hypothesis that HCV itself might contribute to hepatic iron accumulation. Although the precise mechanism remains unclear, HCV may directly or indirectly influence the expression of iron regulatory proteins, which in turn play a central role in the maintenance of iron intracellular homeostasis.[17]

Because the HCV core protein enhances the formation of ROS,[10,18] hepatic iron overload may accelerate ROS-induced hepatocyte injury caused by persistent HCV infection. In fact, in chronic hepatitis C patients carrying the hereditary hemochromatosis gene (*HFE*) mutation referred to as C282Y, serum ferritin levels were found to be higher, hepatocyte iron staining was more commonly observed, and hepatic fibrosis was more advanced than in homozygous normal patients with chronic hepatitis C.[19–22] Therefore, it is quite reasonable to hypothesize that iron reduction therapy, including phlebotomy and dietary iron restriction, may ameliorate the activity of chronic hepatitis C and prevent its progression to cirrhosis.

Phlebotomy for chronic hepatitis C

The beneficial effects of phlebotomy for patients with chronic hepatitis C have been previously reported. Hayashi et al.[23] reported that in 10 patients who underwent phlebotomy, serum ALT levels decreased in all patients (from 152 ± 49 to 55 ± 32 U/l). According to a report by Kato et al.,[24] serum ALT levels significantly improved in 34 patients after 6-year iron reduction therapy (from 150 ± 73 to 35 ± 11 U/l). A randomized controlled study reported by Yano et al.[25] (in this issue of the *Journal of Gastroenterology*) is noteworthy due to its clarification of the efficacy of phlebotomy for the improvement of serum ALT levels in Japanese patients with chronic hepatitis C. Although long-term histological changes were not investigated in the present study, it is expected that sustained improvement in ALT levels would reverse the progression of fibrosis.

This study[25] also demonstrated the high level of safety of phlebotomy for chronic hepatitis C patients. Clinicians occasionally hesitate to introduce interferon therapy, especially in elderly chronic hepatitis C patients and patients with disease complicated by hematological abnormalities, diabetes mellitus, severe systemic arteriosclerosis, and other disorders. The results of 6-month interferon/ribavirin combination therapy remain unsatisfactory for Japanese patients suffering from chronic HCV infection. Therefore, we expect that phlebotomy would be a useful and safe therapy to employ as a substitute for long-term interferon administration.

Further perspectives

It will be important to investigate whether long-term phlebotomy might prevent the progression of hepatic fibrosis and the emergence of hepatocellular carcinoma. A related issue would be the potential of phlebotomy therapy to induce the regression of hepatic fibrosis and to prevent progression to hepatocellular carcinoma in patients with HCV-related cirrhosis. To solve these questions, further long-term randomized controlled studies are needed. Moreover, in Japanese patients with chronic hepatitis C, the association between *HFE* gene mutation and the development of hepatic fibrosis and hepatocellular carcinoma should be investigated.

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Ribavirin-Induced Pure Red-Cell Aplasia during Treatment of Chronic Hepatitis C

TO THE EDITOR: Interferon and ribavirin in combination are the standard treatment for chronic hepatitis C. Hematologic abnormalities, including thrombocytopenia and anemia, are major side effects.[1] Ribavirin is closely associated with hemolytic anemia.[2] We report a case of severe anemia due to acute pure red-cell aplasia during combination therapy, which rapidly improved after the discontinuation of ribavirin.

A 61-year-old man was admitted for treatment of chronic hepatitis C. He had received a blood transfusion after hemorrhoidectomy at the age of 30 years. Abnormal results on liver-function tests and antibody to hepatitis C virus (HCV) had been detected at a health checkup when the man was 55 years of age. His body weight was 75 kg, and physical examination showed only mild hepatomegaly. Laboratory tests demonstrated elevated alanine aminotransferase levels. The hemoglobin level and reticulocyte count were normal. A test for HCV RNA by the polymerase chain reaction was positive at a level above 850,000 IU per milliliter; the genotype was 1b. A liver biopsy showed chronic inflammation with portal fibrosis.

Treatment with interferon alfa-2b (Intron A, 6 million units) and ribavirin (Rebetol, 800 mg) was started. Eight weeks after the initiation of the treatment, the ribavirin dose was reduced to 600 mg per day because the hemoglobin level had decreased from 15.5 g per deciliter to 8.0 g per deciliter. Three weeks later, however, the hemoglobin level dropped to 6.0 g per deciliter even after the reduction in the dose of ribavirin. The reticulocyte count dropped from 7.8×10^4 per microliter to 0.2×10^4 per microliter. During the treatment, no changes in the indirect bilirubin, lactate dehydrogenase, or haptoglobin level were observed.

Bone marrow examination at week 12 showed mild hypocellularity without any morphologic abnormalities and a selective depletion of erythroid precursor cells (Fig. 1). On the basis of these findings, a diagnosis of acute pure red-cell aplasia was made, and ribavirin was discontinued. Thereafter, the anemia and reticulocytopenia improved and had normalized by week 24. Administration of interferon was continued for 24 weeks and resulted in a sustained virologic response.

Acute pure red-cell aplasia is characterized by rapidly progressive anemia with reticulocytopenia and is caused by viral infection, certain drugs, and nutritional disorders.[3] Ribavirin induced dose-related anemia, erythroid hypoplasia, and vacuolization of erythroid precursors in rhesus monkeys, which disappeared after the discontinuation of ribavirin.[4,5] We believe that our patient had acute pure red-cell aplasia caused by ribavirin used in the treatment of chronic hepatitis C. When anemia develops during treatment with interferon and ribavirin, the possibility of ribavirin-induced pure red-cell aplasia should be considered, and careful monitoring of the reticulocyte count is needed.

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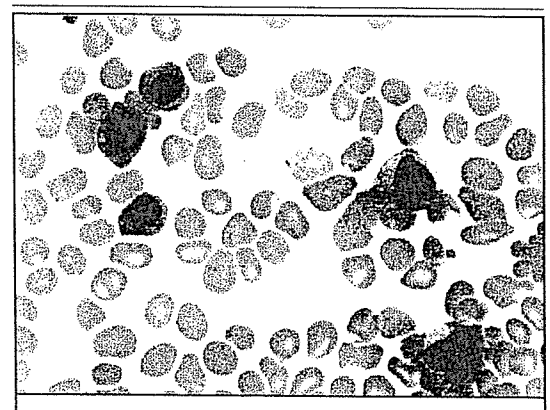

Figure 1. Findings on Microscopical Examination of Bone Marrow 12 Weeks after the Initiation of Combination Treatment with Interferon and Ribavirin (Wright–Giemsa Stain, ×1000).

The nuclear cell count was 8.6×10^4 per microliter (normal range, 10×10^4 to 25×10^4 per microliter), and the ratio of myeloid to erythroid precursors was 5.8 (normal range, 2 to 4). No morphologic abnormalities were found in precursor cells.

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Interferon treatment improves survival in chronic hepatitis C patients showing biochemical as well as virological responses by preventing liver-related death

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SUMMARY. Interferon therapy for chronic hepatitis C reduces the risk of hepatocellular carcinoma, especially among virological and biochemical responders. However, little is known about the effect of interferon therapy on mortality. We studied the long-term effect of interferon therapy on mortality in patients with chronic hepatitis C. For this retrospective cohort study, 2954 patients with chronic hepatitis C were recruited, of whom 2698 received interferon therapy and 256 did not. The effect of interferon therapy on survival was assessed by standardized mortality ratio (SMR) based on published mortality data for the general Japanese population and by risk ratio calculated by proportional hazard regression. Over 6.0 ± 2.2 years follow-up, death from liver-related diseases was observed in 69 (68%) of 101 deaths among interferon-treated patients and in 42 (81%) of 52 deaths among untreated patients. Compared with the general population, overall mortality was high among untreated patients (SMR: 2.7; 95% CI: 2.0–3.6) but not among interferon-treated patients (SMR: 0.9; 95% CI: 0.7–1.1). Liver-related mortality was extremely high among untreated patients (SMR: 22.2; 95% CI: 16.0–30.0) and less among interferon-treated patients (SMR: 5.5; 95% CI: 4.3–6.9). The risk of death from all causes was lower for interferon-treated than untreated patients (risk ratio: 0.47; 95% CI: 0.261–0.836; $P = 0.01$). The risk of death from liver-related diseases was significantly lower for sustained virological responders (risk ratio: 0.04; 95% CI: 0.005–0.301; $P = 0.002$) compared with untreated patients, but not for nonsustained virological responders. Sustained biochemical responders (risk ratio: 0.03; 95% CI: 0.004–0.230; $P < 0.001$) and transient biochemical responders (risk ratio: 0.18; 95% CI: 0.063–0.532; $P = 0.002$) showed a significantly reduced risk of death from liver-related death, whereas biochemical nonresponders did not. Hence interferon treatment improved survival in chronic hepatitis C patients showing a biochemical as well as a virological response by preventing liver-related deaths.

Keywords: chronic hepatitis C, interferon, liver-related mortality, multivariate analysis, standardized mortality ratio.

Abbreviations: HCC, hepatocellular carcinoma; SMR, standardized mortality ratio.

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INTRODUCTION

Hepatitis C virus (HCV) infection rarely resolves spontaneously once it becomes chronic [1]. Consequently, most patients in Japan with chronic HCV infection are likely to progress steadily to liver cirrhosis and hepatocellular carcinoma (HCC), which develops approximately 30 years after blood transfusion [2–4]. HCC is one of the most common malignancies, especially in Southeast Asia, and a major cause of death for patients with chronic HCV infection. In the early 1990s, interferon was introduced worldwide as a therapy for patients with chronic hepatitis C and was effective in inducing normalization of serum alanine aminotransferase (ALT) [5,6], eliminating HCV RNA [7,8], and improving liver histological findings [9–11] in patients with chronic hepatitis C.

To evaluate the effect of interferon therapy on the incidence of HCC and the risk of mortality for chronic hepatitis C patients, a randomized controlled trial is needed. However, a prospective randomized trial with untreated control patients is ethically impossible, because interferon therapy has already been established as a standard treatment for patients with chronic hepatitis C. Therefore, almost all chronic hepatitis C patients, except for cases with medical conditions such as depression, autoimmune disease and severe diabetes mellitus, have been treated with interferon in Japan. Recently, several investigators have reported this therapy as being effective for reducing the incidence of HCC among patients who showed normalization of ALT during and after interferon therapy, as well as among those in whom HCV was eradicated [12–17]. However, a reduced risk of HCC does not necessarily lead to improvement in survival. Indeed, little is known about the effects of interferon therapy on the mortality of patients with chronic hepatitis C. Several investigators [14, 18–23] have tried to evaluate the impact of interferon therapy on mortality. Four of these studies indicated that interferon therapy significantly reduced the mortality of compensated HCV-related cirrhotic patients [18,20] or of chronic hepatitis C patients including patients with compensated cirrhosis [21,23]. However, lack of analysis on response to interferon [18,20–23] or lack of information on disease-specific mortality [20,21] has made it difficult to evaluate the benefits of interferon for survival. Recently, Yoshida *et al.* [24] demonstrated that interferon therapy improved survival by preventing liver-related deaths of chronic hepatitis C patients showing a sustained virological response . However, whether a biochemical response to interferon therapy results in a reduced risk of mortality has not been investigated.

We conducted a multi-centre, large-scale, retrospective cohort study of patients with chronic hepatitis C, who had been enrolled at the end of 1997 at participating hospitals in order to analyse the effect of interferon therapy on the incidence of HCC. The aim of the present study was to examine the effect of interferon therapy on the mortality and causes of death among chronic hepatitis C patients.

PATIENTS AND METHODS

Patients

We recruited chronic hepatitis C patients from four previous studies which were conducted to assess the effect of interferon therapy on the incidence of HCC [12,14,15,17]. All patients meeting the following criteria were included in this study: (i) histological diagnosis of chronic hepatitis or cirrhosis; (ii) no history of clinical signs at entry into the study of complications of cirrhosis, i.e. ascites, jaundice, encephalopathy, or variceal bleeding; (iii) no evidence of HCC at entry into the study as assessed by ultrasonography and/or computed tomography; (iv) absence of serum hepatitis B surface antigen; (v) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis; (vi) absence of excessive alcohol consumption (>80 g/day); and (vii) absence of human immunodeficiency virus antibodies, as described previously [12,14,15,17]. A total of 3025 patients who met these criteria and whose initial sera tested positive for anti-HCV as determined by either first- or second-generation ELISA (Ortho Diagnostics, Tokyo, Japan) and HCV RNA were included in the study. The sera of patients who had been diagnosed as non-A, non-B hepatitis before anti-HCV testing became available (i.e. before 1989) had been frozen at −80 °C and were retrospectively assayed.

Of the 3025 chronic hepatitis C patients, 2762 had received interferon after 1987, when interferon became available in Japan. Interferon-treated patients received a 4–12-month course of interferon therapy, which was initiated within 1 month of liver biopsy. The remaining 263 patients did not undergo interferon therapy or any other antiviral therapy, including almost all patients with biopsy-proven chronic hepatitis who had refused interferon treatment due to adverse effects, lack of time for therapy, or their inability to undergo treatment as a consequence of depression, severe diabetes mellitus or other medical conditions.

Criteria for biochemical and virological responses to interferon therapy

The biochemical response during the follow-up up to 6 months after the completion of interferon therapy was defined according to previously described criteria with minor modifications [8,9]. In the sustained response group, ALT levels decreased to the normal range during therapy and remained within that range up to 24 weeks after therapy without any abnormal elevation. In the transient response group, ALT levels decreased to the normal range by the end of therapy, remained normal during therapy but returned to abnormal levels during the 24 weeks following interferon therapy. In the no-response group, ALT levels did not decrease to the normal range, or fluctuated during therapy and the subsequent 24 weeks. Both biochemical transient

and nonresponders were designated as nonsustained biochemical responders.

A sustained virological response was defined as HCV RNA negativity at more than 6 months after the cessation of interferon therapy. Patients showing positive HCV RNA at the same time were designated as nonsustained virological responders.

Histological evaluation

Liver biopsy was carried out before interferon therapy in all cases. Specimens were fixed in formaldehyde and embedded in paraffin. The sections were stained with haematoxylin-eosin and Azan-Mallory and analysed by two pathologists without any knowledge of the clinical and laboratory data. Histological findings were scored according to the classification of Desmet *et al.* [25].

Follow-up

The starting date of the follow-up for both the interferon-treated and untreated groups was defined as the date of liver biopsy. Biochemical examinations including α-fetoprotein and abdominal ultrasonography were carried out before interferon therapy and every 3–6 months thereafter at the outpatient clinic of the respective hospitals. The end of the follow-up was the date of death or the latest confirmation of survival. Follow-up data on the patients were obtained from the participating hospitals. Follow-up data that were not available from the hospitals were collected from the resident registry of the local municipal office. Death from liver-related disease was defined as death from HCC, liver failure determined by the presence of one or more of ascites, jaundice and hepatic encephalopathy, or variceal bleeding diagnosed on the basis of endoscopic findings of patients presenting with upper gastrointestinal haemorrhage.

Five untreated patients were observed for over 162 months, which corresponded to the longest period of observation of those treated with interferon. In these subjects, only the follow-up data up to 162 months were considered. Seventy-one patients whose follow-up period was shorter than 12 months were excluded from the study. The final numbers of study subjects were 2698 for the interferon-treated group and 256 for the untreated group.

Informed consent was obtained from each patient included in the study. The study protocol was in accordance with the Helsinki Declaration of 1975 (revised in 1983) and approved by the Ethical Committee of the Osaka University Graduate School of Medicine.

Statistical analysis

The chi-square test was used to compare the frequency of gender between the interferon-treated and untreated groups. The difference in age at liver biopsy and ALT between the two groups, expressed as median, was assessed for significance with the Student's *t*-test. The Wilcoxon rank-sum test was used to compare the distribution of age at liver biopsy and histological staging. Cumulative survival curves were determined with the Kaplan–Meier method, and the log-rank test was used to compare the cumulative survival rates.

The observed number of deaths was compared with the expected number, which was calculated by applying sex, 5-year age, 5-year calendar time, and cause-specific mortality rates for the general population in Japan, as prepared by the Statistics and Information Department, Japan Ministry of Health and Welfare [26]. The standardized mortality ratio (SMR) was expressed by dividing the observed number of deaths by the expected number of deaths. The standard error and the 95% CI of SMR were estimated by assuming Poisson's distribution, and differences in mortality between the study cohort and the general population were considered to be significant if the CI did not include unity.

Survival was also analysed by using Cox proportional hazards regression controlling for age (continuous variable), gender, stages of liver fibrosis (stage: 0/1/2/3/4) and time at liver biopsy (1991/1992). Risk ratios attributable to biochemical sustained, transient and no responses and to virological sustained and nonsustained responses were calculated in comparison with no treatment by using dummy variables.

Data analysis was performed with the SAS/PC statistical package (SAS Institute, Cary, NC, USA). All reported *P*-values were two-sided and $P < 0.05$ was considered to be significant.

RESULTS

Patient characteristics at entry

Of the 2698 patients treated with interferon, 901 (33.3%) had a sustained biochemical response, 701 (26.0%) a transient biochemical response and the remaining 1096 patients (40.6%) were classified as biochemical nonresponders. Serum HCV RNA remained negative at more than 6 months after cessation of interferon therapy in 738 (81.9%) of the sustained biochemical responders, designated as sustained virological responders, whereas serum HCV RNA remained positive in 133 (14.8%). Serum HCV RNA was not examined after the termination of interferon therapy in 30 sustained biochemical responders, who were excluded from the analysis according to virological responses to interferon. Positive HCV RNA after interferon therapy was detected in all of the biochemical transient and nonresponders.

The demographic and clinical features of interferon-treated patients according to virological and biochemical responses to interferon and of untreated patients at the time of enrolment are summarized in Table 1. Untreated patients were significantly older than interferon-treated patients ($P = 0.04$), but frequency distribution of age at liver biopsy

Table 1 Characteristics of interferon-treated patients according to virological and biochemical responses to interferon and of untreated patients

	Interferon-treated							
	Virological response		Biochemical response					
	SVR (n = 738)	non-SVR (n = 1930)	SBR (n = 901)	TBR (n = 701)	BNR (n = 1096)	Total (n = 2698)	Untreated (n = 256)	P-value
Median age (range)	51 (20–72)	54 (20–76)	52 (20–73)	53 (20–75)	54 (20–76)	53 (20–76)	54 (21–72)	0.04
Age at biopsy (%)								
≤49	337 (45.7)	687 (35.6)	392 (43.5)	277 (39.5)	369 (33.7)	1038 (38.5)	75 (29.3)	0.12
50–59	240 (32.5)	759 (39.3)	303 (33.6)	280 (39.9)	428 (39.1)	1011 (37.5)	123 (48.9)	
≥60	161 (21.8)	484 (25.1)	206 (22.9)	144 (20.5)	299 (27.3)	649 (24.1)	58 (22.7)	
Sex (M/F)	507/231	1210/720	595/306	440/261	703/393	1738/960	157/99	0.32
Median ALT (U/L), SD	91	92	87	79	103	92	98	0.57
(range)	(7–1110)	(11–1195)	(7–1110)	(13–1195)	(13–828)	(7–1195)	(9–563)	
Stage of fibrosis (%)								
0	5 (0.7)	11 (0.6)	7 (0.8)	4 (0.6)	5 (0.9)	16 (0.6)	9 (3.5)	0.34
1	259 (35.1)	476 (24.7)	337 (37.4)	228 (32.5)	190 (17.3)	755 (28.0)	84 (32.8)	
2	263 (35.6)	614 (31.8)	297 (33.0)	238 (34.0)	349 (31.8)	884 (32.8)	40 (15.6)	
3	189 (25.6)	725 (37.6)	235 (26.1)	209 (29.8)	471 (43.0)	915 (33.9)	93 (36.3)	
4	22 (3.0)	104 (5.4)	25 (2.8)	22 (3.1)	81 (7.4)	128 (4.7)	30 (11.7)	

SVR, sustained virological responders; SBR, sustained biochemical responders; TBR, transient biochemical responders; BNR, biochemical nonresponders; ALT, alanine aminotransferase.

and the stages of liver fibrosis, gender and ALT did not differ significantly. In sustained biochemical responders, the ratio of male patients and median ALT levels were significantly higher for patients with HCV eradication than for those without it ($P < 0.001$, each), whereas median age and the frequency distribution of the stages of liver fibrosis were not significantly different between the two groups.

Follow-up data

The mean period of observation (total cases: 6.0 ± 2.2 years) of the interferon-treated and untreated patients was 5.8 and 8.0 years, respectively, with the former being significantly shorter than the latter ($P = 0.0001$) because interferon therapy was not introduced in Japan until 1987.

Table 2 Follow-up data for interferon-treated patients according to virological and biochemical responses to interferon and for untreated patients

	Interferon-treated						
	Virological response		Biochemical response				
	SVR (n = 738)	non-SVR (n = 1930)	SBR (n = 901)	TBR (n = 701)	BNR (n = 1096)	Total (n = 2698)	Untreated (n = 256)
Mean period of observation, year (SD)	5.7 (2.0)	5.8 (1.9)	5.6 (2.0)	5.7 (1.8)	5.9 (1.9)	5.8 (1.9)	8.0 (3.4)
No. of deaths	7	94	10	10	81	101	52
Liver-related deaths	1	68	1	5	63	69	42
Death from HCC	1	57	1	4	53	58	31
Death from other liver diseases	0	11	0	1	10	11	11
Liver-unrelated deaths	9	26	9	5	18	32	10

SVR, sustained virological responders; SBR, sustained biochemical responders; TBR, transient biochemical responders; BNR, biochemical nonresponders; HCC, hepatocellular carcinoma.

The sustained virological responders, nonsustained virological responders, sustained biochemical responders, transient biochemical responders and biochemical nonresponders were observed for a mean of 5.7, 5.8, 5.6, 5.7 and 5.9 years, respectively (Table 2).

We identified 153 deaths from all causes during the follow-up. The 153 patients who died consisted of 10 sustained biochemical responders (seven of whom were sustained virological responders and three of whom were sustained biochemical responders without HCV eradication), 10 transient biochemical responders, 81 biochemical nonresponders and 52 cases without interferon treatment. Death from all causes did not occur in 30 sustained biochemical responders whose serum HCV RNA was not examined after cessation of interferon therapy. Death from liver-related disease was identified in 111 (73%) of the 153 patients who died: only one death (10%) from liver-related disease (death from HCC) was found among sustained responders with HCV eradication, five (50%) among transient biochemical responders (death from HCC in four cases), 63 (78%) among biochemical nonresponders (death from HCC in 53 cases) and 42 (81%) among untreated patients (death from HCC in 31 cases) (Table 2).

Cumulative survival

The cumulative survival rates from all causes of death were found to be significantly higher for interferon-treated than for untreated patients ($P < 0.001$) (Fig. 1a) The respective 5-year survival rates of interferon-treated and untreated groups were 97.8 and 95.3%, and the 10-year survival rates 87.2 and 77.1%. The cumulative survival rates for sustained virological responders were significantly higher than for nonsustained virological responders ($P < 0.001$) (Fig. 1b), with 5-year survival rates of 99.5 and 97.1%, and 10-year survival rates of 97.8 and 81.9%, respectively. The cumulative survival rates for sustained biochemical responders were significantly higher than for nonsustained biochemical responders ($P < 0.001$). When nonsustained biochemical responders were divided into transient biochemical responders and biochemical nonresponders, the cumulative survival rates for the transient biochemical responders were significantly higher than for the biochemical nonresponders ($P < 0.001$) (Fig. 1c). The respective cumulative survival rates for sustained biochemical responders, transient biochemical responders and biochemical nonresponders were 99.2, 99.1 and 95.8% at the end of the fifth year and 97.8, 97.6 and 72.6% at the end of the 10th year. Among sustained biochemical responders, the cumulative survival rates for sustained virological responders and sustained biochemical responders without HCV eradication were 99.5 and 99.2% at the end of fifth year and 97.8 and 99.2% at the end of the 10th year, showing no statistical significance ($P = 0.18$).

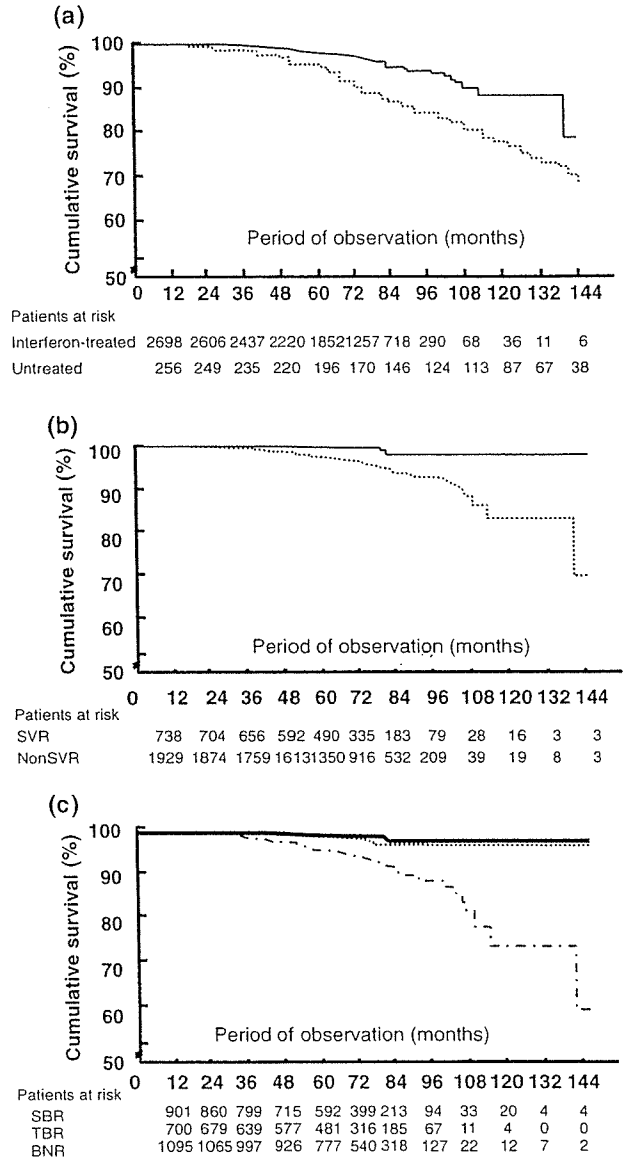

Fig. 1 Cumulative survival rates from all causes of death for patients with chronic hepatitis C. (a) For interferon-treated patients (solid line) and untreated patients (dotted line). (b) According to the virological response to interferon therapy; sustained virological responders (SVR) (solid line) and nonsustained virological responders (non-SVR) (dotted line). (c) In terms of the biochemical responses to interferon, sustained biochemical responders (SBR) (solid line), transient biochemical responders (TBR) (dotted line) and biochemical nonresponders (BNR) (dash-and-dot line).

Standardized mortality ratio

Differences in mortality among interferon-treated and untreated patients from the general population were further assessed by calculating SMR, the ratio of the observed number of deaths to the expected number. Overall mortality

Table 3 Standardized mortality ratios (SMR) in patients with chronic hepatitis C according to virological and biochemical responses to interferon

	Overall deaths			Liver-related deaths			Liver-unrelated deaths		
	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)
Untreated	52	19.2	2.7 (2.0–3.6)	42	1.9	22.2 (16.0–30.0)	10	17.3	0.6 (0.3–1.1)
Interferon-treated	101	112.7	0.9 (0.7–1.1)	69	12.6	5.5 (4.3–6.9)	32	100.0	0.3 (0.2–0.5)
Virological response									
Sustained (HCV RNA negative)	7	29.8	0.2 (0.1–0.5)	1	3.3	0.3 (0.0–1.7)	6	26.5	0.2 (0.1–0.5)
Nonsustained (HCV RNA positive)	94	82.2	1.1 (0.9–1.4)	68	9.2	7.4 (5.8–9.4)	26	73.0	0.4 (0.2–0.5)
Biochemical response									
Sustained response	10	36.5	0.3 (0.1–0.5)	1	4.0	0.3 (0.0–1.4)	9	32.5	0.3 (0.1–0.5)
Transient response	10	27.5	0.4 (0.2–0.7)	5	3.2	1.6 (0.5–3.7)	5	24.3	0.2 (0.1–0.5)
No response	81	48.8	1.7 (1.3–2.1)	63	5.4	11.6 (8.9–14.9)	18	43.3	0.4 (0.3–0.7)

Difference from the expected number of deaths was considered significant if 95% CI of SMR did not include unity.

for untreated patients (SMR: 2.7; 95% CI: 2.0–3.6) but not for the interferon-treated patients (SMR: 0.9; 95% CI: 0.7–1.1) was significantly higher than for the general population. Liver-related mortality was high for untreated patients (SMR: 22.2; 95% CI: 16.0–30.0) and also for interferon-treated patients, although to a lesser degree (SMR: 5.5; 95% CI: 4.3–6.9) (Table 3). For sustained virological responders overall mortality was low (SMR: 0.2; 95% CI: 0.1–0.5), and liver-related mortality (SMR: 0.3; 95% CI: 0.0–1.7) was equivalent to that for the general population. In contrast, liver-related mortality was high for nonsustained virological responders (SMR: 7.4; 95% CI: 5.8–9.4).

Sustained and transient biochemical responders showed a low overall mortality compared with that for the general population (SMR: 0.3; 95% CI: 0.1–0.5, and SMR: 0.4; 95% CI: 0.2–0.7, respectively), whereas overall mortality was high for biochemical nonresponders (SMR: 1.7; 95% CI: 1.3–2.1). Liver-related mortality was not high for sustained and transient biochemical responders (SMR: 0.3; 95% CI: 0.0–1.4, and SMR: 1.6; 95% CI: 0.5–3.7, respectively) compared with that for the general population, but it was high for biochemical nonresponders (SMR: 11.6; 95% CI: 8.9–14.9) (Table 3). Overall and liver-related mortality for sustained biochemical responders without HCV eradication was equivalent to that for the general population (SMR: 0.5; 95% CI: 0.1–1.5, and SMR: 0.0; 95% CI: 0.0–6.1, respectively).

Interferon-treated patients had a statistically lower risk of liver-unrelated death than the general population (SMR: 0.3; 95% CI: 0.2–0.5), whereas untreated patients did not (SMR: 0.6; 95% CI: 0.3–1.1).

Multivariate analysis

The effect of interferon on the risk of death was assessed by Cox proportional hazards regression controlling for age, gender, score of liver fibrosis and time at liver biopsy. Interferon therapy significantly reduced the risk of overall death to a ratio of only 0.47, in comparison with no treatment. When patients were classified according to virological responses to interferon, sustained virological responders showed reduced risks of overall death (risk ratio: 0.14; 95% CI: 0.056–0.352; $P < 0.001$) and liver-related death (risk ratio: 0.04; 95% CI: 0.005–0.301; $P = 0.002$) compared with untreated patients, whereas nonsustained virological responders did not. Similarly, sustained biochemical responders showed a lower risk of death from all causes (risk ratio: 0.16; 95% CI: 0.069–0.354; $P < 0.001$) and liver-related diseases (risk ratio: 0.03; 95% CI: 0.004–0.230; $P < 0.001$). Transient biochemical responders had a high, but still significantly reduced risk of overall death (risk ratio: 0.19; 95% CI: 0.083–0.445; $P < 0.001$) and liver-related death (risk ratio: 0.18; 95% CI: 0.063–0.532; $P = 0.002$), whereas the risk for nonresponders and untreated patients did not

Table 4 Risk of death in patients with chronic hepatitis C according to virological and biochemical responses to interferon

	All causes of deaths			Liver-related deaths		
	Risk ratio	95% CI	*P*-value	Risk ratio	95% CI	*P*-value
Untreated	1.00			1.00		
Interferon-treated	0.47	0.261–0.836	0.010	0.59	0.312–1.097	0.095
Virological response						
Sustained (HCV RNA negative)	0.14	0.056–0.352	<0.001	0.04	0.005–0.301	0.002
Nonsustained (HCV RNA positive)	0.59	0.327–1.057	0.08	0.76	0.402–1.417	0.380
Biochemical response						
Sustained response	0.16	0.069–0.354	<0.001	0.03	0.004–0.230	<0.001
Transient response	0.19	0.083–0.445	<0.001	0.18	0.063–0.532	0.002
No response	0.78	0.432–1.393	0.394	1.02	0.543–1.900	0.962

Adjusted for age, sex, score of liver fibrosis and period at liver biopsy.

change (Table 4). The risk of overall death for sustained biochemical responders without HCV eradication was lower than for untreated patients, although it did not reach a statistical significance (risk ratio: 0.31; 95% CI: 0.09–1.07; $P = 0.06$).

DISCUSSION

We previously demonstrated that interferon treatment could reduce the risk of HCC development in patients with chronic hepatitis C [12]. Following this, five retrospective studies [13–17] showed a similar effect of interferon on the risk of HCC, especially for virological and biochemical responders. These results suggest that interferon therapy for chronic hepatitis C can prevent the development of HCC, possibly leading to improvement in long-term survival. However, only a few previous studies have assessed the effects of interferon therapy on survival [18–24], and whether interferon therapy also reduces mortality from liver-related disease in patients with chronic HCV infection has not been thoroughly investigated. It is also still unclear what type of response to interferon results in the improvement of long-term survival.

To evaluate the effect of interferon therapy on the risk of mortality for chronic hepatitis C patients, a randomized controlled trial should be carried out. However, a prospective randomized trial with untreated control patients is ethically impossible, because interferon therapy has already been established as the standard modality for patients with chronic hepatitis C. Only two randomized controlled trials of a small number of HCV-related cirrhotic cases have evaluated the effect of interferon therapy on mortality [19,21], but with discrepant results. In contrast, large-scale prospective and retrospective cohort studies [23,24] indicate that interferon therapy for HCV-related cirrhosis or chronic hepatitis C improves long-term survival. In particular, Yoshida *et al.* [24] demonstrated in their recent retrospective cohort study that interferon therapy improved survival of chronic hepatitis C patients by preventing liver-related deaths. However, its beneficial effect was considered to be limited to patients with a sustained virological response.

As ours is a retrospective cohort study, it may be subject to several biases. The interferon-treated and untreated groups had different demographic characteristics, including age and gender. These factors were adjusted for multivariate regression analysis and considered when calculating SMR by applying the corresponding mortality for the general population. Severity of chronic liver disease was adjusted by using the stage of liver fibrosis for multivariate analysis. As the time of liver biopsy of untreated patients was earlier than for interferon-treated patients, mortality for untreated patients may be generally higher than for interferon-treated patients. To avoid this bias, we adjusted the time at liver biopsy for multivariate analysis, and 5-year time-specific mortality rates for the general population were prepared in the SMR analysis. Moreover, the number of untreated patients was small, because most Japanese chronic hepatitis C patients, except for cases with medical problems, have been treated with interferon. However, the relatively small number of untreated patients in comparison with the large number of interferon-treated patients is not likely to have resulted in a substantial overestimation of the effect of interferon therapy on survival as several of the biases already mentioned were controlled in the analyses.

When we compared the observed mortality with the expected mortality for the matched general population by calculating SMR, we were able to demonstrate that chronic hepatitis C patients had higher overall and liver-related mortality than the general population, and that the majority of deaths were liver-related. However, interferon-treated patients had a significantly lower risk of liver-unrelated mortality, whereas untreated patients did not. This may represent a selection bias in the use of interferon therapy, which included patients with no medical problems

except for having chronic liver diseases. However, our multivariate regression analysis clearly showed that interferon therapy reduced the risk of liver-related death in virological responders by 96% and in biochemical responders by 82–97%. These findings indicate that a significant reduction in the risk of death from all causes for patients treated with interferon, shown in the analysis of SMR, was not caused by a selection bias but is mainly attributable to the prevention of liver-related death by interferon therapy.

Our multivariate analysis made it clear that the risks of overall and liver-related deaths for chronic hepatitis C patients displaying a sustained virological response were 86 and 96% lower than for untreated patients. The risk reduction for sustained biochemical responders was almost equal to that for sustained virological responders. Similarly, the SMR analyses showed that liver-related mortality for these patients was equivalent to that for the general population. Thus, and as expected, when patients treated with interferon belong to the sustained virological or biochemical response group, they appear to have the highest long-term survival rate.

Of nonsustained virological responders, the risk of death from all causes and liver-related diseases for transient biochemical responders was significantly lower than for untreated patients, but higher than for sustained biochemical and virological responders. The same effects of interferon therapy on survival were observed in the SMR analyses. Although the follow-up period was not sufficiently long for a reliable and accurate examination of mortality, we would like to emphasize that the risk of death from all causes and liver-related diseases was significantly lower for chronic hepatitis C patients for whom interferon was effective in normalizing ALT than for patients who did not receive interferon, even when HCV was not eradicated. However, the risk of death from all causes and liver-related diseases was not reduced in biochemical nonresponders.

In conclusion, the findings reported here indicate that interferon therapy improves long-term survival in chronic hepatitis C patients showing a biochemical as well as a virological response by preventing liver-related deaths.

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Prediction of treatment outcome with daily high-dose IFN α-2b plus ribavirin in patients with chronic hepatitis C with genotype 1b and high HCV RNA levels: relationship of baseline viral levels and viral dynamics during and after therapy

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Abstract

Data on 334 patients with HCV genotype 1b and high viral levels were extracted from two multicenter double-blind studies conducted in Japan comparing IFN α-2b plus ribavirin ($n = 209$) with IFN α-2b alone ($n = 125$) for 24 weeks. HCV RNA assay was conducted before and 4, 12, and 24 weeks after the start and 4, 12, and 24 weeks after the end of treatment. Both sustained viral response (SVR) rate and relapse rate after the end of treatment were analyzed in relation to baseline viral levels and the time of first disappearance of virus. In the combination treatment group, the percentage of patients who were HCV RNA-negative within 4 weeks decreased with increase in baseline viral levels (i.e. 42%, 15%, and 11% were HCV RNA-negative in the groups exhibiting <500, 500 to <850, and ≥850 kcopies/mL, respectively). In the IFN monotherapy group, the response rates were lower at 13%, 15%, and 1%, respectively. Disappearance of virus within 12 weeks after the start of combination treatment was indicative of higher probability of SVR. The risk of relapse was more highly correlated with the timing of initial viral disappearance than with baseline HCV levels; it was 4.8 and 10.3 times higher in patients who became HCV-negative at 4–12 and 13–24 weeks compared with in those who were HCV-negative within 4 weeks.

Keywords: Chronic hepatitis C; Interferon α-2b; Ribavirin; Multicenter randomized double-blind study

1. Introduction

Global consensus obtains that PEG-interferon (PEG-IFN) plus ribavirin combination therapy is the treatment of choice

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for chronic hepatitis C (CHC). That the duration of treatment should be 12 months for hepatitis C virus (HCV) genotype 1 and 6 months for other genotypes is also nearing consensus [1,2]. High-dose daily IFN monotherapy in patients with CHC was originally reported from Japan [3], and since then several reports of better efficacy using high-dose daily IFN therapy similar to that used in Japan plus ribavirin have appeared in both the USA and Europe [4–12].

Recently, much attention has been focused on the relationship between the timing of HCV RNA negativity and antiviral efficacy of IFN therapy. Many reports have investigated this relationship in patients receiving IFN or PEG-IFN and ribavirin combination therapy [13–18]. In Japan, the age of patients with CHC is increasing, and both nonresponders to previous IFN therapy and IFN-treatment-naïve patients usually are given high doses of IFN. In two Japanese studies of high-dose IFN therapy, SVR including in patients with HCV with genotype other than 1 was observed in 27.5% (316/1148) [19] and 30.6% (313/1022) [20], respectively, whereas in the USA and Europe where IFN 3 MIU is normally administered three times/week, SVR was observed in 6–19% even in patients undergoing treatment for 1 year [21–23]. For this reason, Japanese nonresponders to prior IFN therapy cannot be considered the same as non-Japanese patients, and hence direct application of the results of trials conducted outside Japan to Japanese patients is of limited use.

It has also been reported that reducing HCV relapse after the end of treatment enhances the efficacy of combination therapy [24]. Longer-term combination treatment has been confirmed to reduce the rate of HCV relapse after the end of drug administration [22,23], but the mechanism of this effect is not clear. The present study was performed to examine the relationship between the timing of disappearance of HCV RNA and HCV eradication in Japanese patients receiving IFN plus ribavirin combination therapy. Moreover, we attempted to clarify factors related to relapse after the end of treatment by analyzing the relationship between the time of HCV eradication and baseline HCV levels.

2. Materials and methods

2.1. Patient selection

Two randomized comparative studies of IFN α-2b plus ribavirin were conducted using IFN (-2b monotherapy as control; 1 in patients with HCV genotype 1b CHC with high viral levels (the most difficult CHC patients to treat) [25] and 1 in nonresponders and relapsers to previous IFN therapy [26] who are thus in urgent medical need. No bias was observed in patient distribution between groups in these studies (data not shown). Both studies were conducted after approval by the institutional review boards of each medical institution and informed consent was obtained in writing from each patient. IFN α-2b (Intron A, Schering Plough, Kenilworth, NJ) was administered six times/week for 2 weeks at a dose of 6 or 10 MIU and then three times/week for 22 weeks at a dose of 6 MIU. Ribavirin (Rebetol, Schering Plough, Kenilworth, NJ) was administered for 24 weeks at a dose of 600 mg/day (three capsules) in patients weighing <60 kg and 800 mg/day (four capsules) in those whose weight was ≥60 kg. The control group received IFN (-2b together with ribavirin placebo capsules. From these two clinical studies, we extracted data on patients with HCV genotype 1 and high viral levels and retrospectively analyzed the time of initial HCV RNA negativity, the percentage of patients with sustained viral negativity after the end of treatment, and the percentage of patients who relapsed after the end of treatment. The database subjected to retrospective analysis included data on sex, age, body weight, extent and activity of liver tissue lesion, history of IFN therapy, HCV RNA level, aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin, white blood cells (WBC), red blood cells (RBC), platelet count, and serum creatinine. Virological response was defined as qualitative negative by qualitative Amplicor assay (Mitsubishi Kagaku BCL, Tokyo, Japan). In addition, HCV quantitative analysis was conducted by Amplicor HCV monitor method (Mitsubishi Kagaku BCL, Tokyo, Japan) with a detection limit of 100 copies/mL.

Qualitative and quantitative analyses of HCV RNA were performed immediately before and 4, 12, and 24 weeks after the start and 4, 12, and 24 weeks after the end of treatment. Viral levels ≥100 kcopies/mL were considered high. Genotype was determined immediately before the start of treatment by RT-PCR (Mitsubishi Kagaku BCL, Tokyo, Japan). All liver tissue was evaluated by the same examiner.

2.2. Enrollment and exclusion criteria

Enrollment criteria for the two studies were: (1) abnormal ALT and HCV RNA-positive in tests conducted within 12 weeks before the start of treatment; (2) HCV genotype 1, and HCV genotype 2 nonresponders and relapsers to prior IFN therapy; (3) age 20–64 years; (4) hemoglobin ≥12 g/dL and platelet count ≥100,000 mm^{-3} within 12 weeks before the start of treatment; (5) availability to stay in hospital for 4 weeks after the start of treatment; and (6) agreement to take contraceptive measures during and for 6 months after the end of treatment. Patients with the following characteristics were excluded: (1) pregnant or possibly pregnant and lactating women; (2) depression tendency; (3) severe complications; (4) hepatitis C complicated by other types of hepatitis; (5) liver cirrhosis or cancer as diagnosed in tests conducted within 12 weeks before the start of treatment; (6) history of hepatic encephalopathy, rupture of esophageal varices, or ascites; (7) HIV coinfection; (8) taken antiviral therapy or immunotherapy within 12 weeks before the start of treatment; (9) previous ribavirin therapy; and (10) history of allergy to IFN or nucleoside analogues.

2.3. Subgroup analysis

Baseline HCV RNA levels were categorized into three groups: 100 to <500; 500 to <850; and ≥850 kcopies/mL as determined by Amplicor HCV monitor assay. Disappearance of virus and relapse were judged by qualitative Amplicor assay. The time of initial HCV RNA negativity was recorded as the measurement time point (4, 12, and 24 weeks after the start of treatment) at which negativity was first observed; the time of initial relapse was the three measurement time point (4, 12, and 24 weeks after the end of treatment) at which HCV RNA was first detected in patients who achieved HCV RNA negativity during the treatment period. Patients who remained HCV RNA-negative for 6 months after the end of treatment were considered to have achieved SVR.

2.4. Statistical analysis

HCV RNA negativity rate, SVR rate, and relapse rate were compared by baseline viral load between the combination treatment and IFN monotherapy groups by Mantel–Haenzel test using modified RIDIT scores after the lack of interactions in efficacy was confirmed by the Breslow–Day test. Logistic regression analysis was used to identify factors contributing to initial HCV RNA negativity and SVR. The degree of risk of relapse was analyzed using the proportional hazards and grouped exponential models. Intergroup differences in patient profiles were tested by Fisher's exact test, Wilcoxon–Mann Whitney test, and Mantel–Haenzel test. $P < 0.05$ was regarded as statistically significant (two-sided). All calculations were performed by SAS program version 6.12 (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics

Table 1 shows the main characteristics of the 209 patients in the combination treatment and 125 patients in the IFN monotherapy groups. In the study in nonresponders and relapsers to previous IFN therapy [26] 41 and 40 patients were allocated to the combination treatment and IFN monotherapy groups, respectively; in the study in patients with genotype 1b and high viral titers, the numbers were 168 and 85, respectively (i.e. 2:1 randomization) [25]. A total of 107 patients (51%) in the combination treatment and 68 (54%) in the IFN monotherapy group had HCV RNA levels ≥850 kcopies/mL. About half of patients in both treatment groups were relapsers after previous IFN therapy. Forty-nine patients (23%) in the combination treatment and 24 (19%) in the IFN monotherapy group had not received prior IFN therapy. No imbalance was observed in background variables between the two groups.

Table 1
Baseline patient characteristics

	IFN + ribavirin	IFN	P value
No. of patients	209	125	–
Sex (male/female)	164/45	94/31	0.503[a]
Mean age (years)	48	49	0.539[b]
Viral load (kcopies/mL)			0.792[c]
Low (<500)	23.0% (48)	24.8% (31)	
Moderate (500 to <850)	25.8% (54)	20.8% (26)	
High (≥850)	51.2% (107)	54.4% (68)	
Previous IFN therapy			0.295[ad]
Treatment-naive	23.4% (49)	19.2% (24)	
Relapsers	50.7% (106)	50.4% (63)	
Nonresponders	22.5% (47)	30.4% (38)	
Unknown	3.3% (7)	0	

[a] Fisher test.
[b] *U*-test.
[c] Mantel–Haenzel test.
[d] Excluding unknown.

3.2. Response to therapy

The SVR rate was 18% (38/209) with IFN and ribavirin combination therapy and 2% (2/125) with IFN monotherapy. The results of subgroup analysis by baseline viral levels are shown in Table 2. Patients receiving IFN and ribavirin combination therapy had a significantly higher chance for SVR than those receiving IFN monotherapy at any baseline viral level.

3.3. Initial viral negativity

In patients with viral titers of 500 to <850 kcopies/mL, initial viral negativity occurred in 20% by the first 4 weeks, in 46% by 12 weeks, and in 17% by 24 weeks of treatment in the combination therapy group (Fig. 1a). In the IFN monotherapy group the figures were 15%, 31%, and 12%, respectively (Fig. 1b). In patients with viral titers of ≥850 kcopies/mL, the HCV negativity rates at the same time points were 11%, 50%, and 20%, respectively, in the combination therapy group (Fig. 1a) and 1%, 28%, and 15%, respectively, in the monotherapy group (Fig. 1b). The time to initial viral negativity was slightly earlier in patients with viral titers of <500 kcopies/mL (42%, 25%, and 6% at the same time points, respectively) than in those with ≥500 kcopies/mL in the combination therapy group (Fig. 1a). Logistic regression analysis indicated that low HCV RNA levels and high ALT and creatinine levels before treatment are factors related to achieving HCV RNA negativity by week 4 of combination therapy. High baseline creatinine level was associated

Table 2
SVR rate by baseline viral load

Viral load (kcopies/mL)	IFN + ribavirin ($n = 209$)	IFN ($n = 125$)	P value
Low (<500)	29% (14/48)	0 (0/31)	0.001
Moderate (500 to <850)	17% (9/54)	8% (2/26)	0.001
High (≥850)	14% (15/107)	0 (0/68)	0.001

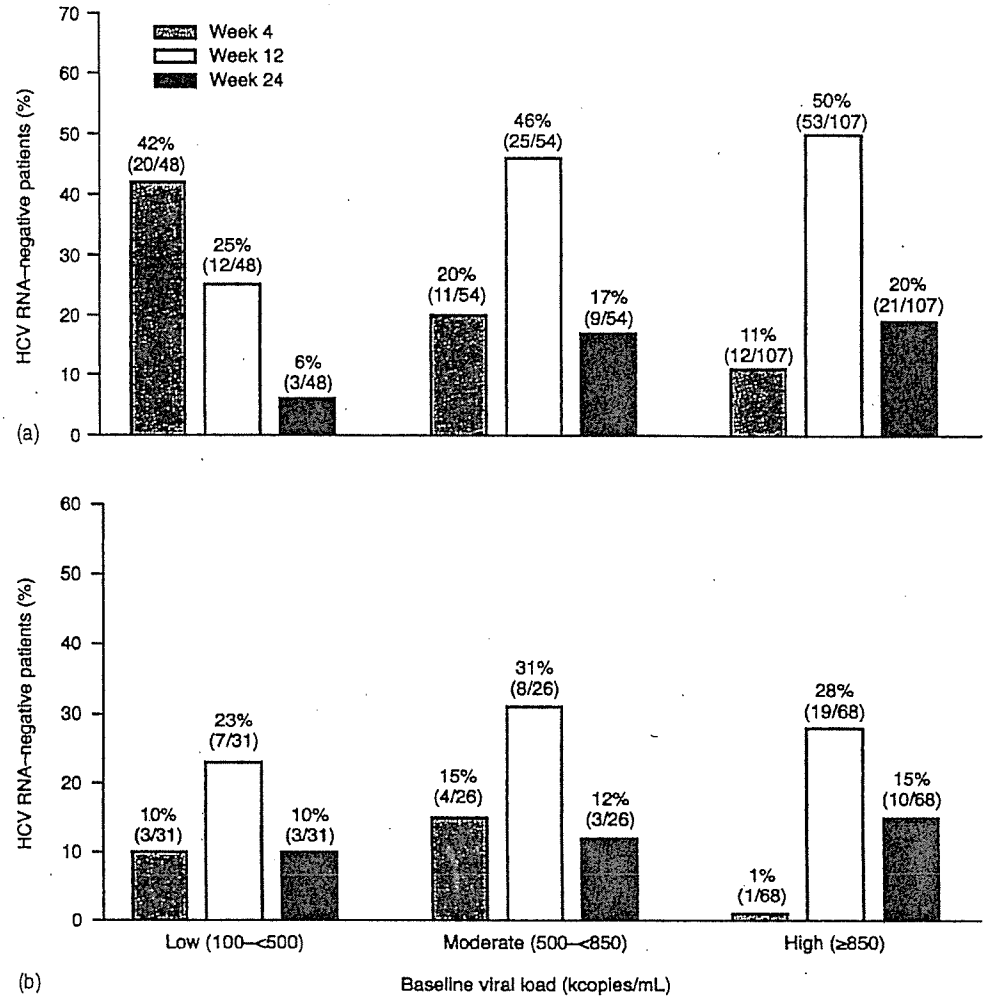

Fig. 1. Percentage of patients testing HCV RNA negative receiving combination therapy (a) and monotherapy (b). Patient numbers are in parentheses.

with achieving HCV RNA negativity by week 12 of treatment

3.4. SVR by baseline viral level and timing of initial viral negativity

Fig. 2 shows the SVR rate with respect to the timing of initial viral disappearance for each baseline HCV level. In patients with <500 kcopies/mL, SVR was observed only in those HCV RNA-negative within 4 weeks after the start of combination treatment, with a high SVR rate of 70% (14/20). However, in patients HCV RNA-negative by week 12 or 24, SVR was not observed in either treatment group. In the IFN monotherapy group, three patients were HCV RNA-negative within 4 weeks and the SVR rate was 0% (0/3). Among patients with 500 to <850 kcopies/mL, SVR was observed in 55% (6/11) and 12% (3/25) of patients HCV RNA-negative within 4 and 12 weeks of the start of combination treatment, respectively, and in none of the nine patients HCV RNA-negative within 24 weeks. Among patients treated with IFN alone, SVR was observed only in 50% (2/4) of patients HCV RNA-negative within 4 weeks. In patients with ≥850 kcopies/mL at baseline, SVR was observed in the combination treatment group in 42% (5/12) and 19% (10/53) of patients HCV RNA-negative within 4 weeks and 12 weeks, respectively. However, SVR was not seen in any of the 21 patients HCV RNA-negative within 24 weeks. SVR was not observed in patients with viral levels ≥850 kcopies/mL treated with IFN alone.

In patients HCV RNA-negative within 4 weeks, low baseline viral levels and high body weight were factors contributing to SVR; in those who were HCV RNA-negative within 12 weeks, low baseline viral levels and high baseline platelet count were contributing factors.

3.5. Relapse rate after end of treatment by baseline viral levels

In the combination treatment group, the relapse rate was 60% (21/35), 80% (36/45), and 83% (71/86) and in the IFN (-2b alone group 100% (13/13), 86% (13/15), and 100% (30/30) in patients with baseline viral levels <500, 500 to

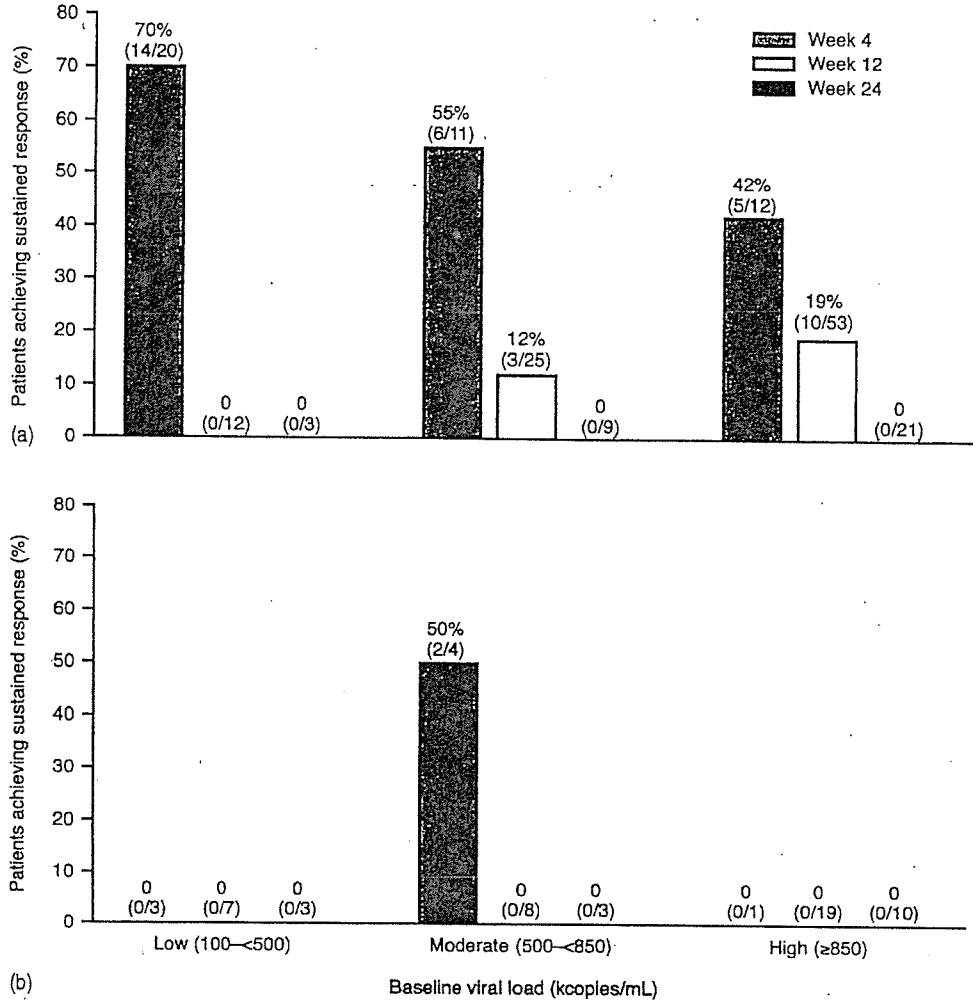

Fig. 2. Sustained response rate in patients receiving combination therapy (a) and monotherapy (b). Patient numbers are in parentheses.

<850, and ≥850 kcopies/mL, respectively (Table 3). Patients in the combination treatment group were 3.7 times (95% CI 1.9–7.3; $P < 0.001$) less likely to relapse than those in the IFN monotherapy group. If the probability of relapse in patients with <500 kcopies/mL is set at 1, then probability was 1.5 (95% CI 0.8–2.9) in patients with 500 to <850 kcopies/mL and 2.0 (95% CI 1.1–3.5) in patients with ≥850 kcopies/mL. However, baseline viral level was not a significant factor ($P = 0.3441$) with regards to risk of relapse. On the other hand, patients HCV RNA-negative within 12 and 24 weeks after the start of treatment were 4.8 (95% CI 2.8–8.1) and 10.3 (95% CI 5.4–19.7) times, respectively, more likely to relapse after the end of treatment than patients HCV RNA-negative within the first 4 weeks ($P < 0.001$).

Table 3
Rate of relapse at 6 months by baseline viral load

Viral load (kcopies/mL)	IFN + ribavirin ($n = 209$)	IFN ($n = 125$)	P value
Low (<500)	60% (21/35)	100% (13/13)	0.001
Moderate (500 to <850)	80% (36/45)	87% (13/15)	0.001
High (≥850)	83% (71/86)	100% (30/30)	0.001

The relative risk of relapse after the end of treatment by initial viral level and time of first viral negativity is shown in Table 4. The risk of relapse in the combination group versus in the IFN monotherapy group was significantly lower by a factor of 0.3 (95% CI 0.1–0.9) and 0.5 (95% CI 0.3–0.8) in patients HCV RNA-negative within 4 weeks ($P = 0.032$) and 12 weeks ($P = 0.011$), respectively. Regarding baseline HCV levels, in patients HCV RNA-negative by 12 weeks, the

Table 4
Relative risk of relapse at 6 months (95% CI)

	First HCV RNA-negative test result		
	4 weeks	12 weeks	24 weeks
Treatment group			
IFN + ribavirin	0.3 (0.1–0.9)*	0.5 (0.4–0.9)*	1.2 (0.6–2.2)
IFN	1	1	1
Baseline viral load (kcopies/mL)			
Low (<500)	1	1	1
Moderate (500 to <850)	0.9 (0.4–2.7)	0.6 (0.4–1.2)	0.8 (0.3–2.3)
High (≥850)	2.1 (0.8–5.6)	0.5 (0.3–0.9)†	0.9 (0.4–2.2)

* $P < 0.05$ vs. monotherapy.
† $P < 0.05$ vs. low baseline viral load.

hazard for relapse was significantly higher (odds ratio: 0.5; $P = 0.021$) in patients with ≥850 kcopies/mL than in those with <500 kcopies/mL. In patients HCV RNA-negative by 24 weeks, no effect on hazard for relapse was observed by treatment group or viral levels.

The relationship between baseline HCV levels and the time of relapse by time of initial HCV negativity is shown in Table 5. The relapse rate in patients HCV RNA-negative within 4 weeks with combination treatment was ≤18% at 4, 12, and 24 weeks after the end of treatment regardless of baseline viral level. In the IFN(-2b alone group, almost all patients relapsed soon after the end of treatment even when HCV RNA-negativity occurred within the first 4 weeks after the start of treatment. The circumstance of relapse in patients HCV RNA-negative within 12 weeks of the start of treatment differed from that in patients HCV RNA-negative within 4 weeks. In those receiving combination treatment, relapse was seen within 4 weeks in 11 (92%), 13 (52%), and 30 (57%) patients whose baseline viral levels were <500, 500 to <850, and ≥850 kcopies/mL, respectively. Relapse within 12 weeks was seen in 1 (8%), 5 (20%), and 13 (26%) patients, respectively. However, even with combination treatment, most patients who first became HCV RNA-negative after 12 weeks from the start of treatment relapsed within 4 weeks after the end of treatment (data not shown)

4. Discussion

In Japan, various IFN regimens for the treatment of CHC have been tried. Under the Japanese health insurance system, the duration of treatment was restricted to 6 months at the time that the present study was conducted. Standard treatment comprises high doses of IFN (6–10 MIU) administered daily in the initial stage of treatment followed by further doses at three times/week for ≤6 months with the aim of eradicating the virus [3]. In 1998, remarkable improvement in efficacy was reported when ribavirin is added to IFN α-2b [21–23], and clinical studies of IFN α-2b plus ribavirin combination therapy were initiated in Japan. Outside Japan, the standard treatment regimen with IFN α-2b was 3 MIU administered three times/week; for combination therapy, ribavirin was added to this standard regimen. The clinical studies in Japan were likewise conducted with ribavirin (600 or 800 mg/day depending on body weight) added to the standard Japanese regimen. When SVR rates by baseline viral levels were compared, combination therapy was superior to monotherapy at all viral levels. A number of reports have been published concerning the timing of first HCV RNA disappearance and its effect on the SVR rate [13–18,27]. To date, however, no study of the SVR rate analyzed in relation to HCV RNA levels has been published. The present study suggests that the timing of first disappearance of HCV RNA is significantly affected by baseline HCV RNA levels. In patients with low baseline viral levels (100 to <500 kcopies/mL), 42% became HCV RNA-negative in comparison with only 11% with high viral levels (≥850 kcopies/mL) following 4 weeks' combination therapy. The study suggests that >4 weeks' treatment is required to achieve HCV RNA negativity in patients with viral levels >500 kcopies/mL. Moreover, with IFN alone the proportion of patients achieving HCV RNA negativity within 4 weeks was especially low among those with HCV genotype 1b and high viral levels; >4 weeks' treatment is required to achieve HCV RNA negativity in this group. Vrolijk et al. [5] reported that when ribavirin was administered in combination with IFNα-2b, HCV RNA negativity was observed by week 4 in nearly half of patients and all patients achieved SVR when treatment was continued for 1.5 years. Tassopoulos et al. [8] reported that when ribavirin was administered in combination with 10 MIU IFNα-2b for 8 weeks, almost half of patients achieved HCV RNA negativity. Treatment was continued thereafter for 48 weeks, and the final SVR rate was roughly 25%. The differences between these studies conducted outside Japan and our results may be explained by the high viral levels in our patients. We also noted that low HCV RNA, high ALT, and high creatinine levels before the start of dosing were factors associated with early disappearance of HCV RNA after treatment was initiated. High serum creatinine levels are related to high serum ribavirin concentrations [28], and this may explain early HCV RNA disappearance.

Kasahara et al. [29] compared the results of 6-month and 1-year treatment and reported that judging from the degree of improvement in ALT, longer duration of treatment with IFN monotherapy may inhibit relapse after the end of treatment. However, no significant difference of SVR rate in CHC patients with genotype 1b and high viral levels between 52 weeks and 78 weeks treatment with IFN monotherapy was reported [30]. Poynard et al. [22] reported that the relapse rate with combination therapy after 48 weeks of treatment in patients not previously treated with IFN and including patients with HCV genotypes other than genotype 1 was significantly lower than after 24 weeks of treatment. McHutchison et al. [23] also reported a similar trend. Portal et al. [6] compared the relapse rate in HCV genotype 1 patients with high viral levels treated with IFN plus ribavirin for 1 year or IFN plus ribavirin for 6 months followed by IFN monotherapy for 6 months, and observed a significantly higher relapse rate in the latter group, indicating the importance of duration of combination treatment in reducing the relapse rate. Although our study of 6-month combination treatment in genotype 1 patients was not adequate to analyze the effects of duration of treatment, our analysis of HCV RNA levels in relation to relapse revealed that relapse is much more likely in patients with high rather than low baseline viral levels. Furthermore, compared with in patients who were HCV RNA negative within 4 weeks, the relative risk for relapse is significantly higher in patients HCV RNA-negative at both 4–12 weeks and 13–24 weeks after the start of treatment. Relative risk of relapse is also reduced by about 0.5 with combination therapy compared with monotherapy. Moderate antiviral effects of ribavirin remaining in the body for long periods after end of

Table 5
Relapse rate by baseline HCV level in patients HCV RNA-negative within 4 weeks and within 12 weeks

	Baseline viral load in patients receiving combination therapy (kcopies/mL)			Baseline viral load in patients receiving monotherapy (kcopies/mL)		
	Low (<500)	Moderate (500 to <850)	High (≥850)	Low (<500)	Moderate (500 to <850)	High (≥850)
(a) HCV RNA-negative within 4 weeks						
Relapse						
4 weeks	15% (3/20)	18% (2/11)	17% (2/12)	67% (2/3)	50% (2/4)	100% (1/1)
12 weeks	5% (1/20)	9% (1/11)	17% (2/12)	0 (0/3)	0 (0/4)	0 (0/1)
24 weeks	10% (2/20)	18% (2/11)	17% (2/12)	0 (0/3)	0 (0/4)	0 (0/1)
Unknown	–	–	8% (1/12)	33% (1/3)	–	–
Total	30% (6/20)	45% (5/11)	55% (7/12)	100% (3/3)	50% (2/4)	100% (0/1)
(b) HCV RNA-negative within 12 weeks						
Relapse						
4 weeks	92% (11/12)	52% (13/25)	57% (30/52)	100% (7/7)	100% (8/8)	84% (16/19)
12 weeks	8% (1/12)	20% (5/25)	26% (13/53)	0 (0/7)	0 (0/8)	5% (1/19)
24 weeks	0 (0/12)	4% (1/25)	0 (0/53)	0 (0/7)	0 (0/8)	5% (1/19)
Unknown	–	12% (3/25)	–	–	–	5% (1/19)
Total	100% (12/12)	88% (22/25)	81% (43/53)	100% (7/7)	100% (8/8)	100% (19/19)

Intergroup within 4 weeks: $P = 0.0319$; time $P = 0.0026$, intergroup within 12 weeks: $P = 0.0109$; time $P = 0.0001$.

treatment might explain the better end-of-treatment response with combination therapy [31]. Although HCV eradication may not be expected in patients HCV positive at 12 weeks, combination therapy should be continued so as to suppress liver inflammation and progression of liver cirrhosis. Moreover, the duration of treatment should be 12 months in patients with genotype 1 and high viral levels.

Many attempts have been made to improve the efficacy of combination therapy. Extending the dosing period from 6 months to 1 year does not affect the HCV RNA negativity rate at the end of treatment [22,23]; improved efficacy with the longer course is attributed to decreases in relapse rate after the end of treatment [32]. Thus it is necessary to conduct a prospective study to determine the optimal duration of combination treatment after HCV RNA becomes negative to improve the efficacy of IFN plus ribavirin combination therapy.

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