Interferon Therapy Reduces the Risk for Hepatocellular Carcinoma: National Surveillance Program of Cirrhotic and Noncirrhotic Patients with Chronic Hepatitis C in Japan

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Background: Previous studies on the effect of interferon therapy on the incidence of hepatocellular carcinoma have not sufficiently assessed degree of liver fibrosis, a major risk factor for hepatocellular carcinoma.

Objective: To evaluate the effect of interferon therapy on incidence of hepatocellular carcinoma, adjusting for risk factors, including the degree of liver fibrosis.

Design: Retrospective cohort study.

Setting: Seven university hospitals and one regional core hospital in Japan.

Patients: 2890 patients with chronic hepatitis C who had undergone liver biopsy since 1986. Of these patients, 2400 received interferon and 490 were untreated.

Measurements: The degree of liver fibrosis was assessed from stage F0 (no fibrosis) to stage F4 (cirrhosis). Response to interferon was determined virologically and biochemically. Screening for development of hepatocellular carcinoma was performed periodically during an average follow-up of 4.3 years. Effect of interferon therapy on the risk for hepatocellular carcinoma was analyzed by using Cox proportional hazards regression.

Results: Hepatocellular carcinoma developed in 89 interferon-treated patients and in 59 untreated patients. Among untreated patients, the annual incidence of hepatocellular carcinoma increased with the degree of liver fibrosis, from 0.5% among patients with stage F0 or F1 fibrosis to 7.9% among patients with stage F4 fibrosis. The cumulative incidence in treated and untreated patients differed significantly for patients with stage F2 fibrosis ($P = 0.0128$) and for those with stage F3 fibrosis ($P = 0.0011$). In multivariate analysis, interferon therapy was associated with a reduced risk for hepatocellular carcinoma (adjusted risk ratio, 0.516 [95% CI, 0.358 to 0.742]; $P < 0.001$), especially among patients with sustained virologic response (risk ratio, 0.197 [CI, 0.099 to 0.392]), among those with persistently normal serum alanine aminotransferase levels (risk ratio, 0.197 [CI, 0.104 to 0.375]), and among those with alanine aminotransferase levels less than two times the upper limit of normal (risk ratio, 0.358 [CI, 0.206 to 0.622]).

Conclusions: Interferon therapy significantly reduces the risk for hepatocellular carcinoma, especially among virologic or biochemical responders.

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Hepatitis C virus (HCV) infection rarely resolves spontaneously once it becomes chronic (1). Most patients remain asymptomatic for a long period, with liver cirrhosis developing after approximately 30 years (2, 3). Chronic hepatitis C with cirrhosis is a major risk factor for hepatocellular carcinoma (4–7). It has been previously shown that the risk increases with the degree of liver fibrosis (5).

Interferon is the only agent known to be effective against HCV infection (8–10). It induces a sustained virologic response in 15% to 30% of patients (11–14). Responders usually show biochemical and histologic improvement (9, 11, 15). Recently, interferon therapy in patients with chronic hepatitis C and cirrhosis was shown to be associated with a reduced incidence of hepatocellular carcinoma (16). Because most patients treated with interferon do not have cirrhosis, we included noncirrhotic as well as cirrhotic patients in our analysis of the effect of interferon therapy on the incidence and prevention of hepatocellular carcinoma.

A national surveillance program, the Inhibition of Hepatocarcinogenesis by Interferon Therapy (IHIT) Study, was begun in 1994 as a multicenter, large-scale, retrospective cohort study supported by the Japan Ministry of Health and Welfare as one of the Comprehensive 10-Year Strategy for Cancer Control Projects (17). In this program, patients with chronic hepatitis C who have undergone liver biopsy at one of eight participating institutions are enrolled and followed periodically for development of hepatocellular carcinoma by using several imaging techniques. We analyzed the incidence of hepatocellular carcinoma as of February 1998 by using multivariate proportional hazards regression.

Methods

Patients

The IHIT Study Group approved the design of this study on 21 September 1994. All patients who were positive by a second-generation HCV antibody assay and who had undergone liver biopsy since

1986 at one of the eight participating institutions were enrolled. Patients who were participants in interferon trials for non-A, non-B chronic hepatitis (18–21) and in whom anti-HCV seropositivity was confirmed by using stored sera were also included; these patients had undergone liver biopsy in 1986 or later. Patients were excluded if at the time of liver biopsy they presented with hepatocellular carcinoma or other liver diseases, such as chronic hepatitis B, alcoholic liver disease, autoimmune hepatitis, or primary biliary cirrhosis.

The minimum follow-up was established as 1 year for two reasons. First, if hepatocellular carcinoma is detected within 1 year after liver biopsy, the possibility that the cancer was present at the time of liver biopsy cannot be ruled out. Second, interferon therapy must be started within 1 year after liver biopsy according to Japanese health insurance rules.

By February 1998, 3223 patients who fulfilled the inclusion criteria were registered. Of these patients, 333 were excluded from the analysis: 161 patients (5.0%) transferred to other hospitals without follow-up, and the follow-up period after liver biopsy was less than 1 year for 172 patients (5.3%). Thus, 2890 patients were included in the present analysis. **Figure 1** shows the schema for patient selection.

Interferon therapy was given to 2400 patients; 490 patients did not receive treatment (control group). Interferon therapy was initiated within 1 year after liver biopsy (within 6 months in 93% of patients); 84% of patients received interferon-α, 14% received interferon-β, and 2% received a combination of interferon-α and interferon-β. The median total dose was 480 MU (first quartile, 324 MU; third quartile, 702 MU), and the median duration of administration was 160 days (first quartile, 94 days; third quartile, 168 days). Once interferon therapy was started, a patient was included in the interferon treatment group even if therapy was discontinued because of adverse events or other reasons. The 490 patients who did not receive interferon chose this course of action voluntarily on the basis of concerns about adverse effects; lack of time for therapy; or physician recommendation, which took into account depression, severe diabetes mellitus, or other medical conditions.

Serum HCV load was quantitatively determined at the time of liver biopsy by using various commercial and in-house assays. Because it is difficult to correlate the results of different assay methods, only data obtained with two widely used assays, the branched-DNA probe assay (22) and competitive reverse-transcription polymerase chain reaction (RT-PCR) (23), were used. HCV RNA genotype was determined by RT-PCR using genotype-specific primers (24) or by serologic grouping of serum antibody (25), assuming that genotypes 1a and 1b correspond to serologic group 1 (genotype 1) and genotypes 2a and 2b correspond to serologic group 2 (genotype 2) (11).

Histologic Evaluation

Liver biopsy specimens were evaluated by a representative pathologist at each institution (a total of eight pathologists were involved) and were scored for the stage of liver fibrosis and grade of inflammatory activity according to the classification of Desmet and colleagues (26). Stage of fibrosis was assessed from stage F0 (no fibrosis) to stage F4 (cirrhosis), and grade of inflammatory activity was

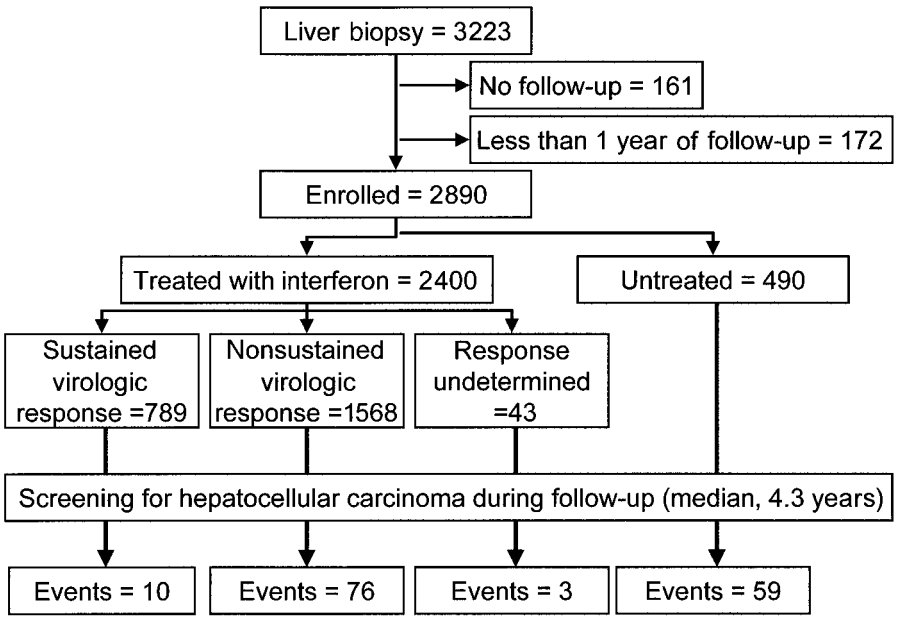


Figure 1. Schema for patient selection.

scored from grade A1 (mild) to grade A3 (severe). To confirm interobserver concordance in scoring, a subsequent blind and independent examination of 350 randomly selected liver biopsy specimens was conducted by two of the eight pathologists.

Definition of Interferon Response

Virologic and biochemical criteria were used to define response to interferon therapy. Hepatitis C virus RNA was used as a marker of virologic response and was determined by RT-PCR. A virologic sustained response was defined as HCV RNA negativity more than 6 months after termination of interferon therapy; positivity at the same time point was considered a nonsustained response (27). Patients with nonsustained response included those who had temporary disappearance of viremia followed by relapse. In patients treated before the availability of RT-PCR, virologic response was determined by using sera stored at −30 °C or collected afterward.

The serum alanine aminotransferase (ALT) level was used as a marker of biochemical response to interferon therapy. Sustained biochemical response was defined as persistently normal serum ALT levels more than 6 months after termination of interferon therapy; nonsustained response was defined as elevated serum ALT levels at the same time point. Nonsustained response was subdivided into two categories: "mildly elevated" for a serum ALT level less than two times the upper limit of normal and "highly elevated" for a serum ALT level two or more times the upper limit of normal.

Screening for Hepatocellular Carcinoma

Patients were examined for hepatocellular carcinoma by abdominal ultrasonography at least every 6 months. If hepatocellular carcinoma was suspected on the basis of ultrasonographic results, additional procedures, such as computed tomography, magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy, were used to confirm the diagnosis.

Statistical Analysis

Statistical analysis was performed by using SAS software, version 6.12 (SAS Institute, Inc., Cary, North Carolina). Interobserver concordance of histologic scoring was evaluated by using the Spearman correlation coefficient. Differences between two groups were evaluated by using the unpaired Student *t*-test or the Mann–Whitney U-test. Categorical data were compared by using the chi-square test or the Fisher exact probability test. Cumulative incidence curves were determined with the Kaplan–Meier method, and the differences between groups were assessed by using the log-rank test. We used the Cox proportional hazards regression analysis to examine the effect of interferon therapy on the incidence of hepatocellular carcinoma. Because virologic and biochemical responses were mutually dependent, the risk ratio for hepatocellular carcinoma

Table 1. Demographic and Clinical Characteristics*

Characteristic	Untreated Patients	Interferon-Treated Patients	*P* Value
Patients, *n*	490	2400	
Sex, *n*			<0.05†
Male	270	1531	
Female	220	869	
Age, *y*	53.6 ± 11.2	49.5 ± 11.3	NS‡
Fibrosis stage, % *(n)*			<0.05†
F0	1.0 (5)	1.9 (45)	
F1	31.6 (155)	27.7 (665)	
F2	33.5 (164)	37.3 (896)	
F3	12.0 (59)	23.5 (564)	
F4	21.8 (107)	9.6 (230)	
ALT level, *IU/L*	78 ± 70	105 ± 84	<0.05‡
AST level, *IU/L*	69 ± 48	78 ± 60	<0.05‡
Albumin level, *g/L*	40 ± 4	42 ± 4	<0.05‡
Bilirubin level, *μmol/L (mg/dL)*	14 ± 5 (0.82 ± 0.32)	13 ± 6 (0.77 ± 0.34)	<0.05‡
Platelet count, × 10^9/*L*	158 ± 62	169 ± 58	<0.05‡
α-Fetoprotein level, *μg/L*	21 ± 67	18 ± 66	NS§
HCV load			<0.05†
Low‖	58	530	
High¶	113	480	
HCV genotype			NS†
Type 1	98	1177	
Type 2	43	496	

* Unless otherwise indicated, data are given as the mean ± SD. ALT = alanine aminotransferase; AST = aspartate aminotransferase; HCV = hepatitis C virus; NS = not significant.
† Chi-square test.
‡ Student *t*-test.
§ Mann–Whitney U-test.
‖ Below the median value.
¶ At or above the median value.

was calculated separately for these factors. The risk ratio attributable to categorical data, such as stage of liver fibrosis and serum ALT level, was calculated by using dummy variables. A P value less than 0.05 was considered statistically significant.

Results

Patient Characteristics

The demographic and clinical features of patients at the time of their enrollment are summarized in **Table 1**. The frequency distribution of the stages of liver fibrosis differed between interferon-treated patients and untreated patients. Most laboratory values also differed between the two groups. However, differences in laboratory values between treated patients and untreated patients were not significant at the same stage of fibrosis. This indicated the need to adjust for stage of liver fibrosis, which was done in the following analyses.

Histologic Evaluation

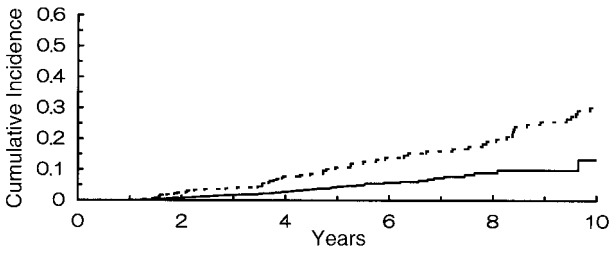
The concordance in scores for stage of fibrosis and grade of inflammatory activity determined at each institution and by the two representative pathologists was strong, with Spearman coefficients ranging from 0.897 to 0.918 for stage of fibrosis and from 0.878 to 0.849 for grade of inflammatory activity. The original score was sustained by at least one of the two pathologists in 319 of 350 cases for fibrosis staging and in 320 of 350 cases for grading inflammatory activity.

Response to Interferon Therapy

Response to interferon therapy was determined in 2357 (98.2%) of the 2400 interferon-treated patients. Response was not determined in 43 patients because of insufficient follow-up (<6 months) after termination of therapy. A sustained virologic response was achieved in 789 patients (33.5%). The response rate was similar regardless of the type of interferon used (32.3%, 34.5%, and 25.6% for interferon-α, interferon-β, and the combination of the two, respectively). A sustained biochemical response was achieved in 984 patients (41.7%). Two hundred sixty patients (11%) had sustained biochemical response but nonsustained virologic response; that is, they had persistently normal serum ALT levels but tested positive for HCV RNA.

Cumulative Incidence of Hepatocellular Carcinoma

By February 1998, the median follow-up was 1575 days (4.3 years) after liver biopsy (first quartile, 1132 days; third quartile, 1959 days) for all 2890 patients, 1596 days (first quartile, 1172 days; third quartile, 1943 days) for interferon-treated patients, and 1467 days (first quartile, 1001 days; third quartile, 2458 days) for untreated patients. There were 85 dropouts (60 interferon-treated patients and 25 untreated patients) who failed to undergo abdominal ultrasonography during the last 2 years because of relocation; in the cumulative incidence analysis, these patients were censored at the time of the last ultrasonography.



Untreated patients with HCC	0	17	34	42	54
Patients at risk	490	337	156	114	88
Interferon-treated patients with HCC	0	37	73	84	88
Patients at risk	2400	1872	781	200	48

Figure 2. Cumulative incidence of hepatocellular carcinoma (*HCC*) among patients treated with interferon (*solid line*) and untreated patients (*dotted line*). $P < 0.001$ by the log-rank test. The number of hepatocellular carcinoma events and patients at risk at each time point are shown below the graph.

During follow-up, hepatocellular carcinoma developed in 89 interferon-treated patients and 59 untreated patients (**Figure 1**). The cumulative incidence differed significantly between the interferon-treated patients and untreated patients (**Figure 2**). Because the two groups had different demographic and clinical profiles, especially with respect to the frequency distribution of the stages of fibrosis, the cumulative incidence was compared after patients were stratified according to stage of liver fibrosis (**Figure 3**). The difference was not significant among patients with less advanced liver fibrosis (stage F0 or F1), but it was significant among patients with stage F2 or F3 fibrosis, and it approached significance among patients with stage F4 fibrosis.

The annual incidence of hepatocellular carcinoma was calculated by the person-years method, with patients stratified according to stage of liver fibrosis (**Table 2**). Among the untreated patients, the annual incidence increased with degree of liver fibrosis, from 0.5% among patients with stage F0 or F1 fibrosis to 7.9% among patients with stage F4 fibrosis (cirrhosis). The annual incidence was lower among interferon-treated patients than among untreated patients at the same stage of liver fibrosis.

Further stratification of the interferon-treated patients according to virologic response showed that the incidence was lower especially among patients with sustained virologic response (**Table 2**). Among cirrhotic patients (stage F4 fibrosis) with sustained

Table 2. Annual Incidence of Hepatocellular Carcinoma*

Fibrosis Stage	Untreated Patients	Interferon-Treated Patients		
		All†	Sustained Virologic Response	Nonsustained Virologic Response
	% (n/n)			
F0/F1	0.45 (3/160)	0.08 (2/710)	0.11 (1/257)	0.07 (1/443)
F2	1.99 (11/164)	0.54 (16/896)	0.10 (1/316)	0.78 (15/568)
F3	5.34 (13/59)	1.95 (38/564)	1.29 (7/163)	2.20 (30/389)
F4	7.88 (32/107)	4.16 (33/230)	0.49 (1/53)	5.32 (30/168)
Total	3.17 (59/490)	1.10 (89/2400)	0.38 (10/789)	1.41 (76/1568)

* Calculated by using the person-years method.
† Includes 43 interferon-treated patients whose response to interferon was not determined.

virologic response, the annual incidence of hepatocellular carcinoma was only 0.5%.

Multivariate Analysis

In multivariate analysis, after adjustment for stage of liver fibrosis, sex, and age, interferon therapy was found to be associated with a risk ratio for hepatocellular carcinoma of 0.516 (95% CI, 0.358 to 0.742) (**Table 3**). Multivariate analysis also confirmed that the risk increased with degree of liver fibrosis; it was 24 times higher in patients with stage F4 fibrosis than in those with stage F0 or F1 fibrosis. This result is compatible with the rough annual incidence values given above. Not only stage F4 (cirrhosis) but also stage F3 and F2 fibrosis were associated with a significantly increased risk compared with stage F0 or F1 fibrosis. Other strong risk factors were male sex and increasing age.

Fibrosis stage, sex, and age remained strong when laboratory data at enrollment were included as variables in the multivariate analysis, whereas laboratory variables, except for platelet count and α-fetoprotein level, were not strong predictors. Hepatitis C virus load and HCV genotype were assessed among 984 patients in whom both values were available; these variables were not found to be risk factors for hepatocellular carcinoma ($P > 0.2$ and $P = 0.1467$, respectively). In contrast, low HCV load (below the median value compared with at or above the median value) and HCV genotype 2 (compared with genotype 1) were significant predisposing factors for sustained virologic response in multivariate logistic regression (data not shown).

Most patients who had sustained virologic response also had sustained biochemical response. Patients with nonsustained virologic response had various serum ALT levels after interferon therapy (**Table 4**). Because virologic and biochemical re-

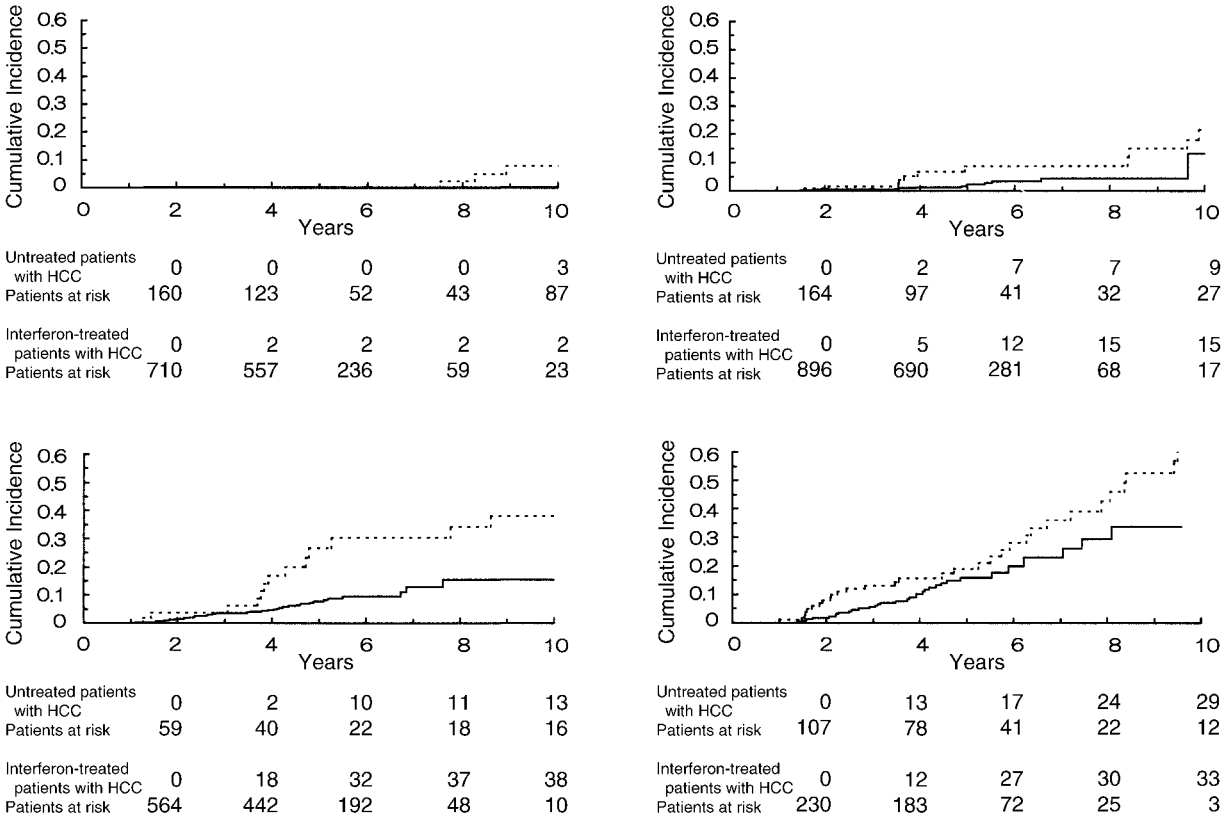


Figure 3. Cumulative incidence of hepatocellular carcinoma (*HCC*) among patients treated with interferon (*solid line*) and untreated patients (*dotted line*), stratified by stage of liver fibrosis. Top left. Patients with stage F0 or F1 fibrosis. $P > 0.2$. **Top right.** Patients with stage F2 fibrosis. $P = 0.0128$. **Bottom left.** Patients with stage F3 fibrosis. $P = 0.0011$. **Bottom right.** Patients with stage F4 fibrosis. $P = 0.0573$. All P values were obtained by using the log-rank test. The number of hepatocellular carcinoma events and patients at risk at each time point are shown below each graph.

Table 3. Risk Factors for Hepatocellular Carcinoma*

Risk Factor	Risk Ratio (95% CI)	*P* Value
Sex (male compared with female)	1.551 (1.100–2.187)	0.012
Age (by every 1 year)	1.063 (1.041–1.085)	<0.001
Stage of fibrosis (compared with stage F0/F1)		
F2	4.431 (1.704–11.522)	0.002
F3	13.097 (5.194–33.021)	<0.001
F4	24.011 (9.638–59.815)	<0.001
Interferon treatment (compared with no treatment)	0.516 (0.358–0.742)	<0.001

* Risk ratios for development of hepatocellular carcinoma (148 events among all 2890 patients) were calculated by using Cox proportional hazards regression analysis.

sponses are associated, they were evaluated in independent analyses. Adjusted risk ratios for hepatocellular carcinoma were calculated compared with untreated patients. The reduction in risk was similar among patients with sustained biochemical response (normal ALT levels) and those with sustained virologic response (**Table 5**). Among 260 who had normal serum ALT levels and also tested positive for HCV RNA, the reduction in risk for hepatocellular carcinoma was similar to that in patients with sustained virologic response.

Multivariate analysis also showed that compared with untreated patients, the risk for hepatocellular carcinoma was reduced in patients with mildly elevated ALT levels (less than two times the upper limit of normal). However, patients with highly elevated ALT levels (two or more times the upper limit of normal) remained at high risk, as did untreated patients. Seventy percent of interferon-treated patients had highly elevated serum ALT levels before therapy, but the proportion decreased significantly to 31% after therapy ($P < 0.001$).

Discussion

In the IHIT Study, patients were included only if they had undergone liver biopsy, because degree of liver fibrosis is strongly associated with risk for hepatocellular carcinoma and should be adjusted for, as confirmed above. Randomized, controlled trials would be an alternative approach, but these may not be ethically feasible. Two randomized, controlled trials have examined the relation between interferon therapy and development of hepatocellular carcinoma (16, 28), but these trials involved only cirrhotic patients.

Evaluation of liver fibrosis is especially important when patients are stratified according to response to interferon therapy, because patients with advanced liver fibrosis are both resistant to interferon therapy and more likely to develop hepatocellular carcinoma. However, previous cohort studies did not stratify the degree of liver fibrosis (29–31) or stratified it only into two ranks (32). In contrast, the IHIT Study used four ranks and showed a significant difference in cumulative incidence of hepatocellular carcinoma between interferon-treated patients and untreated patients with stage F2 or F3 fibrosis.

We found no effect of interferon therapy in patients with mild liver fibrosis (stage F0 or F1), among whom the incidence of hepatocellular carcinoma was low even in the untreated patients. However, the annual incidence increased with time among untreated patients with stage F0 or F1 fibrosis and may reach statistical significance with a longer follow-up period. Among cirrhotic patients (those with stage F4 fibrosis), the difference between treated and untreated patients approached but did not attain statistical significance. This may be due to low efficacy of interferon therapy in these patients. Because the incidence was low among cirrhotic patients showing a virologic sustained response, the effect of interferon therapy would become significant if it were more effective in these patients.

Although several previous studies indicated a relation between risk for hepatocellular carcinoma and HCV genotype (33, 34), we did not confirm this association. We also did not find alcohol consumption to be associated with risk for hepatocellular carcinoma. However, only 2.0% of patients drank more than 80 g of alcohol per day during follow-up,

Table 4. Relation between Biochemical and Virologic Response*

Virologic Response	Sustained Biochemical Response (Normal Serum ALT Level)	Nonsustained Biochemical Response		
		Mildly Elevated Serum ALT Level†	Highly Elevated Serum ALT Level‡	Total
	← *n (%)* →			
Sustained response (HCV RNA negative)	724 (91.7)	59 (7.5)	6 (0.8)	789 (100)
Nonsustained response (HCV RNA positive)	260 (16.6)	592 (37.8)	716 (45.7)	1568 (100)
Total	984 (41.7)	651 (27.6)	722 (30.6)	2357 (100)

* ALT = alanine aminotransferase; HCV = hepatitis C virus.
† Value less than two times the upper limit of normal.
‡ Value two or more times the upper limit of normal.

Table 5. Relation of Interferon Response to Risk for Hepatocellular Carcinoma*

Type of Response	Patients, *n*	Risk Ratio (95% CI)†	*P* Value
Virologic response			
Sustained	789	0.197 (0.099–0.392)	<0.001
Nonsustained	1568	0.631 (0.434–0.918)	0.016
Biochemical response			
Sustained	984	0.197 (0.104–0.375)	<0.001
Nonsustained			
Mildly elevated	651	0.358 (0.206–0.622)	<0.001
Highly elevated	722	0.910 (0.616–1.344)	>0.2
Sustained virologic response			
Sustained	789	0.250 (0.131–0.478)	<0.001
Nonsustained with normal ALT level	260	0.271 (0.086–0.856)	0.026

* ALT = alanine aminotransferase.
† Risk ratio for hepatocellular carcinoma (145 events among 2847 patients) was calcuated by using Cox proportional hazards regression analysis, excluding 43 patients whose response to interferon was not determined. Risk ratios are adjusted for age, sex, and stage of liver fibrosis.

and data may not be sufficient to allow evaluation of the effect of alcohol consumption.

Patients with sustained biochemical response in spite of viremia were at reduced risk for hepatocellular carcinoma compared with patients with sustained virologic response. Moreover, the degree of risk differed between patients with mildly elevated ALT levels and those with highly elevated ALT levels. Thus, reduced risk seems to be associated not only with disappearance of viremia but with amelioration of hepatic inflammation. This finding is compatible with those a previous study of postoperative patients with hepatocellular carcinoma, which found that recurrence was more frequent among patients with high serum ALT levels (>80 IU/L; that is, approximately two times the upper limit of normal) (35).

It has been reported that hepatic inflammation may recur in viremic patients with once-normalized ALT levels (36) and that the recurrence may be accompanied by a reverted risk for hepatocellular carcinoma. In our study, the risk did not increase during 4 years of follow-up among these patients (data not shown); however, longer follow-up is necessary to determine whether this effect is transient.

In conclusion, risk for hepatocellular carcinoma was found to be strongly associated with stage of liver fibrosis and was reduced by interferon therapy among patients with stage F2 or F3 fibrosis. An adjusted risk ratio of 0.539 (CI, 0.374 to 0.779) was attributed to interferon therapy. Sustained virologic and biochemical responses were associated with a further reduction in risk. Patients with serum ALT levels less than two times the upper limit of normal were also at reduced risk for hepatocellular carcinoma.

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