

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

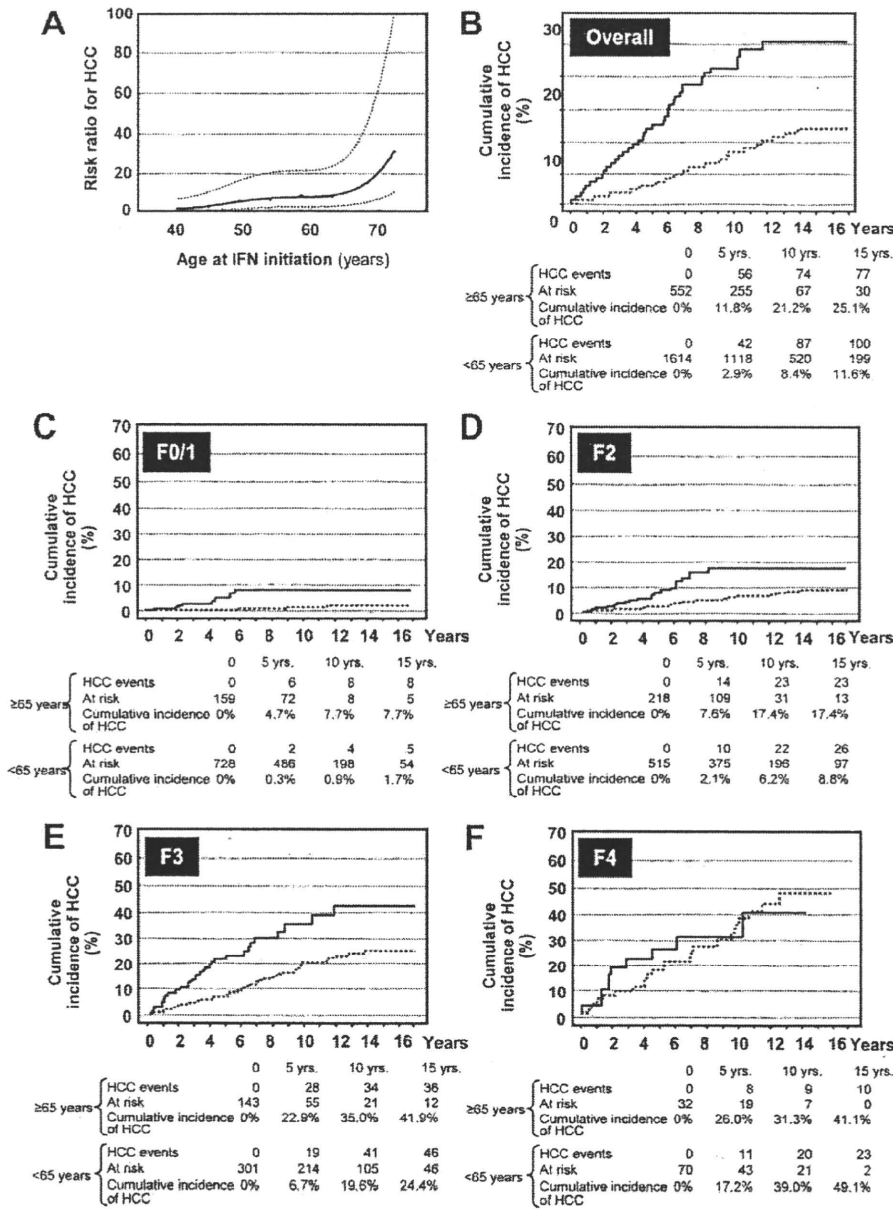


Fig. 2. Effect of aging on the risk for HCC. (A) Risk ratio (solid line) and 95% CI (dotted lines) for the risk of HCC according to age. To show the age-dependent relationship, a multivariate Cox proportional hazard model was used after adjustment for gender, stage of liver fibrosis, body mass index, 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/m² versus 23.0 ± 3.1 kg/m², $P < 0.001$), 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

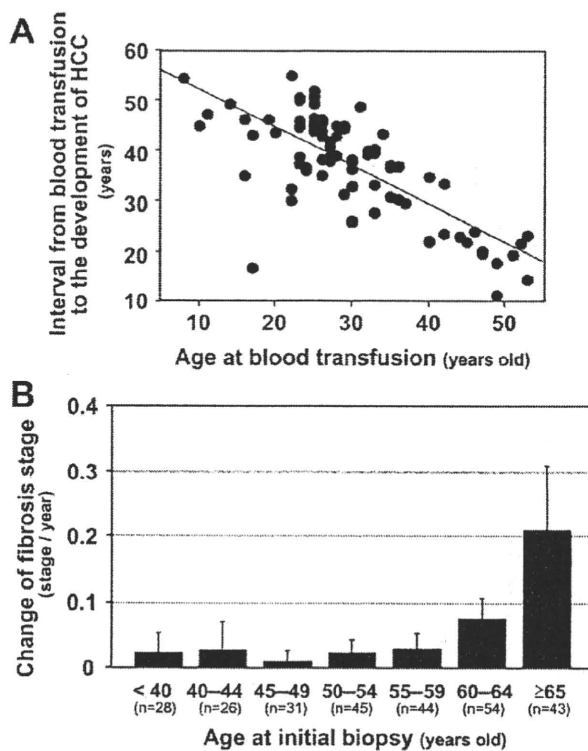


Fig. 3. (A) Relationship between the interval from blood transfusion to development of HCC and the age at blood transfusion ($n = 92$). A significant and strong negative correlation was observed ($r = -0.74$, $P < 0.001$). (B) Change in fibrosis staging over time. A total of 271 patients who had not 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).

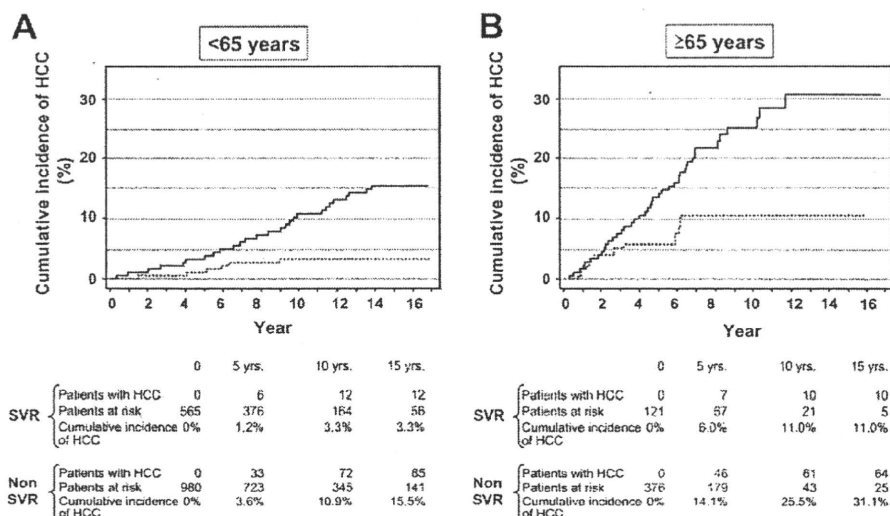


Fig. 4. Cumulative incidence of HCC after interferon therapy among SVRs (dotted lines) and non-SVRs (solid lines) according to age. (A) Younger patients (<65 years). The cumulative incidence of HCC was significantly higher in SVR than in non-SVR (log-rank test, $P < 0.001$). (B) Older patients (≥ 65 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,

Table 3. Factors Associated with HCC After IFN Therapy

Risk Factor Value	Univariate Analysis		Multivariate Analysis	
	Risk Ratio (95% CI)	P Value	Risk Ratio (95% CI)	P Value
Age (by every 10 year)	2.2 (1.8-2.7)	<0.001	3.0 (1.9-4.8)	<0.001
Sex				
Female	1		1	
Male	1.2 (0.9-1.6)	0.2	2.0 (1.0-3.8)	0.04
BMI (by every 10 kg/m ²)	2.0 (1.2-1.3)	0.005	1.1 (0.4-3.5)	0.8
Fibrosis stage				
F0/F1/F2	1		1	
F3/F4	5.4 (3.9-7.5)	<0.001	2.5 (1.2-4.9)	0.01
Degree of steatosis				
<10%	1		1	
≥10%	4.5 (3.0-6.9)	<0.001	3.5 (1.9-6.4)	<0.001
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 (0.3-1.2)	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 100/ μ L)	0.1 (0.03-0.3)	<0.001	0.1 (0.01-2.2)	0.2
RBC (by every 10 ⁶ / μ L)	0.5 (0.4-0.7)	<0.001	1.8 (0.7-4.4)	0.2
Platelet counts (by every 10 ⁶ / μ L)	0.3 (0.2-0.4)	<0.001	0.6 (0.3-1.5)	0.3
Baseline AFP (by every 10 ng/mL)	1.0 (0.9-1.1)	0.2	1.3 (1.0-1.7)	0.04
Post IFN AFP (by every 10 ng/mL)	1.2 (1.1-1.3)	<0.001	1.9 (1.5-2.4)	<0.001
HCV load (by every 100 KU/mL)	1.0 (0.9-1.0)	0.4	1.0 (1.0-1.1)	0.06
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.

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.⁶⁻¹¹ 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 reduc-

tion 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 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/m ²)	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	
1	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 (0.03-2.0)	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.

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^{24,25} and liver disease progression in patients with chronic HCV infection.²⁶ 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 of reducing coffee consumption occurs in older patients, it is unclear whether increased risk for HCC in older patients is an effect of this habitual change in older patients. However, the majority (68%) of Japa-

nese patients who have HCV (n = 1058) drink less than 1 cup of coffee per day, and only 7.6% consume more than 3 cups of coffee per day.²⁷ Therefore, it is unlikely that 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,²⁸ but this finding is still controversial.^{29,30} 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,²⁸ 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.

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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 ours¹⁰ 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 proposed¹⁶ 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

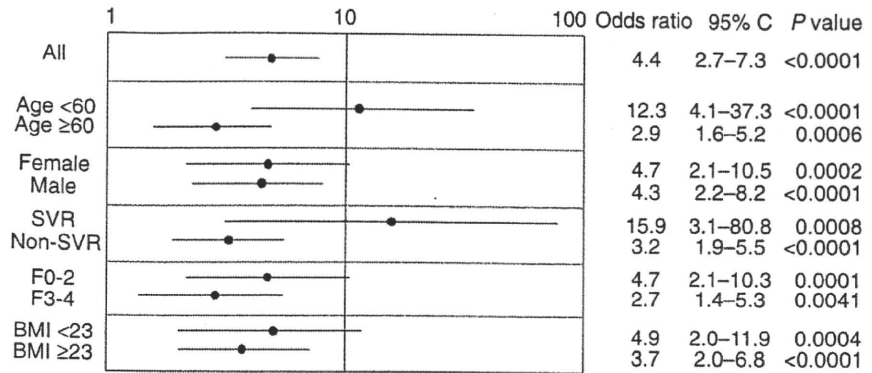
Background factors for steatosis

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 $\geq 10\%$) 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.

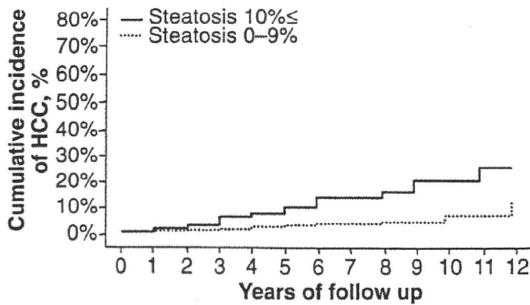
The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed and the possible mechanism has been discussed.¹⁶ There are several cohort studies on this topic but their results are conflicting. The first report included 20 patients with SVR to IFN, 51 patients 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	$\geq 10\%$ 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/m ²)	≥ 23 vs <23	1.69 (1.02-2.86)	0.043

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

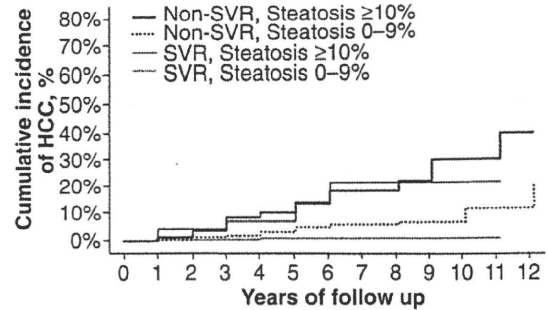
(a) **Entire cohort**



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

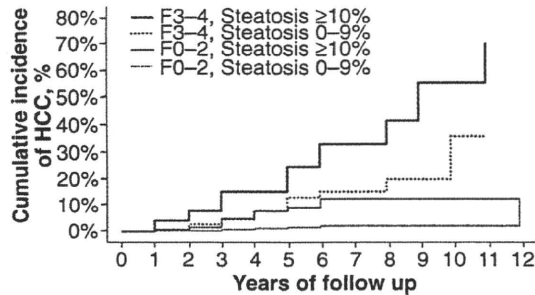
(b) **Stratified by response to IFN therapy**



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

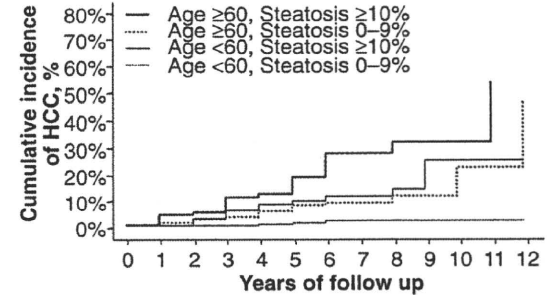
(c) **Stratified by stage of fibrosis**



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

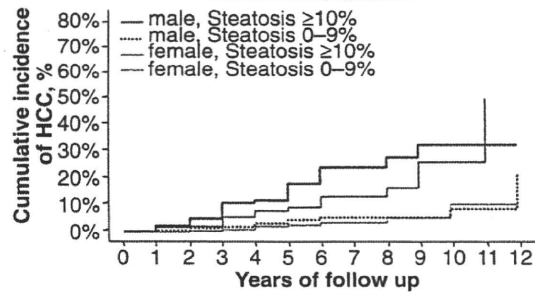
(d) **Stratified by age**



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

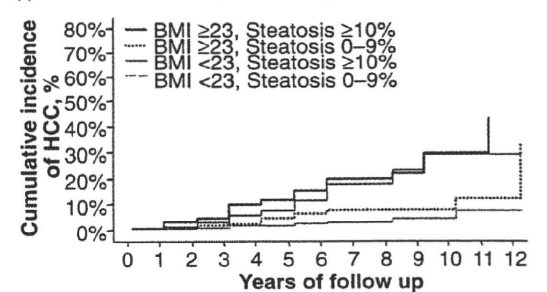
(e) **Stratified by gender**



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/m² and < 23 kg/m² 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 liver²² 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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Liver Cancer Working Group Report

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Hepatocellular carcinoma is a highly prevalent disease in many Asian countries, accounting for 75–80% of victims worldwide. The incidence of hepatocellular carcinoma varies enormously across Asia, but tends to follow the incidences of hepatitis B infection and liver cirrhosis. The incidence and etiology of hepatocellular carcinoma in Japan are different from the rest of Asia, but similar to that in Western countries because hepatitis C infection is the main etiological factor in Japan. Hepatitis B virus vaccination programs are showing great success in reducing hepatitis B virus-related hepatocellular carcinoma. Screening program improves detection of early hepatocellular carcinoma and has some positive impact on survival, but the majority of hepatocellular carcinoma patients in Asia still present with advanced hepatocellular carcinoma. Long-term outcomes following treatment of even early/intermediate or advanced disease are often unsatisfactory because of a lack of effective adjuvant and systemic therapies. Various clinical practice guidelines for hepatocellular carcinoma have been established and are in use. Clinical diagnosis of hepatocellular carcinoma by imaging diagnosis is replacing diagnosis of hepatocellular carcinoma by pathological confirmation. New imaging and treatment techniques are continuously being developed and guidelines should be updated every 3 or 4 years, incorporating new evidence. New molecularly targeted therapies hold great promise. Sorafenib is the first systemic therapy to demonstrate prolonged survival vs. the placebo in patients with advanced hepatocellular carcinoma. Various other new molecularly targeted agents are currently under investigation.

Key words: liver cancer – epidemiology – etiology – diagnosis – treatment

INTRODUCTION

The Liver Cancer Working Group report was divided into seven topics: (i) epidemiology and etiology in Asian countries; (ii) proportions of early, intermediate and advanced stages of hepatocellular carcinoma (HCC); (iii) surveillance systems and prediction of HCC development; (iv) recent developments in imaging diagnosis; (v) pathological development of early HCC, especially consensus between Asia and the West; (vi) current status of treatment

strategies; (vii) future perspectives, especially in regard to sorafenib; and other molecularly targeted agents.

EPIDEMIOLOGY AND ETIOLOGY

Liver cancer, or HCC, is endemic in Asia. It is expected that around 75–80% of HCC cases worldwide develop in Asia (Fig. 1) (1). In most Asian countries, HCC is ranked from number 1 to number 5 among the leading causes of death. In

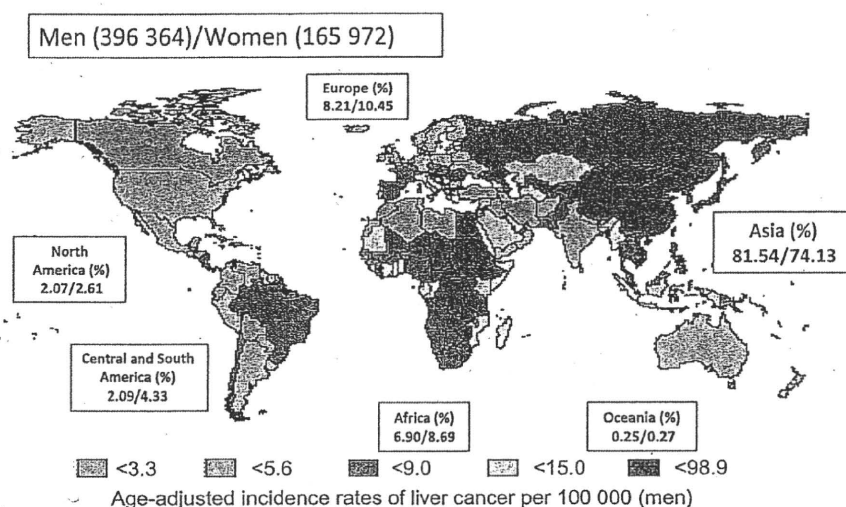


Figure 1. Liver cancer in the world (Curado et al. IARC Press, 2010).

Mainland China and Taiwan, the incidence of HCC has been increasing in the past 30 years, but in Japan, the incidence has been relatively stable during that period (2). In Korea, particularly in the male population, the incidence of HCC decreased slightly in the past 10 years. The primary etiological factor in Asia is hepatitis B. As exemplified by Korea, hepatitis B virus (HBV) accounts for 70–75% of HCC cases and hepatitis C virus (HCV) accounts for 10–15% (3). In Hong Kong, 80% of HCC cases are caused by HBV, and around 7% are caused by HCV. Japan is unique in the etiology of HCC in Asia because almost two-thirds of cases are caused by HCV and only 15% are related to HBV (2,4–6). Taiwan appears to be in between. In the early 1980s, HBV was the dominant cause of HCC in Taiwan, accounting for 88% (4), but in the past 30 years, HCV increased significantly and now accounts for more than 30%. HBV remains the predominant cause, but because of a vaccination program that was started in 1984, Taiwanese younger than 25 years old will have a carrier rate of around 1%. Thirty years from now, HBV-related HCC will decrease dramatically in Taiwan and in other countries that have adopted a nationwide HBV vaccination program (7). Regarding the age distribution of HCC, in all countries in which HBV is the dominant cause, the median age is around 55 years old. Statistics for Japan, which is characterized by HCV, show that the median age is about 10 years older.

In conclusion, HCC in the Asia-Pacific region accounts for 75–80% of victims worldwide. The incidence of HCC is on the rise in some countries, such as mainland China and Taiwan, but it is plateauing and decreasing slightly in some countries, like Japan. Except in Japan, HBV is the major etiology of HCC. The proportion of HCV has increased significantly in the past 30 years in Taiwan. Because of successful vaccination, the incidence of HBV-related HCC will decrease dramatically by 2040 (8).

PROPORTIONS OF EARLY, INTERMEDIATE AND ADVANCED HCC

There are various staging systems for HCC, with each system having its pros and cons and no consensus regarding which system is the best. The Barcelona Clinic of Liver Cancer, BCLC, system (9,10) is quite widely used in the West and in many clinical trials. The BCLC system stages patients into very early stage, early stage, intermediate stage, advanced stage and end stage according to the tumor size, vascular invasion, the tumor nodule number and the presence of metastasis. The BCLC system also provides a guideline for treatment according to the stage of HCC. Basically, patients with very early-stage or early-stage HCC are considered for curative treatment, either resection, liver transplantation or local ablation. Patients with intermediate-stage HCC, mainly those with multinodular disease, will be eligible for transarterial chemoembolization (TACE), and patients with advanced-stage disease showing portal invasion or distant metastasis will be considered for sorafenib or recruitment to clinical trials.

In addition to the BCLC, the Japanese TNM staging system (11) is quite widely used in Japan and Korea. This staging system takes into account three criteria for the T stage, i.e. whether the tumor is solitary or multiple, the tumor size, ≤ 2 cm or > 2 cm, and the presence of any vascular or bile duct invasion. Patients are thus classified as T1, T2, T3 or T4. For N and M, it is similar to other TNM staging systems, based on the presence of lymph node or distant metastasis. By integrating Japanese TNM stage and Child–Pugh grade, Japan Integrated Staging system was developed (12) and widely used in Japan and Korea.

The current distribution of HCC based on the BCLC system is quite similar in Hong Kong and Korea, with about 30–40% of patients having early-stage disease, about 20–30% having intermediate-stage disease and about 30% having advanced-stage disease. In Japan, the proportion of early-stage HCC is very high: about 65%, whereas only 5% of

patients present with advanced-stage disease (5). Japan is thus quite different from the rest of the Asia-Pacific region, probably because of its very well-established surveillance system.

But even within a country, there can be a significant variation between regions, as exemplified by Taiwan. In northern Taiwan, about 58% of patients have early-stage HCC, whereas in the southern part, the rate is only 35.2%. This is probably related to differences in the popularity of surveillance due to cultural, social and economic differences between the populations in the north and south of Taiwan. Data generated in Japan and Korea, using the Japanese TNM staging system, are similar to the BCLC staging results and show that Japan has a higher number of patients with early-stage HCC compared with Korea.

The disease stage obviously affects the treatment modality. For early-stage cancers, curative treatments like surgery or ablation are generally implemented, whereas TACE is performed for intermediate-stage disease and systemic therapy for advanced disease. Comparison between Hong Kong and Japan shows a dominance of ablation and surgery in Japan, whereas in Hong Kong, the percentage of patients amenable to ablation is limited. Even for TACE, the proportion of patients is higher in Japan than in Hong Kong, where a large proportion of patients have advanced disease and receive systemic therapy. For early-stage disease, curative treatment is the first choice, and about 38% of patients in Hong Kong and 65% in Japan are amenable to curative treatments. For intermediate-stage HCC, the rates are 22% in Hong Kong and 30% in Japan, and for advanced-stage disease, the rates are 40% in Hong Kong and 5% in Japan.

BCLC staging has important predictive power for overall survival. Data for more than 3000 patients in Hong Kong show very good stratification of overall survival in terms of the stage. Survival data from Yonsei University (Korea) show a very similar stratification. For patients with early HCC, the 5-year survival rate is now more than 50%, whereas for patients with advanced-stage disease, the 5-year survival is <5%, showing a great difference in the survival outcomes. In some countries, like Korea, evidence points to some recent improvement in the overall survival of HCC patients: comparison between 1993 and 2005 shows that the 5-year survival has improved from 10.7% to 18.9% in the most recent 5-year period.

In conclusion, there is a significant variation in the distribution of early, intermediate and advanced stages of HCC among Asia-Pacific countries, with the highest proportion of early HCC in Japan. Curative treatment for early-stage HCC is associated with the 5-year survival >50%, while the prognosis of advanced-stage HCC remains dismal. These results underscore the importance of early diagnosis by means of surveillance of high-risk patients.

SURVEILLANCE SYSTEMS AND PREDICTION OF HCC

A Hong Kong study proved that a screening program can improve survival by increasing the chance of treatment in

the screened group (13). Unfortunately, in Hong Kong, the percentage of patients with HCC diagnosed by screening is low, but it has increased slightly, from 29% in 1991–1997 to 33% in 1998–2004 (14). There is no government-funded surveillance program for HCC in Hong Kong or other parts of China. Korea, however, established a national surveillance program in 2003, with the target population being those over 40 years of age, with liver cirrhosis or an HBV or HCV carrier (15). Taiwan has a similar surveillance program in place, and a different testing interval is applied depending on whether the subject has cirrhosis or not: 3–6 months for cirrhosis, but 6–12 months for non-cirrhosis. There is no age limitation for surveillance of HBV carriers in Taiwan, but in Korea, the government recommends over 40 years. The surveillance program in Japan is slightly different: it selects super high-risk patients, meaning liver cirrhosis B or C, and applies a shorter interval for examination, every 3 or 4 months, and test for more tumor markers (three tumor markers, including AFP, AFP-L3 and DCP) (16,17). The surveillance programs in Korea and China prefer a 6-month interval. Japanese surveillance program also recommends CT or MRI every 6–12 months for improving sensitivity. Thus, there are some differences in HCC surveillance among Asia-Pacific countries, including the candidates for surveillance and the age limit for HBV carriers. As surveillance tools, ultrasonography and AFP are still the standards, but there is a need to know whether more tumor markers will improve the sensitivity. A study investigated whether the surveillance interval is important for improving the survival. The group with a surveillance interval of within 6 months showed better survival than that of more than 6 months.

It is important to predict the development of HCC by quantitative risk estimation. An individualized prediction model is possible by combining multiple risk factors into a comprehensive risk expression. A study identified eight independent risk factors, and a special formula was established to calculate the relative risk factors. This model enables identification of the high- and low-risk groups.

In conclusion, HCC surveillance can detect early tumors and increase the chance of a curative approach. All patients at risk of developing HCC with potentially curative treatment available are recommended for regular surveillance. At present, ultrasonography and the serum AFP test at 6-month intervals are the standard surveillance tools. To improve the detection rate of early-stage HCC, the benefit of additional tests and a shorter surveillance interval should be confirmed by a randomized clinical trial in Asia. The application of individualized prediction model to surveillance programs may improve the cost-effectiveness by focusing on the high-risk group.

RECENT DEVELOPMENTS IN IMAGING DIAGNOSIS

Various clinical practice guidelines for HCC are being implemented around the world, including in Europe, Korea, America, Japan and the Asia-Pacific region. In accordance

with those guidelines, the use of dynamic imaging, such as contrast-enhanced ultrasound (US), CT and MRI, is increasing and becoming more important, whereas application of biopsy is decreasing. Angiography and fusion imaging are other imaging tools that are available for the diagnosis of HCC. These tools are based on different imaging techniques. US is the first step for imaging diagnosis of HCC in accordance with the guidelines. If a nodule is found by US examination, the next technique to be used depends on the size of the mass. For a nodule that is <1 cm in diameter, follow-up study is usually recommended. If the nodule is >2 cm in diameter, one further imaging examination, such as contrast-enhanced US, CT or MRI, is sufficient to make a diagnosis of HCC with specific findings. Specific findings consist of a hypervascular nature in the arterial phase of imaging, and a washout pattern in the equilibrium phase. Diagnosis of HCC by dynamic imaging (contrast-enhanced ultrasonography, CT or MRI) is based on the enhancement pattern according to time sequence or phase. Overt HCC shows high attenuation in the arterial phase, indicating the hypervascular nature of the tumor, iso-attenuation in the portal-venous phase and low attenuation in the equilibrium phase, indicating a rapid washout pattern. These comprise very specific findings for the diagnosis of HCC.

In the APASL Guideline 2009 for imaging diagnosis of HCC, US is a screening test, not a diagnostic test for confirmation. US can detect a nodule but cannot characterize it. However, contrast-enhanced US is as sensitive as dynamic CT or dynamic MRI for the diagnosis of HCC (18). When using a US contrast agent for the diagnosis of HCC, the

arterial phase and equilibrium phase show a rapid wash-in and washout pattern, which are characteristic findings for overt HCC. Dynamic CT or dynamic MRI is recommended as a first-line diagnostic tool for HCC when a screening test is abnormal. The hallmark of HCC in a CT scan or MRI is the presence of arterial enhancement followed by washout of the tumor in the portal-venous and/or delayed phases. In the diagnostic algorithm for hypervascular masses, typical HCC can be diagnosed by imaging regardless of the size of the detected tumor if a typical vascular pattern—arterial enhancement with portal-venous washout—is obtained on dynamic CT, dynamic MRI or contrast-enhanced US. In the diagnostic algorithm for hypervascular nodules, US is the initial screening method. If a nodule is detected by US, the nodule is then characterized by dynamic CT or MRI. Further characterization is usually performed by Kupffer cell imaging, including Sonazoid-enhanced US, or gadolinium-ethoxybenzyl-diethylene triamine pentaacetic acid (Gd-EOB-DTPA) MRI (Fig. 2) (19). In the diagnostic algorithm for hypovascular masses, nodular lesions showing an atypical imaging pattern, such as iso- or hypovascularity in the arterial phase, or arterial hypervascularity alone without portal-venous washout, should undergo further examination or close follow-up (Fig. 3). Recently, new imaging techniques are being developed, including volume US using various contrast agents, US elastography (20), volume CT, dual energy CT for perfusion CT, diffusion-weighted MRI, MRI elastography, etc. The efficacy of these techniques in diagnosing HCC is being evaluated.

In conclusion, various clinical practice guidelines including diagnostic algorithm for HCC have been established and

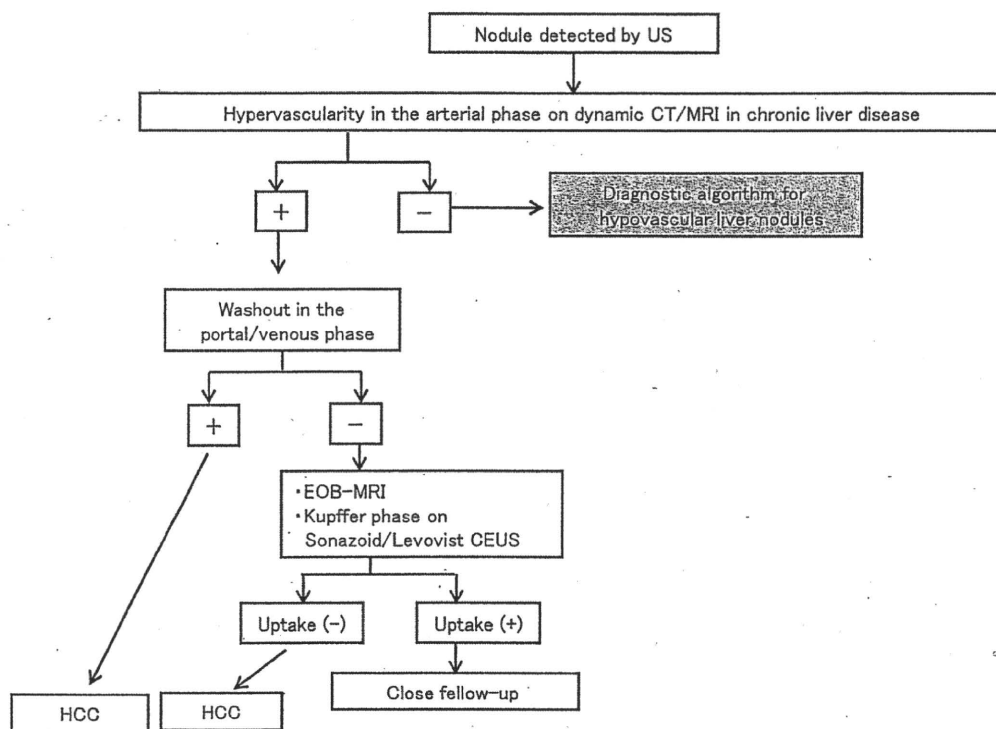


Figure 2. Diagnostic algorithm for hypervascular nodule (APASL Guideline). US, ultrasound; HCC, hepatocellular carcinoma.