patients in SVR was significantly higher than that of females (61.3 vs. 37.3%, $p < 0.00001$).

RVR, cEVR and SVR rates were significantly higher in patients with two or more aa mutations in the ISDR compared to patients having no or one aa substitution in that region (20 vs. 11%, $p = 0.044$; 71 vs. 52%, $p = 0.0021$; 66 vs. 49%, $p = 0.0054$, respectively). AA substitution at core position 70 resulted in significantly lower rate of cEVR, LVR, ETR, and SVR (40 vs. 63%, $p = 0.000037$; 51 vs. 81%, $p < 0.00001$; 56 vs. 83%, 41 vs. 57%; $p < 0.00001$, $p = 0.0031$, respectively). Although the patients with the wild type aa at core 91 showed significantly higher rates of RVR and cEVR, the rate of SVR was not significantly higher in those patients ($p = 0.054$).

SVR rates were 30% for patients with no or one aa substitution in the ISDR and the core aa 70 substitution, and were significantly lower compared to those with the wild type aa core 70 (Fig. 1). These findings were not confirmed in patients with no or one aa substitution in the ISDR and the core aa 91 substitution (Fig. 1).

Factors affecting SVR by univariate analysis

Univariate analysis identified nine parameters that influenced non-SVR significantly: female gender, older age, advanced staged liver fibrosis, high viral load, low serum Alb level, low PLT count, no or one aa substitution in the ISDR, aa substitution at core aa 70, and low adherence to RBV (Table 2). The frequency of steatosis and HOMA-IR were significantly ($p = 0.0057$, $p < 0.00001$, respectively) lower in patients with SVR compared with non-SVR (data not shown). However, these factors were not entered in the multivariate analysis because of the absence of the data in many cases.

Factors affecting RVR, cEVR, and SVR by multivariate logistic regression analysis

Multivariate analysis identified four parameters that influenced RVR independently: low HCV RNA load, low serum ALT level, two or more aa mutations in the ISDR and the wild type aa at core position 91 (Table 3).

Concerning cEVR, male gender, mild fibrosis stage, low HCV RNA load, two or more aa mutations in the ISDR, and the wild type aa at core positions 70 and 91 were independent predictors (Table 3).

Concerning SVR, male gender ($p < 0.0001$), low HCV RNA load ($p = 0.013$), high PLT count ($p = 0.0019$), two or more aa mutations in the ISDR ($p = 0.024$), and wild type core aa 70 ($p = 0.0045$) were found to be independent predictors (Table 3).

The predictive values of the combination of gender, PLT count, ISDR and core aa 70 are shown in Fig. 2a. In male patients having PLT of $<15 \times 10^4/mm^3$, and, no or one aa substitution in the ISDR, the SVR rate was 68% when core 70

Table 2 Univariate analysis to identify the factors of SVR

Factors	Negative of HCV RNA after 24 weeks		*p* value
	(−)	(+)	
No. of patients	214 (52.3%)	195	
Gender			
Male	157 (61.3%)	99	<0.00001
Female	57 (37.3%)	96	
Age			
Median (range)	52.5 (18–75)	58 (20–74)	<0.00001
<60 years	155 (58.1%)	112	0.0018
60 years≤	59 (41.5%)	83	
Age: <60 years			
Male	118 (63.4%)	68	0.010
Female	37 (45.7%)	44	
Age: 60 years≤			
Male	39 (55.7%)	31	0.0011
Female	20 (27.8%)	52	
F stage			
F0–2	190 (58.5%)	135	0.000013
F3–4	25 (29.8%)	59	
Grade (A factor)			
A0–1	138 (56.8%)	104	0.130
A2–3	81 (48.5%)	86	
HCV RNA load 0 week (KIU/mL)			
Median (range)	1300 (100–5000<)	1700 (130–5000<)	0.016
ALT 0 week (IU/L)			
Median (range)	66 (16–391)	67 (19–504)	0.892
BMI			
Median (range)	23.0 (17.3–32.4)	23.25 (16.1–33.9)	0.714
Alb (g/dL)			
Median (range)	4.0 (3.2–5.2)	3.8 (3.0–4.8)	0.0088
LDL-C (mg/dL)			
Median (range)	94.5 (31–185)	97.5 (30–182)	0.611
T-Chol (mg/dL)			
Median (range)	169.5 (85–257)	170 (103–273)	0.511
PLT count ($\times 10^4/mm^3$)			
Median (range)	18.2 (8.7–39.9)	15.1 (8.0–31.9)	<0.00001
<15	54 (36.5%)	94	<0.00001
15≤	160 (61.3%)	101	
Amino acid mutation of ISDR			
0–1	156 (48.6%)	165	0.0054
2≤	58 (65.9%)	30	
Amino acid substitution of core 70			
Wild	166 (57.0%)	125	0.0031
Mutant	48 (40.7%)	70	
Amino acid substitution of core 91			
Wild	141 (56.2%)	110	0.054
Mutant	73 (46.2%)	85	
PEG-IFN adherence			
<80%	35 (42.2%)	48	0.063
80%≤	154 (53.8%)	132	
Ribavirin adherence			
<80%	55 (43.3%)	72	0.048
80%≤	132 (54.5%)	110	

Table 3 Multivariate logistic regression analysis to identify independent predictive factors of RVR, cEVR, and SVR

	Odds ratio	95% CI	*p* value
RVR factors selected by stepwise method			
F stage			
F0–2/F3–4	2.924	0.988–8.696	0.053
HCV RNA load 0 week (KIU/mL)			
<1000/1000≤	2.151	1.130–4.082	0.020
ALT 0 week (IU/L)			
<60/60≤	2.165	1.127–4.149	0.020
Amino acid mutation of ISDR			
2≤/0–1	2.371	1.187–4.735	0.014
Amino acid substitution of core 91			
W/M	2.137	1.021–4.464	0.044
cEVR factors selected by stepwise method			
Gender			
Male/female	1.912	1.209–3.021	0.0055
F stage			
F0–2/F3–4	2.079	1.133–3.817	0.018
HCV RNA load 0 week (KIU/mL)			
<1000/1000≤	1.608	1.002–2.577	0.049
PLT count ($\times 10^4$/mm^3)			
15≤/<15	1.427	0.882–2.309	0.148
Amino acid mutation of ISDR			
2≤/0–1	2.512	1.407–4.485	0.0018
Amino acid substitution of core 70			
W/M	2.513	1.508–4.184	0.0004
Amino acid substitution of core 91			
W/M	1.965	1.241–3.115	0.004
SVR factors selected by stepwise method			
Gender			
Male/female	3.704	2.132–6.410	<0.0001
F stage			
F0–2/F3–4	1.812	0.888–3.690	0.103
HCV RNA load0 week (KIU/mL)			
<1000/1000≤	2.024	1.163–3.534	0.013
PLT count ($\times 10^4$/mm^3)			
15≤/<15	2.469	1.394–4.372	0.0019
Amino acid mutation of ISDR			
2≤/0–1	2.148	1.107–4.170	0.024
Amino acid substitution of core 70			
W/M	2.415	1.316–4.444	0.0045
Amino acid substitution of core 91			
W/M	1.433	0.828–2.481	0.199
PEG adherence (%)			
80≤/<80	1.562	0.834–2.926	0.164

W Wild, *M* Mutant

was a wild type but only 16% in patients with mutant at core 70. In female patients, no or one aa substitution in ISDR and $<15 \times 10^4$/mm^3 of PLT count, the SVR rates were as low as 10 or 8%, irrespective of aa substitution at core 70. SVR was only 24% in patients with substitution of core aa 70 even when the PLT count was $\geq 15 \times 10^4$/mm^3. In this study, the combination analysis of PLT count, ISDR, and core aa substitution was useful for predicting non-SVR.

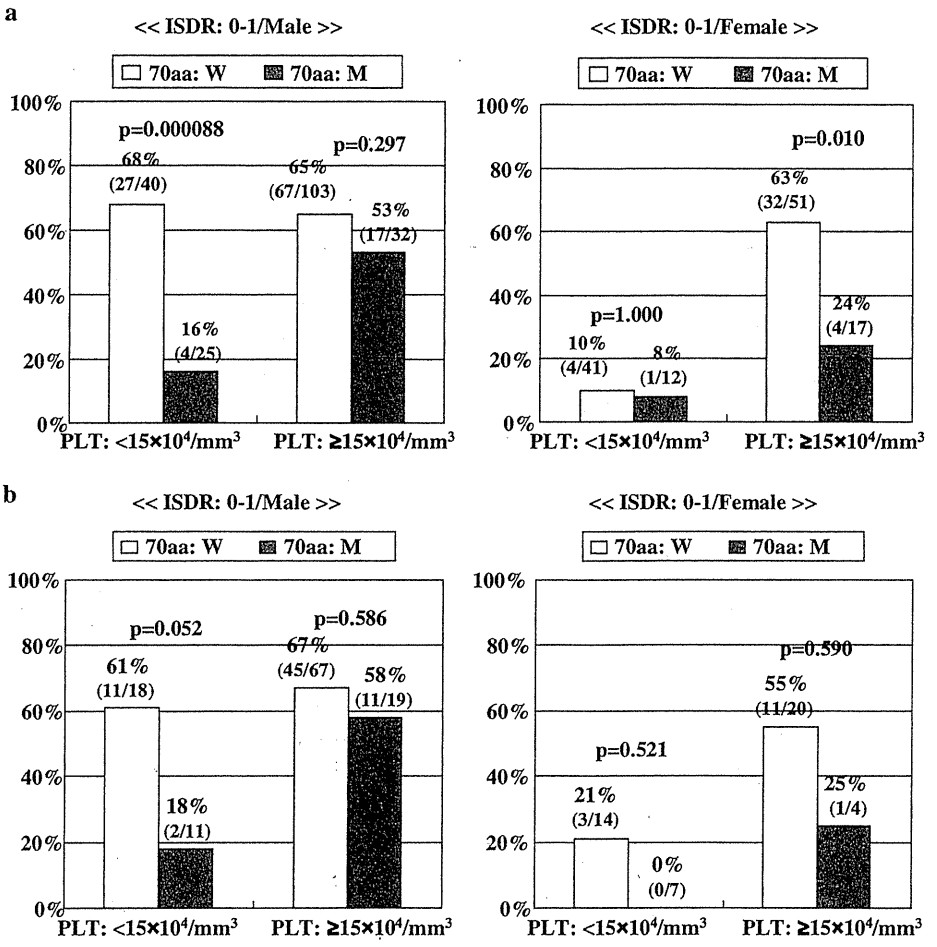

Fig. 2 Relationship between SVR rate and amino acid substitutions in the ISDR and core amino acids 70 and 91, PLT counts and gender difference. The two figures of **a** show the results of *Study 1* and the two figures of **b** show the results of *Study 2*. In male patients with no or only one amino acid (aa) substitution in the ISDR and PLT count of less than $15 \times 10^4/mm^3$, the SVR rate was 68% in those with wild type core aa 70, but only 16% in patients with mutant type of core aa 70, which is significantly different ($p = 0.000088$). There were no significant differences between wild type and mutant type of core aa 70 in the patients with no or one aa substitution in the ISDR and PLT count of over $15 \times 10^4/mm^3$. By contrast, in female patients with no or one aa substitution in the ISDR, there were no significant differences between wild type and mutant type of core aa 70 with PLT count of less than $15 \times 10^4/mm^3$, but there were significant differences between wild type and mutant type of core aa 70 with PLT counts of less than $15 \times 10^4/mm^3$ (**a**). For the patients maintaining over 80% adherences to both PEG-IFN and RBV, in males having no or one aa substitution in the ISDR and PLT counts of less than $15 \times 10^4/mm^3$, a wild type of core aa 70 could predict SVR with a positive predictive value (PPV) of 61% and negative predictive value (NPV) of 82% ($p = 0.052$). However, in male patients with PLT counts of over $15 \times 10^4/mm^3$, core aa 70 was not a useful marker for predicting SVR and non-SVR. The number of female patients with no or one aa substitution in ISDR was too small to reach a definite conclusion (**b**)

Study design 2

The basic features of 201 patients achieving 80% adherences to both PEG-IFN and RBV are as follows: the females were significantly ($p = 0.00006$) older than the males. Iron deposition in liver tissue, alcohol abuse, BMI, serum albumin level, serum ferritin level, and PLT count were significantly higher in males than females. Inflammatory activity was significantly ($p = 0.046$) higher in females than males (data not shown).

AA substitutions in the ISDR were as follows; in males 33 (22.3%) had two or more aa substitutions, in females 8 (15.1%) had two or more aa substitutions. The analysis of core aa position 70 and 91 sequences showed no significant differences in aa substitutions of either core aa 70 or 91 between males and females (data not shown).

In patients less than 60 years of age, SVR rate was significantly higher ($p = 0.0042$) in males than females, but no significant difference was noted between males and females over 60 years old. However, the number of patients over 60 years was small (Table 4).

Table 4 Univariate analysis to identify the significantly different factors between SVR and non-SVR (201 patients received over 80% adherences of both PEG-IFN and RBV)

Factors	Negative of HCV RNA after 24 weeks		*p* value
	(−)	(+)	
No. of patients	111 (55.2%)	90	
Gender			
Male	93 (62.8%)	55	0.00037
Female	18 (34.0%)	35	
Age			
Median (range)	51 (18–70)	56 (23–74)	0.00025
<60 years	91 (60.3%)	60	0.014
60 years≤	20 (40.0%)	30	
Age: <60 years			
Male	79 (66.4%)	40	0.0042
Female	12 (37.5%)	20	
Age: 60 years≤			
Male	14 (48.3%)	15	0.243
Female	6 (28.6%)	15	
F stage			
F0–2	103 (60.9%)	67	0.0012
F3–4	8 (25.8%)	23	
Grade (A factor)			
A0–1	80 (59.3%)	55	0.189
A2–3	31 (47.0%)	35	
HCV RNA load 0 week (KIU/mL)			
Median (range)	1300 (110–5000<)	1280 (130–5000<)	0.351
ALT 0 week (IU/L)			
Median (range)	74 (16–268)	67.5 (19–504)	0.752
BMI			
Median (range)	23.1 (17.3–31.0)	23.6 (16.1–33.9)	0.626
Alb (g/dL)			
Median (range)	3.95 (3.3–5.2)	3.9 (3.0–4.8)	0.079
LDL-C (mg/dL)			
Median (range)	96 (31–185)	97.5 (30–182)	0.865
T-Chol (mg/dL)			
Median (range)	170 (85–248)	170 (105–273)	0.624
PLT count ($\times 10^4$/mm^3)			
Median (range)	18.9 (8.7–30.9)	15.55 (7.2–28.4)	0.00003
<15	23 (35.9%)	41	0.00024
15≤	88 (64.2%)	49	
Amino acid mutation of ISDR			
0–1	84 (52.5%)	76	0.159
2≤	27 (65.9%)	14	
Amino acid substitution of core 70			
Wild	91 (61.5%)	57	0.0037
Mutant	20 (37.7%)	33	
Amino acid substitution of core 91			
Wild	73 (60.3%)	48	0.083
Mutant	38 (47.5%)	42	

Virological responses and aa substitution

The rates of RVR, cEVR, LVR, ETR and SVR in males and females were 12.5 versus 11.3% ($p = 1.000$), 59.6 versus 43.4% ($p = 0.053$), 74.3 versus 50.0% ($p = 0.0018$), 76.2 versus 66.7% ($p = 0.198$), and 62.8 versus 34.0% ($p = 0.00037$), respectively (data not shown). The backgrounds and characteristics of SVR and non SVR patients are shown in Table 4. There were significant differences in gender (male vs. female; $p = 0.00037$), age (<60 years vs. ≥60 years; $p = 0.014$), F stage (F0-2 vs. F3,4; $p = 0.0012$), PLT count ($<15 \times 10^4/mm^3$ vs. $15 \times 10^4/mm^3 \leq$; $p = 0.00024$), and substitution of core aa 70 (wild type vs. mutant, $p = 0.0037$) between SVR and non-SVR patients. The distribution of fatty change in liver tissue (≤10% vs. 11–33% vs. 34%≤; $p = 0.046$) and the grade of HOMA-IR (1.7 vs. 3.9, $p = 0.0018$) were significantly different between SVR and non-SVR (data not described in Table 4).

Factors affecting SVR by multivariate logistic regression analysis

Male gender ($p = 0.0006$), mild fibrosis stage ($p = 0.027$), and wild type of core aa 70 ($p = 0.043$) were independent predictors of SVR.

Valuable markers for predictions of sustained virological response to peginterferon and ribavirin therapy

Two or more aa mutations in the ISDR, wild type core aa 70, $\geq 15 \times 10^4/mm^3$ of PLT count, and male gender were selected statistically as independent predictors of SVR. We show here SVR rates of the patients having over 80% adherences to both PEG-IFN and RBV (Fig. 2b). In males having no or one aa substitution in the ISRD and PLT count of $<15 \times 10^4/mm^3$, wild type core aa 70 could predict SVR with a positive predictive value (PPV) of 61% and negative predictive value (NPV) of 82% ($p = 0.052$). In females, the SVR rate was very low in those who had substitution of core aa 70, but there was no significant difference between patients with wild type and substitution of core aa 70. The number of female patients was too small to provide a definite conclusion.

Discussion

The present multivariate logistic regression analysis revealed that male gender, low HCV RNA load, high PLT count, and two or more aa mutations in the ISDR and wild type core aa 70 were independent predictors for SVR. PLT count significantly decreased corresponding to the progression to the stage of liver fibrosis in CHC [9, 30, 31].

It has been considered that the low adherence level to PEG-IFN/RBV is a major cause of a significantly lower SVR rate in females and older patients [32]. The percentage of patients having over 80% adherences to both PEG-IFN and RBV was significantly lower in females than males, however, differences in the adherence to PEG-IFN/RBV between males and females were not independent predictive factors of non-SVR.

A recent report from Japan showed six or more mutations in the variable region 3 (V3) of nonstructural protein 5A (NS5A) plus upstream flanking region NS5A (aa 2334–2379), referred to as the IFN/RBV resistance determining region (IRRDR), was a useful marker for predicting SVR, but the ISDR sequence was not valuable for predicting SVR [33]. However, the number of subjects in that study was too small ($n = 45$) to reach an acceptable conclusion.

To elucidate the factors affecting low SVR rate in older female patients, we performed a multivariate logistic regression analysis using patients who achieved ≥80% adherence to both PEG-IFN and RBV. Male gender, stage of mild liver fibrosis, and wild type core aa 70 were independent predictors of SVR. In this study, blood concentration of RBV was determined in fewer than 50% of cases during treatment. Thus we cannot exclude the possibility of the effect of the blood concentration of RBV during treatment on the low SVR rate in females and older patients.

From the present analysis, it was clear that ALT, BMI, Alb, T. Chol, and adherence to RBV differed significantly between males and females, however, these factors were not independent predictors of SVR. There is a report that steatosis is an important cofactor that reduces the SVR rate in genotype 1 infected patients [34], however, such an effect was not seen in this study. Thus we could not identify the factors associated with a significantly lower SVR rate in females than males.

In the present multivariate logistic regression analyses, patients having wild type core aa 91 had significantly higher rates of RVR and cEVR, but not SVR, and patients with wild type core aa 70 had significantly higher rates of cEVR and SVR, but not RVR. Patients having two or more aa substitutions in the ISDR had significantly higher rates of RVR, cEVR, and SVR. Although several possibilities have been considered concerning the effects of aa substitutions of core protein on SVR in PEG-IFN/RBV therapy for CHC patients, the exact mechanisms have not yet been elucidated.

Recent reports have indicated that low serum IP-10 (interferon-γ inducible protein 10 kDa) [35], a higher HCV-specific CD8 cell proliferation potential [36], and a high ratio of Th1/Th2 [37] are good predictors of SVR to

PEG-IFN/RBV therapy. These results indicate the importance of immunological status and immunological response to treatment in patients difficult to treat with PEG-IFN/RBV therapy for CHC.

The present univariate analyses revealed that there were many factors relating to RVR, cEVR, and SVR including LDL-C, HOMA-IR, fatty change in liver tissue, and hyaluronic acid, however some of these factors had not been examined in some participating institutes. We consider that we must perform a prospective mass study using many factors including immunological aspects, viral factors, disease status, and therapeutic aspects to elucidate the reason that older female patients are resistant to a combination of PEG-IFN and RBV therapy in CHC with a high viral load genotype 1b.

In conclusion, our results demonstrated that wild type core aa 70, two or more aa mutations in the ISDR, low viral load, high PLT counts, and male gender are useful markers for predicting SVR.

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