Table 2. Outcomes of Patients With Tuberculosis Sequelae With or Without Anti-HCV

	Anti-HCV(+) (n = 147)	Anti-HCV(−) (n = 155)
Duration of follow-up (years)		
Mean ± SD	5.5 ± 3.3	5.9 ± 3.4
(range)	(0.2-13.3)	(0.1-13.3)
Alive	51 (36%)	63 (43%)
Dead	92 (64%)	82 (57%)
Due to tuberculosis sequelae	61 (42%)	66 (46%)
Due to liver disease	8 (6%)	0
Other causes	23 (16%)	16 (11%)
Unknown	4 (3%)	10 (6%)

NOTE: Anti-HCV(+): One hundred nineteen HCV RNA-positive patients and 28 anti-HCV by recombinant immunoblot assay (RIBA)-positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by ELISA or RIBA in serum.

erage annual mortality from liver disease from study entry in anti-HCV–positive patients was 9.8 per 1,000 person-years.

There was no significant difference between the 2-, 5-, and 10-year overall survival probabilities from study entry of the patients with anti-HCV (84%, 60% and 35%, respectively), compared with those of the patients without anti-HCV (85%, 66% and 44%, respectively) ($P = .12$) (Fig. 1). The patients with anti-HCV, however, had significantly lower cause-specific survival probabilities for liver disease from study entry than did those without it: 99%, 96%, and 92%, at 2, 5, and 10 years, respectively ($P < .005$) (Fig. 2).

Of the liver-related deaths in the eight patients with anti-HCV (six men, two women), seven were caused by HCC and one by massive bleeding from esophageal varices (Table 3). All of the patients were positive for HCV RNA and showed abnormal ALT values at entry. None had a history of heavy alcohol use. They had received their transfusions at the ages of 25 to 41 years, had been diagnosed with HCC at 65 to 75 years, and died at 66 to 76 years. The period between transfusion and diagnosis of HCC was 33 to 45 years. Of the seven patients with HCC, two died within 1 year of diagnosis, two within 2 years, and two within 3 years. The remaining patient with HCC, who had been treated successfully with percutaneous ethanol injection therapy, died of recurrent HCC after 7 years. Seven of the eight patients who died of liver disease had been diagnosed with cirrhosis by histological and/or clinical examinations.

Details of the causes of death of patients from "other diseases" are shown in Table 4.

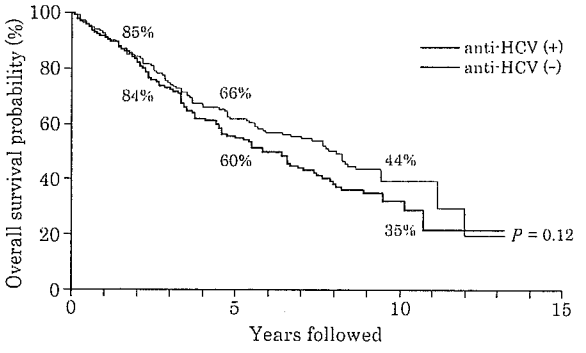

Fig. 1. The cumulative overall survival curves from study entry in anti-HCV-positive patients (**solid line**) and the anti-HCV-negative controls (**dotted line**). The 2-, 5-, and 10-year overall survival probabilities for the subjects were 84%, 60%, and 35%, respectively, and those for the controls were 85%, 66%, and 44%, respectively. The differences between groups was not significant ($P = .12$). Abbreviation: anti-HCV, antibody to HCV; NOTE: Anti-HCV(+): HCV RNA positive or anti-HCV by recombinant immunoblot assay (RIBA) positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by EIA or RIBA in serum.

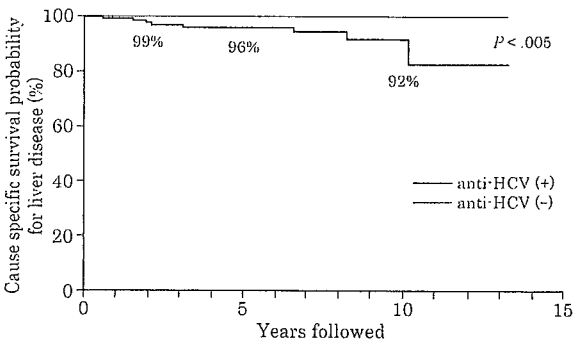

Fig. 2. The cause-specific survival curves for liver disease from study entry in the anti-HCV-positive patients (**solid line**) and the anti-HCV-negative controls (**dotted line**). The anti-HCV-positive patients showed significantly lower cause-specific survival probabilities for liver disease ($P < .005$), 99%, 96%, and 92% at 2, 5, and 10 years, respectively, than the controls. Abbreviation: anti-HCV, antibody to HCV; NOTE: Anti-HCV(+): HCV RNA positive or anti-HCV by recombinant immunoblot assay (RIBA) positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by EIA or RIBA in serum.

Discussion

In Japan, the surgical treatment of pulmonary tuberculosis, such as thoracoplasty or lobectomy, was common in the 1950s until the early 1960s; more than 20,000 patients per year underwent such surgeries during this time.[19] These operations required a large volume of blood by transfusion.[15] Between 1951 and 1967, such blood was obtained commercially. It has been documented that hepatitis was acquired posttransfusion in up to 50% to 80% of patients who had undergone a major operation that involved the use of commercial blood, including those undergoing surgery for pulmonary tuberculosis.[15,16] We could not examine other risk factors for HCV infection than transfusion, so we are unable to completely discount

Table 3. Patients With Anti-HCV Who Died of Liver Disease

Case No.	Sex	Age at Transfusion	Age at Diagnosis of HCC	Age at Death	HCV RNA	ALT (IU/L)	Esophageal Varices	Liver Histology	Causes of Death
1	F	25	65	66	(+)	72	(+)	Unknown	HCC
2	F	26	71	73	(+)	59	(+)	Cirrhosis	HCC
3	M	27	66	66	(+)	37	(+)	Unknown	HCC
4	M	31	64	71	(+)	71	(+)	Unknown	HCC
5	M	33	68	70	(+)	68	(−)	Cirrhosis	HCC
6	M	35	75	76	(+)	139	(−)	Cirrhosis	HCC
7	M	36	–	70	(+)	95	(+)	Cirrhosis	Varices rupture
8	M	41	75	75	(+)	41	Unknown	Unknown	HCC

NOTE: ALT at the entry is shown.
Abbreviations: F, Female; M, Male; HCV RNA, hepatitis C virus ribonucleic acid; HCC, hepatocellular carcinoma.

potential routes such as sexual exposure, medical procedures, reused needles, or intravenous drug use. However, judging from the results that 78% of the patients received transfusion between 1951 and 1967, and the prevalence of anti-HCV was significantly higher in patients who received transfusion at that time than in those receiving it after 1967, it is reasonable to assume that, most of the patients were infected with HCV via a blood transfusion. Furthermore, it is not surprising that 49% of our patients with tuberculosis sequelae were positive for anti-HCV antibody and 39% for HCV RNA; the prevalence of the HCV-RNA–positive patients might be underestimated, because their samples were stored only at −20°C.

Table 4. Details of the Reasons for Death in Patients Who Died of "Other Causes"

Cause of Death	Anti-HCV(+)	Anti-HCV(−)
Cerebral vascular diseases	4	3
Myocardial infarction	1	1
Rupture of aortic aneurysm	1	1
Renal failure	2	1
Myelodysplastic syndrome	1	0
Multiple organ failure	0	1
Sudden death of unknown etiology	2	0
Senile decay	0	3
Accident	1	0
Brain tumor	1	0
Malignancy		
Thyroid cancer	0	1
Lung cancer	2	0
Esophageal cancer	1	0
Gastric cancer	2	1
Colon cancer	2	2
Gallbladder cancer	0	1
Pancreatic cancer	1	0
Multiple myeloma	1	0
Malignant lymphoma	1	1

NOTE: Anti-HCV(+): HCV RNA positive or anti-HCV by recombinant immunoblot assay (RIBA) positive patients. Anti-HCV(−): Patients who were negative for anti-HCV by ELISA, or RIBA in serum.
Abbreviation: anti-HCV, antibody to HCV.

In the United States, patients with chronic HCV infection die more frequently of decompensated cirrhosis than of HCC.[20] By contrast, in Japan, HCC has been the major cause of death in this patient group for a long time. This trend is growing increasingly stronger, and the proportion of death by HCC among all causes of death in patients with cirrhosis and chronic HCV infection has reached 81%.[21] Hamada et al.[22] reported that the age of the patient and duration of infection were independent risk factors affecting the development of HCC, and of them, age was the more significant. On average, approximately 30 years pass between the time of transfusion and the time of diagnosis of HCC.[1,3,22] According to a nationwide survey in Japan,[23] the mean age at such diagnosis was 63.0 years for males and 66.5 years for females (75% of all patients were anti-HCV–positive). Ninety-two percent of HCC patients were 60 years or older at the time they were diagnosed with HCC.[22] In the west of Scotland, age-specific incidence of HCC in men aged over 55 years increased dramatically between 1975 and 1985, particularly among those aged 75 to 84 years, but not in those younger than 55 years; the major etiology was considered to be HCV infection.[24] The patients in our study survived an average of 34 years after having received blood transfusion in early adulthood (at a mean age of 31 years); at the time of entry into the study they had become relatively elderly (average age, 65 years). Accordingly, they were considered to be at high risk for carcinogenesis in view of age and duration of infection.

When investigating the prognosis of liver disease in HCV-infected persons, it would be preferable to start studying patients at the time of infection and continue until they reach old age several decades later, when the risk becomes high for developing HCC. However, it is difficult to carry out such a prospective study. A retrospective cohort study on older HCV-infected persons, for whom a considerable period has elapsed between the presumed

time of their infection and their enrollment into the study, and who were selected with as little bias as possible, could serve as a good alternative to clarify, relatively accurately, the eventual outcome of their liver disease, although the study would miss liver-related events that occurred before entry. Previous retrospective studies identified patients referred for liver disease.[1-5] The patients in our study were recognized at a chest clinic while receiving treatment of sequelae to pulmonary tuberculosis, which had placed them at risk for HCV infection through transfusion. Thus, the advantage of this study was that selection bias would be less likely. Moreover, only two patients received antiviral treatment.

In our study, eight of the anti-HCV–positive patients and none of the anti-HCV–negative patients died of liver disease. The average annual mortality from liver disease from study entry of HCV-infected patients was 9.8 per 1000 person-years, and the cause-specific survival probabilities for liver disease from study entry were 99%, 96% and 92% at 2, 5 and 10 years, respectively. The mortality from liver disease might be underestimated, because chronic illnesses, including tuberculosis sequelae, might confound recognition of liver disease complication, and in some patients the cause of death was obtained from death certificates.

We showed by univariate analysis that the cause-specific survival probability for liver disease was significantly lower in patients with anti-HCV than in those without anti-HCV antibody. Subsequently, we should examine survival by using a Cox proportional hazards regression analysis. Nevertheless, in our study, this analysis was inappropriate, because none of the patients without anti-HCV died of liver disease. However, the patients in both groups were selected on the same conditions, and the background was not different between the two groups, as shown in Table 1. We could therefore conclude that HCV infection was probably an independent risk factor for the death of liver disease. Then, we showed that the overall mortality from study entry was not significantly different between the two patient groups. Because the overall mortality was extremely high owing to a large number of deaths from tuberculosis sequelae in both groups, the impact on mortality of liver-related deaths resulting from HCV infection would have been underestimated. In fact, of the deaths unrelated to tuberculosis sequelae, death from liver disease accounted for 26%.

Many of the patients in our study who died of liver disease died due to HCC, as the previous report from Japan had shown.[21] All the patients were positive for HCV RNA. None of the patients positive for anti-HCV and negative for HCV RNA died of liver disease, and there was no excess liver-related mortality in those patients. Although ALT values at entry were within normal limits in 49% of the HCV RNA–positive patients, no patient who died of liver disease showed normal ALT values.

Several studies reporting on the prognosis of liver disease in cases of chronic HCV infection have looked at patients infected in childhood (average age, 0-8 years)[8,9] or early adulthood (average age, 19-28 years).[10-13] At a median or average of 14 to 35 years after infection, clinical or histological cirrhosis was found in 0% to 8% of patients,[8-13] and no or mild histological fibrosis in 81% to 87%.[8,9,11,12] End-stage liver disease developed with an incidence of 3.1 per 1,000 person-years,[13] and liver-related mortality was 0 to 0.4%,[8,10] with a much better prognosis than was seen in our study. The difference is likely to be attributable to the difference in age of the patients at the time of the investigations. The patients in these studies were young, with an average age of 20 to 45 years. Our study showed that the eight patients who died of liver disease had apparently been infected in early adulthood (25-41 years) and died after they had reached old age (66-76 years). These results suggest that for patients who were infected in early adulthood, the long-term prognosis of liver disease, once they reach old age, is not good. It has been reported that fibrosis begins to accelerate at 50 years of age[25] and that the evolution from chronic hepatitis to cirrhosis occurs more frequently and rapidly in patients aged 50 years or older than in those younger than 50 years.[26] Accordingly, young individuals infected with HCV, and having a favorable course, may undergo rapid progression of the fibrosis once they reach middle age and then develop severe liver disease, including HCC, when they reach old age.

Seeff et al.[14] reported the long-term mortality over approximately 25 years in 222 patients with posttransfusion hepatitis C, with an average age of 49 years, of whom approximately 77% were considered to have been positive for HCV RNA in serum. Their report is the only study that includes a control group and also deals with individuals who had reached old age at the end point of observation. Liver-related mortality was significantly higher among the cases than among the control group of matched, transfused, and nonhepatitis patients (4.1% vs. 1.3%, respectively). However, the all-cause mortality was not different between the two groups (67.1% vs. 65.0%, respectively). Furthermore, the mortality attributed to chronic hepatitis C infection was only about 3%, and liver disease was considered to be a relatively minor cause of patient death. Our study showed distinctly high mortality from liver disease compared with that observed in their study, although both studies dealt with older patients. The reason for the differences in the prognosis between

their study and ours is unclear. It might be related to the difference in duration of infection, which was apparently longer in our patients than in theirs. In our patients, past tuberculosis infection, past treatment for tuberculosis, or tuberculosis sequelae might contribute to more rapid progression of liver disease. Another influencing factor might be differences in race, and this aspect warrants further study.

In conclusion, for the 147 HCV-infected patients (average age, 65 years), of whom 81% were positive for HCV RNA, with tuberculosis sequelae who had received blood transfusion at a younger age, liver-related mortality from study entry was high at 9.8 per 1,000 person-years. Among the deaths unrelated to tuberculosis sequelae, death of liver disease was the most frequently reported cause.

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HEPATOLOGY

Risk factors for the development of hepatocellular carcinoma among patients with chronic hepatitis C who achieved a sustained virological response to interferon therapy

HAJIME TOKITA,* HIDEO FUKUI,* AKIHISA TANAKA,* HIROSHI KAMITSUKASA,* MICHIYASU YAGURA,* HIDEHARU HARADA* AND HIROAKI OKAMOTO†

**Department of Gastroenterology, National Tokyo Hospital, Tokyo and †Division of Virology, Department of Infection and Immunity, Jichi Medical School, Tochigi-Ken, Japan*

Abstract

Background and Aim: Hepatitis C virus (HCV)-infected patients who responded to interferon (IFN) treatment with clearance of serum HCV RNA may rarely develop hepatocellular carcinoma (HCC). The aim of the present study was to elucidate the risk factors for liver carcinogenesis among such patients.

Methods: In total, 126 patients with chronic hepatitis C (CHC) who achieved a sustained virological response (SVR) to IFN monotherapy, which was defined as the absence of detectable HCV RNA in the serum at 6 months after completion of treatment, were enrolled and possible risk factors for HCC were analyzed.

Results: During the observation period of 66 ± 36 months after cessation of IFN treatment, five (4.0%) of the 126 patients developed HCC. The cumulative incidence of HCC at 3, 5 and 10 years was estimated to be 0.9, 4.7 and 7.5%, respectively. The cumulative incidence of HCC was significantly higher among patients with severe fibrosis (F3 or F4) than among patients with no or mild fibrosis (F0 to F2) in the liver before treatment ($P = 0.007$); among patients with alcohol intake of ≥ 27 g/day than among patients with that of < 27 g/day ($P = 0.015$); and among patients who were ≥ 65 years old than among patients who were < 65 years old at the start of treatment ($P = 0.026$).

Conclusions: Patients with CHC who had severe fibrosis, who had regularly taken moderate amounts of alcohol, or who were ≥ 65 years at the start of IFN treatment should be carefully followed to detect small and controllable HCC, even after eradication of HCV.

Key words: hepatocellular carcinoma, interferon, retrospective cohort study, risk factor, sustained virological response.

INTRODUCTION

In Japan, hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths. Approximately 30 000 patients died of HCC in 2002, and 70–80% of these cases were associated with hepatitis C virus (HCV) infection. It has been demonstrated that HCC frequently develops during the advanced stages of chronic hepatitis C (CHC). Thus, it is considered that preventing the progression of CHC would reduce the risk for developing HCC. Interferon (IFN), administered with or without ribavirin, has been widely used for the treatment of CHC patients. Many investigators have reported that IFN treatment is effective for reducing the serum alanine aminotransferase (ALT) level, reducing and eliminating HCV RNA from the circulation, and improving liver histology in CHC patients.[1–5] There is accumulating evidence that a sustained virological response (SVR) to IFN therapy, defined as the absence of serum HCV RNA at follow-up 6 months after the end of treatment, is highly predictive of long-term remission of the disease.[1–3] Furthermore, the long-term outcome of HCV-infected patients who achieved a SVR to IFN treatment has been shown to be excellent with

Correspondence: Hiroaki Okamoto, Division of Virology, Department of Infection and Immunity, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-Machi, Tochigi-Ken 329-0498, Japan. Email: hokamoto@jichi.ac.jp

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improvement of liver fibrosis.[1–5] Therefore, it would seem unlikely that patients who have responded to IFN therapy with loss of HCV RNA subsequently develop liver cirrhosis or HCC. However, the development of HCC among CHC patients with a SVR to IFN therapy has been reported.[4–10] Although risk factors for the development of HCC among CHC patients who underwent IFN therapy have been described in previous studies,[4,5,11–14] it remains unclear as to whether particular subsets of patients with a SVR to IFN therapy should be carefully followed for the early diagnosis of HCC. In the present study, we investigated the risk factors for the development of HCC among CHC patients who had undergone IFN monotherapy and had a SVR.

METHODS

Patients

In total, 126 histologically proven CHC patients who received IFN treatment at the Department of Gastroenterology, National Tokyo Hospital, between January 1992 and December 2001 and achieved a SVR, were enrolled in this retrospective study. In the present study, a SVR was defined as negativity for detectable HCV RNA in the circulation at 6 months after the end of IFN treatment, using a polymerase chain reaction (PCR) assay with a sensitivity of at least 100 copies (50 IU) per ml.[15,16] The exclusion criteria were as follows: patients with hepatitis B surface antigen, indicating ongoing infection of hepatitis B virus (HBV); patients who were complicated with autoimmune hepatitis; patients who had been diagnosed as having HCC and were cured; and patients with late relapse of HCV infection. No patient had concurrent infection of human immunodeficiency virus type 1. The diagnosis of chronic HCV infection was based on continuous positivity for second-generation antibodies to HCV (Abbott Japan, Tokyo, Japan) and positivity for serum HCV RNA[17] for more than 6 months before IFN treatment was started. All patients underwent liver biopsy just before IFN treatment was started. Histological staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] in which staging is defined as follows: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis). The 126 patients underwent IFN-α monotherapy for 24 ± 3 weeks (range: 9–30 weeks). The total dose of IFN was 722 ± 188 million units (range: 430–980 million units). They received 6–10 million units of IFN-α daily for 2–4 weeks, followed by 6–10 million units of IFN-α three times a week.

The following parameters were assayed in each patient just before IFN therapy was started: serum levels of aspartate aminotransferase (AST) (normal range: 9–31 IU/L) and ALT (normal range: 4–34 IU/L), platelet count (normal range: 15–30 × 10^4/μL), antibody against hepatitis B core antigen (anti-HBc, enzyme immunoassay, Abbott Japan), and HCV genotype. When a serum sample was positive for anti-HBc (inhibition percentage ≥ 70%), the serum diluted at 1:200 was also assayed for anti-HBc. The HCV genotype was determined by the method described previously.[19] This study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of National Tokyo Hospital. Informed written consent was obtained from each patient.

Follow-up and diagnosis of hepatocellular carcinoma

Follow-up of patients was performed by blood examinations including ALT, AST, qualitative detection of HCV RNA, α-fetoprotein (AFP: normal < 10 ng/mL), lectin-reactive AFP (AFP-L3: normal < 15%) and vitamin K absence or antagonist II (PIVKA II: normal range: 0–39 mAU/mL) at regular intervals of within 6 months. Imaging diagnosis was performed at least twice a year by ultrasonography (US) or computed tomography (CT). In all patients studied, serum samples were continuously negative for HCV RNA after the end of IFN treatment using Amplicor HCV v2.0 (Nippon Roche, Tokyo, Japan). The diagnosis of HCC was made using liver imaging (US, CT or magnetic resonance imaging) and/or angiography. In patients whose angiogram did not demonstrate a typical hypervascular image of HCC, microscopic examination of liver specimens obtained by echo-guided fine needle biopsy was performed. Consequently, in all patients with a SVR who developed HCC, a histological diagnosis of HCC was made using surgically resected specimens and/or biopsied specimens.

Detection of hepatitis B virus DNA

The presence of HBV DNA was determined by the method described previously.[20] Briefly, nucleic acids were extracted from 100 μL of serum using a commercially available kit (SMITEST EX-R&D; Genome Science, Tokyo, Japan), and were tested for HBV DNA by nested PCR using primers derived from the well-conserved areas in the S gene region of the HBV genomes of all eight genotypes (A to H) and Perkin-Elmer AmpliTaq DNA polymerase (Roche Molecular Systems, Branchburg, NJ, USA). The first-round PCR (94°C for 2 min before the start of cycling; 94°C for 30 s; 55°C for 30 s; 72°C for 90 s, with an additional 7 min in the last cycle) was performed for 35 cycles with primers HB095 (sense: 5′-GAG TCT AGA CTC GTG GTG GAC-3′) and HB184 (antisense: mixture of two sequences, 5′-CGA ACC ACT GAA CAA ATG GCA CCG C-3′ and 5′-CGC ACC ACT GAA CAA ATT GCA C-3′). The second-round PCR for 25 cycles was carried out under the same conditions as the first-round PCR except for extension for 60 s with primers HB097 (sense: 5′-GAC TCG TGG TGG ACT TCT CTC-3′) and S2-2 (antisense: 5′-GGC ACT AGT AAA CTG AGC CA-3′). The amplification product of the first-round PCR was 461 base pairs, and that of the second-round PCR was 437 base pairs.

Statistical analyses

The Kaplan-Meier method was used to calculate the cumulative incidence of HCC and the log-rank test was used to compare the cumulative incidence of HCC between two groups. Differences were considered to be statistically significant at $P < 0.05$. Data are presented as mean ± standard deviation (SD).

RESULTS

The baseline characteristics of the 126 patients at the start of IFN treatment (baseline) who subsequently achieved a SVR to IFN treatment are summarized in Table 1. All patients showed virological clearance and biochemical normalization 6 months after the end of treatment. During the observation period of 66 ± 36 months (range: 7–139 months) after the end of IFN treatment, the sera continued to be negative for HCV RNA in all 126 patients. However, five patients (4.0%) developed HCC. The cumulative incidence of HCC at 3, 5 and 10 years was estimated to be 0.9, 4.7 and 7.5%, respectively. The baseline characteristics of the five patients who developed HCC are presented in Table 2. All five patients who developed HCC were males, whose age ranged from 51 to 70 years at the start of IFN treatment. Four patients were assumed to have contracted HCV infection from a blood transfusion, and one patient was assumed to have contracted HCV infection from home medical therapy. The age at which the five patients were assumed to have contracted HCV infection ranged from 21 to 49 years, and the duration of persistent HCV infection was estimated to be 13–48 years. None of the five patients were heavy drinkers, which was defined as alcohol intake of 80 g or more per day, but four patients (80%) were moderate drinkers who took 27–54 g alcohol per day. The HCV genotype was 1b in one patient, 2a in three patients, and 2b in the remaining patient. Four patients (80%) were positive for anti-HBc but negative for the antibody in the serum diluted at 1:200, indicating that the titer of anti-HBc was too low to support ongoing HBV infection.

Table 1 Characteristics of the 126 patients who achieved a sustained virological response to interferon (IFN) monotherapy

Age at the start of IFN treatment (years)	53 ± 14 (range: 19–75)
Sex (male/female)	78/48
Alcohol intake	
Drinkers (≥ 27 g/day)	42 (33%)
Heavy drinkers (≥ 80 g/day)	15 (12%)
Observation period after IFN treatment (months)	66 ± 36 (range: 7–139)
Development of HCC	5 (4%)
Laboratory data before IFN treatment	
AST (IU/L)	86 ± 74 (range: 12–498)
ALT (IU/L)	119 ± 105 (range: 11–612)
Platelet count ($\times 10^4/\mu L$)	17.3 ± 6.3 (range: 5.6–36.4)
HCV genotype (genotype 1/genotype 2)	44/82
Positive for anti-HBc	49 (39%)
Positive for anti-HBc (diluted at 1:200)	5 (4%)
Liver histology before IFN treatment	
F0	2 (2%)
F1	45 (36%)
F2	47 (37%)
F3	29 (23%)
F4	3 (2%)

The normal range of platelet count is 15–30 × $10^4/\mu L$. Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] ALT, alanine aminotransferase (normal range: 4–34 IU/L); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥ 70%); AST, aspartate aminotransferase (normal range: 9–31 IU/L); HCC, hepatocellular carcinoma.

Table 2 Baseline characteristics of five patients with a sustained virological response who developed hepatocellular carcinoma (HCC) after interferon (IFN) treatment

Case	Sex	Age at the start of IFN treatment (years)	Assumed cause of HCV infection (duration of infection, years)	Before IFN treatment: Alcohol intake	HCV genotype	Anti-HBc	AST (IU/L)	ALT (IU/L)	Liver histology
1	Male	51	Blood transfusion (13)	27 g/day	2a	Positive	58	76	F3
2	Male	63	Home medical therapy (14)	54 g/day	2a	Positive	57	99	F3
3	Male	69	Blood transfusion (48)	None	1b	Positive	105	141	F3
4	Male	66	Blood transfusion (38)	27 g/day	2a	Positive	66	74	F2
5	Male	70	Blood transfusion (36)	54 g/day	2b	Negative	61	82	F3

Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] ALT, alanine aminotransferase (normal range: 4–34 IU/L); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥ 70%); AST, aspartate aminotransferase (normal range: 9–31 IU/L); HCV, hepatitis C virus.

Furthermore, none had detectable HBV DNA in the circulation. The stage of liver fibrosis at baseline was F3 in four patients (80%) and F2 in the remaining one patient.

The laboratory data at the time of diagnosis of HCC and pathological characteristics of the HCCs in the five patients are presented in Table 3. HCC was detected 54 ± 27 months (range: 25–99 months) after the end of IFN treatment. Four patients (80%) had a single HCC tumor and one patient (case 3) had three definable tumors. The size of a HCC tumor ranged from 8 to 30 mm in diameter, and the tumor was pathologically diagnosed as 'moderately differentiated' in four patients (80%) and 'well differentiated' in the patient with three tumor nodules (case 3). At the time of diagnosis of HCC, one patient (case 5) had a slightly elevated serum AFP level; however, no other HCC-related markers (AFP-L3 and PIVKA II) were elevated in any of the patients, including case 5. The histological findings of non-tumor liver tissues obtained at the time of diagnosis of HCC had remarkably improved in each patient compared with the histological findings at baseline. The stage of liver fibrosis had improved from F3 to F2 in case 1; from F3 to F1 in cases 2, 3 and 5; and from F2 to F1 in case 4.

Univariate analyses with the Kaplan-Meier method and the log-rank test were performed to compare the cumulative incidence of HCC with regard to various possible risk factors including age at baseline, sex, alcohol intake, laboratory data at baseline (AST, ALT, platelet count, HCV genotype, and anti-HBc) and the degree of liver fibrosis at baseline (Table 4). These factors were stratified into two groups, and the cumulative incidences of HCC between the two groups were compared. The cumulative incidence of HCC was significantly higher among the 32 patients with severe fibrosis (F3 or F4) than among the 94 patients with no fibrosis (F0) or mild fibrosis (F1 or F2) in the liver tissues before IFN treatment ($P = 0.007$, Fig. 1); among the 42 patients with alcohol intake of ≥ 27 g per day than among the 84 patients with alcohol intake of < 27 g per day ($P = 0.015$); and among the 28 patients who were

Table 3 Characteristics of hepatocellular carcinoma (HCC) in five patients who achieved a sustained virological response to interferon (IFN) therapy

Case	Months between the end of IFN therapy and the detection of HCC	HCC			Laboratory and histological data at the time of diagnosis of HCC				
		Number	Size (mm in diameter)	Differentiation	AFP (ng/mL)	AFP-L3 (%)	PIVKA II (mAU/mL)	Anti-HBc antibody	Liver histology
1	25	One	15	Moderate	2.1	0	25	Positive	F2
2	99	One	16	Moderate	1.8	0	28	Positive	F1
3	53	Three	8, 15, 30	Well	2.3	0	18	Positive	F1
4	42	One	23	Moderate	2.6	0	19	Positive	F1
5	52	One	20	Moderate	14.9	0	25	Negative	F1

Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] AFP, α-fetoprotein (normal < 10 ng/mL); AFP-L3, lectin-reactive AFP (normal < 15%); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥70%); PIVKA II, vitamin K absence or antagonist II (normal range: 0–39 mAU/mL).

Table 4 Risk factors associated with the development of hepatocellular carcinoma (HCC) in patients who achieved a sustained virological response to interferon (IFN) therapy

Factor	Comparison	*P*-value (log-rank test)
Age at the start of IFN treatment (years)	≥ 65 *vs* < 65	0.026
Sex	Male *vs* female	0.059
Alcohol intake	≥ 27 g/day *vs* < 27 g/day	0.015
	≥ 80 g/day *vs* < 80 g/day	0.447
Laboratory data before IFN treatment		
AST (IU/L)	≥ 80 *vs* < 80	0.446
ALT (IU/L)	≥ 80 *vs* < 80	0.890
Platelet count ($\times 10^4/\mu L$)	≥ 15.0 *vs* < 15.0	0.326
HCV genotype	Genotype 1 *vs* genotype 2	0.428
Positive for anti-HBc	Positive *vs* negative	0.097
Positive for anti-HBc (diluted at 1:200)	Positive *vs* negative	0.646
Liver histology before IFN treatment	F0, F1 and F2 *vs* F3 and F4	0.007

The normal range of platelet count is 15–30 $\times 10^4/\mu L$. Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] ALT, alanine aminotransferase (normal range: 4–34 IU/L); anti-HBc, antibody against hepatitis B core antigen (positive inhibition ≥ 70%); AST, aspartate aminotransferase (normal range: 9–31 IU/L); HCV, hepatitis C virus.

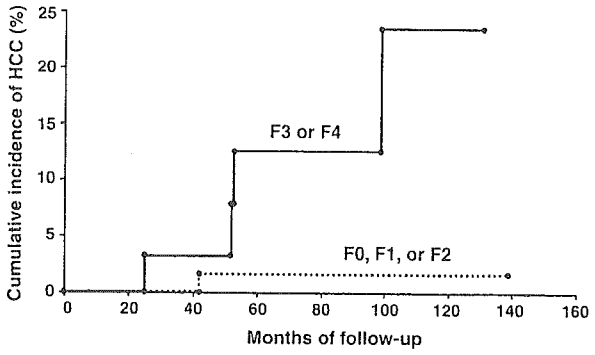

Figure 1 Cumulative incidence of hepatocellular carcinoma (HCC) among 32 patients with severe fibrosis (F3 or F4) and among 94 patients with no or mild fibrosis (F0 to F2) of the liver before interferon treatment who subsequently achieved a sustained virological response using the Kaplan-Meier method and the log-rank test. Staging of chronic hepatitis was based on the scoring system proposed by Desmet *et al.*[18] in which staging is defined as follows: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis). $P = 0.007$ (log-rank test).

≥ 65 years old than among the 98 patients who were < 65 years old at the start of IFN treatment ($P = 0.026$). In addition, the cumulative incidence of HCC tended to be higher among the 78 males than among the 48 females ($P = 0.059$), and among the 49 patients with anti-HBc than among the 77 patients without anti-HBc in their sera before IFN treatment ($P = 0.097$).

DISCUSSION

Patients with CHC who have achieved a SVR to IFN treatment are likely to be considered cured. However, the development of HCC in patients who had a SVR to IFN therapy for a long period of time has recently been reported. In these case reports, HCC developed in the CHC patients with a SVR at 72 months,[6] 77 months,[9] 80 months,[7] and 90 months[10] after the end of IFN therapy. In the present study, five (4.0%) of the 126 patients who had achieved a SVR to IFN therapy developed HCC at 25 months, 42 months, 52 months, 53 months, and 99 months after the end of IFN therapy. Although the growth pattern varies among tumors, the tumor volume doubling time (growth rate) has been estimated to range from 1 to 20 months (median: 6 months),[21] and it has been estimated that the length of time between the occurrence of HCC and the time point at which the HCC tumor has grown to a diameter approximately 1 cm, when it is detectable by conventional US or CT, is more than 72 months.[13] Thus, it seems likely that four of five patients who developed HCC in the present study had had undetectable HCC before IFN therapy. However, it is difficult to distinguish between de novo HCC and HCC that developed before or during IFN therapy. It has been reported that poorer differentiation of a HCC tumor is associated with a shorter doubling time of HCC.[22] Considering the differentiation of the tumor in our five HCC patients, four of the five were moderately differentiated and one was well differentiated. One patient with a SVR in the present study developed moderately differentiated HCC 99 months after the end of IFN therapy. Thus, it is important to investigate the risk factors for the development of HCC among patients with a SVR, separate from those without a SVR.

Many previous studies have revealed various risk factors for the development of HCC among patients with CHC. The risk factors thus far reported are: no history of IFN therapy;[4,11,23] no response to IFN therapy;[4,5,11–14] older age;[4,5,11–14,23,24] male sex;[4,5,12–14,23,24] past history of blood transfusion;[25] heavy alcohol intake;[21,25,26] severe fibrosis of the liver;[4,5,11,13,14,25] high histological activity score;[11] portal inflammation;[12] HCV genotype 1b;[24] high HCV RNA level;[12] lower platelet count;[13,14,23] high serum AFP level;[23] high serum γ-glutamyl transpeptidase level;[5] low serum albumin level;[25] and high serum ALT level.[23] However, it seems unlikely that all of these risk factors are applicable to patients who have achieved a SVR, for early diagnosis and treatment of HCC, because the incidence of HCC among patients with a SVR is very low compared with that among patients who did not achieve a SVR.[4,5]

In the present study, the risk factors for HCC were analyzed among patients with CHC, focusing on those who achieved a SVR to IFN monotherapy, and the following three factors were found to be statistically significant: severe fibrosis (F3 or F4) of the liver before IFN treatment; alcohol intake of 27 g or more per day; and age of 65 years or above at the start of IFN treatment. Of note, a moderate amount of alcohol intake (≥27 g/day) was significantly associated with the development of HCC in patients with a SVR in this study. Although it is well documented that excessive alcohol intake is one of the important risk factors for the development of HCC in patients with CHC,[16,21,25] the effect of lower levels of alcohol consumption is still unclear.[16] Some investigators have pointed out the effect of light drinking on HCV-associated liver disease.[26–29] In the present study, 15 patients with excessive alcohol intake of ≥80 g/day had not developed HCC within the observation period of 26–127 months. This might indicate the existence of a synergistic effect between excessive alcohol intake and other risk factors for HCC. Multivariate analysis (e.g. Cox proportional hazards model) was not performed in this study, because the number of patients who developed HCC were too few ($n = 5$) to draw a plausible conclusion. Therefore, extended studies are required to determine whether or not these three risk factors are independent risk factors for HCC among CHC patients with sustained loss of serum HCV RNA after completion of IFN treatment.

In the current study, we did not examine HCV RNA in the liver tissues at 6 months after completion of IFN treatment when HCV RNA was not detectable in the circulation. Therefore, we cannot rule out the possibility of HCV persistence in the liver tissues of the five patients who developed HCC. However, the histological findings of non-tumor liver tissues obtained at the time of diagnosis of HCC had improved markedly in each patient as compared with those at baseline.

A weak relationship between positivity of anti-HBc and the development of HCC was observed in the present study, although it was not statistically significant ($P = 0.097$). The role of resolved or occult HBV infection in promoting the development of HCC in patients with CHC is highly controversial. Some investigators have emphasized its role in hepatocarcinogenesis,[30–32] and others have reported evidence that does not support this.[33,34] Undoubtedly, the accumulation of CHC patients with a SVR who subsequently developed HCC is necessary to elucidate whether or not the presence of isolated anti-HBc is a risk factor for the development of HCC in CHC patients with a SVR.

In Japan, public health insurance has covered IFN α-2b plus ribavirin therapy[35] since January 2002 and peginterferon α-2a monotherapy[36] since January 2004. Furthermore, combination therapy of peginterferon α-2b and ribavirin[37] is now available. These alternative therapies were demonstrated to be more effective than IFN monotherapy in CHC patients with HCV genotype 1 infection, in those with high HCV viral load in the circulation, and in those with severe fibrosis of the liver.[35–37] However, such patients are also at high risk for developing HCC, and it is very likely that the number of patients who develop HCC even after clearance of serum HCV RNA following more effective IFN therapy administered with or without ribavirin, may increase in the future, indicating the necessity of careful follow-up of such patients.

In conclusion, CHC patients who respond to IFN monotherapy or combination therapy should be followed as closely as possible, even after eradication of HCV, paying special attention to those who had severe fibrosis (F3 or F4) in the liver, those who had taken moderate amounts of alcohol (≥ 27 g/day), and those who were ≥ 65 years at the start of IFN treatment, to detect small and controllable HCC.

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Factors regarding increase of platelet counts in chronic hepatitis C patients with sustained virological response to interferon—Relation to serum thrombopoietin levels

Michiyasu Yagura*, Akihisa Tanaka, Hajime Tokita, Hiroshi Kamitsukasa, Hideharu Harada

Department of Gastroenterology, National Hospital Organization Tokyo Hospital, 3-1-1, Takeoka, Kiyose-city, Tokyo 204-8585, Japan

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Abstract

Thrombocytopenia is frequently found in patients with chronic liver disease, and associated with advanced fibrosis stage and with decreased liver function. Serum thrombopoietin (TPO) levels also decrease as the disease progresses from mild fibrosis to cirrhosis. On the other hand, platelet counts increase associated with improvement of fibrosis in chronic hepatitis C (CH-C) patients with sustained virological response (SVR) to interferon (IFN) therapy. Then, we studied if the increase of platelet counts in SVR associate with elevated TPO production or a reduction of spleen size. Liver fibrosis, spleen size, serum TPO levels, albumin, zinc turbidity test (ZTT), platelet counts were compared in fifteen CH-C patients with SVR before and after IFN therapy.

Results: Albumin increased from 4.2 ± 0.3 to 4.3 ± 0.3 g/dl ($p = 0.067$), ZTT decreased from 17.7 ± 5.9 to 8.9 ± 3.9 K-U ($p < 0.001$), platelet counts increased from $15.5 \pm 6.8 \times 10^4$ to $19.9 \pm 5.8 \times 10^4/\mu l$ ($p < 0.01$) and serum TPO levels increased from 1.65 ± 0.94 to 2.06 ± 1.22 fmol/ml ($p = 0.073$). Spleen size was measured by ultrasonography, and the spleen index was calculated by multiplication of the long and short axes from hilus, which decreased from 14.6 ± 5.0 to 10 ± 3.1 ($p < 0.001$) after IFN therapy.

In conclusion, increase of platelet counts in SVR may be related to the reduction of spleen size and increased serum TPO levels associated with improvement of fibrosis after IFN therapy.

Keywords: Thrombopoietin; Platelet counts; Sustained virological responder; Chronic hepatitis C; Interferon

1. Introduction

Chronic hepatitis C (CH-C) progresses to cirrhosis insidiously after hepatitis C virus (HCV) infection accompanying with the gradual decline in the platelet counts. A low platelet count is an accurate marker of hepatic fibrosis and an excellent predictive noninvasive marker of cirrhosis in the absence of clinical, biological, endoscopic or ultrasonographic signs of portal hypertension [1]. Saito et al. [2] reported that platelet counts significantly correlated with the fibrotic stage, that is, F1, 19.2; F2, 17.2; F3, 13.2; F4, 7.8 ($\times 10^4/\mu l$). The main cause of thrombocytopenia has been attributed to an increased sequestration and pooling of platelets by an enlarged spleen secondary to portal hypertension, especially in severe case such as liver cirrhosis [3,4]. This theory has long been controversial and studies of platelet-turnover have yielded conflicting results [5]. Other mechanisms, such as an autoimmune [6] or viral megakaryocyte infection [7], have also recently been postulated in patients with HCV infection.

Thrombopoietin (TPO) is the most potent and specific cytokine for the growth and maturation of megakaryocyte and platelet production [8–10]. TPO messenger RNA (mRNA) transcripts have been found predominantly in the liver with lesser amounts also detected in the kidneys, bone marrow and spleen [11]. Most TPO is bound to receptors, c-Mpl, on

* Corresponding author. Tel.: +81 424 91 2111; fax: +81 424 94 2168.
E-mail address: Kansyoukaki1949@aol.com (M. Yagura).

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platelet and the serum level is low. When thrombocytopenia develops, binding receptors decrease and serum TPO level increases. Elevated TPO level stimulates megakaryopoiesis and results in increasing platelet production [12–15]. Kawasaki et al. [16] and Adinolfi et al. [17] reported that serum TPO levels were decreased, as the disease progressed from mild fibrosis to cirrhosis, in patients with chronic hepatitis and liver cirrhosis. Moreover, the former authors identified a correlation between serum TPO levels and prothrombin activity, thus suggesting that low TPO levels might be expression of decreased liver function even in patients with chronic hepatitis.

We previously reported that platelet counts increased associated with improvement of fibrosis in CH-C patients with sustained virological response (SVR) to interferon (IFN) [18]. The aim of this study was to look for possible factors regarding the increase of platelet counts, such as serum TPO levels, spleen size, liver histology and liver function test in CH-C patients with SVR to IFN therapy.

2. Patients and methods

2.1. Patients

We studied 15 CH-C patients with SVR (undetectable HCV RNA after IFN therapy) (14 male, 1 female; mean age 59.7 ± 6.9 years old) who were biopsied before and after IFN therapy. Time interval between two times of liver biopsy was mean 7.6 ± 2.0 years (range 3.4–10.7 years). Histological degree of liver fibrosis was graded according to the severity as follows; F1 stage is expansion of portal area, F2 stage is bridging fibrosis without lobular distortion, F3 stage is lobular distortion, F4 stage is cirrhosis. Fibrosis score of each patients before IFN therapy was as follows; 1 patient had F1, 4 patients had F2 and 10 patients had F3. Platelet counts, albumin, zinc turbidity test (ZTT), serum TPO levels and spleen size were compared before and after IFN therapy, respectively. A peripheral blood sample was obtained on the day of liver biopsy.

2.2. TPO assay

Samples were stored at −80 °C until analyzed. The serum TPO were measured by a commercially available sandwich enzyme-linked immunosorbant assay (ELISA) using a monoclonal antibody and a polyclonal antibody to recombinant human TPO as previously described. Normal range: male, 0.79 ± 0.35 fmol/ml; female, 0.70 ± 0.26 fmol/ml [8].

2.3. Spleen size

Spleen size was measured by ultrasonography, and the spleen index (SI) was calculated by multiplication of the long and short axes from hilus.

2.4. Statistical analysis

The data are expressed as mean ± S.D. Wilcoxon signed rank test were used for statistical analysis according to the data analyzed. For all tests, $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Changes of albumin, ZTT and platelet counts before and after IFN therapy

Mean albumin increased from 4.2 ± 0.3 to 4.3 ± 0.3 g/dl ($p = 0.067$), ZTT significantly decreased from 17.7 ± 5.9 K-U to 8.9 ± 3.9 K-U ($p < 0.001$) and platelet counts significantly increased from 15.5 ± 6.8 × 10^4 to 19.9 ± 5.8 × 10^4/μl ($p < 0.01$), respectively (Fig. 1).

3.2. Changes of serum TPO levels before and after IFN therapy

Mean serum TPO levels increased from 1.65 ± 0.94 to 2.06 ± 1.22 fmol/ml ($p = 0.073$) after IFN therapy (Fig. 2). Serum TPO levels increased after IFN therapy in 12 out of 15 patients.

3.3. Changes of fibrosis score before and after IFN therapy

Fibrosis score improved after IFN therapy as follows; from F1 to F1 in one patient, from F2 to F1 in four patients, from F3 to F1 in five patients, from F3 to F2 in three patients and from F3 to F3 in two patients in which the width of fibrous septum became narrower, respectively (Fig. 3).

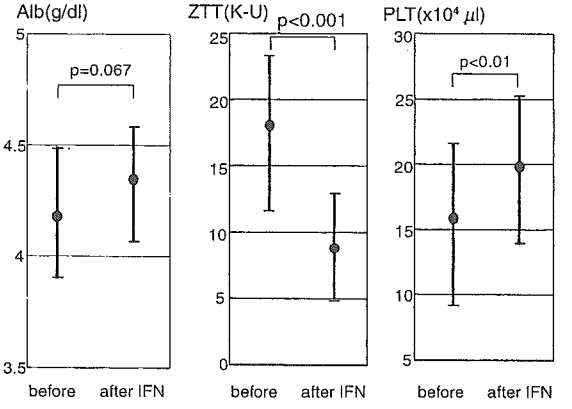

Fig. 1. Changes of albumin, ZTT and platelet counts before and after IFN therapy. Mean albumin increased from 4.2 ± 0.3 to 4.3 ± 0.3 g/dl ($p = 0.067$), ZTT significantly decreased from 17.7 ± 5.9 K-U to 8.9 ± 3.9 K-U ($p < 0.001$) and platelet counts significantly increased from 15.5 ± 6.8 × 10^4 to 19.9 ± 5.8 × 10^4/μl ($p < 0.01$) after 7.6 ± 2.0 years of IFN therapy, respectively.

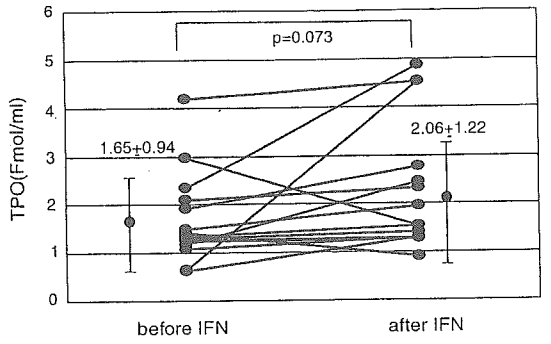

Fig. 2. Changes of serum TPO levels before and after IFN therapy. Serum TPO levels increased from 1.65 ± 0.94 to 2.06 ± 1.22 fmol/ml ($p = 0.073$) after 7.6 ± 2.0 years of IFN therapy.

3.4. Changes of spleen size before and after IFN therapy

Mean spleen index (SI) decreased from 14.6 ± 5.0 to 10 ± 3.1 ($p < 0.001$) after 7.6 ± 2.0 years of IFN therapy, which equaled to the rate of decrease of 0.7 ± 0.2 times (range 0.50–0.97 times) as compared to that before IFN therapy (Fig. 4).

3.5. Correlation between platelet counts and serum TPO levels or spleen index (SI) in the rate of change before and after IFN therapy

There was no significant correlation between platelet counts and serum TPO levels in the rate of increase nor between the rate of increase of platelet counts and the rate of decrease of SI; correlation coefficient = 0.143 ($p = 0.189$) and 0.214 ($p = 0.127$), respectively.

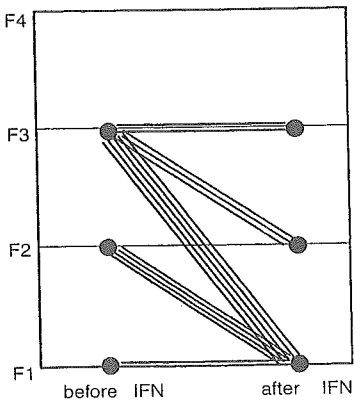

Fig. 3. Changes of fibrosis score before and after IFN therapy. Fibrosis score improved after IFN therapy as follows; from F1 to F1 in one patient, from F2 to F1 in four patients, from F3 to F1 in five patients, from F3 to F2 in three patients and from F3 to F3 in two patients in which the width of fibrous septum became narrower after 7.6 ± 2.0 years of IFN therapy, respectively.

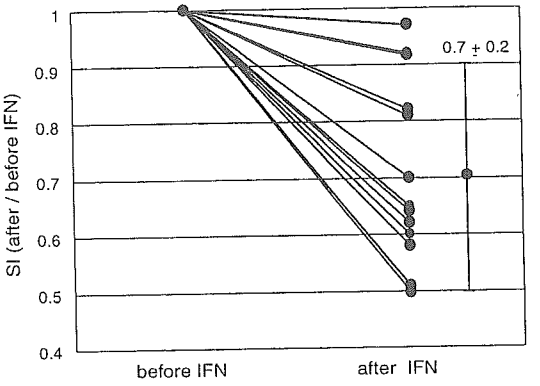

Fig. 4. Changes of spleen size before and after IFN therapy. Spleen index (SI) was calculated by multiplication of the long and short axes from hilus on ultrasonography, and decreased from 14.6 ± 5.0 to 10 ± 3.1 ($p < 0.001$), which equaled to the rate of decrease of 0.7 ± 0.2 times (range 0.50–0.97 times) as compared to that before IFN therapy.

4. Discussion

Thrombocytopenia is frequently found in patients with chronic liver disease, and associated with advanced fibrosis stage and with decreased liver function. Moreover, thrombocytopenia is included in one of risk factors for development of hepatocellular carcinoma (HCC) concomitant with male gender, age 55 years or older, prothrombin activity 75% or less and anti-HCV positivity [19], and alcohol, tobacco and obesity as synergistic risk factors [20]. Kubo et al. [21] reported that the proportion of patients with multicentric HCC was significantly higher among patients with low platelet counts (below $10^5/mm^3$) than patients with a higher count. These findings suggest that platelet counts are relevant to not only development of HCC but also prognosis of HCC. From this point of view, we studied on the significance of SVR in which platelet counts increased and incidence of HCC decreased as compared with those of NR [18]. Further, serum TPO levels decrease as the disease progresses from mild fibrosis to cirrhosis [16,17]. In the present study we studied if the increase of platelet counts in CH-C patients with SVR to IFN therapy associate with elevated TPO production or a reduction of spleen size. There have been no reports regarding the relationship between platelet counts and the change of spleen size after IFN therapy. Bizollon et al. [22] reported that biochemical and virological responder to combination therapy of ribavirin and IFN associated with marked histological improvement and Shiota et al. [23] reported that serum TPO levels increased following SVR to IFN therapy in patients with chronic hepatitis C. Itoh et al. [24] showed a significant decrease in fibrosis stage paralleled by an increase in platelet count and serum TPO levels in patients with SVR to IFN therapy after 4 years of follow-up. We also recently reported that the increase of platelet counts and serum albumin and decrease of ZTT associated with improvement of fibrosis in CH-C patients with SVR to IFN therapy [18]. In the present study, we demonstrated that a reduction of spleen size and the

increase of serum TPO levels were relevant factors regarding the increase of platelet counts in SVR. We investigated factors relating to the reduction rate of SI after IFN therapy, such as fibrosis stage, albumin, ZTT, platelet counts, TPO, age and SI before IFN therapy, and the ratio of the value after IFN therapy in those items to the value before IFN therapy; however, no significant difference was seen between two groups—one group with less than 10% ($n = 3$) and the other group over 30% ($n = 10$) of the reduction rate. Serum TPO levels did not increase in 3 out of 15 patients after IFN therapy regardless of the decrease of spleen size in all patients. These may show that the decrease of spleen size have a stronger influence on the increase of platelet counts than the increase of serum TPO levels. The increase of albumin and decrease of ZTT also seems to be the result from the regeneration and the amelioration of necroinflammatory change of hepatic cells, respectively.

Chen-Wei et al. [25] described that serum TPO levels elevation response to consensus interferon (CIFN) therapy is higher in SVR than in nonresponder, which means less hepatic fibrosis and better hepatic function reserve in SVR. Therefore, the serum TPO response to CIFN-induced thrombocytopenia may possibly serve as a marker for the severity of hepatic fibrosis. Koruk et al. [26] reported a positive correlation between serum TPO and albumin levels in patients with LC, revealing that serum TPO concentration may decrease with deterioration of protein producing ability of liver in LC. Kato et al. [27] showed a 30–40% reduction of total liver TPO mRNA content and thrombocytopenia in patients with cirrhosis compared to patients without cirrhosis. Giannini et al. [28] also demonstrated that serum TPO levels was correlated to liver functional impairment evaluated by means of [^{13}C]aminopyrine breath test and liver fibrosis.

From these findings, it is assumed that improvement of liver fibrosis induced by IFN therapy resulted in the increase of serum TPO levels and serum albumin, and further the decrease of spleen size leading to the increase of platelet counts. However, we did not evaluate other factors regarding thrombocytopenia such as anti-platelet-autoantibodies, platelet-turnover, measurement of portal hyperpressure. Therefore, these mechanisms could not be completely ruled out.

In conclusion, it appears that the increase of platelet counts in CH-C patients with SVR relates to both the reduction of spleen size and the increased TPO levels associated with improvement of liver histology after IFN therapy.

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今月の症例

ペグインターフェロンα-2a導入後早期にBasedow病を発症したC型慢性肝炎の1例

独立行政法人国立病院機構横浜医療センター臨床研究部
前出幸子　森下慶一　岩村国博　高山敬子　塚田百合子
岸野真衣子　清水　健　松島昭三　小松達司　笠木陽子

概要　症例は23歳，女性，C型慢性肝炎の治療のため，pegylated interferon α-2a（PEG-IFNα-2a）を投与したところ，治療開始約1カ月後にBasedow病を発症した．IFN治療による甲状腺機能亢進症の多くは無痛性甲状腺炎であり，Basedow病は稀である．また本症例は，その発症時期が非常に早期であったことからも興味深い症例と考え報告する．〔日内会誌　94：2600～2602，2005〕

Key words：C型慢性肝炎，ペグインターフェロンα-2a，Basedow病

症　例

患者：23歳女性．主訴：全身倦怠感．既往歴・家族歴：特記事項なし．輸血歴：なし．生活歴：アルコール焼酎水割り3杯/日×5年間．タバコ15本/日．現病歴：2003年12月全身倦怠感があり，近医受診．肝機能障害を指摘され，12月初旬当科入院．入院時AST 267IU/*l*，ALT 507IU/*l*，T-bil 0.6mg/dl，HCV抗体陽性，HCV genotype2a，HCV-RNA 410KIU/ml，肝生検では慢性肝炎（F1/A1）の所見であった．本人の希望により一時退院後，2月下旬よりPEG-IFNα-2a180μg週1回の投与を開始した．IFN開始前の甲状腺ホルモン値は正常範囲，thyroid test，microsome testは400倍であった．AST，ALTは速やかに改善した．WBC減少のため6回目よりPEG-IFNα-2aは90μgに減量．3月下旬頃より全身倦怠感と眼痛が出現し，3月下旬精査加療目的で入院となった．入院時現症：血圧；100/60mmHg，脈拍；109bpm整，体温；37.2℃．眼瞼結膜貧血なし，眼球結膜黄染なし．頸部に弾性硬の甲状腺腫大を認めた．心音，呼吸音正常．腹部腸蠕動音正常，圧痛なし，肝，脾触知せず．四肢浮腫なし．手指の振戦を認めた．入院時検査所見：尿所見異常なし，Hb 13.0g/dl，WBC 1,700/μl（Neut 47%，Ly 14%，Mo 10%，Bas 0%，Eos 1%），Plt 14.4×10⁴/μl，T.Bil 0.6mg/dl，AST 94IU/*l*，ALT 172IU/*l*，ALP 271IU/*l*，γGTP 30IU/*l*，T-chol 107mg/dl，TG 35mg/dl，TSH 0.005μIU/*l*，fT_3 20.51pg/ml，fT_4 5.29ng/dl，TSH受容体抗体（TRAb）20IU/*l*，超音波検査では甲状腺のびまん性腫大と血流増加を認めた（図1）．

臨床経過（図2）

本症例はPEG-IFNα-2a投与開始5週後に甲状腺機能亢進症状と思われる全身倦怠感，眼痛を認め，TSHの低下，fT3，fT4の著明な高値，TRAb陽性，甲状腺超音波検査所見などよりBasedow病と診断した．後日保存血清により測定した結果では，治療開始後4週の時点でTSHの低下，

〔平成16年10月9日　第521回関東地方会推薦〕

Chronic hepatitis C with early complication of Grave's disease during the treatment of pegylated interferon α-2a.

Yukiko Maede, Keiichi Morishita, Kunihiro Iwamura, Yukiko Takayama, Yuriko Tsukada, Maiko Kishino, Takeshi Shimizu, Shouzou Matsushima, Tatsuji Komatsu and Youko Kasagi : Department of clinical research ; National Hospital Organization Yokohama Medical Center, Yokohama.

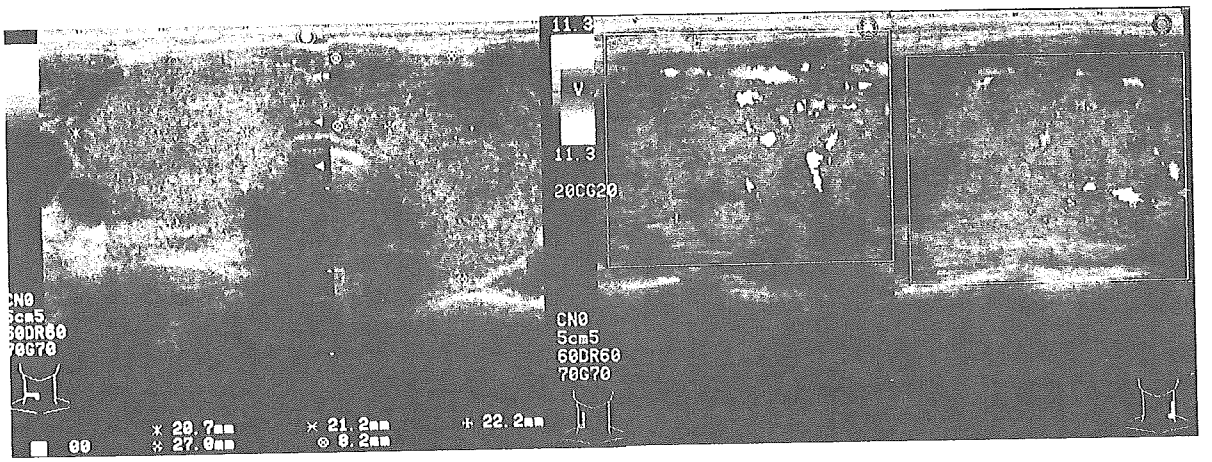

図1．甲状腺エコーではRt；67.5×20.7×25.5mm，Lt；58.0×22.2×20.9mm，びまん性に腫大しており，表面はやや不整，内部均一であった．カラードップラーエコーでは内部血流は増加（火焔状）していることよりBasedow病と診断．

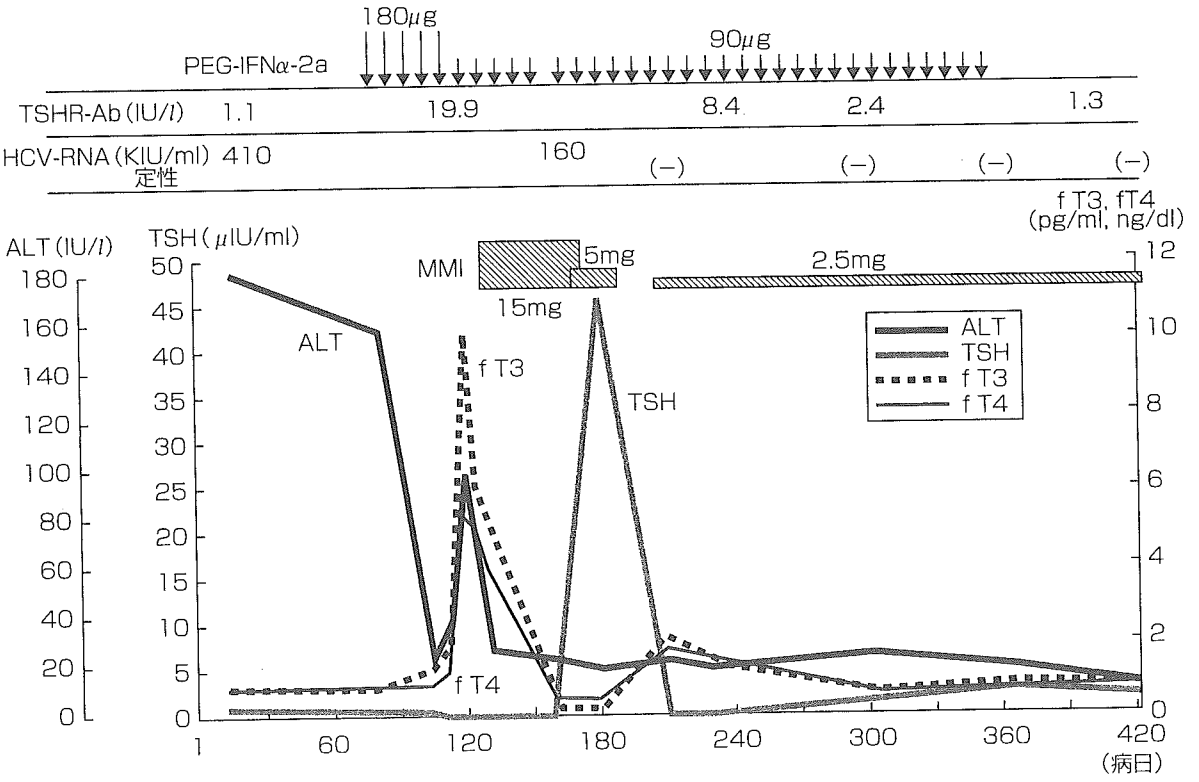

図2．臨床経過

fT3，fT4の上昇がみられた．また治療開始前のTRAbは陰性（1.0IU/l未満）であった．チアマゾール（MMI）15mg/日から投与開始し，症状は改善．MMI漸減し，現在5mgの隔日投与で機能正常である．またPEG-IFNα-2aは予定通り48週間投与し，治療終了6カ月後までAST，ALT

は正常，HCV-RNAも陰性で経過している．

考　察

IFN治療による甲状腺機能異常の出現頻度は10%前後といわれている．女性，甲状腺疾患の家族歴，治療前の甲状腺自己抗体陽性例で，甲状腺機能異常が出現する可能性が高い[1,2]．本症例ではIFN投与前の甲状腺機能は正常であったが，thyroid test，microsome testが陽性であり，慎重にIFNを投与した．IFN治療誘発性の甲状腺機能異常は甲状腺機能低下症や無痛性甲状腺炎が多く，Basedow病は稀である[3,4]．甲状腺機能異常の病因としては，免疫修飾作用が主と考えられるが，その他IFNαによる直接の甲状腺機能抑制作用や，IL-6を介して甲状腺ホルモンの末梢代謝に及ぼす影響も報告されている[5]．今までの報告では，IFN投与開始からBasedow病の出現時期まで，ほとんどの症例が3カ月以上経過しており，その平均は1年との報告もある[4,6]．本症例は，IFN投与開始後約1カ月で甲状腺機能亢進症が出現しており，これまでの報告に比べ非常に早期である．このことはPEG-IFNα-2a製剤（あるいはPEG-IFN製剤）に特徴的なことなのか，本症例が特殊なケースなのか，興味深い点である．IFN長期（1年以上）投与症例の増加に伴い，今後Basedow病発症例も増加することが予想される．本例のように早期に診断し，適切に治療することによりIFN治療も継続可能であることから，自覚症状の有無にかかわらず甲状腺機能検査を定期的に施行することは重要であると思われる[7]．

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Immunomodulatory effects of selective leucocytapheresis as a new adjunct to interferon-α2b plus ribavirin combination therapy: a prospective study in patients with high plasma HCV viraemia

K. Sawada[1,5], N. Masaki,[2] S. Hayashi,[3] M. Zeniya,[4] T. Ishikawa,[4] H. Takahashi,[4] K. Ohnishi,[5] K. Fukunaga,[5] N. Hara,[6] T. Yamamoto,[6] T. Hada,[6] and G. Toda[4] [1]*Department of Gastroentrology, Fujimoto Hospital Medicine;* [2]*International Medical Center of Japan;* [3]*Disaster Medical Center of Japan;* [4]*Division of Gastroenterology and Hepatology, Jikei University School of Medicine;* [5]*Department of Gastroentrology; and* [6]*Department of Internal Medicine, Hyogo College of Medicine, Tokyo, Japan*

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SUMMARY. Efficacy of interferon-α2b (IFN) + ribavirin (IFN/RBV) combination in patients with high plasma hepatitis C virus (HCV) is very poor. Dysregulated CD4+ /CD8+ T cells is involved in both impaired cell-mediated immunity and resistance to IFN. Adsorptive granulocytes and monocytes apheresis (GMA) can remove infected leucocytes which are extrahepatic HCV reservoirs and also has been associated with intriguing immunomodulation and increases in CD4+ T cells. Our aim was to see if GMA enhances the efficacy of IFN/RBV. Twenty-four patients, 13 IFN resistant and 11 IFN naive were enrolled. Seventeen were genotype 1b and 7 were 2a or 2b. Mean plasma HCV-RNA was 612.9 (100–850) kIU/mL and alanine aminotransferase, 108 (41–373) U/L. GMA was performed with Adacolumn at one session/day for five consecutive days and IFN/RBV was started within 24 h after the last GMA session. Daily 6 million units of IFN, six times/week for 2 weeks and then three times/week for 22 weeks were given with RBV (600–800 mg/day/patient). Patients were followed for 6 months. GMA was associated with a significant increase in lymphocyte counts, complement activation fragment C3a and falls in tissue necrosis factor-alpha, and IL-8 produced by peripheral blood leucocytes. At week 24, 20 of 24 patients (83%) were HCV negative and by end of follow-up (week 49), the remission was sustained in 14 of 24 patients (58%) including 100% of patients with 2a or 2b. In conclusion, enhanced efficacy of IFN/RBV following GMA might be attributed to a more efficient immune function and a renewed IFN signaling towards HCV.

Keywords: chronic hepatitis C, complement activation fragments, granulocyte and monocyte adsorptive apheresis, interferon-α2b, lymphocytes, ribavirin.

INTRODUCTION

Hepatitis C virus (HCV) has an estimated worldwide prevalence of 170 million cases, including 2 million in Japan [1,2]. The clinicopathological features of the infected population include persistently elevated alanine aminotransferase (ALT) levels or normal liver function. The natural history of HCV shows that infection with relatively mild disease may progress to liver cirrhosis and hepatocellular carcinoma (HCC) during 20–30 years [1–4]; the infection becomes chronic in 50–85% of cases [5].

The treatment of HCV is currently [1,5,6] based on a combination of interferon-alpha (IFN) with ribavirin (RBV). With this regimen, a failure to eradicate HCV occurs in most patients infected by genotype 1b who present with a high viral load [5–7]. Factors associated with HCV resistance to IFN/RBV combination are not fully understood yet, but dysregulated functional T cells (CD4+/CD8+ T cells) is thought to be involved in both impaired cell-mediated immunity against HCV and resistance to anti-HCV drug therapy [8–11]. Thus, IFN resistance is thought to play a role at the early stages of infection, while a qualitative and

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GMA, granulocyte and monocyte apheresis; Hb, haemoglubin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon-alpha; IFN/RBV, IFN-α2b in combination with ribavirin; RT nested-PCR, reverse transcription nested polymerase chain reaction; SOCS, suppressor of cytokine signaling; SRL, special research laboratory; TNF-α, tissue necrosis factor-alpha; WBC, white blood cell counts.

Correspondence: Dr Koji Sawada MD, PhD, Department of Gastroenterology, Fujimoto Hospital Medicine, 3-15-27 Konda Habikino, Osaka 583-0857, Japan. E-mail: sawachi@trust.ocn.ne.jp