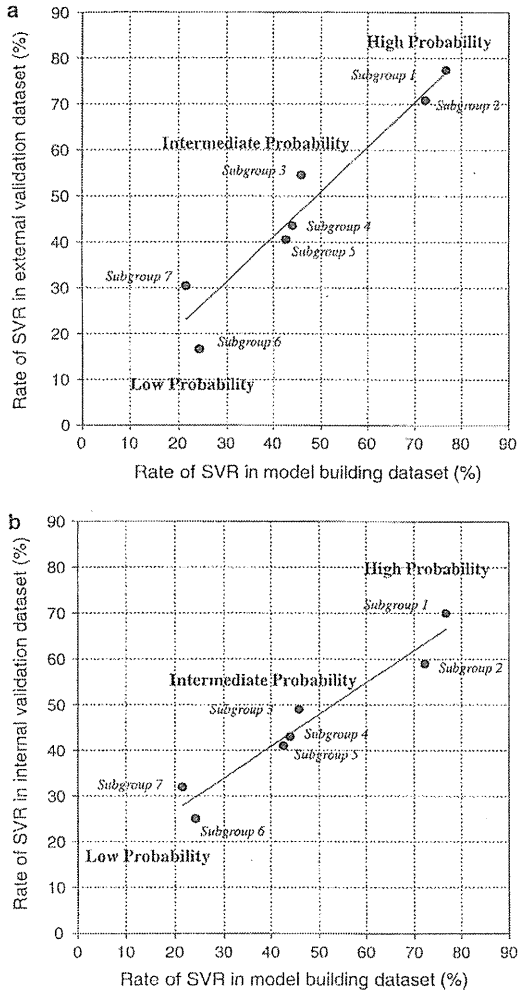

Fig. 2 Validation of the decision tree analysis by an internal and external validation dataset: subgroup-stratified comparison of the SVR rate. The rate of SVR in each subgroup was plotted. The *X* axis represents the model building, and the *Y* axis represents the validation datasets. a Internal validation and b external validation. There was a close correlation between the model building and the internal validation dataset (correlation coefficient $r^2 = 0.925$) and between the model building and the external validation dataset (correlation coefficient $r^2 = 0.936$)

original dataset used for model building. Each patient in the external validation set was allocated to subgroups 1–7 using the flow-chart form of the tree. The rates of SVR were 70% for subgroup 1, 59% for subgroup 2, 49% for subgroup 3, 43% for subgroup 4, 41% for subgroup 5, 25% for subgroup 6, and 32% for subgroup 7. The rates of SVR for each subgroup of patients were closely correlated

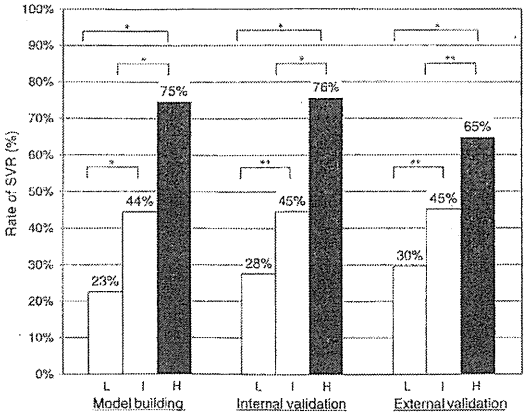

Fig. 3 Comparison of SVR rates between groups divided by the decision tree. The rate of SVR was compared among the 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes*, indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively). The rate of SVR was significantly different among the 3 groups. $*p < 0.0001$, $**p < 0.001$

between the model-building dataset and the validation dataset ($r^2 = 0.936$) (Fig. 2b).

Construction of 3 groups according to the probability of SVR

Seven subgroups were reconstructed into 3 groups according to their predicted rates of SVR: the high probability group consisted of subgroups 1 and 2, the intermediate probability group consisted of subgroups 3, 4 and 5, and the low probability group consisted of subgroups 6 and 7. The rate of SVR was significantly different among the 3 groups (Fig. 3). The rate of SVR in the high probability group was consistently high: 75% for model building patients, 76% for internal validation patients and 65% for external validation patients. Conversely, the rate of SVR in the low probability group was consistently low: 23% for model building patients, 28% for internal validation patients and 30% for external validation patients. The rate of SVR in the intermediate probability group was 44% for model building patients, 45% for internal validation patients and 45% for external validation patients. Since 28–32% of patients were classified as high probability and 30–32% were classified as low probability, roughly 60% of patients were classified as having either a high or low probability of achieving SVR.

Effect of dose reductions of PEG-IFN and RBV on SVR

The cumulative dose of PEG-IFN and RBV was not included as a variable of analysis since the present study

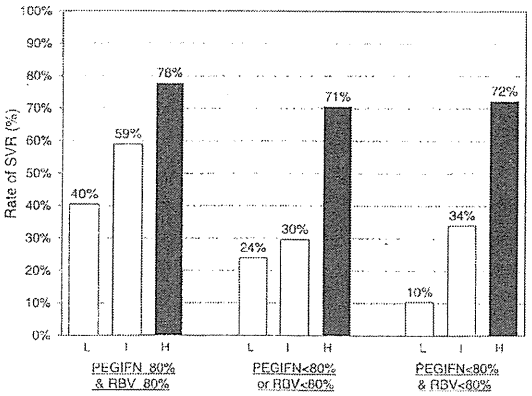

Fig. 4 Comparison of SVR rates among groups stratified by drug adherence. The 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes* indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV. The good adherence group (≥80% planned dose of both PEG-IFN and RBV) had a higher rate of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) but not in the high probability group ($p = 0.53$)

aimed to develop a pre-treatment model for the prediction of response. To analyze the possible effect of drug reductions on the result of the decision tree analysis, 3 groups of patients divided by the decision tree analysis (low, intermediate and high probability group) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV (Fig. 4). Even after adjustment for adherence, 3 groups of patients still had low, intermediate and high probability of achieving SVR, respectively. Of note, the good adherence group (≥80% planned dose of both PEG-IFN and RBV) had higher rates of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) probability group, but not in the high probability group ($p = 0.53$).

Factors associated with SVR by multivariate logistic regression analysis

We also explored the factors associated with SVR using a standard statistical analysis. By univariate analysis, age, gender, serum albumin, creatinine, alanine aminotransferase, GGT, red blood cell count, hemoglobin, hematocrit, platelet count and AFP were found to be associated with SVR (Table 2). HCVRNA load was not associated with SVR. By multivariate analysis, age, gender, GGT and platelet count were found to be independently associated with SVR (Table 3). Of note, AFP, which was selected as a significant predictor of response in the decision tree analysis, was not found to be an independent response predictor in the standard multivariate analysis. This indicates a unique feature of the decision tree analysis; i.e., it could identify significant predictors that specifically apply to selected patients, in this case patients younger than 50 years old.

Relationships between decision tree model and stage of fibrosis or HCV RNA load

Liver biopsy was performed in 664 patients. The distribution of fibrosis in three probability groups differed significantly. Advanced fibrosis (F3 or F4) was higher in the low probability group (39%) compared to the intermediate probability group (13%) ($p < 0.0001$) and to the high probability group (6%) ($p < 0.0001$). Advanced fibrosis was also higher in the intermediate group compared to the high probability group ($p = 0.01$). AFP was significantly associated with liver fibrosis stage: medians of AFP levels were 4.9, 5.9, 13.0 and 18.6 for F1, F2, F3 and F4, respectively ($p < 0.0001$, Spearman's rank correlations). Lower platelet counts correlated with advanced fibrosis stages (data not shown). The SVR rate was higher in the high probability group compared to the intermediate or low probability group after stratification by HCV RNA load. Among patients with low HCVRNA load (<400,000 IU/ml), the rate of SVR was 93, 59 and 50% for the high, intermediate and low probability group, respectively ($p = 0.002$ for high vs. intermediate and $p < 0.001$ for high vs. low probability groups). Among patients with a high HCVRNA load (≥400,000 IU/ml), the rate of SVR was 73, 42 and 21% for the high, intermediate and low probability group, respectively ($p < 0.001$ for high vs. low, high vs. intermediate and intermediate vs. low probability groups).

Discussion

Currently, the combination of PEG-IFN and RBV is the recommended therapy for chronic HCV infection. The rate of SVR with 48 weeks of therapy is around 50% in patients with HCV genotype 1b and a high HCV RNA titer [2, 3]. To date, the virological response during therapy is the most reliable means for predicting the likelihood of SVR [2, 24, 25]. More potent therapy, such as a triple combination of protease inhibitor, PEG-IFN and RBV, is being evaluated in clinical trials but is not readily available [26, 27]. Under the circumstances, pre-treatment prediction of the likelihood of SVR may be useful for both patients and physicians to support clinical decisions as to whether to start PEG-IFN/RBV therapy or delay treatment until a new more effective therapy becomes available.

Table 2 Comparison of pre-treatment factors between patients with and without sustained virological response (SVR) among the model building dataset ($n = 506$)

	SVR ($n = 240$)	Non-SVR ($n = 266$)	p
Age (years)	54 (25–75)	60 (36–73)	<0.0001
Male gender[a]	151/240 (63%)	171/266 (41%)	<0.0001
Body mass index (kg/m^2)	22.5 (16.8–32.0)	22.6 (15.5–33.3)	0.244
Albumin (g/dl)	4.1 (3.2–5.0)	4 (2.7–4.9)	0.004
Creatinine (mg/dl)	0.7 (0.44–1.14)	0.69 (0.39–1.47)	<0.0001
AST (IU/l)	59 (11–370)	61 (17–261)	0.457
ALT (IU/l)	58 (11–413)	53 (11–316)	0.031
GGT (IU/l)	31 (10–322)	43 (12–328)	0.005
Total cholesterol (mg/dl)	175 (87–297)	171 (73–274)	0.184
Triglyceride (mg/dl)	105 (36–474)	105 (33–294)	0.992
White blood cell count (/μl)	4,600 (2,200–10,900)	4,425 (1,800–10,810)	0.479
Neutrophils (/μl)	2,507 (667–7,870)	2,423 (900–7,281)	0.321
Red blood cell count (/μl)	455 (336–577)	441 (313–564)	0.001
Hemoglobin (g/dl)	14.3 (10.2–17.6)	13.9 (9.4–17.9)	0.004
Hematocrit (%)	42.1 (13.3–53.7)	41.2 (30.7–52.0)	0.031
Platelets (10^9/l)	178 (81–380)	142 (60–320)	<0.0001
AFP (ng/ml)	4.3 (0.9–680)	6.4 (1.9–468)	0.041
HCVRNA (10^3 IU/ml)	1,400 (100–5,100)	1,700 (100–5,100)	0.659
Fibrosis stage: F3–4[a]	21/198 (11%)	52/219 (24%)	<0.0001

Data expressed as median (range) unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *AFP* alpha-fetoprotein

[a] Data expressed as number/available data (percentage)

Table 3 Multivariate logistic regression analysis for factors associated with sustained virological response (SVR)

	Odds	95% CI	p value
Age (years)	0.96	0.94–0.98	0.001
Platelets (10^9/l)	1.09	1.04–1.14	<0.0001
ALT (IU/l)	1.01	1.00–1.01	0.001
GGT (IU/l)	0.99	0.98–0.99	<0.0001
Male gender	2.92	1.87–4.55	<0.0001

GGT gamma-glutamyltransferase

Using the data mining analysis, we constructed a simple decision tree model for the pre-treatment prediction of response to PEG-IFN/RBV. The analysis highlighted 5 variables relevant to response: age, gender, platelet count, AFP and GGT. Classification based on these variables identified subgroups of patients with high probabilities of achieving SVR among difficult to treat genotype 1b chronic hepatitis C patients. The reproducibility of the model was confirmed by the independent internal and external validation datasets. An advantage of the decision tree analysis over traditional regression models is that the decision tree model is user-intuitive and can be readily interpreted by medical professionals without any specific knowledge of statistics. Patients can be allocated to specific subgroups with a defined rate of response simply by following the flow-chart form. Using this model, an estimate of the response before treatment can be rapidly obtained, which may facilitate clinical decision making. Thus, this model could be readily applicable to clinical practice.

According to the results of the decision tree analysis, patients were categorized into 3 groups: the rate of SVR was 23–30% for the low probability group, 44–45% for the intermediate probability group and 65–76% for the high probability group. About 30% of patients were each categorized in the high and low probability group and the remaining 40% of patients in the intermediate probability group. These results support the evidence-based approach for selecting an optimum treatment strategy for individual patients. For example, patients in the high probability group may be the most suitable candidates for PEG-IFN/RBV therapy, while patients in the low probability group may be advised to wait for a future therapy, such as the combination of protease inhibitor, PEG-IFN and RBV. However, the estimation of low probability should not be used to preclude patients from therapy, and the final decision should be made on a case-by-case basis, taking into consideration the acceptance by the patient of a low likelihood of response and the potential risk of disease progression while waiting for a future therapy.

Another important finding was that poor adherence to drugs lowered the rate of SVR in the low and intermediate probability groups, which implies that effort should be made to maintain ≥80% of the planned dose of PEG-IFN and RBV in those patients. On the other hand, the rate of SVR was high irrespective of drug adherence in the high probability group. Whether shorter duration of therapy is sufficient in this group of patients should be confirmed in future study.

The variables used in the decision tree have been previously reported to associate with the efficacy of IFN therapy. Younger age and male gender are associated with a favorable response [28]. Lower platelet count is a hallmark of advanced fibrosis in chronic hepatitis C and is reported to be associated with poor response to IFN [29]. AFP is usually used for the screening or the diagnosis of hepatocellular carcinoma, but recent studies suggest an association between higher AFP levels and poor response to IFN therapy [30–33]. Previous report speculated that higher expression of AFP by hepatic progenitor cells may be associated with non-response to therapy [30]. Another report speculated that AFP levels predict poor response to therapy through the underlining link to advanced liver fibrosis [31]. Our data support the latter speculation since advanced fibrosis was associated with elevation of AFP levels. Fibrosis of the liver is an important predictor of response, but we did not include this factor in the decision tree analysis since liver biopsy may not always be available in general practice. As a result, two predictive factors that correlate with fibrosis stage (platelet counts and AFP) were selected in the model, and three probability groups reflected the different distribution of fibrosis stage. GGT is reported to be associated with insulin resistance and hepatic steatosis [34–37], a factor that confers resistance to IFN therapy [38–44]. What is unique to the present study is the visualization of response probability by combining these factors and its high reproducibility revealed by a high-quality validation of the model by internal and external validation datasets that were completely independent of the model building dataset. Since factors used in the model were clinical parameters that are readily available by the usual workup of patients, this model could be immediately applicable to clinical practice without imposing costs for additional examinations.

A potential limitation of this study is that data mining analysis has an intrinsic risk of showing relationships that fit to the original dataset but are not reproducible in different populations. Although internal and external validations showed that our model had high reproducibility, we recognize that further validation on a larger external validation cohort, especially in populations other than Japanese, may be necessary to further verify the reliability of our model.

In conclusion, we built a pre-treatment model for the prediction of virological response to PEG-IFN/RBV. Because this decision tree model was made up of simple variables, it can be easily applied to clinical practice. This model may have the potential to support decisions about patient selection for PEG-IFN/RBV based on a possibility of response weighed against the potential risk of adverse events or costs.

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Original Article

Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy

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Aim: Hepatic steatosis is linked to development of hepatocellular carcinoma (HCC) in non-viral liver disease such as non-alcoholic steatohepatitis. The present study aimed to assess whether hepatic steatosis is associated with the development of HCC in chronic hepatitis C.

Methods: We studied a retrospective cohort of 1279 patients with chronic hepatitis C who received interferon (IFN) therapy between 1994 and 2005 at a single regional hospital in Japan. Of these patients, 393 had a sustained virological response (SVR) and 886 had non-SVR to IFN therapy. After IFN therapy, these patients were screened for development of HCC every 6 months. The average period of observation was 4.5 years.

Results: HCC developed in 68 patients. The annual incidence of HCC was 2.73% for patients with a steatosis grade of 10% or greater and 0.69% for patients with a steatosis grade of 0–9%. On multivariate analysis, higher grade of steatosis was a significant risk factor for HCC independent of older age, male sex, higher body mass index (BMI), advanced fibrosis stage and non-SVR to IFN therapy. The adjusted risk ratio of hepatic steatosis was 3.04 (confidence interval 1.82–5.06, $P < 0.0001$), which was higher than that of older age (1.09), male sex (2.12), non-SVR to IFN (2.43) and higher BMI (1.69).

Conclusion: Hepatic steatosis is a significant risk factor for development of HCC in chronic hepatitis C independent of other known risk factors, which suggest the possibility that amelioration of hepatic steatosis may prevent hepatocarcinogenesis.

Key words: hepatocellular carcinoma, interferon, steatosis, virological response.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide and its incidence has been increasing. This recent increase in HCC incidence may likely be attributed to the higher prevalence of non-alcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV) infection.[1]

Non-alcoholic fatty liver disease is characterized by hepatic steatosis with or without inflammation in the absence of excessive alcohol consumption. Several studies have indicated the etiological association between NAFLD and development of HCC.[2–4] Other studies have shown that obesity or diabetes, a common etiology of non-alcoholic hepatic steatosis, is associated with development of HCC.[5–7] Although the mechanism of carcinogenesis in NAFLD has not been determined, an animal model showed that obesity-related hepatic steatosis leads to the development of hepatic

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hyperplasia, suggesting the possibility that hepatic steatosis is a pre-malignant condition.[8]

Another important etiological agent for HCC is HCV infection. Because steatosis is a common pathological feature of HCV-infected patients,[9] the important question is whether steatosis influences the progression of liver disease in hepatitis C, by analogy with NAFLD. Several studies, including ours[10] indicated that hepatic steatosis promotes the progression of hepatic fibrosis.[11–15] The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed[16] and was confirmed in two studies[17,18] while another study failed to show such an association.[19] The present study was conducted to analyze the association between hepatic steatosis and development of HCC in a large cohort of chronic hepatitis C patients, which enabled to adjust for known risk factors for HCC.

METHODS

Patients

A TOTAL OF 1437 chronic hepatitis C patients were treated with interferon (IFN) at Musashino Red Cross Hospital between October 1994 and October 2005. Among them, 1279 patients who fulfilled the following inclusion criteria were enrolled in this study: (i) positive for HCV RNA by reverse-transcription polymerase chain reaction before IFN therapy; (ii) absence of other causes of liver disease, such as co-infection with hepatitis B virus, autoimmune hepatitis or primary biliary cirrhosis; (iii) had undergone liver biopsy within the 12 months prior to IFN treatment; (iv) were followed for more than 1 year after the completion of IFN therapy; and (v) absence of HCC during and within 1 year after the completion of therapy. A total of 158 patients were excluded: two patients who were positive for hepatitis B surface antigen, 97 patients lacking liver biopsy, 53 patients with less than 1 year's duration of follow up, and six patients who developed HCC within 1 year of the completion of IFN therapy. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee.

Patients were followed up by regular visits to our hospital every 1–3 months. Six patients died of liver-unrelated disease (two patients with gastric cancer and one patient each with lung cancer, colon cancer, pancreatic cancer and leukemia). There were 122 patients who were lost to follow up because of relocation. We included their data in the analysis, censored at the time of their last visit. The start of follow up was defined as the date of completion of first IFN therapy and the end of follow up was defined as the date of diagnosis of HCC or the date of the last visit. The average period of follow up was 4.5 years.

Clinical characteristics and laboratory data were collected at the most recent time point before liver biopsy. Diabetes mellitus was diagnosed based on a fasting plasma glucose concentration that exceeded 126 mg/dL, a casual plasma glucose concentration that exceeded 200 mg/dL, or the need for insulin or oral anti-hyperglycemic drugs. Information regarding alcohol consumption was obtained through an interview. Body mass index (BMI) was calculated using the following formula: weight in kilograms/height in meters squared. The baseline clinical features of patients at enrollment are summarized in Table 1.

Histological examination

Liver biopsy specimens were obtained from all patients before therapy. The median length of liver biopsy specimens was 13 mm (range 10–42 mm) and median number of portal tracts was 11 (range 4–30). Histological findings were re-evaluated recently by three independent pathologists who were blinded to the clinical details to ensure consistency over time. Fibrosis and activity were scored according to the METAVIR scoring system.[20] Fibrosis was staged on a scale of 0–4: F0 (no fibrosis); F1 (mild fibrosis: portal fibrosis without septa); F2 (moderate fibrosis: few septa); F3 (severe fibrosis: numerous septa without cirrhosis); and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of 0–3: A0 (no activity); A1 (mild activity); A2 (moderate activity); and A3 (severe activity). Percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis and graded on a scale of 0%, 1–9%, 10–29% and 30% or greater as reported previously.[10] All three pathologists assigned the same scale in 85% of cases for fibrosis staging, 87% for inflammation grading and 95% for steatosis grading. If there was discordance, the scores assigned by two pathologists were used for the analysis.

Screening for HCC

At enrollment, no patient had HCC or any suspicious lesion on abdominal ultrasonography or computed tomography. Patients were examined for HCC by abdominal ultrasonography or computed tomography at least every 6 months. Suspicious lesions were examined further by a triphasic contrast-enhanced computerized tomography or magnetic resonance imaging,

Table 1 Clinical characteristics of patients

Male, *n* (%)	643 (50%)
Age (years)	54.2 ± 11.9
BMI (kg/m^2)	23.4 ± 3.1
Alcohol consumption ≥20 g/day, *n* (%)	44 (3%)
Diabetes Mellitus, *n* (%)	197 (15%)
AST level (IU/L)	68.9 ± 45.3
ALT level (IU/L)	92.9 ± 75.9
GGT level (IU/L)	41.2 ± 38.2
Platelet count (×10^{10}/L)	16.4 ± 5.2
HCV genotype, *n* (%)	
1b	873 (68.2%)
2a	236 (18.4%)
2b	139 (10.9%)
3	2 (0.2%)
Not determined	29 (2.3%)
Histological findings	
Grade of activity, *n* (%)	
A0	154 (12%)
A1	574 (45%)
A2	441 (34%)
A3	110 (9%)
Stage of fibrosis, *n* (%)	
F0	24 (2%)
F1	591 (46%)
F2	378 (30%)
F3	242 (19%)
F4	44 (3%)
Grade of steatosis, *n* (%)	
0%	384 (30%)
1–9%	543 (42%)
10–29%	215 (17%)
≥30%	137 (11%)
SVR to interferon therapy, *n* (%)	393 (31%)
Development of HCC, *n* (%)	68 (5%)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

angiography or tumor biopsy to confirm the diagnosis. Diagnostic criteria of HCC on radiological findings were hyper-vascularity at angiography or hyper-attenuation at triphasic contrast-enhanced computerized tomography or magnetic resonance imaging during the hepatic arterial phase.

Statistical analysis

The SPSS software package ver. 15.0 was used for statistical analysis. Categorical data were analyzed using Fisher's exact test. Continuous variables were compared with Student's *t*-test. The time for the development of HCC was defined as the time from the completion of IFN therapy to the time of diagnosis. Annual incidence of HCC was calculated using the person-years method. Effect of hepatic steatosis on time to development of HCC was analyzed by the Kaplan–Meier method and log-rank test, after stratification by age, sex, BMI, degree of fibrosis and response to IFN therapy, as well as multivariate analysis using Cox proportional hazards regression analysis. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Background factors for steatosis

PATIENTS WITH A steatosis grade of 10% or greater were older (53.6 ± 12.6 vs 56.0 ± 9.8, $P = 0.001$), had a higher BMI (23.0 ± 3.0 vs 24.6 ± 3.3, $P < 0.0001$), higher frequency of diabetes (12% vs 24%, $P < 0.0001$), higher serum levels of aspartate aminotransferase (AST) (66 ± 46 vs 75 ± 43, $P = 0.002$), γ-glutamyltransferase (GGT) (37 ± 52 vs 52 ± 33, $P < 0.0001$), total cholesterol (173 ± 32 vs 179 ± 33, $P = 0.005$), triglycerides (123 ± 56 vs 145 ± 68, $P < 0.0001$), and a lower serum level of albumin (4.2 ± 0.3 vs 4.1 ± 0.3, $P = 0.005$) and lower platelet counts (16.6 ± 5.2 vs 15.7 ± 5.1, $P = 0.007$). Histological grade of activity (A2–3: 39% vs 54%, $P < 0.0001$), and stage of fibrosis (F3–4: 18% vs 34%, $P < 0.0001$) were higher. The proportion of non-sustained virological response (SVR) to IFN also was higher (35% vs 19%, $P < 0.0001$). These results indicate that hepatic steatosis in hepatitis C is related to metabolic factors and associated with other risk factors for the development of HCC such as older age, advanced stage of fibrosis, and non-SVR to IFN therapy.

Factors associated with the development of HCC

Hepatocellular carcinoma developed in 68 patients during follow up. An overall annual incidence of HCC development was 1.19% by person-years. The annual incidence of HCC development by person-years was higher in patients with higher grade of steatosis: 0.45% for patients without steatosis, 0.78% for patients with 1–9% of steatosis, 2.30% for patients with 10–29% of steatosis, and 3.56% for patients with 30% of steatosis. The relative risk of hepatic steatosis (grade of ≥10%) for HCC development was 4.39 (95% confidence interval 2.66–7.26, $P < 0.0001$). The difference remained significant, even after stratification for other risk factors such as IFN therapy, stage of fibrosis, age, sex and BMI (Fig. 1). When analyzed by the multivariate Cox proportional hazards regression method, a higher grade of steatosis,

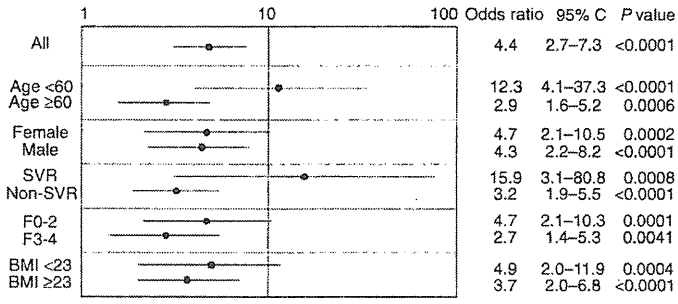


Figure 1 Relative risk differences of hepatocellular carcinoma (HCC) among patients with and without steatosis. The relative risk of hepatic steatosis (grade ≥10%) for HCC development was analyzed, after stratification for other risk factors such as interferon (IFN) therapy, stage of fibrosis, age, sex and body mass index (BMI). SVR, sustained virological response.

older age, male sex, higher BMI, an advanced stage of fibrosis and non-SVR to IFN therapy were independent risk factors associated with the development of HCC (Table 2). The adjusted risk ratio of hepatic steatosis was 3.04 (95% confidence interval 1.82–5.06, $P < 0.0001$). The presence of diabetes and consumption of ethanol were not significant. Figure 2(a) shows the Kaplan–Meier curve of the time to development of HCC in the entire cohort. The cumulative incidence of HCC was significantly higher with hepatic steatosis of 10% or greater. To adjust for other risk factors, patients were stratified according to response to IFN therapy, stage of fibrosis, age, sex and BMI. The difference remained significant, even after stratification for these confounding factors (Fig. 2b–f). Three patients died after the development of HCC. All were over 60 years old, and had significant steatosis. The impact of hepatic steatosis on the survival rate could not be analyzed due to the small number of death.

DISCUSSION

IN THIS STUDY, we have shown that the presence of significant steatosis is an independent risk factor for the development of HCC in chronic hepatitis C. Our study involved the largest number of patients, compared to previous reports, and this enabled us to adjust for other known risk factors for HCC. The impact of steatosis on HCC development remained significant even after adjusting for other risk factors such as older age, male sex, higher BMI, advanced fibrosis and non-SVR to IFN therapy. These findings indicate the need of intensive surveillance for HCC in patients with significant steatosis and provide an argument for therapeutic interventions aimed at reducing steatosis, in order to reduce the risk of HCC.

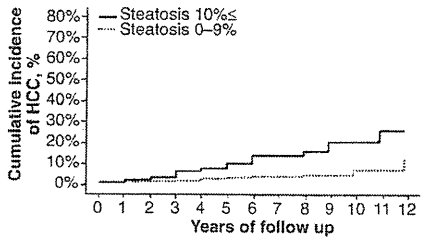
The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed and the possible mechanism has been discussed.[16] There are several cohort studies on this topic but their results are conflicting. The first report included 20 patients with SVR to IFN, 51 patients with non-SVR to IFN and 90 patients who did not receive IFN therapy.[17] In this cohort of 161 patients, older age, absence of IFN therapy, cirrhosis and steatosis were associated with HCC development. Another study involved 25 patients with HCC and an equal number of patients who did not develop HCC, matched for

Table 2 Multivariate analysis of risk factors for hepatocellular carcinoma

Predictor		Odds ratio (95% CI)	*P*-value
Age	By every 10 years	1.09 (1.05–1.13)	<0.0001
Sex	Male vs female	2.12 (1.28–3.51)	0.004
Stage of fibrosis	F3–4 vs F0–2	4.30 (2.59–7.14)	<0.0001
Grade of steatosis	≥10% vs <10%	3.04 (1.82–5.06)	<0.0001
Response to IFN	Non-SVR vs SVR	2.43 (1.13–5.23)	0.023
Diabetes	Present vs absent	0.75 (0.42–1.33)	0.319
Ethanol consumption (g/day)	≥20 vs <20	0.50 (0.07–3.60)	0.478
BMI (kg/m2)	≥23 vs <23	1.69 (1.02–2.86)	0.043

BMI, body mass index; CI, confidence interval; IFN, interferon; SVR, sustained virological response.

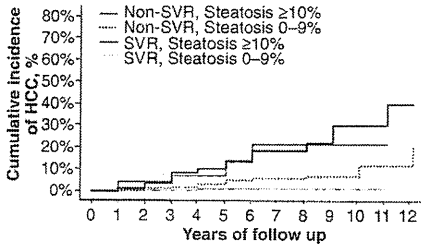
(a) **Entire cohort**



Number of patients at risk

Steatosis 0–9%	927	824	620	503	320	227	161	117	77	49	27	10
Steatosis ≥10%	352	271	207	157	113	83	54	48	32	17	9	1

(b) **Stratified by response to IFN therapy**



Number of patients at risk

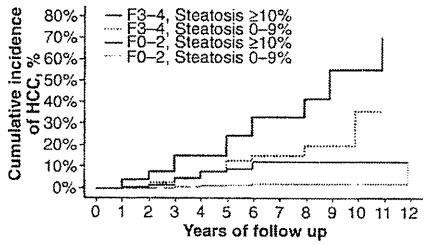
SVR

Steatosis 0–9%	326	254	204	153	81	55	33	21	15	10	5	0
Steatosis ≥10%	67	50	34	22	14	10	4	4	4	2	2	0

Non-SVR

Steatosis 0–9%	601	507	416	350	239	172	128	96	62	39	22	10
Steatosis ≥10%	285	221	173	135	99	73	50	44	28	15	7	1

(c) **Stratified by stage of fibrosis**



Number of patients at risk

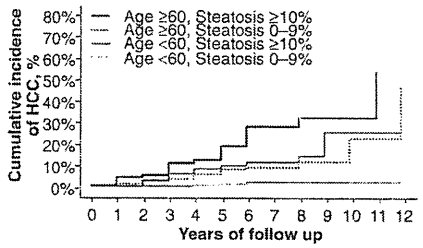
F0–2

Steatosis 0–9%	759	623	509	415	266	188	137	99	64	39	25	10
Steatosis ≥10%	234	190	146	107	77	55	37	32	19	11	6	1

F3–4

Steatosis 0–9%	118	81	61	50	36	28	17	16	13	6	3	0
Steatosis ≥10%	168	138	111	88	54	39	23	18	13	10	2	0

(d) **Stratified by age**



Number of patients at risk

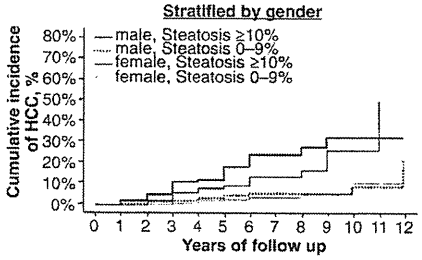
Age <60

Steatosis 0–9%	549	457	367	298	188	148	111	83	53	33	19	7
Steatosis ≥10%	193	154	111	83	61	48	34	31	23	12	6	1

Age ≥60

Steatosis 0–9%	378	304	253	205	132	79	50	34	24	16	8	3
Steatosis ≥10%	159	117	96	74	52	35	20	17	9	5	3	0

(e) **Stratified by gender**



Number of patients at risk

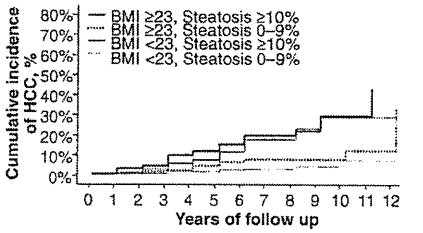
Male

Steatosis 0–9%	470	389	319	265	169	126	90	65	46	30	17	7
Steatosis ≥10%	173	134	98	73	54	40	21	21	15	8	6	1

Female

Steatosis 0–9%	457	372	301	238	151	101	71	52	31	19	10	3
Steatosis ≥10%	179	137	109	84	59	43	33	27	17	9	3	0

(f) **Stratified by BMI**



Number of patients at risk

BMI ≥23

Steatosis 0–9%	417	346	269	213	129	94	66	49	31	19	8	4
Steatosis ≥10%	226	176	137	101	71	55	34	33	20	10	5	0

BMI <23

Steatosis 0–9%	510	415	351	290	191	133	95	68	46	30	19	6
Steatosis ≥10%	126	95	70	56	42	28	20	15	12	7	4	1

Figure 2 Cumulative incidence of hepatocellular carcinoma (HCC) among patients with steatosis (solid line) and without steatosis (dotted line), stratified by other risk factors. The cumulative incidence of HCC was (a) significantly higher in patients with a steatosis grade of 10% or greater ($P < 0.0001$ by the log-rank test), even after (b) stratification by the response to interferon therapy ($P < 0.0001$ for sustained virological response [SVR] and non-SVR by the log-rank test), (c) stratification by the stage of fibrosis ($P < 0.0001$ for F0–2 and $P = 0.0036$ for F3–4 by the log-rank test), (d) stratification by age ($P = 0.0001$ for age ≥60 and $P < 0.0001$ for age <60 by the log-rank test), (e) stratification by sex ($P < 0.0001$ for men and women by the log-rank test), and (f) stratification by body mass index (BMI) ($P < 0.0001$ for BMI ≥23 kg/m^2 and <23 kg/m^2 by the log-rank test). The number of patients at risk is shown below each graph.

age, sex, HCV genotype and stage of fibrosis.[19] In this study, only ALT and albumin were identified as predictors of HCC and steatosis was not. The authors acknowledged the small size of the cohort as a limitation and emphasized the need for larger cohort studies. The third study analyzed explanted liver from cirrhotic patients who underwent liver transplantation and included 32 patients with HCC and 62 patients without HCC.[18] The authors found that older age, higher α-fetoprotein levels and steatosis were significantly associated with HCC. The major advantage of this study was the standardization of fibrosis stage to cirrhosis. On the other hand, a limitation was the retrospective nature of the study; steatosis was evaluated after the diagnosis of HCC, when cirrhosis already was present (fibrosis stage F4). Because steatosis has been reported to decrease once cirrhosis has developed, this study may have underestimated the grade of steatosis present prior to the development of HCC. Thus, we cannot simply apply their findings to a clinical setting where biopsies are usually obtained before the development of cirrhosis and years before the development of HCC. Based on that background, the principal aim of this study was to analyze the association between hepatic steatosis and the development of HCC in chronic hepatitis C patients, adjusting for known risk factors. We found that steatosis was an independent risk factor by the multivariate Cox proportional hazards regression analysis and by the Kaplan–Meier method and log-rank test after stratification by other risk factors. To our surprise, the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI.

How steatosis contributes to the development of HCC remains unclear. Several studies including ours,[10] indicated that hepatic steatosis promotes the progression of hepatic fibrosis,[11–15] which potentiates the risk of HCC indirectly. On the other hand, the ob/ob mouse model of NAFLD showed that hepatic neoplasia developed in the absence of advanced fibrosis, supporting the concept that metabolic abnormalities related to obesity initiate the neoplastic process.[8] Leptin, an adipocytokine related to steatosis in chronic hepatitis C,[21] was shown recently to be mitogenic in human liver[22] and thus may be a link between steatosis and HCC development. Otherwise, steatosis may be responsible for increased lipid peroxidation and reactive oxygen species which induce genetic damage.[23–25] Another study showed that mice transgenic for the HCV core gene developed hepatic steatosis early in life and thereafter HCC which indicates that the HCV core protein has a chief role in the development of both steatosis and HCC development.[26] The precise mechanism of the association between steatosis and carcinogenesis needs further investigation.

The higher incidence of HCC in patients with significant steatosis has important clinical implications. The most important question is whether therapeutic interventions aimed at reducing steatosis could reduce the risk of HCC in chronic hepatitis C. Because the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI, we hypothesize that modification of lifestyle and the amelioration of hepatic steatosis may efficiently prevent hepatocarcinogenesis in patients having concomitant risk factors. Apparently, further prospective studies focusing on this point are necessary. Weight reduction may provide an important treatment strategy because one study indicated that weight reduction in chronic hepatitis C leads to a reduction in steatosis and an improvement in fibrosis despite the persistence of HCV infection.[27] Alternatively, insulin resistance may be another target of therapy because a study showed that the administration of pioglitazone led to metabolic and histological improvement in subjects with non-alcoholic steatohepatitis.[28] A limitation of the present study was that data for the plasma insulin concentration was not available and thus insulin resistance could not be assessed. Whether insulin resistance plays a role in hepatocarcinogenesis or its amelioration could improve steatosis and ultimately prevent development of HCC in chronic hepatitis C awaits future investigation.

Another important finding of the present study was that steatosis was a significant risk factor for the development of HCC in patients with SVR to IFN therapy. Thus, steatosis may play a role in carcinogenesis in patients who have cleared HCV. Several studies have shown that the incidence of HCC is reduced but not eliminated in those with SVR to IFN.[29–31] Because the predictors of HCC development in SVR patients have not been established to date, steatosis may be used to identify patients who need intensive surveillance and long-term follow up, even after the clearance of HCV. In conclusion, we showed that hepatic steatosis is significantly associated with the development of HCC in chronic hepatitis C independent of age, sex, BMI, degree of fibrosis and response to previous IFN therapy. Steatosis may be a useful marker for identifying patients at higher risk for HCC. Further studies are needed to evaluate the hypothesis that therapeutic interventions aimed at reducing steatosis may prevent hepatocarcinogenesis.

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Effect of Aging on Risk for Hepatocellular Carcinoma in Chronic Hepatitis C Virus Infection

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An increase in the aging population is an impending problem. A large cohort study was carried out to determine the influence of aging and other factors on hepatocarcinogenesis in patients treated with interferon. Biopsy-proven 2547 chronic hepatitis C patients registered at our referral center since 1992 were included. Of these, 2166 were treated with interferon-based therapy. Incidences of hepatocellular carcinoma (HCC) associated with interferon were analyzed by Kaplan-Meier and person-years methods for an average follow-up of 7.5 years. Factors associated with HCC risk were determined by Cox proportional hazard analysis. HCC developed in 177 interferon-treated patients. The risk for HCC depended on age at primary biopsy and increased more than 15-fold after 65 years of age. Even when stratified by stage of fibrosis, the cumulative and annual incidences of HCC were significantly higher in older patients than in younger patients ($P < 0.001$) at the same stage of fibrosis, except for cirrhosis. Progression of fibrosis over time was significantly accelerated in older patients. The impact of viral eradication on HCC prevention was less significant in older patients than in younger patients. Multivariate analysis confirmed that age, gender, liver fibrosis, liver steatosis, total cholesterol level, fasting blood sugar level, baseline and postinterferon alpha-fetoprotein level, and virological response to interferon were independent risk factors associated with HCC. Aging was the strongest risk factor for a nonvirological response to interferon-based antiviral therapy. *Conclusion:* Elderly patients are at a higher risk for HCC. Hepatitis C viral eradication had a smaller effect on hepatocarcinogenesis in older patients. Patients should therefore be identified at an earlier age and treatment should be initiated. (HEPATOLOGY 2010;52:518-527)

Primary liver cancer is the third most common cause of cancer mortality worldwide,[1] and hepatocellular carcinoma (HCC) is one of the most frequent primary liver cancers.[2,3] Infection with hepatitis C virus (HCV) is a common cause of chronic hepatitis, which progresses to HCC in many patients.[4] The prevalence of older patients has been increasing in Japan, and this is an impending problem in other countries where viral spread has occurred more recently.[5] The number of Americans older than 65 years is expected to double by the year 2030.[6] In Western Europe, people older than 65 years already constitute 15%-18% of the population[7]; thus, aging patient who is chronically infected with HCV is

Abbreviations: AFP, alpha-fetoprotein; HBc, hepatitis B core; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; SVR, sustained virological response.

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one of the most important issues confronted by physicians.

Viral eradication with interferon-based therapy for chronic hepatitis C has been shown to prevent HCC by studies conducted in Japan and Italy.[8-11] However, this finding is controversial according to another study conducted in Europe and Canada,[12] in which viral eradication did not significantly reduce the risk for HCC in 479 consecutively treated patients. The likelihood of development of HCC among interferon-treated patients is difficult to determine because of the paucity of adequate long-term cohort studies. Moreover, in patients who are treated with interferon the effect of certain factors, including aging, on the risk for HCC remains unclear. Furthermore, the benefit of viral eradication with interferon-based therapy, including pegylated interferon and ribavirin combination therapy, in older patients remains unknown. To further clarify this, we conducted a large-scale, long-term cohort study and analyzed the influence of aging and other host and virological factors in patients treated with interferon.

Patients and Methods

Patients. Consecutive patients (n = 2547) chronically infected with HCV who underwent liver biopsy between 1992 and January 2008 at our referral center were enrolled. Of these, 2166 patients were treated with interferon-based antiviral therapy, whereas 381 patients did not receive interferon treatment (Fig. 1). All patients had histologically proven chronic hepatitis or cirrhosis. HCV infection was proven in all patients by identification of HCV RNA. Patients with a history of HCC, autoimmune hepatitis, or primary biliary cirrhosis were excluded. We also excluded patients who had a history of excessive alcohol consumption (50 g/day) and confirmed alcohol abstinence during follow-up. No patient was positive for hepatitis B surface antigen or antihuman immunodeficiency virus antibody. Written informed consent was obtained from all patients. The study was approved by the Ethical Committee of Musashino Red Cross Hospital in accordance with the Declaration of Helsinki.

Histological Evaluation. A liver biopsy specimen was obtained laparoscopically using 13G needles. When laparoscopy was impossible, ultrasound-guided liver biopsy was performed with 15G needles (n = 254). The mean length of the specimen was 18 mm (range 12-40 mm), and the mean number of portal tracts was 17 (range 8-34). Liver biopsy specimens were scored by board-certified pathologists for stage of fibrosis and grade of inflammatory activity according to the classification of Desmet et al.[13] Additional macroscopic pathological information was obtained from laparoscopic findings. The percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis. In this study, superimposed nonalcoholic steatohepatitis (NASH) was defined as a central pattern of colocalization of hepatic steatosis and hepatocyte ballooning with pericellular/perisinusoidal fibrosis or Mallory hyaline.

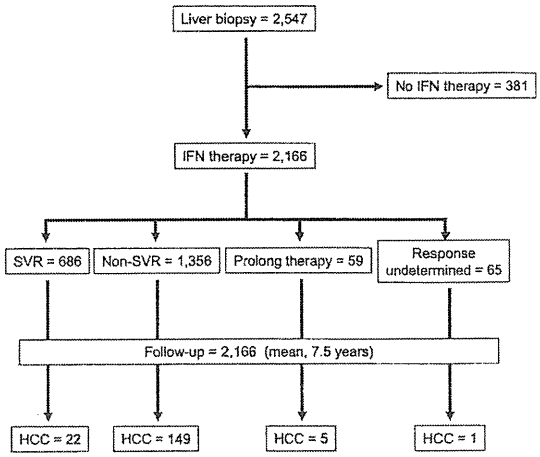


Fig. 1. Clinical outcomes of the patients enrolled in the present study. HCC, hepatocellular carcinoma; SVR, sustained virological response.

Interferon Treatment. Among the 2166 patients treated with interferon-based antiviral therapy, 1062 patients received interferon-alpha or beta monotherapy either for 24 weeks (n = 1003) or for 2 to 5 years (n = 59); 386 patients received interferon-alpha and ribavirin combination therapy for 24 weeks; 306 received pegylated interferon-alpha monotherapy for 48 weeks; and 412 received pegylated interferon-alpha and ribavirin combination therapy for 48 weeks. All interferon treatment was initiated within 48 weeks after liver biopsy.

Definitions of Response to Interferon Therapy. A patient negative for serum HCV RNA after the first 6 months of completion of interferon-based therapy was defined as a sustained viral responder. HCV RNA was determined by the qualitative Amplicor or TaqMan HCV assay (Roche Molecular Diagnostics, Tokyo, Japan).

Data Collection and Patient Follow-up. Data on patient characteristics, biochemical data, hematological

data, virological data, histological data, and treatment details were collected at enrollment. Age was determined at primary liver biopsy. Patients were examined for HCC with abdominal ultrasonography, dynamic computed tomography, and/or magnetic resonance imaging every 3-6 months. Serum alpha-fetoprotein (AFP) levels were measured every 1-2 months. This screening program constitutes the standard of care in Japan. To evaluate the effect of interferon-induced AFP reduction on hepatocarcinogenesis, the average AFP level after interferon treatment was calculated in each patient. HCC diagnosis was confirmed with needle biopsy, surgically resected specimens, or typical radiological findings diagnosed by board-certified radiologists. Figure 1 shows the schema for patient follow-up and clinical outcomes.

The start date of follow-up was the date of primary liver biopsy and the endpoint of follow-up was the development of HCC or the latest medical attendance until January 2009. The mean follow-up period was 7.5 years (range 0.5-17 years). The factors associated with development of HCC were retrospectively analyzed.

Change in Fibrosis Staging Over Time. To evaluate change in fibrosis staging over time, 271 patients who had not achieved a sustained virological response (SVR) with interferon therapy underwent a sequential biopsy after the initial biopsy. The interval between the paired biopsies was on average 4.8 years (range 0.7-14 years). The yearly rate of progression of fibrosis was calculated as the change in fibrosis staging divided by the time between paired biopsies.

Statistical Analysis. Categorical data were compared by the chi-square test and Fisher's exact test. Distributions of continuous variables were analyzed with Student's t test or the Mann-Whitney U test for two groups. All tests of significance were two-tailed and a P value of <0.05 was considered statistically significant. The cumulative incidence curve was determined with the Kaplan-Meier method and differences among groups were assessed using the log-rank test. Factors associated with HCC risk and virological response to interferon therapy were determined by the Cox proportional hazard model and logistic regression analysis, respectively. To depict the role of aging in developing risk for HCC, the multivariate Cox proportional hazard model was used after adjusting for stage of liver fibrosis, steatosis, and virological response to interferon. A polynomial regression was used to fit risk ratios for segments of the age distribution. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 11.0 (SPSS, Chicago, IL).

Results

Patient Characteristics. Patient characteristics at the time of enrollment are shown in Table 1. The distribution of stages of liver fibrosis differed between younger and older patients, indicating the need to adjust for stage of liver fibrosis when comparing the two subgroups.

Response to Interferon Therapy. The response to interferon therapy was determined in 2042 (97.2%) of the interferon-treated patients, excluding those who received prolonged interferon treatment at the endpoint. SVR rates are shown in Table 1. The percentage of patients showing SVR was significantly lower in older patients (≥65 years) than in younger patients (<65 years) ($P < 0.001$). Overall response rates to the different types of interferon therapy were as follows: interferon monotherapy, 31.5% (312/992); interferon-alpha and ribavirin combination therapy, 28.6% (108/378); pegylated interferon-alpha monotherapy, 37.9% (108/285); and pegylated interferon-alpha and ribavirin combination therapy, 41.1% (159/387). Response rates in genotype-1 patients (n = 1347) were 20.6% (114/554), 17.9% (29/162), 18.9% (56/297), and 36.8% (123/334), and those in nongenotype-1 patients (n = 565) were 52.2% (163/312), 63.1% (77/122), 65.0% (52/80), and 70.6% (36/51). Overall response rates of interferon and pegylated interferon monotherapy seem to be high because of the high response rates in the nongenotype-1 patients treated with these regimens.

Overall Cumulative Incidence of HCC. During follow-up, HCC developed in 177 interferon-treated patients (Fig. 1). The cumulative incidence of HCC 5, 10, and 15 years after interferon therapy was 4.7%, 11.6%, and 15.5%, respectively. The cumulative incidence in SVR patients was 2.1%, 4.3%, and 4.3%, respectively, which was significantly lower than that in non-SVR patients (5.8%, 14.9%, and 20.2%, respectively; log-rank test, $P < 0.001$).

Effect of Aging on Risk for HCC. The risk ratio determined by multivariate Cox proportional hazards analysis after adjustment for stage of liver fibrosis, degree of liver steatosis, and virological response to interferon demonstrated that the risk for HCC after interferon treatment was age-dependent and increased predominantly when the age at primary liver biopsy was >65 years (Fig. 2A). Hence, we defined older patients as those ≥65 years of age at primary liver biopsy and younger patients as those aged <65 years. As shown in Fig. 2B, the cumulative incidence of HCC was significantly higher in older patients than in younger patients (log-rank test, $P < 0.001$).

Table 1. Characteristics of Patients Enrolled in the Present Study

Characteristics	Total	<65 year	≥65 year	*P* Value*
Patients, n	2166	1614	552	
Sex, n (%)				<0.001†
Male	1080 (49.9)	840 (52.0)	240 (43.6)	
Female	1086 (50.1)	774 (48.0)	312 (56.4)	
Age (SD), year	55.4 (12.1)	51.1 (10.8)	68.4 (2.9)	<0.001‡
BMI (SD), kg/m^2	23.3 (3.1)	23.4 (3.0)	23.3 (3.1)	0.9‡
Fibrosis stage, n (%)				<0.001†
F0	27 (1.3)	24 (1.5)	3 (0.5)	
F1	860 (39.7)	704 (43.6)	156 (28.2)	
F2	733 (33.8)	515 (31.9)	218 (39.5)	
F3	444 (20.5)	301 (18.6)	143 (25.9)	
F4	102 (4.7)	70 (4.3)	32 (5.8)	
%Severe steatosis (≥10%)	27.6	27.1	29.3	0.4†
ALT level (SD), IU/L	95 (18)	101 (119)	76 (58)	<0.001‡
HCV load (SD), KIU/mL	880 (1046)	861 (1016)	924 (1116)	0.2‡
HCV genotype, n (%)				<0.001†
1a	7 (0.3)	5 (0.3)	2 (0.4)	
1b	1414 (69.6)	1036 (68.9)	378 (71.3)	
2a	373 (18.3)	273 (18.2)	100 (18.9)	
2b	211 (10.4)	164 (10.9)	47 (8.9)	
Others	28 (1.4)	25 (1.7)	3 (0.6)	
Duration (SD), year	7.5 (4.4)	8.1 (4.4)	5.8 (3.7)	<0.001‡
IFN regimen, n (%)				<0.001†
IFN mono	1062 (49.0)	833 (51.6)	229 (41.5)	
PEG-IFN mono	306 (14.1)	200 (12.4)	106 (19.2)	
IFN + RBV	386 (17.8)	291 (18.0)	95 (17.2)	
PEG-IFN + RBV	412 (19.0)	290 (18.0)	122 (22.1)	
SVR, n (%)	686 (33.6)§	565 (36.6)‖	121 (24.3)¶	<0.001‡

Unless otherwise indicated, data are given as the mean (SD).

ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; IFN, interferon; N/A, not applicable; PEG, pegylated; RBV, ribavirin; SVR, sustained virological response.

*Comparison between <65 years and ≥65 years.

†Chi-squared test.

‡Student *t* test.

§Virological responses were determined in 2042 patients.

‖Virological responses were determined in 1545 patients.

¶Virological responses were determined in 497 patients.

As shown in Fig. 2C-E, even when stratified by stage of fibrosis the cumulative incidences among patients at stages F0/F1, F2, and F3 were significantly greater in older patients than in younger patients (log-rank test, $P < 0.001$). These differences were not significant among patients with cirrhosis (Fig. 2F, log-rank test, $P = 0.7$).

The annual incidence of HCC after interferon treatment was calculated by the person-years method (Table 2); it increased with the degree of liver fibrosis from 0.2% (F0 or F1) to 4.6% (F4) and was higher among older patients at the same stage of liver fibrosis.

Among the 177 patients with HCC, 92 showed evidence of a single blood transfusion. We analyzed the relationship between duration of infection and age in these 92 patients. A significant and strong negative correlation was found between the interval from blood transfusion to development of HCC and the age of the patients at the time of blood transfusion ($r = -0.74$, $P < 0.001$) (Fig. 3A). The mean duration of chronic infection was 22.0 years in patients who had received blood transfusion at >40 years of age, which was significantly shorter than that in patients who received it at ≤40 years of age (40.6 years, $P < 0.001$).

The presence of cirrhosis at the time of development of HCC, which was defined as having any of the following criteria, was evaluated: (1) histological evidence for cirrhosis, (2) findings of cirrhosis in any radiological study, or (3) presence of marked portal hypertension (i.e., presence of esophagogastric varices). Following this, 142 of the 177 with HCC (80.2%) were diagnosed as having cirrhosis, of which 42 were diagnosed histologically, 69 radiologically, and 31 based on the presence of marked portal hypertension. No significant difference was found in the proportion of patients with cirrhosis between older and younger patients, at the rate of 78.3% (94/120) in older

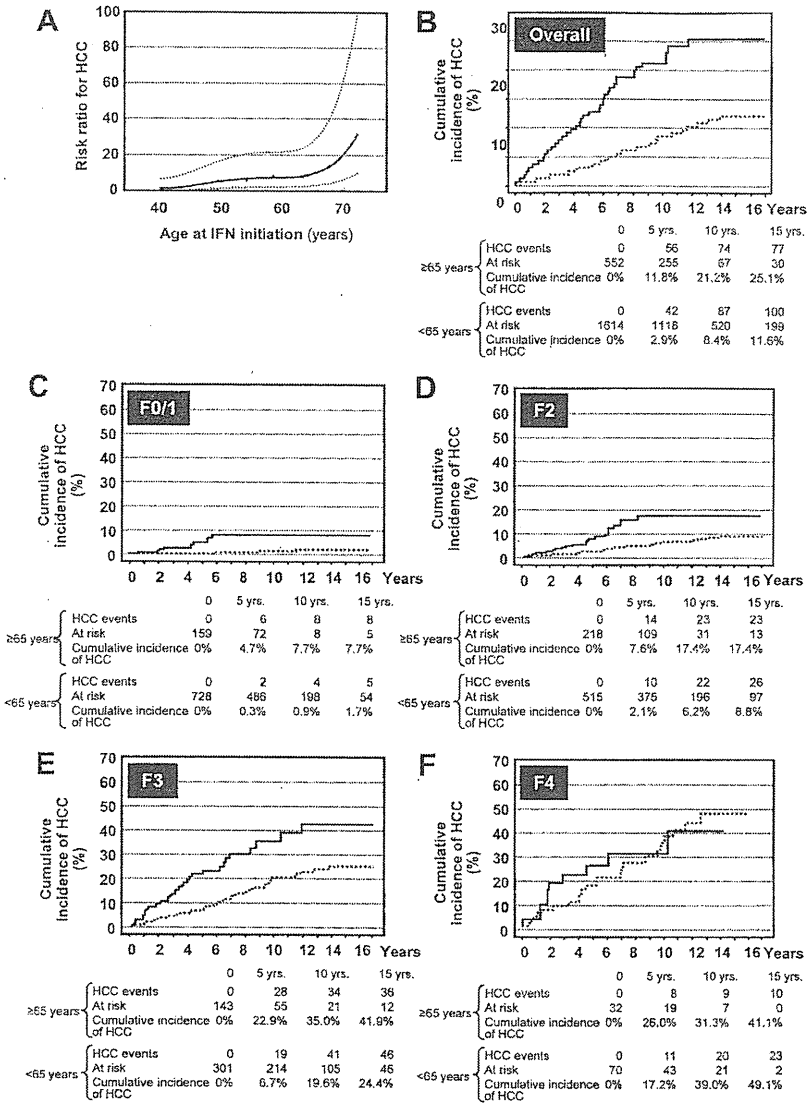


Fig. 2. Effect of aging on the risk for HCC. (A) Risk ratio (solid line) and 95% CI (dotted lines) for the risk of HCC according to age. To show the age-dependent relationship, a multivariate Cox proportional hazard model was used after adjustment for gender, stage of liver fibrosis, body mass index, and virological response to interferon therapy. Curves were fitted using polynomial regression. (B-F) Cumulative incidence of HCC after interferon therapy among younger (<65 years, n = 552, dotted line) and older patients (≥65 years, n = 1614, solid line). (B) Overall data, $P < 0.001$. (C) Patients with stage F0 or F1 liver fibrosis (no or mild fibrosis with portal expansion), $P < 0.001$. (D) Patients with stage F2 liver fibrosis (bridging fibrosis without architectural distortion), $P < 0.001$. (E) Patients with stage F3 liver fibrosis (bridging fibrosis with architectural distortion), $P < 0.001$. (F) Patients with stage F4 liver fibrosis (cirrhosis), $P = 0.7$. All P values were obtained by the log-rank test. The numbers of HCC events and patients at risk at each timepoint are shown below the graphs.

patients and 84.2% (48/57) in younger patients ($P = 0.36$, comparison at the age of HCC development).

Influence of Aging on Progression in Fibrosis Staging Over Time. In 271 patients who underwent paired biopsies, fibrosis staging progressed in 69 patients (25.5%), remained unchanged in 154 (56.8%), and regressed in 48 patients (17.7%). The overall rate of progression of fibrosis in these patients was 0.06 ± 0.02 fibrosis stages per year. Progression of fibrosis over time was significantly accelerated in older patients than in younger patients (0.21 ± 0.10 versus 0.03 ± 0.21 fibrosis stages per year, $P = 0.03$, Mann-Whitney U test) (Fig. 3B).

Effect of Viral Eradication on Risk for HCC in Older Patients. As shown in Fig. 4, the effect of viral eradication on the prevention of HCC was less significant in older patients than in younger patients. The annual incidence was higher among older patients than among younger patients with the same virological response (Table 2).

Influence of Liver Steatosis on Risk for HCC. The cumulative incidence of HCC after interferon therapy was significantly higher in patients with severe steatosis (≥10%) than in those with milder steatosis (at 5, 10, and 15 years: 8.6%, 19.1%, 32.0% versus 1.8%, 4.8%, 7.0%, respectively, log-rank test, $P < 0.001$).

Table 2. Annual Incidence of HCC After IFN Treatment

Factors	Total	<65 Years	≥65 Years
Fibrosis stage			
F0/F1	0.2%	0.1%	0.9%
F2	0.8%	0.6%	1.7%
F3	2.5%	1.8%	4.6%
F4	4.6%	4.4%	5.1%
Total	1.1%	0.8%	2.4%
Degree of liver steatosis			
<10%	0.5%	0.2%	1.4%
≥10%	2.0%	1.8%	3.0%
Virological response			
SVR	0.4%	0.2%	1.3%
Non-SVR	1.4%	1.0%	2.9%

Data were calculated by the person-years method. IFN, interferon; SVR, sustained virological response.

The annual incidence was higher in older patients than in younger patients with the same degree of liver steatosis (Table 2). In patients with severe steatosis (≥10%), superimposed NASH was diagnosed in 6.0% (26/435). Overall, superimposed NASH was significantly associated with hepatocarcinogenesis on univariate analysis (risk ratio, 4.1; 95% confidence interval [CI], 1.8-9.4; $P < 0.001$), but not on multivariate analysis. Superimposed NASH was significantly associated with high body mass index (27.2 ± 4.6 kg/m^2 versus 23.0 ± 3.1 kg/m^2, $P < 0.001$), hyperglycemia (186 ± 67 mg/dL versus 115 ± 39 mg/dL, $P < 0.001$), and advanced fibrosis (F3) (risk ratio, 2.9; 95% CI, 1.4-6.0; $P = 0.005$).

Factors Associated with Hepatocarcinogenesis After Interferon Therapy. Univariate analysis demonstrated factors that increase the risk ratio for the development of HCC (Table 3). Multivariate analysis using Cox proportional hazards regression confirmed that aging was one of the most significant independent factors associated with the development of HCC after interferon therapy. In this analysis, advanced fibrosis, presence of steatosis, male gender, lower total cholesterol level, higher fasting blood sugar level, higher baseline AFP level, insignificant improvement of mean AFP level after interferon therapy, and nonresponse to interferon therapy were also significantly associated with risk for HCC (Table 3).

We identified 22 patients in whom HCC developed even after achieving SVR. Univariate and multivariate logistic regression analyses indicated that both liver steatosis and aging were independently associated with the development of HCC among patients who achieved SVR (n = 686) (Table 4). Anti-HBc was detected in only 4 out of 22 patients and the age distribution was similar among anti-HBc-positive and anti-HBc-negative patients.

Response to Interferon Therapy in Older Patients. Multivariate logistic regression analysis confirmed that aging, female gender, severe liver fibrosis, extremely severe liver steatosis, genotype-1, high HCV load, and nonuse of pegylated interferon and ribavirin were independent risk factors for non-SVR (Supporting Table 1). The odds ratio, determined by multivariate logistic regression analysis after adjustment for these factors, demonstrated that the risk for non-SVR was age-dependent (Supporting Fig. 1). It was also ≈2.5 times higher in patients aged ≥65 years than in those aged <35 years.

In patients with genotype-1b and a high viral load who were treated with pegylated interferon and ribavirin combination therapy, the SVR rate was significantly lower in older patients than in younger patients (<49 years, 59.3%; 50-59 years, 50.5%; 60-65 years, 27.3%; ≥65 years, 25.2%; intention-to-treat analysis). Multivariate logistic regression analysis showed that

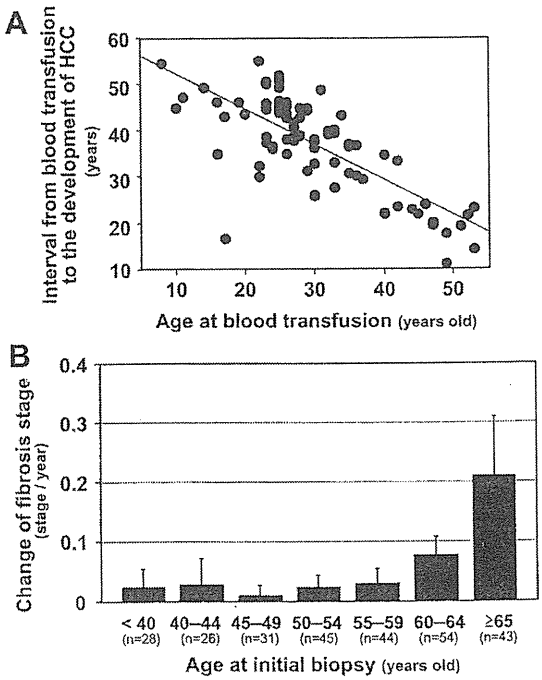
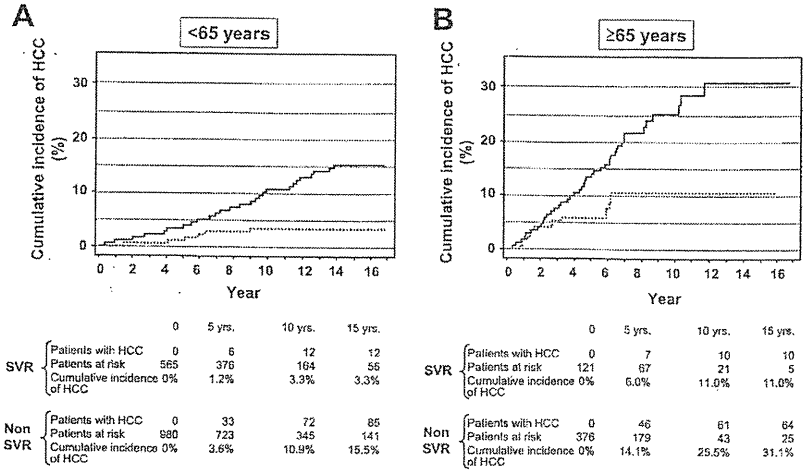


Fig. 3. (A) Relationship between the interval from blood transfusion to development of HCC and the age at blood transfusion (n = 92). A significant and strong negative correlation was observed ($r = -0.74$, $P < 0.001$). (B) Change in fibrosis staging over time. A total of 271 patients who had not achieved SVR by interferon therapy underwent a sequential biopsy after the initial biopsy. The yearly rate of progression of fibrosis was calculated as the change in fibrosis stage divided by the time between the paired biopsies. The yearly rate of progression of fibrosis was significantly higher in older patients (≥65 years) than in younger patients (<65 years) ($P = 0.03$, Mann-Whitney U test).



		0	5 yrs.	10 yrs.	15 yrs.
SVR	Patients with HCC	0	6	12	12
	Patients at risk	565	376	164	56
	Cumulative incidence of HCC	0%	1.2%	3.3%	3.3%
Non SVR	Patients with HCC	0	33	72	85
	Patients at risk	980	723	345	141
	Cumulative incidence of HCC	0%	3.6%	10.9%	15.5%

		0	5 yrs.	10 yrs.	15 yrs.
SVR	Patients with HCC	0	7	10	10
	Patients at risk	121	67	21	5
	Cumulative incidence of HCC	0%	6.0%	11.0%	11.0%
Non SVR	Patients with HCC	0	46	61	64
	Patients at risk	376	179	43	25
	Cumulative incidence of HCC	0%	14.1%	25.5%	31.1%

Fig. 4. Cumulative incidence of HCC after interferon therapy among SVRs (dotted lines) and non-SVRs (solid lines) according to age. (A) Younger patients (<65 years). The cumulative incidence of HCC was significantly higher in SVR than in non-SVR (log-rank test, $P < 0.001$). (B) Older patients (≥65 years). The cumulative incidence of HCC was significantly higher in SVR than in non-SVR (log-rank test, $P = 0.02$). However, the difference between SVR and non-SVR was less in older patients than in younger patients. The number of HCC events and patients at risk at each timepoint are shown below the graphs.

aging was the strongest independent factor contributing to SVR in these patients (data not shown). The odds ratio for the risk of non-SVR was 1.8 for each additional 10 years of age (95% CI, 1.5-2.3, $P < 0.001$).

Discussion

In this large cohort study we demonstrated that aging is significantly associated with the development of HCC in patients treated with interferon. The risk ratio increased predominantly in patients older than 65 years, which was more than 15 times that in patients in their 20s. Aging is becoming the most critical risk factor for the development of HCC. Although liver fibrosis was also an important risk factor, we clearly demonstrated that the risk for hepatocarcinogenesis after interferon treatment was significantly higher in older patients at each stage of liver fibrosis except for cirrhosis. Hence, physicians should be aware that older patients can develop HCC regardless of the stage of fibrosis.

Because the present study included a large cohort, it was difficult to determine the duration of infection in all patients, and this might have affected the risk determination for HCC development. Therefore, we analyzed the relationship between duration of chronic infection and HCC development in patients who underwent a single blood transfusion. We found a significant and strong negative correlation between the interval from blood transfusion to development of HCC and the age of the patients at the time of blood transfusion. Consistent with our results, a previous report with posttransfusion HCV demonstrated that the age of patients, rather than the duration of HCV infection, was more significant for HCC development.[14-16] Therefore, older age and not duration of infection is more likely to influence hepatocarcinogenesis. Moreover, our analysis of sequential biopsy specimens demonstrated that the progression rate of liver fibrosis significantly accelerated in patients aged >65 years. Hence, the progression of fibrosis along with aging may also contribute to the increased risk for hepatocarcinogenesis in older patients.

We further demonstrated that liver steatosis was an independent risk factor for the development of HCC, which was not mentioned in previous reports.[8-11] The presence of steatosis is related to both viral (genotype-3 or HCV core protein) and host metabolic factors.[17,18] In our cohort, most superimposed NASH was associated with host metabolic factors such as high body mass index and hyperglycemia, whereas infection of genotype-3 was only noted in two patients. In vitro experiments have suggested an association between liver steatosis induced by HCV core protein and hepatocarcinogenesis,[19] and have proposed virus-associated steatohepatitis as a new aspect of chronic hepatitis C.[20,21] Because steatosis was likely to be related to hepatocarcinogenesis, patients with chronic hepatitis C, whose liver histology shows superimposed NASH,