Table 1 Baseline characteristics of the estimation cohort

Variables	All	HCC development (+)	HCC development (–)	*P*-value
Number of patients	151	9	142	
Age (years)	62 (22–82)	67 (60–82)	61 (22–80)	0.010†
Male (%)	55	55.6	54.9	1.000‡
BMI (kg/m^2)	23.5 (18.1–36.8)	23.8 (23.3–25.7)	23.4 (18.1–36.8)	0.217†
Habitual drinker (%)	10.6	11.1	10.6	1.000‡
Fibrosis stage (F0–2/F3–4)	115/36	5/4	110/32	0.048‡
Inflammatory grade (A0–1/A2–3)	33/118	0/9	33/109	0.101‡
LSM (kPa)	8.8 (2.8–45.7)	14.8 (9.8–45.7)	8.7 (2.8–34.8)	0.002†
Observation period (days)	722 (189–1378)	688 (189–1217)	733 (190–1378)	0.467†
Genotype 1 (%)	56.3	100	53.5	0.065‡
HCV-RNA (log IU/mL)	6.4 (0.0–7.7)	6.5 (2.9–7.2)	6.3 (0.0–7.7)	0.168†
Albumin (g/dL)	4.1 (3.4–4.8)	4.1 (3.5–4.6)	4.1 (3.4–4.8)	0.390†
ALT (IU/L)	59 (10–410)	75 (27–181)	57 (10–410)	0.467†
Total bilirubin (mg/dL)	0.7 (0.3–1.8)	0.8 (0.5–1.3)	0.7 (0.3–1.8)	0.070†
γGTP (IU/L)	44 (4–517)	75 (31–129)	41 (4–517)	0.120†
Hemoglobin A1c (%)	5.1 (3.7–8.2)	5.1 (3.7–6.1)	5.1 (4.2–8.2)	0.561†
Ferritin (ng/mL)	134 (8–2096)	215 (8–1026)	134 (9–2096)	0.675†
White blood cell count (× 10^3/μL)	4.9 (2.0–10.3)	4.3 (3.0–7.3)	4.9 (2.0–10.3)	0.496†
Hemoglobin (g/dL)	13.8 (8.9–17.5)	13.3 (9.9–17.5)	13.8 (8.9–17.1)	0.376†
Platelet count (× 10^4/μL)	16.3 (5.2–37.0)	9.6 (5.2–19.4)	16.5 (5.8–37.0)	0.004†
Prothrombin time (%)	100 (70–157)	93 (79–120)	102 (70–157)	0.185†
AFP (ng/mL)	6 (1–306)	14 (4–109)	6 (1–306)	0.004†
SVR rate (%)	55	11.1	57.7	0.011‡

Scale data are shown as median (range). *P* values are for comparisons between patients with and without HCC development.
†Mann–Whitney *U* test.
‡Chi-square test.
γGTP, γ-glutamyl transpeptidase; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LSM, liver stiffness measurement; SVR, sustained virological response.

l HCV infection was 56.3%. Following IFN-based antiviral therapy, SVR was obtained in 83 of the 151 patients (55%). During the median follow-up period of 722 days (range 189–1378 days), nine patients (6.0%) developed HCC. The cumulative incidence of HCC estimated using the Kaplan–Meier method was 1.3%, 4.5%, and 9.0% at 1, 2, and 3 years, respectively (Fig. 1). Compared with patients who had not developed HCC, HCC patients were of advanced age and had a high LSM, a high fibrosis stage, a low platelet count, and a low SVR rate (Table 1).

Risk analyses. Univariate analysis revealed that age ($P = 0.029$), LSM ($P = 0.005$), platelet count ($P = 0.002$), AFP ($P = 0.003$), and non-SVR ($P = 0.011$) were associated with HCC development (Table 2). Multivariate Cox logistic regression analysis identified three independent risk factors: LSM ≥ 14.0 kPa (hazard ratio [HR] 5.58, 95% confidence interval [CI] 1.32–23.64, $P = 0.02$), non-SVR (HR 8.28, 95% CI 1.01–68.05, $P = 0.049$), and platelet count < 14.1 × 10^4/μL (HR 5.59, 95% CI 1.14–27.53, $P = 0.034$), Table 3. The 1-, 2-, and 3-year cumulative incidence rates of HCC development in patients with LSM < 14.0 kPa were 0.8%, 2.3%, and 4.6%, respectively, whereas those with LSM ≥ 14.0 kPa were 3.2%, 12.0%, and 22.2%, respectively ($P = 0.005$) (Fig. 2a). The cumulative incidence rates of HCC development in patients with SVR were 0.0%, 2.0%, and 2.0%, respectively, whereas those without SVR were 3.0%, 7.4%, and 17.1%, respectively ($P = 0.011$) (Fig. 2b). The cumulative incidence rates of HCC development in patients with a platelet count ≥ 14.1 × 10^4/μL were 0.0%, 0.0%, and 4.2%, respectively, whereas those with a platelet count < 14.1 × 10^4/μL were 4.0%, 13.4%, and 19.1%, respectively ($P = 0.002$) (Fig. 2c).

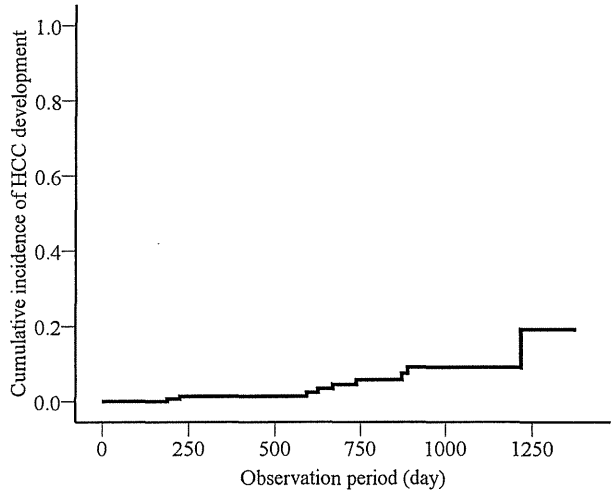

Figure 1 Incidence of hepatocellular carcinoma (HCC) in 151 patients with chronic hepatitis C receiving interferon-based anti-viral therapy estimated using the Kaplan–Meier method.

Table 2 Univariate analysis of factors associated with hepatocellular carcinoma development

Variables	*n*	Cumulative incidence of HCC (%)		*P*-value
		1 year	3 years	
Age (years)				
< 60	63	0.0	0.0	0.029
≥ 60	88	2.3	13.6	
Sex				
Female	68	1.5	12.1	0.910
Male	83	1.2	6.7	
BMI† (kg/m²)				
< 23.8	50	0.0	5.3	0.250
≥ 23.8	42	2.4	6.0	
Habitual drinker				
No	135	0.8	9.6	0.905
Yes	16	6.2	6.2	
Fibrosis stage				
F0–2	115	0.9	6.7	0.228
F3–4	36	2.9	15.0	
LSM (kPa)				
< 14	119	0.8	4.6	0.005
≥ 14	32	3.2	22.2	
ALT (IU/L)				
< 55	71	0.0	4.9	0.123
≥ 55	80	2.5	12.9	
γGTP† (IU/L)				
< 55	83	0.0	5.2	0.057
≥ 55	67	3.0	13.5	
Hemoglobin A1c† (%)				
< 5.5	109	0.9	6.8	0.219
≥ 5.5	25	0.0	18.8	
Ferritin† (ng/mL)				
< 210	74	1.4	10.0	0.175
≥ 210	43	2.3	16.3	
Platelet count (× 10^4/μL)				
≥ 14.1	101	0.0	4.2	0.002
< 14.1	50	4.0	19.1	
AFP† (ng/mL)				
< 10	95	0.0	5.6	0.003
≥ 10	38	4.9	22.3	
SVR				
Yes	83	0.0	2.0	0.011
No	68	3.0	17.1	

†Data not available for all patients.
AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; γGTP, γ-glutamyl transpeptidase; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; SVR, sustained virological response.

Number of risk factors and HCC development. The number of risk factors varied between patients: 12 patients (7.9%) had all three risk factors, 32 patients (21.2%) had two, 50 patients (33.1%) had one, and 57 patients (37.7%) had none of these risk factors (Fig. 3). Patients without these risk factors did not develop HCC during the study period. In patients with 1 or 2 risk factors, the cumulative incidence rates at 1, 2, and 3 years were 1.2%, 3.1%, and 8.2%, respectively, whereas patients with all three risk

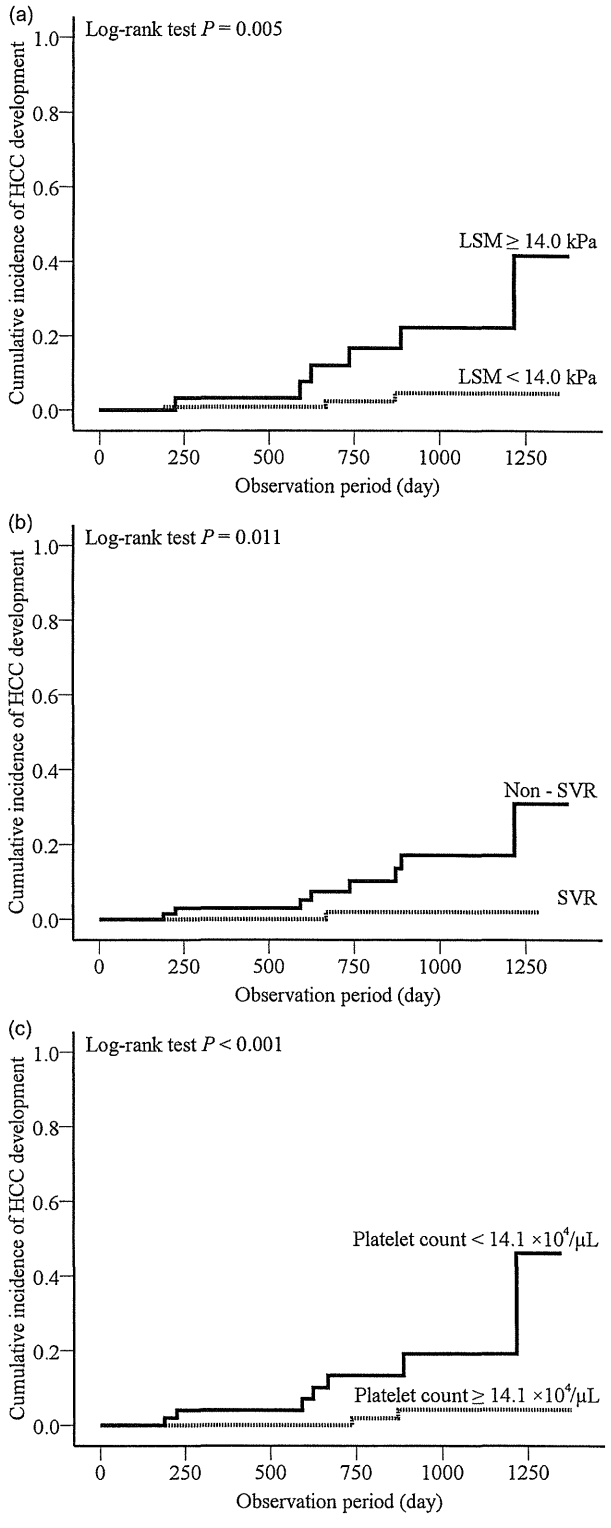

Figure 2 Kaplan–Meier curves comparing the cumulative incidence of hepatocellular carcinoma (HCC) development. Patients were stratified according to liver stiffness measurement (LSM) (a), sustained virological response (SVR) (b), and platelet count (c).

Table 3 Multivariate analysis of factors associated with hepatocellular carcinoma development

Variable		Hazard ratio (95% CI)	*P*-value
LSM (kPa)	< 14.0	1.00	0.020
	≥ 14.0	5.58 (1.32–23.64)	
SVR	SVR	1.00	0.049
	Non-SVR	8.28 (1.01–68.05)	
Platelet count (× 10^4/μL)	> 14.1	1.00	0.034
	≤ 14.1	5.59 (1.14–27.53)	

CI, confidence interval; LSM, liver stiffness measurement; SVR, sustained virological response.

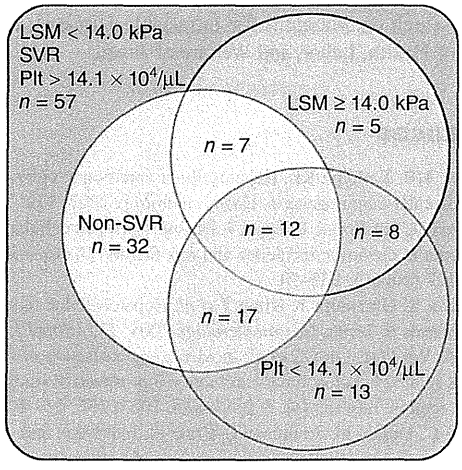

Figure 3 Patient distribution at each risk factor. LSM, liver stiffness measurement; Plt, platelet count; SVR, sustained virological response.

factors had significantly higher cumulative incidence rates (9.1%, 39.4%, and 59.6% at 1, 2, and 3 years, respectively; log-rank test, $P < 0.001$) (Fig. 4).

The relationship between the number of risk factors and HCC development in the validation cohort. Fifty-six patients who received IFN therapy without liver biopsy were enrolled into the validation group for analysis of these three risk factors. The 56 patients (33 male and 23 female) had a median age of 65 years (range 35–79 years) and a median LSM of 8.0 kPa (range 2.6–32.0 kPa). There were no significant differences in clinical, anthropometric, and laboratory findings between the validation and estimation cohorts (data not shown). In the validation cohort, seven patients (12.5%) had all three risk factors, 25 patients (44.6%) had one or two risk factors, and 24 patients (42.9%) had none of these risk factors. Patients without these risk factors did not develop HCC during the study period. In patients with one or two risk factors, and patients with all three risk factors, the cumulative incidence rates at 3 years were 12.7% and 28.6%, respectively. There was also a significant difference in the cumulative incidences of HCC development according to the number of risk factors ($P = 0.037$, Fig. 5).

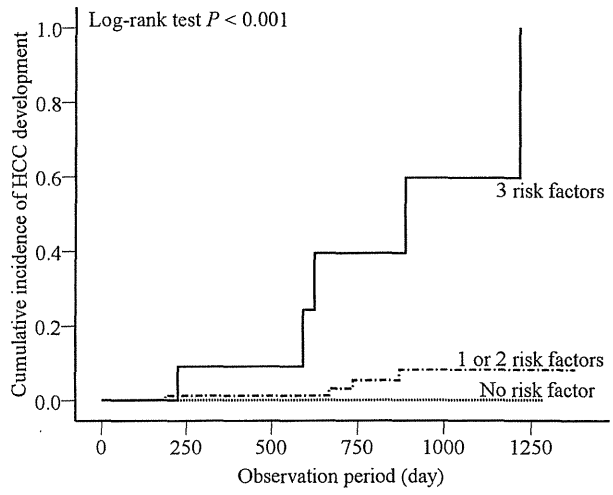

Figure 4 Kaplan–Meier curves comparing the cumulative incidence of hepatocellular carcinoma (HCC) development. Patients were stratified according to the number of risk factors.

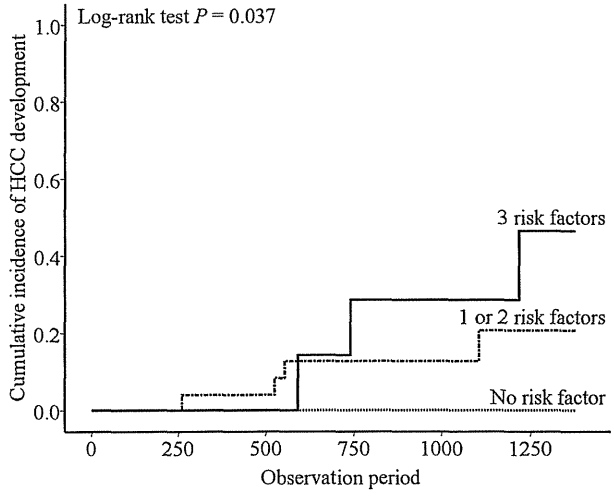

Figure 5 Kaplan–Meier curves comparing the cumulative incidence of hepatocellular carcinoma (HCC) development in the validation cohort. Patients were stratified according to the number of risk factors they had.

Discussion

Patients with liver cirrhosis or pre-existing severe hepatic fibrosis have a higher risk of developing HCC,[2] even after IFN-based therapy with SVR.[9,10] Clinical diagnosis of liver cirrhosis can be easily made in cases showing stigmata of end-stage liver disease, such as ascites, jaundice, variceal bleeding, and hepatic encephalopathy; however, diagnosis becomes difficult if the liver shows compensation, and normal or near-normal laboratory findings. Liver biopsy has been considered the only diagnostic method for the assessment of early compensated cirrhosis, although

several studies have pointed out sampling variability as a potential limitation of biopsy to diagnose cirrhosis.[21,22] Given the importance of assessing the HCC risk factors in managing CHC patients, we evaluated factors that affect the occurrence of HCC in CHC patients receiving IFN therapy, with a special focus on the predictive value of LSM as an alternative to liver biopsy.

Our data identified three risk factors for developing HCC after IFN therapy. Consistent with previous reports,[5–7] we found that failure to achieve SVR was a significant predictor of HCC development among patients receiving IFN therapy. Although it is possible that IFN therapy itself reduces the risk of HCC,[6,7] non-SVR patients had an approximately eightfold higher risk of developing HCC than SVR patients. In addition, we identified both high LSM and low platelet count as significant predictors of HCC development independently of non-SVR. The LSM threshold ≥ 14.0 kPa identified here as a risk factor for HCC is in agreement with previously reported cut-off values for liver cirrhosis,[15,16] further supporting the idea that pre-existing liver cirrhosis increases the risk of HCC development. Similar to LSM, the platelet count reflects the severity of CHC[21] and is used to estimate the degree of fibrosis.[23–25] Previous reports have also shown low platelet counts to represent a risk of HCC.[23,24] Our cohort showed that LSM was sometimes high even in patients without a low platelet count, whereas other patients had a low platelet count without LSM elevation. Such patients are nevertheless at risk of HCC, suggesting that LSM and platelet count indicate advanced fibrosis or compensated cirrhosis in a complementary manner.

In agreement with a previous report, our findings indicate that LSM could be used to stratify the risk of HCC development in CHC patients.[26] Moreover, combination of LSM with platelet count and the IFN-therapeutic effect could be used to stratify the risk of HCC in patients receiving IFN therapy. Patients without all three risk factors had a very low risk of HCC development, and patients with 1 or 2 risk factors had a moderate risk. Conversely, patients with all three risks had an extremely high risk. In clinical practice, frequency of HCC surveillance should be decided based on HCC risk. Indeed, each of these three factors has previously been shown to be associated with the risk of developing HCC. However, here, we have proposed a new, non-invasive risk assessment based on the combination of LSM and two other factors. In the present study, we did not identify advanced histological fibrosis stage F3–4 as a risk factor for HCC likely because of liver biopsy sampling variability because patients were not excluded based on the length of liver biopsy samples, an important factor affecting variability in histological assessment of liver fibrosis.[15] Taken together, these findings suggest that LSM would be more useful than liver biopsy for diagnosis of patients with liver cirrhosis who are at high risk of HCC, especially those with compensated cirrhosis.

Our data indicate patients with all of the three risk factors require the most intensive HCC surveillance; however, this study does have a few limitations. One drawback is that LSM failure and unreliable results occur in some patients. In our cohort, 9.0% of patients who received LSM did not yield reliable results. Because subcutaneous fat attenuates the transmission of share waves and the ultrasonic signals into the liver used to determine LSM, obesity is the principal reason for LSM failure.[27] In addition, it is likely that obesity itself is associated with an increased risk of HCC.[28] As a result, our findings might not reflect the risk of HCC in obese patients. Another recent report demonstrated that a new FibroScan XL probe, designated for use in obese patients, could reduce LSM failure and facilitate reliable results.[29] A study using this new probe will more accurately evaluate the predictive value of LSM for the risk of HCC development.

In conclusion, our findings indicate that LSM, platelet count, and IFN-therapeutic effect could be used to successfully stratify the risk for HCC development in patients receiving IFN-based antiviral therapy and demonstrate the usefulness of LSM before IFN therapy for the management of CHC patients.

Acknowledgment

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SHORT COMMUNICATION

BJC

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Keywords: chemotherapy; 5-fluorouracil; cisplatin; liver neoplasm; hepatocellular carcinoma; propensity score

Effect of hepatic arterial infusion chemotherapy of 5-fluorouracil and cisplatin for advanced hepatocellular carcinoma in the Nationwide Survey of Primary Liver Cancer in Japan

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Background: The efficacy of hepatic arterial infusion chemotherapy for the treatment of advanced hepatocellular carcinoma (HCC) remains unclear.

Methods: The outcome of 476 patients with HCC who underwent hepatic arterial infusion chemotherapy with 5-fluorouracil and cisplatin (HAIC) were compared with 1466 patients who did not receive active therapy.

Results: A survival benefit of the therapy after adjusting for known risk factors was observed (hazard ratio, 0.48; 95% CI, 0.41–0.56; $P<0.0001$). In propensity score-matched analysis ($n=682$), median survival time was longer for patients who underwent chemotherapy (14.0 months) than for patients who did not receive active treatment (5.2 months, $P<0.0001$).

Conclusion: For advanced HCC, HAIC is considered to be an effective treatment.

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Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide (Jemal *et al*, 2011). Screening patients with chronic liver diseases increases the chance that HCC can be diagnosed in the early stage (Kudo *et al*, 2011; European Association for Study of Liver; European Organisation for Research and Treatment of Cancer, 2012; Sherman *et al*, 2012). However, many HCCs are detected at an advanced stage.

According to the treatment algorithm of HCC, patients with advanced disease are candidates for chemotherapy (Kudo *et al*, 2011; European Organisation for Research and Treatment of Cancer, 2012; Sherman *et al*, 2012). Currently, sorafenib is the only chemotherapy proven to be effective for advanced HCC (Llovet *et al*, 2008; Cheng *et al*, 2009). Several other therapies have been evaluated, including hepatic arterial infusion of 5-fluorouracil (5-FU) and cisplatin, which was the most common regimen in Japan (Ueshima *et al*, 2010; Kim do *et al*, 2011; Yamashita *et al*, 2011). However, most of these studies were retrospective and nonrandomised; therefore, its efficacy remains unclear.

The Liver Cancer Study Group of Japan uses questionnaires to collect data from patients with HCC every 2 years, with several minor modifications of the contents since 1965 (Ikai *et al*, 2005, 2007). We used the three most recent sets of data to determine the efficacy of arterial infusion therapy with 5-FU and cisplatin for advanced HCC.

MATERIALS AND METHODS

Data sources. From January 2000 to December 2005, a total of 62 315 patients with primary liver cancer were newly registered by the Liver Cancer Study Group of Japan (Ikai *et al*, 2005, 2007). The cohort was followed up biannually and their clinical outcome was examined. Of these 62 315 patients, 57 445 (92.2%) received a diagnosis of HCC, and 31 743 patients with complete data were selected for this study. Among the patients, 1150 patients initially underwent chemotherapy and 1466 patients received no active therapy (no therapy group). In patients who underwent chemotherapy, 476 (41.4%) underwent arterial infusion chemotherapy with 5-FU and cisplatin using a subcutaneous infusion port (HAIC group). All patients in the HAIC group and in the no therapy group were enrolled in this study (Supplementary Figure 1).

Hepatocellular carcinoma was diagnosed primarily by imaging modalities such as computed tomography (1579, 81.3%), magnetic resonance imaging (257, 13.2%), ultrasonography (1167, 60.1%), and/or angiography (360, 18.5%) with the findings of hyperattenuation at the arterial phase and hypoattenuation at the portal phase and/or tumour staining. A histological diagnosis was made in 4.5% ($n=87$) of the patients. Treatment effect was evaluated by a criteria, 'Treatment effect of the target nodule', outlined by the Liver Cancer Study Group of Japan (Liver Cancer Study Group of Japan, 2003).

All data were provided anonymously. This study was approved by the review board of the Liver Cancer Study Group of Japan.

Statistical analysis. Continuous variables were compared by *t*-test, and categorical variables were compared by χ^2 test. Survival was estimated by the Kaplan–Meier method and compared by the log-rank test.

Univariate and multivariate analyses of the primary cohort ($n=1942$) were carried out using the Cox proportional hazard model. Adjusted hazard ratios for HAIC according to subgroups (prognostic tumour factors in multivariate analysis) were also analysed and presented as a forest plot.

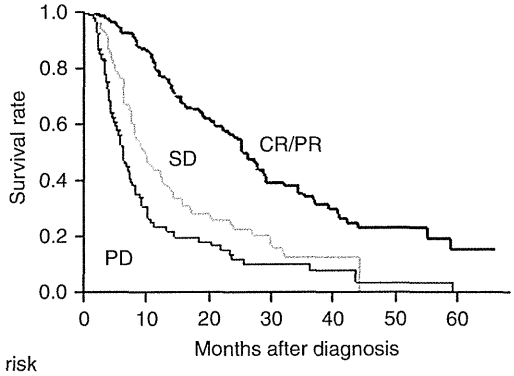
To determine the efficacy of HAIC, propensity score-matching analysis was performed (HAIC, $n=476$; no therapy, $n=1466$). A propensity score for use of HAIC was estimated using a logistic regression model fit with 15 variables: sex, age, hepatitis B surface antigen (HBsAg) positivity, hepatitis C virus (HCV) antibody positivity, alcohol intake, presence of encephalopathy, presence of ascites, total bilirubin, albumin, prothrombin time, maximum tumour size, tumour number, portal vein invasion, extrahepatic metastasis, and α-fetoprotein level. To create a propensity-matched cohort of patients who underwent HAIC or no therapy (1 : 1 match), a nearest-neighbour-matching algorithm with a 'greedy' heuristic was used (Austin and Mamdani, 2006).

The same matching procedure was carried out in patients with Child–Pugh A/B disease and portal vein invasion or more than three tumours, and survival rates for each matched cohort were compared.

RESULTS

Patient characteristics. The HAIC group was significantly younger and had more males than the no therapy group (Supplementary Table 1). Patients in the HAIC group had better liver function but more cases of hepatitis B infection and more advanced tumours than the no therapy group. These differences except follow-up period disappeared after propensity score matching.

Treatment effect of HAIC. In the HAIC group, the response rates were as follows: complete response (CR, $n=19$, 4.0%), partial response (PR, $n=173$, 36.5%), stable disease (SD, $n=112$, 23.6%), progressive disease (PD, $n=129$, 27.2%), and undefined ($n=41$, 8.7%). The 1- and 3-year survival rates according to response were CR/PR (77.7% and 34.6%), SD (44.2% and 13.3%), and PD (23.7% and 10.3%), respectively ($P<0.0001$, Figure 1). All factors including HAIC treatment correlated with prognosis in the univariate analysis (Table 1). Multivariate analysis revealed that HBsAg, more than three tumours, large tumours (>3 cm), distant metastasis, portal vein invasion (VP3 and VP4), and high α-fetoprotein levels (>400 ng ml^{-1}) were associated with poor survival. The VP3 and VP4 indicated tumour invasion to the first-order branches of the portal vein and the invasion to the main trunk of the portal vein, respectively (Liver Cancer Study Group of Japan, 2003). Conversely, Child–Pugh A/B disease (hazard ratio,



Patients at risk

CR/PR	192	136	72	37	20	9	5
SD	112	34	16	9	2	0	0
PD	129	29	12	6	4	2	0

Figure 1. Survival of patients who underwent hepatic arterial infusion of 5-fluorouracil and cisplatin using a subcutaneous infusion port. The 1- and 3-year survival rates and median survival times according to response were as follows: CR/PR (77.7%, 34.6%, 25.8 months), SD (44.2%, 13.3%, 9.5 months), and PD (23.7%, 10.3%, 6.0 months) ($P<0.0001$). Abbreviations: CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease.

Table 1. Risk factors for survival

	Univariate			Multivariate		
Characteristics	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Age >70 years	0.87	0.77–0.98	0.025	1.02	0.89–1.16	0.708
Male sex	1.19	1.04–1.37	0.007	1.04	0.90–1.21	0.541
HBsAg positive	1.47	1.27–1.69	<0.001	1.20	1.01–1.44	0.037
HCV Ab positive	0.78	0.69–0.88	<0.001	0.93	0.81–1.08	0.379
Alcohol intake >90 g day^{-1}	1.18	1.05–1.34	0.006	1.08	0.94–1.23	0.259
Child–Pugh grade A/B	0.51	0.45–0.58	<0.001	0.51	0.45–0.59	<0.001
Total bilirubin >2 mg dl^{-1}	1.99	1.76–2.24	<0.001			
Albumin >3 g dl^{-1}	0.65	0.57–0.73	<0.001			
Prothrombin time >80%	0.71	0.63–0.80	<0.001			
Ascites	2.54	2.26–2.86	<0.001			
Encephalopathy	1.28	1.09–1.50	0.002			
More than three tumours	1.84	1.64–2.07	<0.001	1.47	1.29–1.67	<0.001
Tumour >3 cm	2.26	1.98–2.58	<0.001	1.76	1.51–2.04	<0.001
Distant metastasis	2.11	1.81–2.45	<0.001	1.43	1.22–1.67	<0.001
Portal vein invasion, VP3 and VP4	3.08	2.72–3.47	<0.001	2.28	1.99–2.62	<0.001
AFP >400 ng ml^{-1}	2.35	2.09–2.65	<0.001	1.46	1.28–1.67	<0.001
HAIC/no therapy	0.71	0.62–0.81	<0.001	0.48	0.41–0.56	<0.001

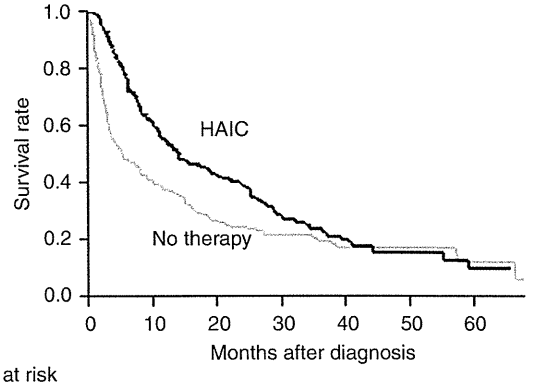
Abbreviations: AFP = α-fetoprotein; CI = confidence interval; HAIC = hepatic arterial infusion chemotherapy with 5-fluorouracil and cisplatin; HBsAg = hepatitis B virus surface antigen; HCV Ab = hepatitis C virus antibody; HR = hazard ratio; VP3 = tumour invasion to the first-order branches of the portal vein; VP4 = tumour invasion to the main trunk of the portal vein.

0.51; 95% confidence interval (CI), 0.45–0.59; $P<0.0001$) and HAIC (hazard ratio, 0.48; 95% CI, 0.41–0.56; $P<0.0001$) were associated with better survival.

An exploratory subgroup analysis of patients who underwent HAIC therapy evaluated six prognostic variables: presence of HBsAg, tumour number, tumour size, presence of extrahepatic metastasis and vascular invasion, and α-fetoprotein levels. Compared with no therapy, HAIC improved survival, regardless of the values of these six prognostic factors (Supplementary Figure 2).

Survival rates of propensity score-matched cohorts. In the propensity score-matched cohort ($n=682$), 198 patients in the HAIC group and 199 patients in the no therapy group died during the observation period. The cause of death was liver related that included death by liver cancer as well as by liver failure in 184 patients (92.9%) in the HAIC group and 180 patients (90.4%) in the no therapy group ($P=0.47$). Median survival times were 14.0 months (HAIC group) and 5.2 months (no therapy group), and survival was significantly higher in the HAIC group ($P<0.0001$) (Figure 2). Hazard ratio of HAIC in this propensity score-matched cohort was 0.60 (95% CI, 0.49–0.73; $P<0.0001$). The same relationship was observed even when the event was limited to liver-related death ($P<0.0001$). Median survival times were 15.4 months (HAIC group) and 7.3 months (no therapy group).

Because most treatment guidelines for HCC recommend chemotherapy for patients with Child–Pugh A/B disease who have portal vein invasion and/or more than three tumours, we analysed the effect of HAIC in patients who met these criteria in the propensity score-matched cohort. In cases of Child–Pugh A/B disease with more than three tumours (370 propensity score-matched patients), median survival times were 13.9 months (HAIC) and 3.7 months (no therapy), and a survival benefit of HAIC treatment was observed ($P<0.0001$; Supplementary Figure 3). The same relationship was also observed in cases of Child–Pugh A/B disease with portal vein tumour thrombus (378 propensity score-matched patients, $P<0.0001$; Supplementary Figure 4). Median survival times were 7.9 months (HAIC) and 3.1 months (no therapy).



Patients at risk

HAIC	341	161	84	42	21	10	5
No therapy	341	84	44	27	16	9	5

Figure 2. Survival of propensity score-matched patients who underwent hepatic arterial infusion of 5-fluorouracil and cisplatin (HAIC) or no active therapy (no therapy). Median survival times were 14.0 months (HAIC) and 5.2 months (no therapy) ($P<0.0001$).

DISCUSSION

Hepatic arterial infusion of cisplatin and 5-FU using a subcutaneous infusion port has been widely used in Japan to treat advanced

HCC because of its relatively high response rate (27.8–57.1%) (Ando *et al*, 2002; Eun *et al*, 2009; Ueshima *et al*, 2010; Kim do *et al*, 2011; Kim *et al*, 2011); however, no randomized control trial has been conducted to demonstrate its effectiveness and survival benefit. Most reports of HAIC were retrospective studies with small numbers of patients. In this study we used data from a large-scale nationwide survey and found that the response rate to HAIC was high (40.5%), survival was prolonged, and response to therapy could be used as a surrogate marker for overall survival. The survival benefit was also observed when only liver-related deaths were treated as 'events'.

Cisplatin interacts with DNA, preferentially binding nucleophilic N7 sites on purine bases (Galluzzi *et al*, 2012). As a consequence, protein–DNA complexes and DNA–DNA inter- and intra-strand adducts are generated, inducing cytotoxicity. Cisplatin also increases the folate concentration in cancer cells, reinforcing the effect of 5-FU through the formation of an inactive ternary complex (Scanlon *et al*, 1986; Kim *et al*, 2002). This synergistic effect of cisplatin and 5-FU is the basis of HAIC therapy.

Our study has some limitations. The information of dose reduction or termination due to drug toxicity is missing. Propensity scores were used to adjust for patient characteristics; however, it is not possible to adjust for all possible confounding factors related to survival, and the exact reasons of no therapy in control group were not known. Another weak point in this study is that performance status was not included as a covariate because two-thirds of the patients enrolled in this study lacked these data. However, the survival benefit of HAIC was observed even when the event was limited to liver-related death and when analysing the most recent database, which included performance status (median observation period 3 months, data not shown). Finally, we did not know the precise regimen used in this study population; however, many studies of HAIC report the administration of low-dose cisplatin (5–20 mg) several times a week, and continuous infusion of 5-FU for a few weeks.

As sorafenib has become the standard treatment for advanced HCC, several randomized controlled trials have been planned to evaluate new drugs using sorafenib as a control (Kudo, 2012). Some of these trials will evaluate HAIC, which will clarify some of the uncertainties of the present study.

In this large-scale retrospective study, we demonstrated the effectiveness of HAIC, although it was difficult to achieve long-term survival because HCCs re-grew even after response to the drugs. Our findings indicate that HAIC could be an alternative therapy for advanced HCC. Further examination of the factors that can predict the therapeutic effect is important for achieving long survival in future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Nationwide Study of 4741 Patients With Non-B Non-C Hepatocellular Carcinoma With Special Reference to the Therapeutic Impact

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Objective: To examine the prognostic factors and outcomes after several types of treatments in patients with hepatocellular carcinoma (HCC) negative for hepatitis B surface antigen and hepatitis C antibody, so-called "non-B non-C HCC" using the data of a nationwide survey.

Background: The proportion of non-B non-C HCC is rapidly increasing in Japan.

Methods: A total of 4741 patients with non-B non-C HCC, who underwent hepatic resection (HR, n = 2872), radiofrequency ablation (RFA, n = 432), and transcatheter arterial chemoembolization (TACE, n = 1437) as the initial treatment, were enrolled in this study. The exclusion criteria included extrahepatic metastases and/or Child-Pugh C. Significant prognostic variables determined by a univariate analysis were subjected to a multivariate analysis using a Cox proportional hazard regression model.

Results: The degree of liver damage in the HR group was significantly lower than that in the RFA and TACE groups. The HR and TACE groups had significantly more advanced HCC than the RFA group. The 5-year survival rates after HR, RFA, and TACE were 66%, 49%, and 32%, respectively. Stratifying the survival rates, according to the TNM stage and the Japan Integrated Staging (JIS) score, showed the HR group to have a significantly better prognosis than the RFA group in the stage II and in the JIS scores "1" and "2." The multivariate analysis showed 12 independent prognostic factors. HR offers significant prognostic advantages over TACE and RFA.

Conclusions: The findings of this large prospective cohort study indicated that HR may be recommended, especially in patients with TNM stage II and JIS scores "1" and "2" of non-B non-C HCC.

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths and fifth most common cancer worldwide.[1,2] Moreover, the incidence and mortality rate have been increasing in the United States and other countries.[3,4] The prominent etiological factors associated with HCC include chronic infection of hepatitis B virus (HBV) and hepatitis C virus (HCV), and chronic alcohol consumption. Although HCV-related HCC is responsible for the greatest proportion of HCC patients in Japan,[5,6] many hepatologists note that the proportion of HCC negative for hepatitis B surface antigen (HBsAg) and hepatitis C antibody (HCVAb), so-called "non-B non-C HCC," is rapidly increasing.[7,8] Indeed, a nationwide follow-up survey by the Liver Cancer Study Group of Japan (LCSGJ) found the proportions of HBV- and HCV-related HCC to have decreased over the previous decade, possibly thanks to the promotion of antiviral therapy, whereas the number of other HCC patients (mostly non-B non-C HCC) have more than doubled during the same period from 6.8% to 17.3%.[9] The exact background or molecular mechanisms for such a sharp increase in the incidence of non-B non-C HCC remain unclear at this point; however, nonalcoholic steatohepatitis (NASH) and metabolic syndrome are suggested to be important risk factors.[10] Nonetheless, it is crucial to elucidate clinicopathological characteristics including the prognostic factors of such patients with non-B non-C HCC at this moment.

Several studies, most of which enrolled around 100 patients or less, have investigated the clinical features of non-B non-C HCC to date.[11–16] However, the impact of the treatment, such as surgical treatment, local ablative therapy, and hepatic arterial embolization, for these patients has not been thoroughly examined. On the contrary, many studies have compared the outcomes after several therapeutic modalities for patients with HCC, and the results have been controversial because of the different therapeutic designs and small sample sizes.[17–21] All these findings prompted a study, clarifying the prognostic factors and the therapeutic impact of several types of treatment for the patients with non-B non-C HCC based on the data of the nationwide follow-up survey by the LCSGJ.

METHODS

A total of 62,321 patients with primary liver cancer were prospectively registered biannually from January 2000 to December 2005 by the LCSGJ using a registration/questionnaire sheet with more than 180 questions. They included 57,450 patients who were clinically diagnosed with HCC using multiple imaging modalities, clinical data, such as tumor markers, and/or histopathological

studies at each institution. Radiofrequency ablation (RFA) began to be more widely used in Japan in 2000. In addition, the data of the Child-Pugh class were requested on the form from the 16th survey. Therefore, the current study used the data from 2000 (16th survey) to 2005 (the latest 18th survey). In this study, 3447 patients for whom the data of hepatitis viral infection status of HBsAg and HCVAb were not available were excluded (Fig. 1), and 9307 of the remaining 54,003 patients with HCC (17.2%) were negative for both HBsAg and HCVAb (defined as "non-B non-C HCC").

The main purpose of this study was to compare the outcomes after hepatic resection (HR), RFA, and transcatheter arterial chemoembolization (TACE) in the non-B non-C HCC patients. The treatment algorithm for HCC proposed by Japanese guideline[22] indicates these 3 types of therapeutic modalities for patients without extrahepatic metastasis in the degree of liver damage A or B. The treatment algorithm[22] is based on 3 factors: "degree of liver damage" defined by the LCSGJ,[23] "number of tumors," and "tumor diameter." However, Child-Pugh class was adopted instead of the degree of liver damage because the former is globally used to evaluate liver function. Accordingly, the patients with extrahepatic metastasis (n = 944) and those in Child-Pugh C (n = 1028) were excluded. The study also excluded the 2192 patients who underwent the treatment other than the 3 types of therapeutic modalities described earlier. In addition, patients lacking outcome data were excluded (n = 402). Finally, 4741 non-B non-C HCC patients were selected in the current cohort study (Fig. 1) and classified according to the primary treatment into the HR group (n = 2,872), the RFA group (n = 432), and the TACE group (n = 1,437). In fact, the majority of Japanese patients with HCC are treated with 1 of the 3 types of treatment modalities, including surgical treatment, local ablative therapy, and hepatic arterial embolization. The questionnaire sheet of LCSGJ subclassified "Surgical treatment" into HR, liver transplantation, and others. "Local ablative therapy" includes RFA, ethanol injection therapy, microwave coagulation therapy, and others. "Hepatic arterial embolization" is subdivided into TACE (anticancer agents and lipiodol followed by gelatin sponge particles; this method was defined as "TACE" in this study), anticancer agents and lipiodol alone, anticancer agents and gelatin sponge particles alone, and others. The current investigation strictly selected HR, RFA, and TACE as the most frequently adopted and well-standardized therapeutic strategy from each type of treatment modality in Japan. Indeed, the 18th survey of LCSGJ found that approximately 97% of "Surgical treatment" was HR, 72% of "Local ablative therapy" was RFA, and 76% of "Hepatic arterial embolization" was TACE.

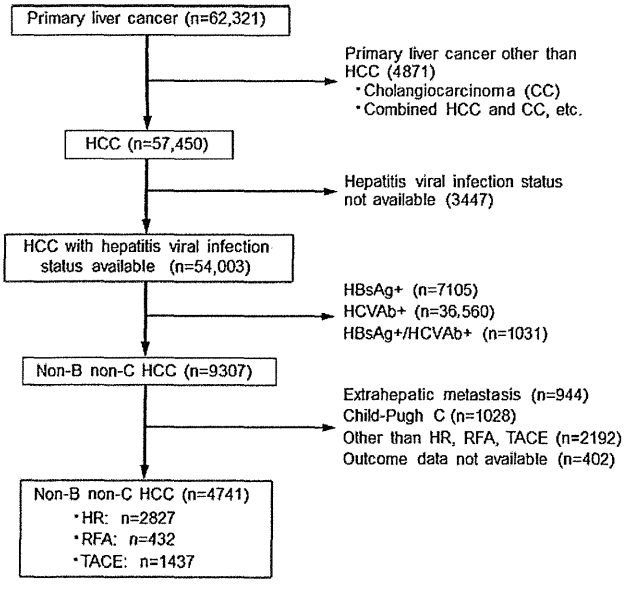

FIGURE 1. Flow chart of the patients with non-B non-C hepatocellular carcinoma (HCC) analyzed in this study.

The patients were prospectively followed up at each institution. Most of the patients have been traditionally observed according to the protocol, similar to the Japanese guidelines,[22] in which ultrasonography and measurement of the tumor markers every 3 or 4 months and enhanced computed tomography or magnetic resonance imaging every 6 or 12 months is recommended. The final prognosis of these registered patients was followed until confirmation of death at every survey.

The clinical characteristics among the 3 treatment groups were summarized in Table 1. All of the 19 variables were significantly different among the groups. Particularly, for the patients in the HR group, the positive percent of habitual alcohol consumption, defined as 86 g or more of ethanol per day over a 10-year period, was significantly lower than that in the RFA and TACE groups. The results of liver function tests, such as indocyanine green retention rate at 15 minutes (ICGR15) and prothrombin activity in this group, were significantly better than those in the RFA and TACE groups. These findings were well coordinated with the status of Child-Pugh class among the 3 groups. On the contrary, the HR and TACE groups had significantly more advanced HCC based on the most of tumor factors, such as the tumor size, tumor markers, and portal venous invasion, than the RFA group. However, the number of tumors in the HR group was the smallest, whereas that in the TACE group was largest. Liver-related deaths, such as those due to liver failure, in the RFA group were more frequently observed, whereas HCC-related deaths were more common in the HR and TACE groups (Table 1).

Statistical Analysis

The clinical characteristics among the 3 treatment groups were compared by either the chi-square test or the Kraskul-Wallis test. The survival rate after each treatment was calculated by the Kaplan–Meier method and then was compared by the log-rank test. The Bonferroni correction was applied for the multiple comparisons. Nineteen clinical variables, including type of treatment were evaluated by univariate analysis using a log-rank test to determine the prognostic factors in the patients with non-B non-C HCC. The survival rates after each treatment were stratified according to the TNM staging system defined by the LCSGJ (Table 2 and Table 3)[23] and the Japan Integrated Staging (JIS) score (Table 4).[24] Because the patients in Child-Pugh C were excluded in this study, JIS score "2" indicated either Child-Pugh class A/stage III or Child-Pugh class B/stage II, JIS score "3" indicated either Child-Pugh class A/stage IVA or Child-Pugh class B/stage III, and JIS core "4" indicated Child-Pugh class B/stage IVA.

Continuous variables were divided into 2 groups according to the median value. Significant variables with a P value less than 0.05 by the univariate analysis were subjected to multivariate analysis using a Cox proportional hazard regression model with backward elimination method.[25] All significance tests were 2-tailed, and a P value less than 0.05 was considered statistically significant. All statistical analyses were performed with the Statistical Analysis System (SAS) version 9.1.3 (SAS Inc, Cary, NC).

RESULTS

The follow-up periods after the treatment of HR, RFA, and TACE were 1.9 ± 1.6 years, 2.3 ± 1.4 years, and 1.5 ± 1.4 years, respectively. The 1-, 3-, and 5-year survival rates of the 4741 patients with non-B non-C HCC were 89%, 70%, and 55%, respectively.

TABLE 1. Clinical Characteristics in the Non-B Non-C HCC Patients Who Underwent 3 Types of Therapeutic Strategies

Variables	HR (n = 2872)	RFA (n = 432)	TACE (n = 1437)	*P*
Age (yr)	67 (50, 79)	68 (53, 81)	69 (53, 83)	<0.001
Sex				<0.001
Male	2332 (81%)	315 (73%)	1124 (78%)	
Female	540 (19%)	117 (27%)	313 (22%)	
Alcohol				<0.001
None	1,652 (58%)	209 (48%)	689 (48%)	
Positive*	874 (30%)	178 (41%)	619 (43%)	
Unknown	346 (12%)	45 (10%)	129 (9%)	
Serum albumin (g/dL)	4.0 (3.2, 4.7)	3.8 (2.9, 4.6)	3.7 (2.8, 4.5)	<0.001
Serum total bilirubin (mg/dL)	0.8 (0.4, 1.5)	1.1 (0.4, 2.4)	1.1 (0.4, 2.3)	<0.001
ICG R15 (%)	15 (4, 32)	26 (8, 52)	25 (5, 56)	<0.001
Prothrombin activity (%)	89 (65, 114)	80 (54, 104)	82 (55, 106)	< 0.001
Esophageal varices				<0.001
None	2231 (78%)	195 (45%)	740 (52%)	
Positive	276 (10%)	152 (35%)	489 (34%)	
Unknown	365 (13%)	85 (20%)	208 (15%)	
Degree of liver damage†				<0.001
A	2368 (83%)	224 (52%)	808 (56%)	
B	409 (14%)	132 (31%)	399 (28%)	
C	10 (0.3%)	14 (3%)	39 (3%)	
Unknown	85 (3%)	62 (14%)	191 (13%)	
Child-Pugh class				<0.001
A	2679 (93%)	316 (73%)	1068 (74%)	
B	193 (7%)	116 (27%)	369 (26%)	
Alpha-fetoprotein (ng/mL)	3491 (15, 16368)	215 (15, 927)	3177 (15, 13605)	<0.001
PIVKA-II (AU/mL)‡	2198 (40, 10000)	501 (40, 10000)	1905 (40, 10000)	<0.001
Tumor number				<0.001
1	2193 (76%)	293 (68%)	679 (47%)	
2	323 (11%)	85 (20%)	256 (18%)	
>3	126 (4%)	28 (7%)	126 (9%)	
Tumor size (mm)	5.8 (1.8, 14)	3.0 (1.1, 6)	5.0 (1.4, 13)	<0.001
Gross classification§				<0.001
Type 1	2362 (82%)	407 (94%)	1181 (82%)	
Type 2	199 (7%)	9 (2%)	160 (11%)	
Type 3	21 (0.7%)	2 (0.5%)	39 (3%)	
Unknown	290 (10%)	14 (3%)	57 (4%)	
Portal venous invasion				<0.001
Negative	2336 (81%)	403 (93%)	1218 (85%)	
Positive	342 (12%)	8 (2%)	179 (10%)	
Unknown	194 (7%)	21 (5%)	76 (5%)	
TNM stage†				<0.001
I	251 (9%)	119 (28%)	160 (11%)	
II	1489 (52%)	189 (44%)	550 (38%)	
III	707 (25%)	75 (17%)	517 (36%)	
IVA	321 (11%)	4 (1%)	74 (5%)	
Unknown	85 (3%)	45 (10%)	136 (10%)	
JIS score				<0.001
0	233 (8%)	87 (20%)	116 (8%)	
1	1423 (50%)	173 (40%)	466 (32%)	
2	732 (26%)	103 (24%)	514 (36%)	
3	374 (13%)	23 (5%)	184 (13%)	
4	25 (1%)	1 (0.2%)	21 (2%)	
Unknown	85 (3%)	45 (10%)	136 (10%)	
Cause of death				<0.001
HCC-related	302 (63%)	41 (38%)	271 (62%)	
Liver-related	69 (14%)	31 (29%)	94 (22%)	
Treatment-related	15 (3%)	2 (2%)	1 (0.2%)	
Others	96 (20%)	34 (32%)	68 (16%)	
Median follow-up period (yr)	1.9 (0.1, 5.1)	2.3 (0.1, 4.7)	1.5 (0.1, 4.3)	<0.001

Data are shown as the median (5 percentile, 95 percentile) unless specified.
*Eighty-six gram of alcohol daily for more than 10 years.
†By the Liver Cancer Study Group of Japan.
‡Questionnaire sheet requested the actual value when it was between 40 and 10,000 AU/mL.
§Type 1, simple nodular type; Type 2, simple nodular type with extranodular growth; Type 3, confluent multinodular type.

TABLE 2. TNM Stage by the Liver Cancer Study Group of Japan

	T Category	N Category	M Category
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
Stage IVA	T4	N0	M0
	T1, T2, T3, T4	N1	M0
Stage IVB	T1, T2, T3, T4	N0, N1	M1

The grade for each category is determined individually, and the staging of the disease is determined according to the aforementioned chart.

M1 indicates presence of distant metastasis; N1: presence of lymph node metastasis.

TABLE 3. T Category of the TNM Stage by the Liver Cancer Study Group of Japan

	T1		T2		T3		T4	
No. tumor: multiple	−	+	−	−	+	+	−	+
Tumor diameter: >2 cm	−	−	+	−	+	−	+	+
Vascular and/or bile duct invasion	−	−	−	+	−	+	+	+

The T category is determined on the basis of the "number," "size," and "vascular and/or bile duct invasion" by the tumor. All multiple tumors, including multicentric tumors and intrahepatic metastatic tumors, are counted.

TABLE 4. Definition and Criteria for the JIS Score

	0	1	2	3
Child-Pugh class	A	B	C	
TNM stage*	I	II	III	IV

JIS score = Child-Pugh class + TNM stage.
*By the Liver Cancer Study Group of Japan.

Prognostic Factors and Survival Rates

Nineteen clinical variables were screened as prognostic factors using a univariate analysis (Table 5). Sex and habitual alcohol intake were not selected as prognostic factors, whereas the remaining 17 variables, including age, serum albumin, serum total bilirubin, ICGR15, prothrombin activity, esophageal varices, degree of liver damage, Child-Pugh class, alpha-fetoprotein, protein induced by Vitamin K absence-II (PIVKA-II), tumor number, tumor size, gross classification, portal venous invasion, TNM stage, JIS score, and type of treatment, were significant prognostic factors. With the Child-Pugh class, 5-year survival rates of grades A and B were 58% and 31%, respectively, with statistical significance ($P < 0.001$; Fig. 2A). The TNM staging system by the LCSGJ[23] revealed that 5-year survival rates in stages I, II, III, and IVA were 66%, 64%, 46%, and 19%, respectively. A good separation, except stage I vs II, was observed (Fig. 2B). The 5-year survival rates based on a JIS score of 0, 1, 2, 3, and 4 were 70%, 67%, 44%, 23%, and 0%, respectively. There was a good separation, except JIS score "0" vs "1" (Fig. 2C). The 5-year survival rates after HR, RFA, and TACE were 66%, 49%, and 32%, respectively (Fig. 2D). There was no significant difference between the HR group and the RFA group ($P = 0.101$).

However, when the survival rates were stratified according to the TNM staging system (Fig. 3), the HR group showed a significantly better prognosis than the TACE group in all 4 stages (stage I to IVA). The RFA group had a significantly better prognosis than the TACE group only in the stage II and III. A comparison between the HR group and the RFA group showed that the HR group had a significantly better prognosis than the RFA group in stage II (Fig. 3B). However, there were no statistically significant differences between the 2 groups in stages I, III, and IVA. The survival rates in the stage II patients were further stratified according to each T category (Table 3) on the basis of the "number of tumors: multiple," "tumor diameter > 2 cm," and "vascular and/or bile duct invasion" by the tumor (Fig. 4). The HR group had a significantly better prognosis than the RFA group in all 3 T categories. The effectiveness of RFA was almost identical to that of TACE in the stage II patients with multiple tumors (Fig. 4A) and only HR could provide long-term survival in the stage II patients with vascular and/or bile duct invasion (Fig. 4C).

Similarly, stratifying survival rates according to the JIS score (Fig. 5) showed that the HR group had a significantly better prognosis than the TACE group in all the 4 scores (JIS score "0" to "3"). The RFA group had a significantly better prognosis than the TACE group only in the JIS score "1" and "3." A comparison between the HR group and the RFA group revealed that the former had a significantly better prognosis than the later in the JIS scores "1" and "2" (Figs. 5B, C). In contrast, the RFA group had an even better prognosis than the HR group in the JIS score "3" (Fig. 5D). The survival rates in the JIS scores "1," "2," and "3" were further stratified according to each criterion (Table 4) on the basis of the "Child-Pugh class" and "TNM stage" (Supplemental Figs 1–3, available at http://links.lww.com/SLA/A388, http://links.lww.com/SLA/A389, and http://links.lww.com/SLA/A390).

Analysis of the Factors Independently Affecting the Survival of Patients

The multivariate initial model provided 11 variables as independent prognostic factors: age, serum albumin, ICGR15, esophageal varices, Child-Pugh class, alpha-fetoprotein, PIVKA-II, tumor size, gross classification, TNM stage, and type of treatment (Supplemental Table 1, available at http://links.lww.com/SLA/A387). Consequently, the multivariate final model showed 12 variables as independent prognostic factors: the 11 variables described earlier and portal venous invasion (Table 6). The stage IVA and gross classification type 3 (confluent multinodular type) had the highest hazard ratio of 3.83 and 2.86, respectively. In particular, the univariate analysis showed no significant difference between the HR group and the RFA group (Table 5), but the multivariate analysis revealed a statistically significant difference (hazard ratio: 1.54, $P = 0.014$) between the 2 groups.

DISCUSSION

In general, it is theoretically difficult to clarify the prognostic factors and therapeutic outcomes after treatments for patients with HCC due to the diversities of tumor stage, degree of chronic liver damage, and therapeutic design, as well as variable etiologic factors of HCC. The present study focused on a relatively small proportion of patients with non-B non-C HCC in Japan, which were further restricted to the patients without extrahepatic metastasis in the Child-Pugh A or B, and which principally met the indications for HR, RFA, and TACE based on the treatment guideline.[22] It was obvious that such strict selection of patients requires huge number of patients to be analyzed. Therefore, the present study used the data of a nationwide follow-up survey by the LCSGJ.

The study first compared the clinical backgrounds among the patients who underwent HR, RFA, or TACE as the initial therapy (Table 1). The degree of liver damage in the HR group was significantly lower than those in the RFA and TACE groups. On the contrary, the HR and TACE groups had significantly more advanced HCC than the RFA group. These findings seem to be consistent with

TABLE 5. Prognostic Factors Determined by the Univariate Analysis in the Patients with Non-B Non-C Hepatocellular Carcinoma

Variables	No. Patient	Survivals (%)			P
		1-yr	3-yr	5-yr	
All	4741	89	70	55	
Age (yr)					
<69	2289	88	72	58	Reference
≥69	2438	90	69	50	0.046
Sex					
Male	3771	88	71	56	Reference
Female	970	91	66	51	0.312
Alcohol					
None	2550	89	69	56	Reference
Positive*	1671	89	72	52	0.907
Serum albumin (g/dL)					
<3.9	2004	85	61	42	Reference
≥3.9	2645	92	76	63	<0.001
Serum total bilirubin (mg/dL)					
<0.8	2004	90	74	61	Reference
≥0.8	2645	88	67	48	<0.001
ICGR15 (%)					
<14	1809	90	75	75	Reference
≥14	1896	89	68	68	<0.001
Prothrombin activity (%)					
<87	2177	88	66	48	Reference
≥87	2239	89	73	61	<0.001
Esophageal varices					
None	3166	90	74	60	Reference
Positive	917	85	58	32	<0.001
Degree of liver damage†					
A	3400	90	90	60	Reference
B	940	85	85	39	<0.001
C	63	68	68	–	<0.001
Child-Pugh class					
A	4063	90	73	58	Reference
B	678	81	51	31	<0.001
Alpha-fetoprotein (ng/mL)					
<15	2638	95	80	63	Reference
≥15	1915	81	57	43	<0.001
PIVKA-II (AU/mL)					
<148	2069	94	79	66	Reference
≥148	2074	84	62	45	<0.001
Tumor number					
1	3165	91	76	62	Reference
>2	1461	84	56	38	<0.001
Tumor size (mm)					
<40	2128	94	77	58	Reference
≥40	2455	85	65	53	<0.001
Gross classification‡					
Type 1	3950	91	73	57	Reference
Type 2	368	70	41	32	<0.001
Type 3	62	55	32	0	<0.001
Portal venous invasion					
Negative	3957	91	73	57	Reference
Positive	493	67	41	24	<0.001
TNM stage†					
I	530	96	83	66	Reference
II	2228	93	78	64	0.121
III	1299	87	62	46	<0.001
IVA	399	64	35	19	<0.001
JIS score					
0	436	97	85	70	Reference
1	2062	94	81	67	0.208
2	1349	87	62	44	<0.001
3	581	71	41	23	<0.001
4	47	49	9	0	<0.001
Type of treatment					
HR	2872	91	77	66	Reference
RFA	432	93	73	49	0.101
TACE	1437	83	55	32	<0.001

*Eighty-six gram of alcohol daily for more than 10 yrs.
†By the Liver Cancer Study Group of Japan.
‡Type 1, simple nodular type; Type 2, simple nodular type with extranodular growth; Type 3, confluent multinodular type.

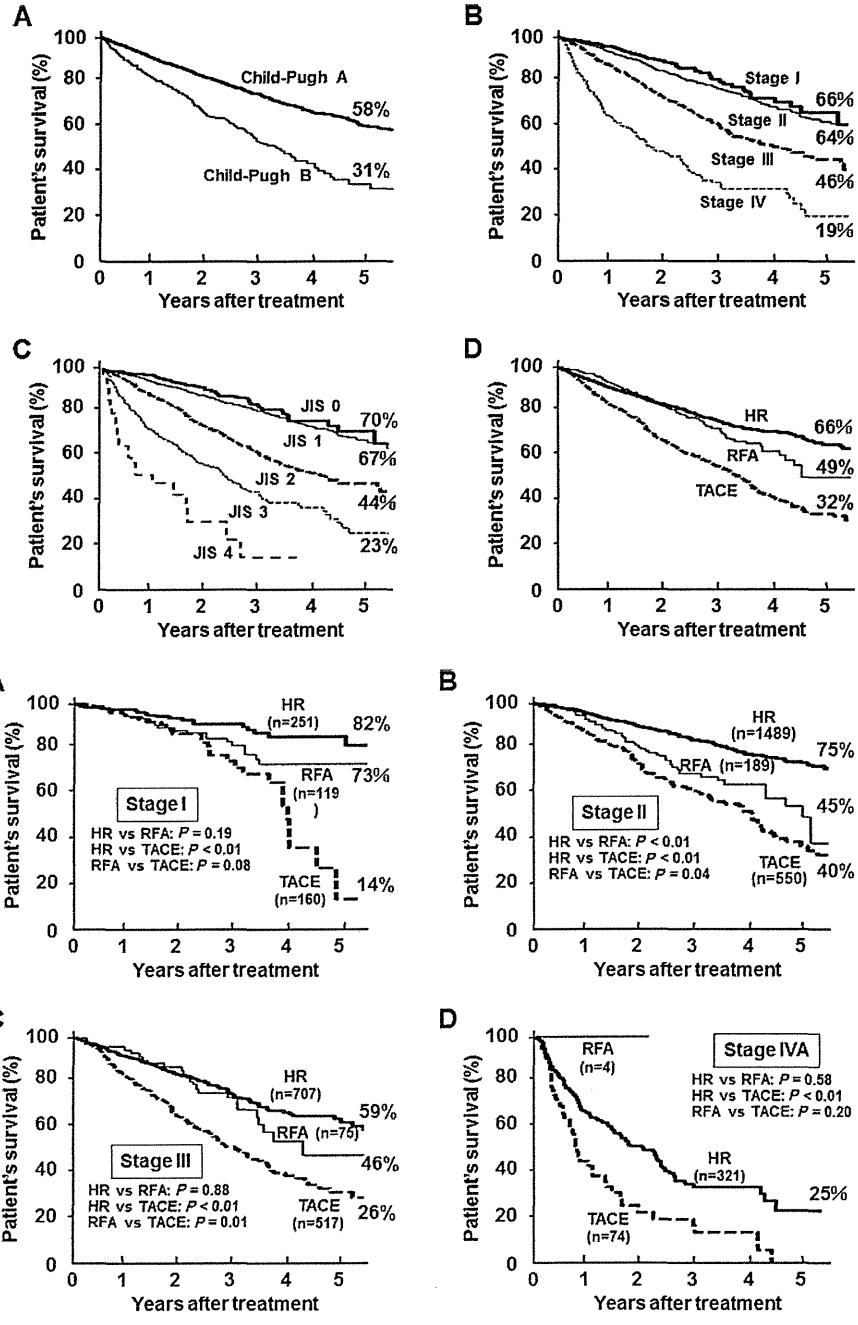

FIGURE 2. Comparisons of the survival rates among liver function, tumor stage, and type of treatment. Survival rates stratified by Child-Pugh A and B (A), staging system according to the Liver Cancer Study Group of Japan (B), JIS score (C), and type of treatment (D). HR vs RFA, $P = 0.30$; HR vs TACE, $P < 0.001$; RFA vs TACE, $P < 0.001$. All comparisons were made the log-rank test with Bonferroni correction.[24]

FIGURE 3. Comparisons of the survival rates among the type of treatment. Survival rates were stratified by stage I (A), stage II (B), stage III (C), and stage IVA (D). All comparisons were made by the log-rank test with Bonferroni correction.

those in other studies that included the patients with HCC of varied etiologies of liver disease. However, none of the previous studies have compared the prognostic factors and therapeutic outcomes after the 3 types of treatment modalities with taking such differences in the clinical backgrounds into consideration, possibly due to the limited number of patients.

The study then analyzed the prognostic factors and found that 17 variables, including types of treatment, were significant prognostic factors. Sex and alcohol abuse were not selected as prognostic factors. Although the synergic action of alcohol and HCV infection on hepatocarcinogenesis has been suggested,[26] alcohol consumption alone may not always affect the progression of HCC. The 5-year survival rate in the TACE group (32%) was significantly poorer, whereas there was no significant difference between the RFA group (49%) and the HR group (66%) in the univariate analysis. The 5-year survival rate after TACE in this series (32%) was almost identical to that (34%) based on the data of same nationwide survey (LCSGJ) during the same periods (January 2000–December 2005) but not restricted to the patients with non-B non-C HCC.[27] Hasegawa et al[18] also used the data of the nationwide survey by LCSGJ and compared the prognosis after surgical resection, RFA, and percutaneous ethanol injection. Their evaluation of more than 7000 HCC patients revealed that the time-to-recurrence rate of surgical resection was significantly better than that of RFA or percutaneous ethanol injection. However, the median follow-up period was only 10.4 months, and they did not provide the 5-year survival rate in their study.

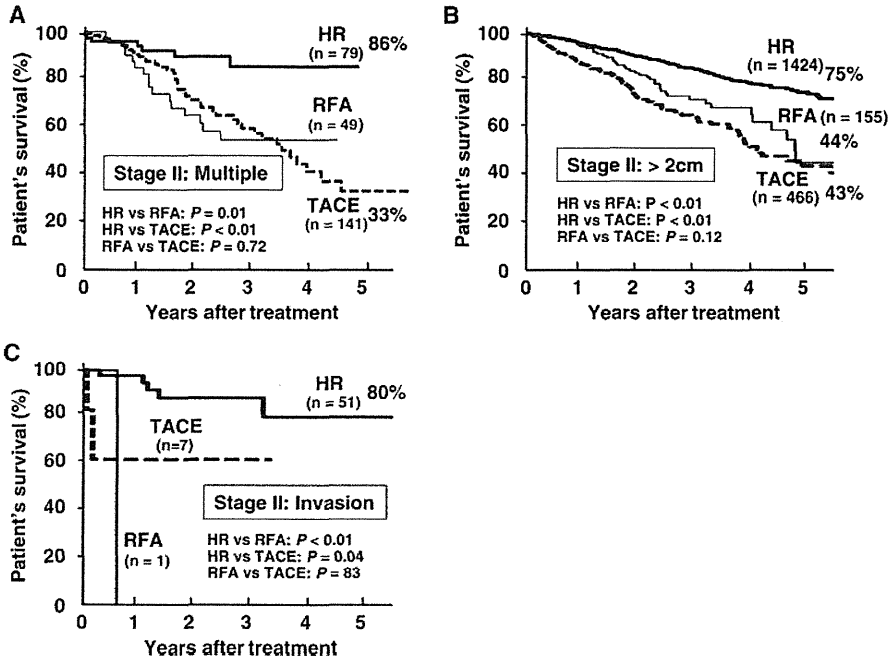

FIGURE 4. Comparisons of the survival rates based on the type of treatment. The survival rates in the stage II were further stratified by number of tumor (A), tumor size (B), and vascular and/or bile duct invasion (C). All comparisons were made by the log-rank test with Bonferroni correction.

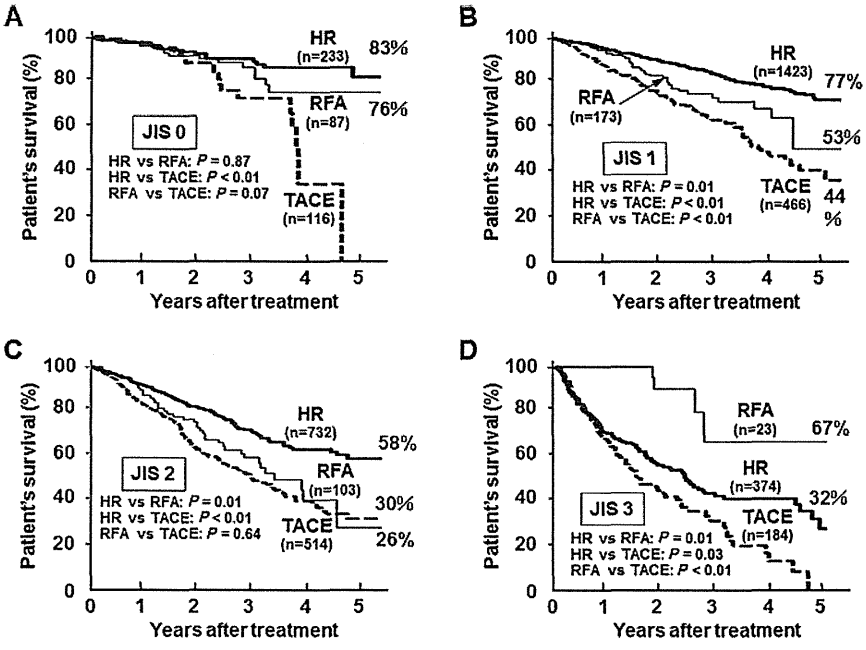

FIGURE 5. Comparisons of the survival rates among the type of treatment. Survival rates were stratified by JIS score "0" (A), JIS score "1" (B), JIS score "2" (C), and JIS score "3" (D). All comparisons were made by log-rank test with Bonferroni correction. [24]

The patients in the TACE group had poorer liver functional reserve and more advanced stage of HCC, thus it would be quite natural that overall survival rate in this group had a poorer prognosis. Because the degree of chronic liver damage and the tumor stage were markedly different among the HR, RFA, and TACE groups, the patients were stratified according to the TNM stage. The study also stratified the patients on the basis of the JIS score.[24] Particularly, the HR group had a significantly better prognosis than the TACE group in all 4 stages and the 4 JIS scores even after the stratifications. On the contrary, the prognosis for the patients in the RFA group did not differ significantly in comparison with those in the TACE group in stages I and IVA and JIS scores "0 and 2." The comparison between the HR group and the RFA group showed the HR group to have a significantly better prognosis than the RFA group only in stage II and in JIS scores "1" and "2." These findings suggest that the HR may not offer prognostic advantages over RFA in the early or far advanced stage of non-B non-C HCC patients. Because the stage II patients included the 3 different types of T categories (Table 3), the survival rates were further stratified on the basis of the T categories (Fig. 4). The HR group had a significantly better prognosis than the RFA group, especially for the patients with multiple tumors and with vascular and/or bile duct invasion. Long-term survival could be expected only after HR in the stage II patients with vascular and/or bile duct invasion (Fig. 4C). Similarly, the survival rates in the

TABLE 6. Independent Prognostic Factors Determined by the Cox Proportional Hazard Regression Analysis With the Backward Elimination Method (Multivariate Final Model)

Variables	No. Patient	Hazard Ratio (95% CI)	*P*
Age (yr)			
<69	1125	Reference	—
≥69	1174	1.37 (1.13, 1.66)	0.001
Serum albumin (g/dL)			
<3.9	939	Reference	—
≥3.9	1360	0.81 (0.66, 0.99)	0.047
ICGR15 (%)			
<14	1129	Reference	—
≥14	1170	1.29 (1.04, 1.59)	0.021
Esophageal varices			
None	1844	Reference	—
Positive	455	1.71 (1.34, 2.17)	<0.001
Child-Pugh class			
A	2032	Reference	—
B	267	1.46 (1.10, 1.92)	0.008
Alpha-fetoprotein (ng/mL)			
<15	1354	Reference	—
≥15	945	1.46 (1.20, 1.79)	<0.001
PIVKA-II (AU/mL)			
<148	1149	Reference	—
≥148	1150	1.60 (1.28, 1.99)	<0.001
Tumor size (mm)			
<40	1015	Reference	—
≥40	1284	1.36 (1.07, 1.74)	0.013
Gross classification*			
Type 1	2105	Reference	—
Type 2	171	1.59 (1.18, 2.12)	0.002
Type 3	23	2.86 (1.48, 5.51)	0.002
Portal venous invasion			
Negative	2068	Reference	—
Positive	231	1.41 (1.04, 1.91)	0.025
TNM stage†			
I	257	Reference	—
II	1168	1.51 (0.97, 2.33)	0.062
III	677	1.96 (1.25, 3.05)	0.003
IVA	197	3.83 (2.27, 6.47)	<0.001
Type of treatment			
HR	1644	Reference	—
RFA	167	1.54 (1.09, 2.19)	0.014
TACE	488	1.56 (1.23, 1.97)	< 0.001

*Type 1, simple nodular type; Type 2, simple nodular type with extranodular growth; Type 3, confluent multinodular type.
†By the Liver Cancer Study Group of Japan.

patients with JIS scores of "1" and "2" were further stratified (Supplemental Figs. 1, 2, available at http://links.lww.com/SLA/A388 and http://links.lww.com/SLA/A389). The effect of HR was observed only in the patients with Child-Pugh class A. Interestingly, the patients in the RFA group (n = 23) in the JIS score "3" subgroup had a significantly better prognosis than the HR group (n = 374). However, after further stratification (Supplemental Fig. 3, available at http://links.lww.com/SLA/A390), there was no statistically significant difference between the 2 groups, possibly because of the small number of patients. A possible therapeutic advantage of RFA in the JIS score "3" patients remains to be confirmed.

Surgical hepatectomy provides better survival and lower recurrence rates than RFA for patients with HCC conforming to the Milan criteria in a randomized clinical trial.[19] The authors considered that segment-based anatomic hepatectomy with at least 1 cm of the rim of nontumor parenchyma eradicates both the primary tumor and intrahepatic micrometastasis. There are 2 types of HCC recurrence; one is "early recurrence" due to intrahepatic metastasis and the other is "late recurrence" due to multicentric hepatocarcinogenesis.[28] Recurrence in non-B non-C HCC are mainly dependent on the advanced tumor factors, such as lager tumor size and portal venous invasion, and thus local control of microscopic intrahepatic metastases is required.[29] The importance of an adequate surgical margin for the non-B non-C HCC has also been reported.[14] Therefore, HR, if a segment-based anatomic hepatectomy is deemed to be possible, should be recommended especially for the patients with stage II or the JIS scores "1" and "2" of non-B non-C HCC. Anatomic hepatectomy with adequate surgical margin may decrease the risk of "early recurrence" of non-B non-C HCC due to intrahepatic metastasis. However, the prediction and prevention of "late recurrence" of non-B non-C HCC due to de novo hepatocarcinogenesis may be difficult, because the background liver diseases can be multifactorial and non-B non-C HCC may develop without displaying any features of severe underlying fibrosis.[29–32] In fact, 13,572 patients underwent HR among the 54,003 total patients for whom the data regarding the hepatitis viral infection status were available (Fig. 1). The incidence of liver cirrhosis based on

the histological examination of resected specimens was 1130 of 2495 patients (45%) with HBV-related HCC, 3666 of 7783 patients (47%) with HCV-related HCC, and 788 of 3040 patients (26%) with non-B non-C HCC, indicating that there was a markedly lower incidence of cirrhosis in the non-B non-C HCC patients. Information regarding the possible etiologies of non-B non-C HCC, such as NASH, diabetes mellitus, autoimmune hepatitis, primary biliary cirrhosis, aflatoxin-B1-contaminated food consumption, and hemochromatosis was not available because of lack of inclusion in the questionnaire sheet of this survey. However, according to the reports describing the recent trend of clinical features in Japanese patients with HCC,[10,33] it is conceivable that a nonnegligible proportion of patients in this study met the criteria for the metabolic syndrome. Potential carcinogenic mediators related to NASH in metabolic syndrome are insulin, lipid peroxidation, free radical oxidative stress, and proinflammatory cytokines.[34–36] Because HCC associated with metabolic syndrome can often develop without significant liver fibrosis,[31,32] metabolic syndrome per se may have a direct oncogenic effect, and it may follow a specific molecular pathway of tumorigenesis different from the usual multistep process: fibrosis-cirrhosis-HCC.[31] In this context, specific strategies for screening "late recurrence" may be required for patients with HCC related to metabolic syndrome, even when underlying chronic liver damage is only minimal.

The molecular mechanisms underlying the individual predisposition to non-B non-C HCC may be different, and a better understanding of these mechanisms will lead to improvements in the prevention and early diagnosis of "late recurrence."[9] Because the number of patients with each etiology is limited, a prospective accumulation of non-B non-C HCC patients including information regarding the possible etiologies is essential, and a nationwide multi-institutional study would be desirable.

Finally, 12 independent prognostic factors, including the type of treatment, were identified by using the Cox proportional hazard regression analysis. There was a significant prognostic advantage of HR not only to TACE but also to RFA. Many studies have compared the outcomes after several therapeutic modalities for patients with HCC,[17–21] most of which compared HR versus RFA, whereas a few studies compared HR versus TACE or RFA versus TACE. This is the first study to compare the prognostic factors and outcomes after 3 types of therapeutic modalities at once. All these findings regarding the non-B non-C HCC patients in Japan may be applicable to the HCC patients in the United States and Western countries where the prominent etiological factors are NASH and metabolic syndrome rather than chronic infection of hepatitis viruses.

Limitations of this study include that the data of TNM staging system of the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) were not available to directly apply the current data to the HCC patients in other countries. However, both the TNM stage by the LCSGJ and the AJCC/UICC were developed on the basis of a survival analysis of patients who underwent HR. Therefore, the applicability of these surgical staging systems to other therapies, such as RFA and TACE, has been a matter of controversy.[37] Comparisons of clinicopathological features and prognostic factors between the non-B non-C HCC and HCC caused by other etiological factors, such as HBV- and HCV-related HCC, are beyond the scope of this study. Because the current study was not prospectively randomized, the treatment polices were not regulated and the effectiveness of each treatment might not be comparable among the different institutions. In addition, although this study used a multivariate analysis to assess the impact of diverse background on outcomes, there are limits to such a statistical approach.

CONCLUSIONS

This large prospective study based on data derived from a nationwide follow-up survey suggested that HR offers prognostic advantage over RFA and TACE although such advantage may depend upon the degrees of chronic liver damage and the tumor stage.

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The Bcl-2 Homology Domain 3 (BH3)-only Proteins Bim and Bid Are Functionally Active and Restrained by Anti-apoptotic Bcl-2 Family Proteins in Healthy Liver*[S]

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Background: A fine balance between the anti- and pro-apoptotic multidomain Bcl-2 family proteins controls hepatocyte apoptosis in the healthy liver.
Results: Disruption of the BH3-only proteins Bim and Bid prevents spontaneous hepatocyte apoptosis in the absence of anti-apoptotic Bcl-2 family proteins.
Conclusion: Hepatocyte integrity is maintained by the well orchestrated Bcl-2 network.
Significance: We demonstrated the novel involvement of BH3-only proteins in the healthy Bcl-2 network of the liver.

An intrinsic pathway of apoptosis is regulated by the B-cell lymphoma-2 (Bcl-2) family proteins. We previously reported that a fine rheostatic balance between the anti- and pro-apoptotic multidomain Bcl-2 family proteins controls hepatocyte apoptosis in the healthy liver. The Bcl-2 homology domain 3 (BH3)-only proteins set this rheostatic balance toward apoptosis upon activation in the diseased liver. However, their involvement in healthy Bcl-2 rheostasis remains unknown. In the present study, we focused on two BH3-only proteins, Bim and Bid, and we clarified the Bcl-2 network that governs hepatocyte life and death in the healthy liver. We generated hepatocyte-specific Bcl-xL- or Mcl-1-knock-out mice, with or without disrupting Bim and/or Bid, and we examined hepatocyte apoptosis under physiological conditions. We also examined the effect of both Bid and Bim disruption on the hepatocyte apoptosis caused by the inhibition of Bcl-xL and Mcl-1. Spontaneous hepatocyte apoptosis in Bcl-xL- or Mcl-1-knock-out mice was significantly ameliorated by Bim deletion. The disruption of both Bim and Bid completely prevented hepatocyte apoptosis in Bcl-xL-knock-out mice and weakened massive hepatocyte apoptosis via the additional *in vivo* knockdown of *mcl-1* in these mice. Finally, the hepatocyte apoptosis caused by ABT-737, which is a Bcl-xL/Bcl-2/Bcl-w inhibitor, was completely prevented in Bim/Bid double knock-out mice. The BH3-only proteins Bim and Bid are functionally active but are restrained by the anti-apoptotic Bcl-2 family proteins under physiological conditions. Hepatocyte integrity is maintained by the dynamic and well orchestrated Bcl-2 network in the healthy liver.

Apoptosis via the intrinsic pathway, which is known as the mitochondrial pathway, is regulated by Bcl-2 family members. These members are divided into two groups as follows: core Bcl-2 family proteins, which possess three or four Bcl-2 homology domains (BH1–BH4)[2] and the Bcl-2 homology domain 3 (BH3)-only proteins (1). The former, which are multidomain proteins, are subdivided into pro- and anti-apoptotic proteins. Pro-apoptotic core Bcl-2 family members, such as Bax and Bak, serve as effector molecules of this apoptotic machinery. Upon activation, these members can form pores to permeabilize the mitochondrial outer membrane. Apoptogenic factors, such as cytochrome *c*, can then be released through this membrane into the cytosol, leading to the activation of the caspase cascade and to cellular demise (2). Anti-apoptotic core Bcl-2 family members, including Bcl-2, Bcl-xL, Mcl-1, Bcl-w, and Bfl-1/A1, inhibit the intrinsic pathway of apoptosis by either directly or indirectly antagonizing Bak/Bax activity (3–5). In the original rheostasis model, cellular life and death are regulated by a balance between these anti- and pro-apoptotic core Bcl-2 family proteins (6). We previously reported that the hepatocyte-specific deletion of the *bcl-x* gene resulted in spontaneous hepatocyte apoptosis, and this effect could be completely prevented by the additional deletion of the *bak* and *bax* genes (7). These findings elucidated the importance of the rheostatic balance of the core Bcl-2 family proteins in controlling hepatocyte apoptosis in the healthy liver.

The BH3-only proteins, which include at least eight members, are considered to function as pro-apoptotic sensors, and these proteins set this rheostatic balance toward apoptosis upon activation by a variety of apoptotic stimuli (8, 9). It has been reported that hepatocyte apoptosis through the activation of these BH3-only proteins is involved in the pathophysiology of various liver diseases (10–12). Alternatively, we previously reported that the slight activation of Bid, which can trigger hepatocyte apoptosis, occurs even in the healthy liver and that the inactivation of Bid partially ameliorated spontaneous hepato-

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[2] The abbreviations used are: BH1–BH4, Bcl-2 homology domains 1–4; SCID, severe combined immune deficiency; ALT, alanine aminotransferase.