入することが望ましい。インターフェロン療法の年齢的な限界は70歳程度と考えられており，それ以上の高齢者の場合は患者個々の状態により判断すべきであろう。高齢者や肝線維化の軽い例では，ウルソデオキシコール酸やグリチルリチン製剤の投与を行いトランスアミナーゼを抑制する。また，瀉血および鉄制限食による除鉄療法も有効である。

今後の展開

近年，免疫抑制剤であるシクロスポリンAの抗ウイルス効果が注目されている[4]。C型肝炎関連の肝移植例では術後高率に肝炎が再燃し，ウイルス量が高値となり，肝線維化の進行が早いため，移植後のインターフェロン療法が必要であると考えられている[5]。肝移植後に免疫抑制剤としてシクロスポリンAが用いられることがあるため，インターフェロンとリバビリンとの3者併用療法を行うことが検討されている。また，シクロスポリンAの類似体で免疫抑制効果が無く，抗ウイルス効果のみを持った薬剤の臨床応用が検討されている[6]。

おわりに

新しいインターフェロン製剤の導入により1b型高ウイルス量の難治性C型肝炎に対する治療効果は著しく向上してきている。今後開発される薬剤の導入によりさらなる治療効果が期待される。しかしながらこれらの治療抵抗性のウイルスもおり，今後ウイルス側のみでなく宿主側の条件も解析し，より効果的な治療法の開発が望まれる。

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Characteristics of Patients with Chronic Hepatitis C who Develop Hepatocellular Carcinoma after a Sustained Response to Interferon Therapy

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BACKGROUND. The objective of the current study was to determine the characteristic features of sustained responders who develop hepatocellular carcinoma after treatment with interferon for chronic hepatitis C.

METHODS. This study included 3626 patients with chronic hepatitis C who had received interferon monotherapy. Cox proportional hazards analysis was used to compare sustained responders who did and did not develop hepatocellular carcinoma, and nonsustained responders who developed hepatocellular carcinoma in a multicenter, retrospective cohort study.

RESULTS. Among 1197 sustained responders, 27 patients developed hepatocellular carcinoma (2.3%). Compared with sustained responders who did not develop hepatocellular carcinoma, patients who developed disease more often were male ($P = 0.0212$), were older ($P = 0.0068$), and had advanced-stage histologic disease before interferon therapy ($P = 0.0345$). Conversely, compared with patients with hepatocellular carcinoma who were not sustained responders, patients who were sustained responders tended to be older at the time of the initiation of interferon therapy ($P = 0.0552$) and at the time hepatocellular carcinoma was detected ($P = 0.0593$), and they also were predominantly male ($P = 0.0507$). The histologic staging and serum aminotransferase levels at the initiation of interferon therapy, the interval to the detection of tumor, and the tumor size showed no significant differences between the two groups.

CONCLUSIONS. Sustained responders in the group at high risk for developing hepatocellular carcinoma after interferon therapy were older, more often were male, and had more advanced histologic disease stage. Such patients should be followed carefully periodically for > 10 years after they complete interferon therapy. *Cancer* **2004;101:1616–22.** © *2004 American Cancer Society.*

KEYWORDS: chronic hepatitis type C, hepatocellular carcinoma, interferon, sustained responder.

In Japan, chronic hepatitis C (CH-C) with advanced histologic staging often progresses to hepatocellular carcinoma (HCC),[1] although patients who are seropositive for antihepatitis C virus (anti-HCV) antibodies or for HCV RNA do not always progress to cirrhosis or HCC.[2,3] Risk factors for developing HCC in patients with CH-C are advanced histologic stage, irregular regeneration of hepatocytes, heavy drinking, higher serum alanine aminotransferase (ALT) levels or lower serum albumin levels, male gender, and older age.[1,4–7] Since 1992, patients with CH-C commonly have been treated with interferon α (IFN-α) or IFN-β, which are covered by public health insurance in Japan. Because IFN improves hepatic inflammation and inhibits the progression of hepatic fibrosis, it

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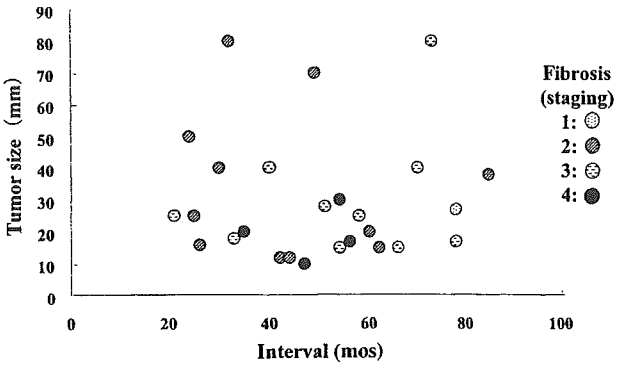

FIGURE 1. The interval from the completion of IFN therapy to the detection of SR HCC statistically did not correlate significantly with the tumor size or hepatic staging.

has been suggested that the incidence of HCC may be reduced by IFN treatment. In fact, IFN therapy reportedly was effective not only for improving liver biochemistry and eliminating HCV RNA but also for reducing the inflammation/fibrosis scores and lowering the risk of HCC, especially in sustained responders (SR patients).[8–14]

Although a significant decrease in the incidence of HCC has been observed in SR patients after IFN therapy,[9–14] HCC is detected in some of them.[15–25] The clinical features of SR patients who develop HCC (SR HCC patients) and the long-term incidence of HCC in SR patients remain unclear, and the optimal duration and frequency of follow-up have not been established. Therefore, we analyzed SR HCC patients to determine their characteristic features compared with SR patients who did not develop HCC (SR non-HCC patients) and non-SRs who developed HCC (non-SR HCC patients).

MATERIALS AND METHODS

Patients

For this study, 3626 patients with CH-C were enrolled (2344 males and 1282 females) who had received IFN therapy between January 1990 and November 2001. Data from these patients were collected from 6 institutions and related hospitals, including 1371 patients from Kyoto Prefectural University of Medicine, 1478 patients from Osaka University, 497 patients from Miyazaki Medical College, 130 patients from Nagoya University, 102 patients from Shinsyu University, and 48 patients from Yamaguchi University. All patients were seropositive for anti-HCV antibodies, positive for serum HCV RNA, and seronegative for hepatitis B virus surface antigen. We excluded patients who had coexisting liver diseases, such as autoimmune hepatitis or primary biliary cirrhosis, and confirmed that they did not abuse alcohol (daily alcohol intake > 60 g of ethanol). No patients were infected with human immunodeficiency virus (HIV). At the time of entry into this study, no patients showed evidence of HCC, as determined by ultrasonography (US) and/or computed tomography (CT) studies. In principle, patients underwent liver biopsy prior to IFN therapy, and the histologic diagnoses were reached according to the classification of Desmet et al.[26] The gender, mean age, and histologic disease stage at the initiation of IFN therapy are shown in Table 1.

TABLE 1
Characteristics of Patients with Chronic Hepatitis C who were Treated with Interferon[a]

Characteristic	Sustained responder	Nonsustained responder	*P* value[b]
No. patients	1197	2429	—
Male:female ratio	776:421	1568:861	0.8826
Age (yrs, mean ± SD)	49.4 ± 11.9	51.2 ± 10.6	< 0.0001
Histologic staging score: No. of patients (%)			
F1	385 (38.6)	522 (25.8)	
F2	322 (32.3)	613 (30.3)	< 0.0001
F3	262 (26.3)	782 (38.6)	
F4	29 (2.9)	109 (5.4)	
Not available	199	403	

SD: standard deviation; IFN: interferon.
[a] All data were determined before interferon therapy.
[b] *P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.

Natural IFN-α, recombinant IFN-α-2a, and recombinant IFN-α-2b were used in this study. In general, the IFN treatment protocol was within the range covered by public health insurance in Japan, namely, 3–10 MU of IFN-α for 24 weeks (daily for 2 weeks and 3 times per week for 22 weeks). In a few patients, administration of IFN-α was prolonged to 52 weeks. In some patients who suffered from severe side effects, the therapy period was shortened. In addition, patients for whom the total dose of IFN was < 200 MU were excluded from the study. Patients who had been treated with peginterferon or IFN/Ribavirin also were excluded. There was no difference noted with regard to the treatment protocol among the institutions and their related hospitals. We checked the laboratory findings at the end of IFN therapy and 6 months later. SR patients were defined as those who demonstrated continuous normal serum ALT levels for 6 months after finishing IFN therapy. The remaining patients were regarded as non-SR patients. The patient population included 1197 SR patients and 2429 non-SR patients.

We followed all patients for at least 1 year after the end of IFN therapy. The mean ± standard deviation

(SD) follow-up was 5.9 years ± 1.9 years. In SR patients, in general, we performed biochemical examinations, which sometimes included α-fetoprotein, every 3–12 months after confirming a sustained response. US and/or CT studies were performed at least once annually. However, because the incidence of HCC in non-SR patients—especially those with advanced-stage disease (fibrotic scores of F3 or F4)–was expected to be higher than that in SR patients, US and/or CT studies were performed every 3–6 months in non-SR patients. This strategy was similar in all of the institutions, and the frequency of radiographic examination was calculated to avoid unnecessary cost and not to miss HCC. However, some SR patients and non-SR patients who skipped or stopped visiting the outpatient clinic and some patients who were followed by their home physicians were not followed sufficiently. The diagnosis of HCC was based on appropriate radiologic findings (hepatic angiography, dynamic CT, magnetic resonance imaging).[27] When it was difficult to determine a final diagnosis with the radiologic findings, a histologic diagnosis was reached by tumor biopsy. In 17 of 27 SR HCC patients, a histologic diagnosis of HCC was obtained by the examination of resected hepatic tumors or biopsied tumor specimens. Patients who were diagnosed with HCC within 1 year after the end of IFN therapy were excluded from this study because of the possibility that a small but detectable HCC was missed before IFN therapy. Written informed consent to receive IFN therapy and to participate in this follow-up study was obtained from all patients, and the ethical committees of the participating institutions approved this study.

Statistical Analysis

Statistical analysis was performed using the SAS/PC statistical package (SAS Institute, Cary, NC). The Fischer exact probability test was used to compare the frequencies of gender. The Wilcoxon two-sample test was used to compare age, histologic staging, serum ALT level, interval between the end of IFN therapy and the detection of HCC, and the size of HCC. The independent risk factors for developing HCC in SR patients were examined by Cox proportional-hazards analysis; the variables were gender, age, histologic stage, and serum ALT level. Patients who had missing data were excluded from this analysis. Each variable was transformed into categorical data comprised of two-sample, ordinal numbers for multivariate analysis. P values were two-sided, and P values < 0.05 were considered statistically significant.

RESULTS

Characteristic Features of SR HCC Patients

During the observation of 3626 patients, HCC was detected in 259 patients; however, 19 patients were excluded, because HCC was detected within 1 year after they completed IFN therapy. The distribution of the remaining 240 HCC patients among the 6 institutions was as follows: 109 patients from Kyoto Prefectural University of Medicine (HCC incidence, 8.0%), 102 patients from Osaka University (HCC incidence, 6.9%), 3 patients from Miyazaki Medical College (HCC incidence, 0.6%), 15 patients from Nagoya University (HCC incidence, 11.5%), 8 patients from Shinsyu University (HCC incidence, 7.8%), and 3 patients from Yamaguchi University (HCC incidence, 6.3%). The incidence of HCC did not differ significantly among the institutions, except for Miyazaki Medical College. partly because hepatic fibrosis was less advanced in patients from this institution compared with patients from the other five institutions. Of 240 patients, 27 were SR patients, and 213 were non-SR patients. The ages of the 240 patients at the initiation of IFN therapy ranged from 37–77 years (mean age ± SD, 59.1 years ± 6.6 years) and varied from 39–83 years (63.6 years ± 6.8 years) at the time HCC was detected.

Among the 27 SR HCC patients, 5 patients consumed ≈ 50 g of ethanol daily. By evaluating liver specimens and biochemical examinations, including γ-glutamyl transferase, we excluded the possibility of alcoholic liver diseases in these patients. Serum HCV RNA was evaluated in the SR HCC patients by reverse transcriptase-polymerase chain reaction analysis. Twenty-six SR HCC patients were complete responders (seronegative for HCV RNA both at the end of IFN therapy and 6 months later), and 1 SR HCC patient was a biochemical responder (seropositive for HCV RNA at the end of IFN therapy). In 1 complete responder who developed HCC, serum HCV RNA became positive 12 months after completing IFN therapy.

No correlation could be found between the interval before HCC was detected, tumor size, or hepatic histologic stage among the SR HCC patients (Fig. 1). HCC that was detected long after discontinuing IFN therapy was not always large, and the patients with large HCC did not always show more advanced stage according to liver histology. The greatest dimensions of the 2 largest SR HCC tumors were 80 mm and were detected 32 months and 73 months after the end of IFN therapy. The greatest dimension of SR HCC found after the longest interval (85 months) was 38 mm.

Tumor tissue samples could be examined from 18 of 27 SR HCC patients. Two samples were categorized

TABLE 2
Comparisons between Sustained Responders with and without Hepatocellular Carcinoma[a]

Characteristic	SR HCC	SR non-HCC	*P* value[b]
No. of patients	27	1170	
Male:female ratio	25:2	751:419	0.0016
Age (yrs, mean ± SD)	60.7 ± 7.5	50.2 ± 12.4	< 0.0001
Serum ALT (IU/L, mean ± SD)	111.7 ± 67.7	122.6 ± 109.9	0.7267
Histologic staging score: No. of patients (%)			
F1	1 (3.7)	384 (39.6)	
F2	11 (40.7)	310 (32.0)	< 0.0001
F3	10 (37.0)	252 (26.0)	
F4	5 (18.5)	24 (2.5)	

SR: sustained responder; HCC: hepatocellular carcinoma; SD: standard deviation; ALT: alanine aminotransferase; IFN: interferon.
[a] All data were determined before interferon therapy.
[b] *P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.

TABLE 3
Factors Associated with the Development of Hepatocellular Carcinoma in Sustained Responders[a]

Characteristic	Risk ratio	95% CI	*P* value
Male vs. female	5.498	1.290–23.439	0.0212
Age	7.378	1.737–31.326	0.0068
Stage of liver disease	2.344	1.064–5.164	0.0345
Serum ALT	1.331	0.606–2.923	0.4768

95% CI: 95% confidence interval; ALT: alanine aminotransferase.
[a] All data were determined before interferon therapy. Statistical analysis was performed using the Cox proportional hazards test. The variable for age was set at < 50 years or ≥ 50 years, the variable for stage was set < F3 or ≥ F3, and the variable for the serum alanine aminotransferase level was set at < 88 UI/L or ≥ 88 UI/L. The variables age and serum alanine aminotransferase level were determined as median data. The variable for stage was set to obtain the largest hazard ratio.

as well differentiated HCC, 11 samples were moderately differentiated HCC, 2 samples were poorly differentiated HCC, and 2 samples were undifferentiated HCC. One sample was the necrotic tissue after transcatheter arterial embolization therapy (TAE). Nontumorous liver tissue samples from 18 patients were evaluated for their fibrosis scores in resected HCC or tumor biopsy specimens. Liver fibrosis scores improved in nine patients, did not change significantly in eight patients, and worsened in one patient.

Sixteen of 27 SR HCC patients underwent partial hepatectomy, and 10 patients were treated with TAE and/or percutaneous ethanol injection therapy. Because one patient changed his hospital after the diagnosis of HCC, we could not know his prognosis.

Comparison between SR HCC Patients and SR Non-HCC Patients

We compared 27 SR HCC patients with 1170 SR non-HCC patients. The SR HCC patients included 25 males (92.6%) and 2 females (7.4%), and the SR non-HCC patients included 751 males (63.5%) and 419 females (35.8%). At the time IFN therapy was initiated, the mean age of the SR HCC patients was 60.7 years ± 7.5 years (range, 37–70 years), whereas the mean age of the SR non-HCC patients was 50.2 years ± 12.4 years (range, 17–73 years). Thus, the SR HCC patients more often were male ($P = 0.0016$) and were older ($P < 0.0001$) compared with the SR non-HCC patients (Table 2).

The fibrotic scores in biopsied liver specimens before IFN therapy for the SR HCC patients included 1 F1 specimen (3.7%), 11 F2 specimens (40.7%), 10 F3 specimens (37.0%), and 5 F4 specimens (18.5%); and the fibrotic scores for the SR non-HCC patients included 384 F1 specimens (39.6%), 310 F2 specimens (32.0%), 252 F3 specimens (26.0%), and 24 F4 specimens (2.5%). The 2 female SR HCC patients both had F4 specimens. Among the total SR population, SR HCC patients had more advanced-stage disease ($P < 0.0001$). The mean serum ALT level at the initiation of IFN therapy was 111.7 IU/L ± 67.7 IU/L in the SR HCC patients and 122.6 IU/L ± 109.9 IU/L in the SR non-HCC patients (Table 2).

Cox proportional-hazards analysis of factors associated with the development of HCC in the SR patients was performed with four variables (gender, age, histologic stage, and serum ALT level). In this analysis, the hazard ratios for age, stage, and serum ALT level were calculated between the two groups. The age variable was set at < 50 years or ≥ 50 years, the fibrotic score (stage) variable was set at < F3 or ≥ F3, and the variable for serum ALT level was set at < 88 IU/L or ≥ 88 IU/L. The variables age and serum ALT level were determined as median data. We chose the variable for stage to obtain the greatest hazard ratio. The SR HCC patients more often were male ($P = 0.0212$, 95%CI, 1.290–23.439), were older ($P = 0.0098$, 95%CI, 1.737–31.326), and had advanced-stage disease according to liver histology ($P = 0.0345$; 95%CI, 1.064–5.164) before IFN therapy. Gender, age, and histologic stage before IFN therapy were considered independent risk factors for the development of HCC (Table 3).

Comparison between SR HCC Patients and Non-SR HCC Patients

We compared the clinical characteristics of the 27 SR HCC patients with the 213 non-SR HCC patients. The non-SR HCC patients included 161 males (75.6%) and 52 females (24.4%). The mean age of the non-SR HCC patients at the initiation of IFN therapy was 58.9 years ± 6.5 years (range, 40–77 years), and the mean age at

TABLE 4
Comparisons between Sustained Responders and Nonsustained Responders among Patients with Hepatocellular Carcinoma

Characteristic	SR	Non-SR	*P* value[a]
No. of patients	27	213	
Male:female ratio	25:2	161:52	0.0507
Age at the initiation of IFN (yrs, mean ± SD)	60.7 ± 7.5	58.9 ± 6.5	0.0552
Age at the detection of HCC (yrs, mean ± SD)	65.1 ± 7.8	63.4 ± 6.7	0.0593
Serum ALT (IU/L)[b]	111.7 ± 67.7	120.5 ± 56.4	0.2027
Histologic staging score: No. of patients (%)[b]			
F1	1 (3.7)	12 (5.6)	
F2	11 (40.7)	36 (16.9)	0.1861
F3	10 (37.0)	135 (63.4)	
F4*	5 (18.5)	30 (14.1)	
Interval (mos, mean ± SD)[c]	49.3 ± 18.2	49.7 ± 24.8	0.7484
Tumor size (mm, mean ± SD)	31.2 ± 20.1	21.3 ± 9.9	0.1573

SR: sustained responder; IFN: interferon; SD: standard deviation; HCC: hepatocellular carcinoma; ALT: alanine aminotransferase.
[a]*P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.
[b]Data were determined before interferon therapy.
[c]The interval was between the completion of interferon therapy and the detection of hepatocellular carcinoma.

time HCC was detected was 63.2 years ± 6.7 years (range, 44–83 years). The mean serum ALT level in the non-SR HCC patients at the start of IFN therapy was 120.5 IU/L ± 56.4 IU/L. The fibrotic scores of biopsied liver specimens obtained from the non-SR HCC patients before IFN therapy included 12 F1 specimens (5.6%), 36 F2 specimens (16.9%), 135 F3 specimens (63.4%), and 30 F4 specimens (14.1%). Thus, concerning gender and age, the SR HCC patients tended to be predominantly male ($P = 0.0507$) and were older (both at the initiation of IFN therapy [$P = 0.0552$] and at the time HCC was detected [$P = 0.0593$]) compared with the non-SR HCC patients; however, the serum ALT levels and the histologic stage before IFN therapy among the SR HCC patients did not differ significantly compared with the non-SR HCC patients (Table 4).

The mean interval between the end of IFN therapy and the detection of HCC for the SR HCC patients was 49.3 months ± 18.2 months (range, 21–85 months), which was not significantly different from that for the non-SR HCC patients (49.7 months ± 24.8 months; range, 12–141 months). The mean greatest dimension of SR HCC was 31.2 mm ± 20.1 mm, which was slightly greater than, but not significantly different from, the mean greatest dimension of non-SR HCC (21.3 mm ± 9.9 mm) (Table 4).

DISCUSSION

In the current study, we compared the clinical characteristics of SR HCC patients with the characteristics of SR non-HCC patients to determine the characteristic features of SR HCC. The incidence of HCC among the 1197 SR patients was 2.3%, and the incidence among the 2429 non-SR patients was 8.8% during the mean follow-up of 5.9 years. In patients with CH-C, aging and advanced hepatic histologic stage reportedly are major risk factors for HCC development.[1,4] This was true for the SR population in our current investigation, because the risk ratio for developing HCC was > 7 times greater in older patients (≥ 50 years) and was more than twice as high in patients who had advanced histologic stage disease (fibrotic score ≥ F3) according to a Cox proportional-hazards analysis. Khan et al. also reported that male gender is an important risk factor for HCC development.[5] In the current study, males were more than five times more likely to develop HCC in the SR population. Thus, older male patients with advanced hepatic fibrosis were considered to be a high-risk group for developing HCC among the SR population (Table 3).

Conversely, compared with the non-SR HCC patients, the SR HCC patients were older at the initiation of IFN therapy ($P = 0.0552$) and at the detection of HCC ($P = 0.0593$), and they were predominantly male ($P = 0.0507$). Although these characteristics may not have differed significantly in the current study, a study of even larger size may show that this indeed is a trend. The histologic staging, the serum ALT level at the initiation of IFN therapy, the interval for the detection of HCC, and the tumor size did not differ significantly between the two groups. The tumor size in SR HCC patients was slightly greater compared with the tumor size in non-SR HCC patients, most likely because of the extended interval of screening for HCC after patients attained a sustained response to IFN therapy (Table 4).

Some previous articles reported that HCV RNA may survive in the hepatic tissues of SR HCC patients[28–30] and may be involved in the carcinogenesis or growth of HCC. Although we could not demonstrate the presence of HCV RNA in tumors and surrounding hepatic tissues from SR HCC patients, eradication of HCV from these tissues, along with the nontumorous hepatic tissues, was confirmed in several previous studies,[15–21] suggesting that the persistence of HCV is not essential for the growth of HCC in SR patients.

To ascertain the time of HCC occurrence, several studies were performed that examined the doubling time (DT) of HCC. Two studies from Japan reported that the DT of HCC measuring < 3 cm in greatest dimension was 93.0 days ± 57.4 days or 195.0 days ± 171.0 days.[31,32] Barbara et al. reported that the DT of HCC measuring < 5 cm in greatest dimension was 204.2 days ± 135 days.[33] Recently, Toyoda et al. re-

ported similar results, assuming that the greatest dimension of occult HCC was 5 mm before IFN therapy.[34] We calculated the growth interval between a single HCC cell and an HCC measuring 1 cm in greatest dimension on the assumption that the DT of HCC was 90 days and concluded that the growth interval may be > 6 years.[8] Because smaller and well differentiated HCCs have a longer DT, the growth interval to reach 1 cm in greatest dimension may be much longer than 6 years. Therefore, it is probable that small HCC may have existed in the liver prior to IFN therapy in the current SR HCC patients.[35]

It cannot be determined with certainty how long SR patients should be followed after they complete IFN therapy. Judging from the results obtained in the current study, we recommend that, when SR patients are male, age > 50 years old, and have F3 or F4 histologic stage, they should be checked by US or CT at least twice per year for > 10 years. Other SR patients with less advanced disease should be checked at least once per year.

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Practice of Interferon Therapy
—Chronic hepatitis C (combination with ribavirin)—

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Abstract: Interferon (IFN) plus ribavirin therapy over 24 weeks resulted in a 20% complete response (CR) in chronic hepatitis C (CH-C) patients who had relapsed on IFN monotherapy in Japan. As we reported previously, the serum amount of HCV RNA decreases biphasically during IFN therapy in CH-C patients. It has been considered that the first phase reflects a direct antiviral effect of IFN and the second phase might reflect antiviral activity and elimination of hepatocytes by apoptosis which might be induced by activated CTL. The second phase might be an important factor in achieving a complete response (CR) in antiviral therapy. Serum HCV RNA level, HCV genotype, amino acid changes in NS5A region, and stage of liver fibrosis are important predictive factors in IFN monotherapy in CH-C patients. However, these factors were not so useful for predicting CR in IFN/ribavirin therapy in the Japanese study. To clarify the predictive factors on IFN/ribavirin in CH-C patients, we are going to study HCV dynamics, changes of Th1/Th2 balance in peripheral blood, and changes of receptors of Th1 and Th2 in peripheral blood on IFN/ribavirin therapy in CH-C patients.

Key words: Chronic hepatitis C; Interferon; Ribavirin; HCV dynamics; First phase; Second phase

Introduction

In Japan, there have been hundreds of thousands of patients with intractable chronic hepatitis C who have not responded to interferon (IFN) therapy in Japan. The combination of IFN/ribavirin, which became covered by the Japanese medical insurance in December 2001, can treat around 20% of such patients. Although only the data from clinical studies are available for the follow-up results for many patients, this paper describes the indications, therapeutic outcomes, and adverse reactions of the combination therapy. The combination

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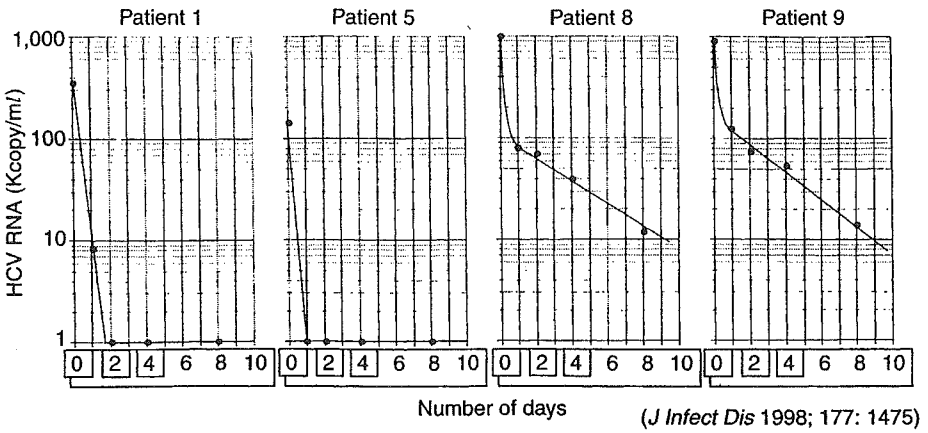

Fig. 1 Blood HCV RNA dynamics during IFN therapy

therapy has been used widely over the past few years in Western countries. The combination of peginterferon (Peg-IFN), a long-acting preparation of IFN, and ribavirin, is now the standard therapy for intractable hepatitis C.

Characteristics of IFN/Ribavirin Combination Therapy

IFN has both antiviral and immunomodulating effects. Ribavirin (Rib) is a nucleic acid derivative with a relatively weak antiviral effect. It mainly acts on the immune system: it inhibits the Th2 system to make the Th1 system relatively superior, and it directly stimulates the Th1 system.[1-3] The IFN/Rib combination therapy produces a significantly higher response rate than the IFN therapy alone in patients with chronic hepatitis C.

We previously examined the blood HCV dynamics after the consecutive administration of IFN in patients with chronic hepatitis C, and reported that the serum amount of virus (amount of HCV RNA) was reduced in a biphasic manner[4]: the serum amount of virus was acutely reduced in the first 24 hours (1st phase, half life: 5 to 7 hours) and then slowly in the subsequent period (2nd phase, half life: 70 to 100 hours) (Fig. 1).[4]

The HCV dynamics after the administration of IFN can be roughly divided into 4 patterns: (1) the HCV RNA amount is sharply reduced to 10 to 1% (or lower) of that of the pretreatment level, and comes close to or falls below the minimum limit of determination in the 1st phase; (2) the HCV RNA amount is reduced to around 10% of that of the pretreatment level in the 1st phase, with a subsequent half life that is 5 to 10 times as long as that of the 1st phase; (3) the HCV RNA amount is significantly reduced in the 1st phase, followed by poor reduction during the second phase, with HCV RNA remaining positive even after several months; and (4) no significant viral reduction during either the 1st or 2nd phase.

It is considered that the 1st phase represents the direct antiviral effect of IFN, while the 2nd phase represents the antiviral effect plus the removal of infected hepatocytes by cytotoxic T lymphocytes (CTL).[5] Hepatitis C with the above (3) or (4) HCV dynamics pattern cannot be eliminated with IFN therapy. It is assumed that the IFN/Rib combination may increase the antiviral effect of IFN and stimulate CTL to remove infected hepatocytes and reduce the half-life of the 2nd phase, resulting in an increase in the rate of marked response. In fact, clinical results supporting this assumption have been reported.[6,7] It is possible that some of the patients who showed the above (2) or (3) pattern after IFN therapy may well respond to the IFN/Rib combination.

Indications

Basically, the indications of the IFN/Rib combination are the same as those of IFN therapy. However, it should be noted that Rib dose-dependently causes hemolytic anemia, and that it is contraindicated in patients with a pretreatment hemoglobin level of 12 g/dL or lower because it reduces hemoglobin by 3 to 4 g/dL, on an average. Other adverse reactions to the combination therapy are similar to those of IFN therapy. However, general malaise, anorexia, alopecia, and eruptions tend to be slightly severer with the combination therapy. Therefore, it is generally contraindicated in patients of 70 years or older, and should be carefully administered in patients of 65 years or older.

Table 1 Therapeutic Efficacy of IFN in 1,370 Patients with Chronic Hepatitis C

Disease stage	Therapeutic efficacy of IFN (biochemical efficacy determination)		
	Marked response	Transient response	No response
F1 (n=229)	101 (44%)	76 (33%)	52 (23%)
F2 (n=710)	236 (33%)	195 (27%)	279 (40%)
F3 (n=383)	85 (22%)	80 (21%)	218 (57%)
F4 (n= 48)	4 (8%)	7 (15%)	37 (77%)
Total 1,370	426 (31%)	358 (26%)	586 (43%)

(Okanoue, T. *et al.*: *Hepatol Res* 2002)

Therapeutic Practice and results

Important predictive factors for the therapeutic efficacy of IFN for chronic hepatitis C include serum viral amount, HCV genotype, and severity of hepatic fibrosis. Table 1 shows the efficacy of IFN in 1,370 patients with hepatitis C by the disease stages (severity of fibrosis). It shows that 31% of the patients markedly responded to IFN.[8] It should be noted that since 10% of the 31% still had viremia, they are so-called biochemically marked responders.

The Japanese clinical study for developing the IFN/Rib combination therapy was performed in intractable cases with a genotype of 1b and HCV RNA amount of 1 Meq/mL or higher. The following 3 groups were set in the study: a group treated with IFN at 6 MU/day (6 MU/day for 2 weeks consecutively followed by thrice weekly) and Rib; a group treated with IFN at 10 MU/day (10 MU/day for 2 weeks consecutively followed by thrice weekly at 6 MU/day) and Rib, and a group treated with IFN alone. All three groups were treated for a total of 24 weeks. The rate of marked responders was 2.3% in the IFN group and around 20% in the IFN 6 MU/day + Rib combination group (Table 2). Rib was administered at a dose of 600 mg/day in patients weighing 60 kg or less and 800 mg/day in those weighing 61 kg or more. The patients who entered the study were intractable cases, and about three quarters had been treated with IFN without sufficient re-

Table 2 IFN/Rib Combination Therapy for Intractable Chronic Hepatitis C (comparison with IFN therapy)

Therapeutic contents	Marked response rate
rIFNα-2b, 6 MU for 2 weeks continuously, followed by 6 MU thrice weekly for 22 weeks plus ribavirin	20.2% (18/89)
rIFNα-2b, 10 MU for 2 weeks continuously, followed by 6 MU thrice weekly for 22 weeks plus ribavirin	17.0% (16/92)
rIFNα-2b, 10 MU for 2 weeks continuously, followed by 6 MU thrice weekly for 22 weeks (IFN alone)	2.3% (2/88)

Patients with a gene type of 1b and large amounts of virus in the blood were treated, and about 75% of them had shown no response to IFN alone.

sults. About 50% of them were very intractable with an HCV RNA amount of 850 KIU/mL or higher. The results obtained were not so different from those obtained in Western countries. In Western countries, IFN/Rib combination therapy is given for 48 weeks in intractable chronic hepatitis C patients,[9] and the combination of Rib and Peg-IFN, a long-acting preparation of IFN, has recently become the first line therapy for intractable chronic hepatitis C.[10,11]

Since the rate of marked response in the IFN/Rib combination therapy is proportional to the blood Rib concentration, Rib should be administered at 1,000 mg/day in patients weighing 75 kg or more.

Predictive Factors of Efficacy

Serum viral amount, genotype, and severity of hepatic fibrosis are important factors that influence the therapeutic efficacy of IFN therapy. The rate of marked response by IFN is almost 0% in patients with a serum viral amount of 850 KIU/mL or more. In contrast, the IFN/Rib combination therapy in patients with a genotype of 1b and a large serum viral amount showed a marked response rate of 19.2% in the group treated with 6 MU/day of INF even in patients with a serum viral amount of 850 KIU/mL or more. This indicates a certain rate of marked response can be expected from the combination therapy in patients with a high viral amount. Although the marked response rate of IFN therapy is significantly reduced as hepatic fibrosis progresses, it is not so reduced as the IFN therapy in patients treated with the combination therapy (F1: 23%, F2: 18%, and F3: 15%). Therefore, it is impossible to accurately predict the efficacy of the IFN/Rib combination therapy, and it is worth trying it in any patients for whom it is indicated.

The presence/absence of blood HCV RNA at 4 or 12 weeks after the start of treatment is an important predictor for the efficacy of the ongoing combination therapy: 56% of those negative for blood HCV RNA determination at 4 weeks recovered completely, and 27% of those positive at 4 weeks but negative at 12 weeks showed marked response. In contrast, there is little possibility of recovery for those positive for HCV RNA at 12 weeks.

We are now examining the relationship between HCV dynamics and Th1/Th2 balance or the expression of Th1 and Th2 cytokine receptors to identify a predictive factor of the efficacy of the IFN/Rib therapy. It is considered important to stimulate the Th1 system by Rib and increase the antiviral effect of IFN by the combination in order to treat patients not responding to IFN therapy with the IFN/Rib combination therapy.

Adverse Reactions

As described above, Rib surely causes hemolytic anemia. Rib should be reduced when Hb falls to 10 g/dL, and returned to the original level when Hb increases. Anemia is most likely to occur 2 to 4 weeks after the start of treatment, although it may progress after 4 weeks.

The IFN/Rib combination therapy should be given carefully to patients with hypertension or diabetes, particularly those with a change of the fundus occuli, because they may develop cerebral hemorrhage during treatment.

Conclusions

IFN-Rib combination therapy is the first line therapy for intractable chronic hepatitis C. However, because much remains to be improved (such as lessening the relatively severe adverse reactions), it should be applied carefully.

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A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase

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***Background/Aims*: Long-term follow-up study was performed to identify the candidates for antiviral therapy for hepatitis C virus (HCV) infection among carriers with persistently normal aminotransferase (ALT ≤ 30 U/L) levels (PNAL).**

***Methods*: One hundred and twenty-nine HCV carriers with PNAL who underwent liver biopsy and had platelet count over 150,000/μl were entered and 69 were followed for over 5 years. Thirty-five patients underwent serial liver biopsies. Serum ferritin and thioredoxin levels were also determined.**

***Results*: Seventeen patients had normal liver histology, 10 had moderate chronic hepatitis and the remainder 102 had mild hepatitis. Serum ferritin and thioredoxin levels were normal. The mean follow-up period for the 69 patients was 8.5 years. Of these 69 patients, 10 had persistently normal ALT levels (group A), 39 had transient elevation of ALT (group B), and 20 changed to symptomatic chronic hepatitis (group C). The rate of progression of fibrosis for groups A, B, and C were 0.05, 0.04, and 0.08, respectively. Hepatocellular carcinoma was not diagnosed in any of the patients.**

***Conclusions*: Around 90% of HCV carriers with PNAL have normal to mild liver histology. This long-term follow-up study demonstrated that 30% of such carriers became candidates for antiviral therapy within 5 years.**

***Keywords*: Hepatitis C virus; Chronic hepatitis C; Asymptomatic HCV carrier; Normal serum ALT; Interferon**

1. Introduction

An estimated 170 million individuals are infected with hepatitis C virus (HCV) worldwide and chronic hepatitis C has recently become the leading cause of liver cirrhosis and hepatocellular carcinoma (HCC) in many countries including Japan. Most HCC develop in patients with advanced staged chronic hepatitis or cirrhosis, and rarely from mild chronic hepatitis type C.

It is thought that type C liver cirrhosis and HCC develop over 20–35 years following HCV infection [1], however, around 25% of patients with chronic HCV infection have normal serum aminotransferase (ALT) levels [2,3]. We reported previously that asymptomatic HCV carriers were predominant among females and that most of them had histologically minimal to mild chronic hepatitis [4]. In that paper, we defined asymptomatic HCV carriers as persistently HCV RNA positive patients with normal serum ALT levels (≤30 U/L) over 1 year. However, it has been reported that HCV carriers with normal serum ALT level had more advanced liver histology compared to HCV carriers with elevated serum ALT [5]. This discrepancy might be attributed to differences in the definition of the normal range of serum ALT used by various centers, however, it is very important to clarify whether HCV carriers with persistently normal ALT level (PNAL) are candidates for antiviral therapy.

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The current normal limit of serum ALT is 40 U/L, however, a recent report from an Italian group demonstrated that the healthy ranges for serum ALT were 30 U/L for men and 19 U/L for women, respectively [6], which are lower than the current values that have been used over the past 15 years. This criterion of normal serum ALT might be reasonable because a few cirrhotic patients have from 30 to 40 U/L of ALT [7].

In Japan, the number of HCC patients with HCV infection has increased since 1975. Antiviral treatment for chronic hepatitis C resulted in the inhibition of hepatic inflammation and progression of hepatic fibrosis and as a consequence the inhibition of the development of HCC [8–13]. Thus, inhibition of HCC is a very important issue in the treatment of patients with chronic hepatitis C. It remains controversial whether asymptomatic HCV carriers are candidates for antiviral therapy because of the low efficacy of treatment and flare-ups post treatment. However, taking into consideration the recent progress in antiviral therapy for chronic hepatitis C patients, the National Institute of Health Consensus Development Conference reported that patients with hepatitis C with normal serum ALT levels are candidates for interferon and ribavirin therapy [14]. Recently, a multicenter, randomized, controlled study for the treatment of patients with chronic hepatitis and persistently normal ALT levels with pegylated interferon alpha and ribavirin for 48 weeks led to eradication in 40% of patients infected with genotype 1b patients [15], which is similar to the results for symptomatic chronic hepatitis C patients [16,17]. However, most HCV carriers with PNAL have minimal to mild liver histology and their prognosis might be very good. Thus, there is some doubt, whether they are candidates for antiviral treatment to inhibit the progression of liver disease and hepatocarcinogenesis.

Recently, it has been reported that oxidative stress is an important factor in the development of HCV-related HCC [18–22] and the HCV core protein may generate oxidative stress via mitochondrial injury [23,24]. It is also demonstrated that iron overload generates oxidative stress, resulting in hepatic injury, and DNA damage and consequently this becomes an important factor for hepatocarcinogenesis [22,25,26].

We report here the biochemical and histological results of 8.5 years of follow-up of HCV carriers with PNAL. The data were analyzed according to the definitions of normal range ($\leq$30 U/L) of serum ALT and platelet count (PLT) over 150,000 μl/ml. We also analyzed the status of oxidative stress using serum ferritin and thioredoxin levels. These results demonstrate the importance of the normal range of serum ALT, oxidative stress and follow-up study to decide the indication for antiviral therapy of HCV carriers with PNAL.

2. Patients and methods

2.1. Eligibility and definition

This study was conducted from January 1990 to August 2004.

HCV carriers with persistently normal ALT levels (PNAL) were defined as those patients who were HCV RNA positive by reverse transcriptase polymerase chain reaction (RT-PCR), had normal serum ALT levels ($\leq$30 U/L) over a 12-months period and on least three different occasions and platelet count of over 15×10^4 μl/ml. Patients positive for hepatitis B surface antigen (HBsAg), previous interferon (IFN) treatment, a history of heavy alcohol abuse, anti-nuclear antibody (ANA) and anti-smooth muscle antibody (ASMA) positivity, patients with overt Diabetes mellitus and obesity (body mass index; over 30 kg/m^2) were excluded from this study.

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and approved by the Ethics Committee of Kyoto Prefectural University of Medicine. Informed consent was obtained from every patient.

2.2. Quantification and determination of HCV RNA and genotyping

Frozen-stored sera from 129 individuals were tested. Serum HCV RNA levels was determined using the AMPLICOR GT HCV MONITOR (Roche Diagnostic Systems, Tokyo, Japan). The detection range of this assay was between 0.5 and 850 KIU/ml, and each sample was measured again after dilution with distilled water. HCV genotypes 1 and 2 were determined by a serologic genotyping assay [27]. Genotypes 1 and 2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds et al. [28].

2.3. Study design

Of the 129 patients who underwent liver biopsy, 69 patients enrolled in this study and followed over 5 years (8 males, 61 females). These patients received blood tests every 4 months for an initial 2 years and then received blood tests every 6 months when they remained still normal ALT. αfetoprotein (AFP) was measured every years in all patients, and all patients underwent ultrasonography every year to detect HCC.

All patients submitted to a liver biopsy using a Menghini needle guided by ultrasonography prior to entry. Formalin-fixed liver specimens were stained with hematoxylin and eosin for morphological evaluation, with Masson's trichrome stain for assessment of fibrosis, and with Perls' Prussian blue stain (from February 1998) for assessment of iron loading. Histological follow-up studies were carried out for 35 patients 3.4–13.4 years (mean: 6.8 years) after the first biopsy.

The histological findings of HCV carriers with PNAL were interpreted and scored according to the classification proposed by Desmet et al. [29] and Ishak et al. [30]. Steatosis is defined having fat droplets in over 10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0 to 4+, based on the scoring system of MacSween et al. [31].

Fasting blood samples were collected in the morning. Serum ALT, blood glucose level, serum ferritin, platelet count (PLT), serum HCV RNA level and HCV genotype were examined in the laboratory of our university hospital, using the standard analytical method; the ULA ALT value was 30 U/L. Serum thioredoxin (TRX) levels were measured with a sensitive sandwich ELISA kit (Fujirebio, Inc., Tokyo, Japan) as described previously [26,32] and of the 129 patients 47 were available for this assay. Blood chemistry was done every 4–6 months during the follow-up period.

2.4. Statistical analysis

Data values are expressed as medians with interquartile ranges. We compared continuous variables using the Mann–Whitney *U*-test. The Kruskal–Wallis test was used for multiple group comparisons, and Spearman correction coefficients were used to examine the relationship between groups. Frequency analysis was performed with the χ^2 test, and Fisher's exact test. *P* values of less than 0.05 were considered significant.

3. Results

3.1. Demographic and clinical features

The demographic and clinical features of the 129 HCV carriers with PNAL are shown in Table 1. Twenty-four were male and 105 were female. No significant differences were noted in age, serum ALT, PLT, and follow-up period between males and females. Serum ferritin levels were 76.1±53.4 ng/ml in male and 60.0±43.3 ng/ml in female. Serum HCV RNA levels were significantly ($P=0.0012$) higher in G1 compared with G2 (648.7±622.5 KIU/ml vs 356.2±628.8 KIU/ml (Table 1).

Characteristics of the 69 patients followed over 5 years are also shown in Table 1. Their mean follow-up period was 8.5±2.4 years.

Of the 105 female patients, 44 had serum ALT levels≦19 U/L and 61 had serum ALT levels of 20–30 U/L at entry. There were no significant differences in their ages, platelet count, serum ferritin levels, serum HCV RNA levels, or BMI (Table 2).

Serum thioredoxin (TRX) levels in these patients were within the normal range, and significantly lower than those of patients with chronic hepatitis and cirrhosis (Table 3).

Table 1
Characteristics of 129 HCV carriers with persistently normal ALT who underwent liver biopsy

	$N=129$	Followed over 5 years ($N=69$)
Follow-up period (years)	5.7±3.6	8.5±2.4
Age (years)	48 (21–77)	45 (29–71)
Male ($N=24$)	49.8±16.4	42.3±14.9
Female ($N=105$)	47.2±12.5	46.63±11.6
Sex (M/F)	24/105	8/61
ALT (U/L)	8–30	9–30
Male ($N=24$)	22.5±5.7	21.1±5.4
Female ($N=105$)	21.6±4.8	22.3±5.1
PLT ($\times10^4$/ml)	15–31	15–31
Male ($N=24$)	20.3±4.4	20.9±5.3
Female ($N=105$)	21.8±4.4	21.2±4.0
Ferritin (ng/ml)	5–225	5–225
Male	76.2±53.5	84.6±59.2
Female	60.0±43.3	66.6±52.5
HCV RNA (KIU/ml)	6–3350	22–2100
G1 ($N=58$)	648.9±622.57*	595.1±561.1** ($N=32$)
G2 ($N=45$)	356.2±628.8	211.0±219.2 ($N=27$)
Mixed and unclassified	6–1994	
BMI (kg/m^2)	16–27	16–27
Male	22.2±1.7	21.9±1.9
Female	21.3±2.2	21.0±2.4

Values were expressed as mean±SD. P values were calculated by Mann–Whitney U-analysis with correction for tie. *$P=0.0012$ (G1 vs G2); **$P=0.0006$ (G1 vs G2).

Table 2
Baseline of female patients between HCV carriers having ≦19 U/L of ALT and HCV carriers showing 20–30 U/L of ALT

	ALT≦19 (U/L)	20<ALT≦30 (U/L)	P value
Number of patient	44	61	
Age (y.o)	44.9±12.5	48.8±12.2	
ALT (U/L)	16.0±2.4	24.3±2.9	<0.0001
PLT ($\times10^4$/μl)	22.0±4.4	21.6±4.3	
HCV RNA (KIU/ml)	400.2±555.1	500.7±541.1	0.3896
BMI (kg/m^2)	21.2±2.3	21.4±2.2	

Values were expressed as mean±SD. P values were calculated by Mann–Whitney U-analysis with correction for tie.

3.2. Liver histology

The results of liver histology for the first biopsy are described in Table 4. Normal liver histology was noted in 17 (14%) subjects, 102 (79%) showed minimal to mild chronic hepatitis, 10 (8%) had moderate chronic hepatitis.

Steatosis was seen in nine patients (7%) and iron loading was noted in 6/50 (12%).

3.3. Follow-up study of laboratory data

Of the 69 patients followed over 5 years (mean±SD: 8.5±2.4 years), 10 (14%) had continuously normal ALT (group A), 39 (57%) showed transient elevation of ALT (group B), and 20 (29%) changed to chronic hepatitis with continuously abnormal serum ALT (group C) (Table 5). Of the 61 female patients, eight were group A, 34 were group B, and 19 were group C. There were no significant differences in age, ferritin levels, serum HCV RNA levels, or BMI among the three groups. However, serum ALT levels were significantly lower in group A compared with group B and C (Table 6). The number of patients having ALT levels≦19 IU/L in these three groups were seven (7/8:87.5%) in group A, 12 (12/34:35.3%) in group B, and three (3/19:15.8%) in group C.

Table 3
Serum thioredoxin (TRX) levels in 47 HCV carriers with PNAL at liver biopsy

	Serum thioredoxin (ng/ml)
HCV carriers with PANL ($n=47$)	27.7 [9.1–38.5]
Chronic hepatitis ($n=124$)	34.5 [8.6–135.6][a]+
Liver cirrhosis ($n=24$)	42.5 [21.4–97.2][a]++
Control ($n=15$)	24.9 [1.3–50.7][a]

*$P=0.0012$ when compared with G2. The overall significance of differences between four groups according to non-parametric Kruskal–Wallis analysis of variance was $P<0.001$. Therefore, the significance of differences between groups was determined by Scheffe's method: +$P<0.01$; ++$P<0.001$, compared to HCV carriers with PNAL.

[a] These data were reported in J Hepatol 2000; 33: 616–622.

Table 4
Liver histology of 129 carriers at the first biopsy

Grade	Stage of liver fibrosis				Total number of patients
	F0	F1	F2	F3	
A0	17 (11)	3 (1)	0	0	20 (12)
A1	24 (21)	75 (62)	2 (2)	0	101 (85)
A2	0	6 (5)	2 (2)	0	8 (7)
A3	0	0	0	0	0
Total	41 (32)	84 (68)	4 (4)	0	129 (104)

Numbers of female patients are given in parentheses.

The stage of liver fibrosis in the 22 female patients with ALT levels≦19 IU/L at entry were F0 ($N=10$) or F1 ($N=12$). The frequency of stage F0 liver histology was slightly higher in group A and B patients compared with group C. However, there were no significant differences among the three groups.

Seven patients from group C had ALT levels over 100 U/L during the follow-up period and received antiviral therapy (five received interferon monotherapy and two received interferon plus ribavirin therapy), and five had a sustained virological response.

3.4. Follow-up study of liver histology

Thirty-five patients submitted to repeat biopsies and five of them a third biopsy. Of the 35 patients, 5 were in group A, 16 in B, and 14 in C. The intervals between the first biopsy and the last biopsy in these three groups were 7.3±2.1 years (group A), 6.8±2.0 years (group B), and 6.1±2.3 years (group C). The changes in stage of live fibrosis are shown in Fig. 1 (group A), 2 (group B), and 3 (group C). Progression of fibrosis stage was noted in 2 of 5 in group A, 5 of 16 in group B, and 6 of 14 in group C, as shown in Figs. 1–3. The median rates of fibrosis progression per year for these three groups were 0.05, 0.04, and 0.08 fibrosis unit, respectively. There were no significant differences in the rate of fibrosis progression per year between group A and B, B and C, and A and C (A vs B; $P=0.6643$, B vs C; $P=0.0699$, A vs C; $P=0.3512$).

Of the 32 female patients who received serial biopsies, 10 had ALT levels≦19 U/L at entry, in four of whom had F0 stage progress to F1. One F0 and five F1 patients showed no changes in their stages during the follow-up periods.

Table 5
Changes of serum ALT in 69 patients followed over 5 years

	No. of patients
Persistently normal (group A)	10 (14%)
Transient elevation (group B)	39 (57%)
Continuous elevation (group C)	20 (29%)

Group A, continuously normal serum ALT during the follow-up period. Group B, serum ALT transiently over 31 U/L during the follow-up period. Group C, serum ALT became continuously abnormal during the follow-up period.

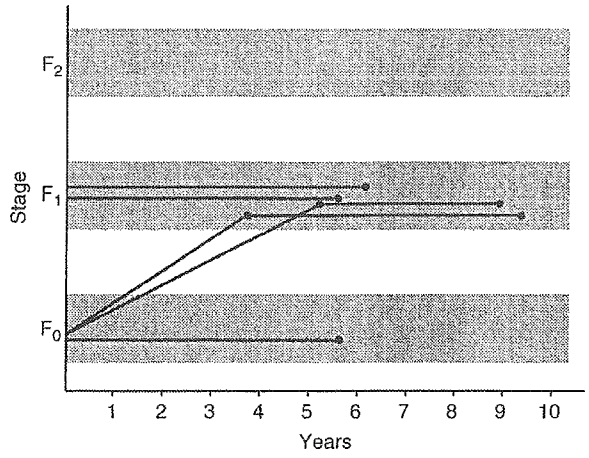

Fig. 1. Follow-up study of liver histology on asymptomatic hepatitis C virus carriers whose alanine aminotransferase levels remained normal during the follow-up period. Five patients with persistently normal serum aminotransferase levels submitted to repeat biopsies and the stage of liver fibrosis progressed from F0 to F1 in two patients after 3.4 and 5 years.

3.5. Follow-up study of AFP and ultrasonography

Three patients in group C showed transient elevation of AFP over 20 ng/ml. No patients in groups A or B had elevations of serum AFP during their follow-up periods. HCC was not detected in any patients by ultrasonography and/or computed tomography. AFP titers in those three patients did not increase further.

4. Discussion

The present study demonstrated several characteristics of HCV carriers with persistently normal ALT levels (PNAL).

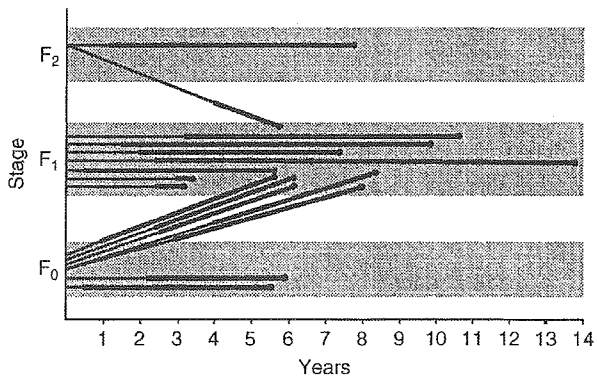

Fig. 2. Follow-up study of liver histology on asymptomatic hepatitis C virus carriers whose alanine aminotransferase levels were transiently elevated during the follow-up period. Sixteen patients with transient elevation of serum aminotransferase levels submitted to repeat biopsies and the stage of liver fibrosis progressed from F0 to F1 after 5.3–8.1 years in five patients. One patient showed the regression of the stage of liver fibrosis from F2 to F1 after 5.5 years. The left side edge of the large bar indicates the initial recording of abnormal serum aminotransferase during follow-up period.

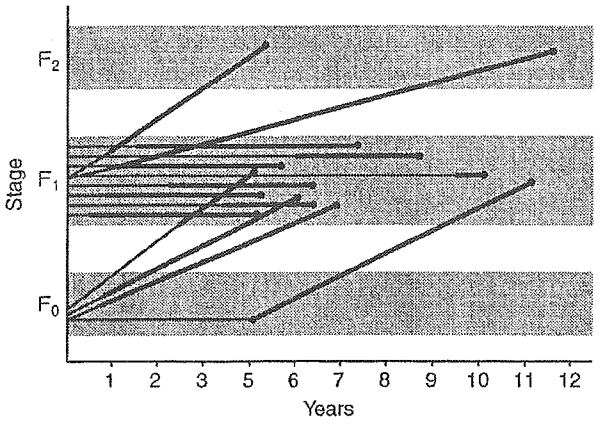

Fig. 3. Follow-up study of liver histology on asymptomatic hepatitis C virus carriers whose alanine aminotransferase levels became persistently abnormal during the follow-up period. Fourteen patients who developed continuously abnormal serum aminotransferase levels submitted to repeat liver biopsies after 4.0–10.3 years. Progression of the stage of liver disease was noted in six patients, of whom four progressed from F0 to F1 and two from F1 to F2 after 4.0–10.3 years. The left side edge of the large bar indicates the initial recording of abnormal serum aminotransferase during follow-up period.

Serum HCV RNA levels were similar to patients with symptomatic chronic hepatitis, however, the frequency of HCV genotype 2 was significantly higher in HCV carriers with PNAL than those with chronic hepatitis C (data not shown here, of 123 patients with chronic hepatitis C in our clinic 75% had genotype 1 and 22% were genotype 2). Females were predominant among the HCV carriers with PNAL compared with chronic hepatitis [4] which is similar to other reports [5,33–35]. Female HCV carriers with continuously normal ALT had significantly lower ALT levels at entry as shown in Table 6. Of the 105 female patients, 44 had ALT levels $\leqq$ 19 U/L and showed mild liver injury compared with carriers with whose ALT levels were 20–30 U/L. However, the progression rate of fibrosis was not significantly different.

The serum ferritin and serum thioredoxin (TRX) levels in HCV carriers with PNAL showed normal ranges and were significantly lower than in chronic hepatitis C patients, as we have reported previously [26]. The frequency and grade of fatty liver and iron loading were quite low compared with chronic hepatitis C patients, also as reported previously [26]. Liver histology was minimal to mild and moderate chronic hepatitis was noted in only around 8% of subjects. Long-term follow-up study demonstrated that 29% of HCV carriers with PNAL developed chronic hepatitis with persistently high serum ALT within 5 years, 57% showed transient elevation of serum ALT, and 14% had continuously normal ALT. There are many reports concerning the natural course of liver fibrosis in chronic hepatitis C patients including patients with normal serum ALT level [5,33–41]. More than half of chronic hepatitis C patients show progression of stage of liver fibrosis from F1 to F2-4 within 10 years and it was previously reported that progression of liver fibrosis in HCV carriers with PNAL was more rapid compared with the present result [5]. The main reason for this discrepancy between the previous reports and the present result might be due to the difference in the definition of the normal range of serum ALT. Poynard et al. [37] reported that the median rate of progression of fibrosis per year was 0.1333 fibrosis unit, which was 1.5–3 times faster than the present results in HCV carriers with PNAL.

These results indicate that HCV carriers with PNAL are in a condition with less oxidative stress [26] and they have a lower risk of cirrhosis and hepatocarcinogenesis compared to chronic hepatitis patients [13,22].

It is well known that the rate of the development of hepatocellular carcinoma (HCC) is correlated with the progression of liver fibrosis; the stage of liver disease [9,11,13]. Sustained low serum ALT also lowers the rate of the development of HCC [9,13,42]. No HCC was detected during the follow-up period in any of the HCV carriers in this study, reflecting the results of previous clinical studies.

Peginterferon and ribavirin administration for 48 weeks resulted in sustained virological response in around 40% of patients with genotype 1 [15], however, this therapy is expensive and induces various side effects.

The present results indicate that most HCV carriers with persistently normal serum ALT have a good prognosis with a low risk of developing hepatocellular carcinoma. Antiviral treatment for these patients should take into consideration the follow-up results of blood chemistry and liver histology.

Table 6
Characteristics of 61 female patients in groups A–C followed over 5 years

	Group A (N=8)	Group B (N=34)	Group C (N=19)
Age (y.o.)	49.6±12.9	44.9±12.5	48.2±8.9
BMI (kg/m^2)	20.8±2.9	20.6±2.1	21.8±2.5
Ferritin (ng/ml)	73.4±33.7	59.3±56.8	76.8±47.1
ALT (U/L)*	15.8±3.2	22.4±4.6	23.9±4.9
HCV RNA (KIU/ml)**	186.5±141.8	474.6±486.0	454.0±575.2

Values were expressed as mean±SD. There were no significant differences in their age, BMI, ferritin, and HCV RNA levels in three groups. *Serum ALT level was significantly lower in group A compared with group B (group A vs group B; P=0.0045) and with group C (group A vs group C; P=0.0003), however, no significant difference was noted between group B and C (P=0.0758). **There were no significant differences in serum amount of HCV RNA between group A and B (P=0.3529) and group A and C (P=0.8676).

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