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 population7; thus, aging patient who is chronically infected with HCV is...
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.\textsuperscript{8-11} However, this finding is controversial according to another study conducted in Europe and Canada,\textsuperscript{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.\textsuperscript{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.

**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).
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 esophageal 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

### 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 (%)               |             |          |          |               |
| Male                     | 1080 (49.9) | 840 (52.0)| 240 (43.6)| <0.001†       |
| Female                   | 1086 (50.1) | 774 (48.0)| 312 (56.4)|               |
| Age (SD), year           | 55.4 (12.1) | 774 (10.8)| 68.4 (2.9) | <0.001†       |
| BMI (SD), kg/m²           | 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.
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).
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 ($\geq 10\%$), 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 $\pm$ 4.6 kg/m$^2$ versus 23.0 $\pm$ 3.1 kg/m$^2$, $P < 0.001$), hyperglycemia (186 $\pm$ 67 mg/dL versus 115 $\pm$ 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 $\approx 2.5$ times higher in patients aged $\geq 65$ 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%; $\geq 65$ years, 25.2%; intention-to-treat analysis). Multivariate logistic regression analysis showed that

| Table 2. Annual Incidence of HCC After IFN Treatment |
|---------------------------------|-----------------|-----------------|-----------------|
| **Factors** | **Total** | **<65 Years** | **$\geq 65$ 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% |
| $\geq 10\%$ | 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.
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. 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. The presence of steatosis is related to both viral (genotype-3 or HCV core protein) and host metabolic factors. 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, and have proposed virus-associated steatohepatitis as a new aspect of chronic hepatitis C. Because steatosis was likely to be related to hepatocarcinogenesis, patients with chronic hepatitis C, whose liver histology shows superimposed NASH,
may be at a higher risk of developing HCC. Further study is necessary to confirm this association in a clinical situation. Because several developed countries are in the midst of a growing obesity epidemic, the risk related to obesity cannot be ignored in patients with chronic hepatitis C who are treated with interferon.

Several retrospective cohort studies have been conducted to evaluate the effect of interferon on the incidence of HCC among patients with chronic hepatitis C.8-11 Our results, obtained from one of the largest cohort studies, confirm the efficacy of viral eradication in preventing HCC. In one study conducted in a Western population, no statistically significant reduction was found in the development of HCC among patients with SVR compared with those without SVR (adjusted hazard ratio, 0.46; 95% CI, 0.12-1.70; \( P = 0.25 \)).12 Because relatively few occurrences of HCC were observed in this cohort, and the duration of follow-up was shorter, the differences in HCC development between patients with and without SVR might be less pronounced.

Interestingly, our results demonstrated that the risk for HCC remains even after achieving SVR in older patients, confirming the findings of previous studies conducted with a smaller number of patients.22,23 The cumulative incidence of HCC during the first 5 years

| Table 3. Factors Associated with HCC After IFN Therapy |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| Risk Factor Value              | Univariate Analysis |              | Multivariate Analysis |              |              |
| Age (by every 10 year)          | 2.2 (1.8-2.7)     | <0.001        | 3.0 (1.9-4.8)     | <0.001        |
| Sex                             | <              |               | 1               |               |
| Female                          | 1              |               | 3.0 (1.9-4.8)     | <0.001        |
| Male                            | 1.2 (0.9-1.6)    | 0.2           | 2.0 (1.0-3.8)     | 0.04          |
| BMI (by every 10 kg/m²)         | 2.0 (1.2-1.3)    | 0.005         | 1.1 (0.4-3.5)     | 0.8           |
| Fibrosis stage                  | <              |               | 1               |               |
| F0/F1/F2                        | 5.4 (3.9-7.5)    | <0.001        | 2.5 (1.2-4.9)     | 0.01          |
| Degree of steatosis             | >              |               | 1               |               |
| <10%                            | 4.5 (3.0-6.9)    | <0.001        | 3.5 (1.9-6.4)     | <0.001        |
| ≥10%                            | 1              |               | 1               |               |
| Esophagogastric varices         | No             | 1             | 1               |               |
| Yes                             | 3.3 (2.0-5.3)    | <0.001        | 1.6 (0.6-4.4)     | 0.3           |
| Virological response            | SVR            | 1             | 1               |               |
| Non-SVR                         | 3.3 (2.1-5.2)    | <0.001        | 2.6 (1.2-5.5)     | 0.001         |
| Genotype                        | Non-1          | 1             | 1               |               |
| 1                               | 1.7 (1.2-2.5)    | 0.006         | 1.0 (0.5-2.3)     | 0.9           |
| Albumin (by every 1 g/dL)       | 0.2 (0.1-0.3)    | <0.001        | 0.6 (0.2-2.2)     | 0.3           |
| ALT (by every 100 IU/L)         | 1.0 (0.9-1.0)    | 0.8           | 0.4 (0.1-1.8)     | 0.6           |
| AST (by every 100 IU/L)         | 1.2 (1.1-1.3)    | 0.001         | 1.1 (0.6-1.8)     | 0.8           |
| γ-GTP (by every 100 IU/L)       | 1.3 (1.1-1.6)    | 0.009         | 0.6 (0.3-1.6)     | 0.3           |
| ALP (by every 100 IU/L)         | 1.3 (1.2-1.5)    | <0.001        | 0.6 (0.4-1.2)     | 0.2           |
| Total bilirubin (by every 1 mg/dL) | 1.6 (1.3-2.1)    | <0.001        | 1.2 (0.6-2.7)     | 0.6           |
| Total cholesterol (by every 100 mg/dL) | 0.3 (0.2-0.6)    | <0.001        | 0.2 (0.1-0.6)     | 0.006         |
| Triglyceride (by every 100 mg/dL) | 0.8 (0.5-1.1)    | 0.2           | 0.1 (0.02-1.1)    | 0.08          |
| Fasting blood sugar (by every 100 mg/dL) | 1.8 (1.5-2.2)    | <0.001        | 1.1 (1.0-1.1)     | 0.04          |
| WBC (by every 1000/µL)          | 0.1 (0.03-0.3)   | <0.001        | 0.1 (0.01-2.2)    | 0.2           |
| RBC (by every 10³/µL)           | 0.5 (0.4-0.7)    | <0.001        | 1.8 (0.7-4.4)     | 0.2           |
| Platelet counts (by every 10⁹/µL) | 0.3 (0.2-0.4)    | <0.001        | 0.6 (0.3-1.5)     | 0.3           |
| Baseline AFP (by every 10 mg/mL) | 1.0 (0.9-1.1)    | 0.2           | 1.3 (1.0-1.7)     | 0.04          |
| Post IFN AFP (by every 10 mg/mL) | 1.2 (1.1-1.3)    | <0.001        | 1.9 (1.5-2.4)     | <0.001        |
| HDV load (by every 100 KIU/mL)  | 1.0 (0.9-1.0)    | 0.4           | 1.0 (1.0-1.1)     | 0.08          |
| IFN regimen                     | IFN monotherapy | 1             | 1               |               |
| IFN + RBV (24 W)                | 1.2 (0.8-1.8)    | 0.4           | 1.5 (0.7-3.2)     | 0.3           |
| PEG-IFN monotherapy (48 W)      | 1.1 (0.6-1.9)    | 0.8           | 1.5 (0.4-5.5)     | 0.6           |
| PEG-IFN + RBV                    | 0.4 (0.2-0.9)    | 0.03          | 1.0 (0.3-3.1)     | 0.9           |

Risk ratios for development of HCC were calculated by Cox proportional hazards regression analysis. AFP, alpha fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γ-GTP, gamma-glutamyltranspeptidase; HCC, hepatocellular carcinoma; IFN, interferon; PEG, pegylated; RBC, red blood cell counts; RBV, ribavirin; SVR, sustained virological response; WBC, white blood cell count.
after completion of interferon therapy was similar between SVR and non-SVR patients in the older age group, and the risk for HCC remained for 9 years after eradication of HCV in our patients. Therefore, HCC patients with SVR who have a risk factor should be screened for at least 5-10 years after the completion of interferon therapy.

It has been reported that coffee consumption has a protective effect against hepatocarcinogenesis and liver disease progression in patients with chronic HCV infection. Because we could not review coffee consumption in all the patients and fewer data were available in the previous literature as to whether a habitual change in older patients affects the increased risk for hepatocarcinogenesis in older patients.

Recently, it was reported that interferon therapy might be less effective in preventing HCC among patients with chronic hepatitis C who are positive for anti-HBc antibody, but this finding is still controversial. In the present study, anti-HBc was only detected in 4 of 22 patients in whom HCC developed after viral eradication, and age distribution was similar among anti-HBc-positive and anti-HBc-negative patients. Because no significant difference in mean age was found between anti-HBc-positive and anti-HBc-negative patients in the recent study conducted in Japan, it is unlikely that previous exposure to hepatitis B virus or occult hepatitis B virus infection is responsible for the difference in risk for HCC between younger and elderly patients found in the present study.

In conclusion, aging has become one of the most important risk factors for HCC. Even after stratification by stage of fibrosis, the risk for HCC after antiviral treatment was significantly higher in older patients, and HCV eradication had a smaller effect on HCC-free survival in older patients. Patients with HCV should therefore be identified at an earlier age and antiviral treatment should be initiated. The present results have potentially important clinical implications for physicians that may influence their decisions about the treatment strategy in individual patients.

Table 4. Factors Associated with Development of HCC After Achieving SVR

| Risk Factor                           | Odds Ratio (95% CI) | P-value |
|---------------------------------------|--------------------|--------|
| **Univariate analysis**               |                    |        |
| Age (by every 10 year)                | 3.2 (1.8-5.5)      | <0.001 |
| Sex                                   |                    |        |
| Female                                | 1                  |        |
| Male                                  | 3.0 (1.0-8.8)      | 0.04   |
| Fibrosis stage                        |                    |        |
| F0/F1/F2                              | 1                  |        |
| F3/F4                                 | 5.9 (2.5-14.0)     | <0.001 |
| Degree of steatosis                   |                    |        |
| <10%                                  | 1                  |        |
| ≥10%                                  | 5.5 (2.0-15.2)     | 0.001  |
| BMI (by every 10 kg/m²)               | 3.2 (0.8-12.6)     | 0.09   |
| ALT (by every 10 IU/L)                | 0.9 (0.7-1.3)      | 0.7    |
| AST (by every 10 IU/L)                | 1.1 (0.9-1.4)      | 0.3    |
| Genotype                              |                    |        |
| Non-1                                 | 1                  |        |
| 10%                                   | 1.2 (0.6-3.0)      | 0.5    |
| HCV load (by every 100 KIU/mL)        | 0.9 (0.8-1.0)      | 0.2    |
| IFN regimen                           |                    |        |
| IFN monotherapy                       | 1                  |        |
| IFN + RBV (24 W)                      | 0.7 (0.2-2.3)      | 0.5    |
| PEG-IFN monotherapy (48 W)            | 0.8 (0.2-3.6)      | 0.8    |
| PEG-IFN + RBV                         | 0.3 (0.3-2.0)      | 0.2    |
| **Multivariate analysis**             |                    |        |
| Age (by every 10 year)                | 2.7 (1.5-5.1)      | 0.002  |
| Sex                                   |                    |        |
| Female                                | 1                  |        |
| Male                                  | 4.1 (0.9-18.9)     | 0.06   |
| Fibrosis stage                        |                    |        |
| F0/F1/F2                              | 1                  |        |
| F3/F4                                 | 2.6 (0.9-7.5)      | 0.08   |
| Degree of steatosis                   |                    |        |
| <10%                                  | 1                  |        |
| ≥10%                                  | 5.6 (1.9-16.5)     | 0.002  |

Odds ratios for SVR were calculated by logistic regression analysis.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HCV, hepatitis C virus; IFN, interferon; HCC, hepatocellular carcinoma; PEG, pegylated; RBV, ribavirin; SVR, sustained virological response.

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