Table 2 Efficacy of combination therapy

	Total patients (n = 220) % (n)	Age <60 years (n = 154) % (n)	Age ≥60 years (n = 66) % (n)	P
SVR rate (intention-to-treat)	36.4 (80/220)	38.3 (59/154)	31.8 (21/66)	0.3589
SVR rate (per-protocol)	43.7 (80/183)	45.0 (59/131)	40.4 (21/52)	0.5671
ETR rate (intention-to-treat)	71.8 (158/220)	71.4 (110/154)	72.7 (48/66)	0.8444
ETR rate (per-protocol)	81.4 (149/183)	79.4 (104/131)	86.5 (45/52)	0.2621
SVR/relapse/NR/discontinuation	80/69/34/37	59/45/27/23	21/24/7/14	0.2834
Ribavirin discontinuation rate	24.5 (54/220)	20.8 (32/154)	33.3 (22/66)	0.0474
Ribavirin dose reduction rate	33.6 (74/220)	29.9 (46/154)	42.4 (28/66)	0.0709
IFN discontinuation rate	16.8 (37/220)	14.9 (23/154)	21.2 (14/66)	0.2540
IFN dose reduction rate	15.9 (35/220)	15.6 (24/154)	16.7 (11/66)	0.8406
Combination therapy discontinuation rate	16.8 (37/220)	14.9 (23/154)	21.2 (14/66)	0.2540

ETR, end of treatment virologic response; IFN, interferon; NR, non-response; SVR, sustained virologic response.

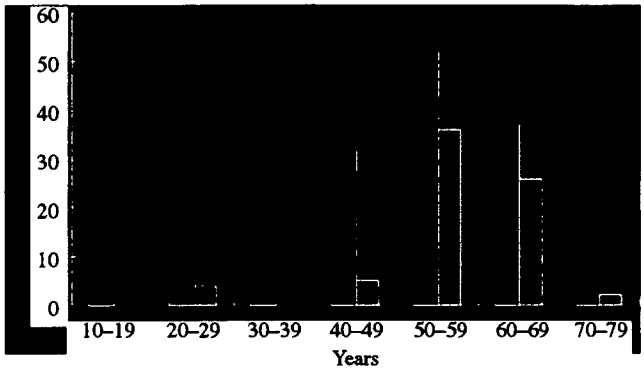

Figure 1 Patient age distribution by decade. (■) Male; (□) female.

using SAS software (SAS Institute, Cary, NC, USA). All P were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Patients were 147 men and 73 women aged 17–71 years (mean ± SD, 53.0 ± 11.1 years). The age distribution of patients treated with combination therapy is shown in Fig. 1. Patients ≥ 60 years comprised 30.0% of the patient population (66/220). The majority of female patients were over age 50 years (87.7%, 64/73). Clinical characteristics of the two study groups are shown in Table 1. The hemoglobin level was significantly lower in patients aged ≥60 years than in patients aged <60 years ($P = 0.0056$). Creatinine clearance in patients aged ≥60 years was worse than that in patients aged <60 years ($P < 0.0001$).

Response to therapy

The ribavirin discontinuation rate was significantly higher in patients aged ≥60 years than in patients aged <60 years ($P = 0.0474$). The dose ribavirin reduction was higher in the patients aged ≥60 years, but the difference did not reach statistical significance (42.4% *vs* 29.9%; $P = 0.0709$). However, the IFN discontinuation and dose reduction rate did not differ significantly between the two groups. The treatment discontinuation rate did not differ significantly between the two groups. As a result, the SVR rate by both intention-to-treat analysis and per-protocol analysis did not differ significantly between the two groups. And ETR rate by both intention-to-treat analysis and per-protocol analysis also did not differ significantly between the two groups (Table 2).

Histologic factor associated with SVR were determined by univariate analysis. The SVR rate of the F0–1 patients was not different from that of the F2–4 patients (49.3% *vs* 47.7%, $P = 0.8490$ by per-protocol analysis; 43.9% *vs* 38.3%, $P = 0.4651$ by intention-to-treat analysis). Factors associated with SVR in combination therapy were determined by multivariate analysis (Table 3). Genotype ($P < 0.0001$, odds ratio 0.074, 95% confidence interval [CI]: 0.030–0.182), and viral load ($P = 0.0002$, odds ratio 1.002, 95%CI: 1.001–1.004]) were significantly associated with SVR, but age was not significantly associated with SVR.

Clinical characteristics of the 66 patients aged ≥60 years who underwent combination therapy and 47 historical control patients aged ≥60 years who underwent monotherapy are shown in Table 4. The SVR rate with combination therapy was significantly higher than that with monotherapy (31.8%, 21/66 *vs* 10.6%, 5/47, $P = 0.0084$ by intention-to-treat analysis; 40.4%, 21/52 *vs* 10.6%, 5/47, $P = 0.0008$ by per-protocol analysis). Treatment discontinuation rate of combination therapy tends to be higher than that of monotherapy, but there was no significant difference between the two groups. This is because the number of patients undergoing monotherapy was small.

Virologic response to combination therapy and to IFN monotherapy in patients with HCV genotype 1 and a high viral load are shown by age group in Fig. 2.

With monotherapy, the SVR rate decreased with age, but with combination therapy, the SVR rates of patients in their 40s, 50s, and 60s and higher were similar. In patients ≥60 years with genotype 1 and a high viral load, the SVR rate with combination therapy was significantly higher than that with monotherapy (27.5% *vs* 6.7%, $P = 0.0322$ by per-protocol analysis).

Virologic responses to combination therapy and to IFN monotherapy in patients with HCV genotype 2 and a high viral load are shown by age group in Fig. 3.

Table 3 Factors associated with SVR to combination therapy ($n = 220$; multivariate analysis)

Variable		Odds ratio (95%CI)	*P*
Sex	Male *vs* female	0.808 (0.365–1.789)	0.5985
Age (years)		1.015 (0.983–1.048)	0.3677
Baseline serum ALT (IU/L)		0.997 (0.992–1.002)	0.1973
Genotype	1 *vs* 2	0.074 (0.030–0.182)	<0.0001
Viral load (KIU/mL)		1.002 (1.001–1.004)	0.0002

ALT, alanine aminotransferase; CI, confidence interval; SVR, sustained virologic response.

Table 4 Treatment efficacy in patients aged ≥60 years

	Combination therapy ($n = 66$)	Monotherapy ($n = 47$)	*P*
Sex ratio (male/female)	38/28	30/17	0.5033
Baseline serum ALT(IU/L)	97.6 ± 62.0	100.0 ± 71.8	0.8536
Genotype (1/2)	54/12	34/13	0.2316
Activity (A0/A1/A2/A3)	1/22/19/4	1/18/25/0	0.1593
Fibrosis (F0/F1/F2/F3/F4)	2/20/11/9/3	2/16/20/7/0	0.1773
SVR rate (intention-to-treat)	31.8 (21/66)	10.6 (5/47)	0.0084
SVR rate (per-protocol)	40.4 (21/52)	10.6 (5/47)	0.0008
SVR/relapse/NR/discontinuation	21/24/7/14	5/23/15/4	<0.0001
Treatment discontinuation rate	21.2 (14/66)	8.5 (4/47)	0.0690

ALT, alanine aminotransferase; NR, non-response; SVR, sustained virologic response.

With combination therapy, the SVR rate was similar for all age groups. In patients ≥60 years with genotype 2 and a high viral load, the SVR rate was significantly higher with combination therapy than with monotherapy (83.3% *vs* 23.1%, $P = 0.0048$ by per-protocol analysis and by intention-to-treat analysis).

Adverse events

For 14 of 74 patients with dose reduction of ribavirin, ribavirin was reduced due to fatigue and anemic symptoms though the hemoglobin levels were above 10 g/dL, which is the level of dose reduction of this study. The combination therapy discontinuation rate was not statistically different between patients aged ≥60 years and those aged <60 years (Table 2). The combination therapy discontinuation rate was higher in combination therapy (21.2%) than in monotherapy (8.5%) among patients aged ≥60 years (Table 4). The reasons for discontinuation of the combination therapy and the times at which the therapy was discontinued are shown in Table 5. If discontinuation of treatment occurred we did not restart therapy after disappearance of the initial symptom or illness. Ribavirin discontinuation was higher in older patients ($P < 0.05$). A serious adverse effect occurred in one patient in each group: infarction of vessel in the retina in the older group and cerebral hemorrhage in the younger group.

Effect of dose reduction and discontinuation of ribavirin or IFN on the SVR rate

Ribavirin dose reduction and discontinuation rates are shown according to age group in Fig. 4. The total of dose reduction and discontinuation rates increased with age. The SVR of patients who completed treatment was 44.7% (51/114). Among patients who had dose reduction, the SVR was 36.5% (19/52). Among patients who discontinued treatment, the SVR was 18.5% (10/54). The SVR was not significantly different between those in whom the dose of ribavirin was reduced and those in whom it was not. Creatinine clearance in patients who needed dose reduction or discontinuation of ribavirin was worse than that in patients who did not (90.2 ± 20.9 mL/min *vs* 107.5 ± 24.2 mL/min, $P < 0.0001$). The SVR in those who completed full treatment was significantly higher than that in those who had reduced-dose IFN (39.5% *vs* 20%, $P = 0.0282$). The SVR in those who completed full treatment was significantly higher than that in those who had discontinued IFN (43.3% *vs* 5%, $P < 0.0001$).

Comparison between 24-week and 48-week treatment

Among the patients with HCV genotype 1, the SVR of 48-week treatment was significantly higher than that of 24-week treatment (48.1% *vs* 24.3%, $P = 0.0148$ by per-protocol analysis; 37.1% *vs* 19.4%, $P = 0.0265$ by intention-to-treat analysis). However, among the patients with HCV genotype 2, the SVR of the 48-week treatment was similar to that of the 24-week treatment (75.0% *vs* 85.0%, $P = 0.4884$ by per-protocol analysis; 75.0% *vs* 81.0%, $P = 0.6997$ by intention-to-treat analysis).

The IFN dose reduction rate for 48-week treatment was significantly higher than that of 24-week treatment (27.3% *vs* 13.1%, $P = 0.0212$). The treatment discontinuation rate for the 48-week course was not statistically different from the 24-week course (20.5% *vs* 17.6%, $P = 0.6621$).

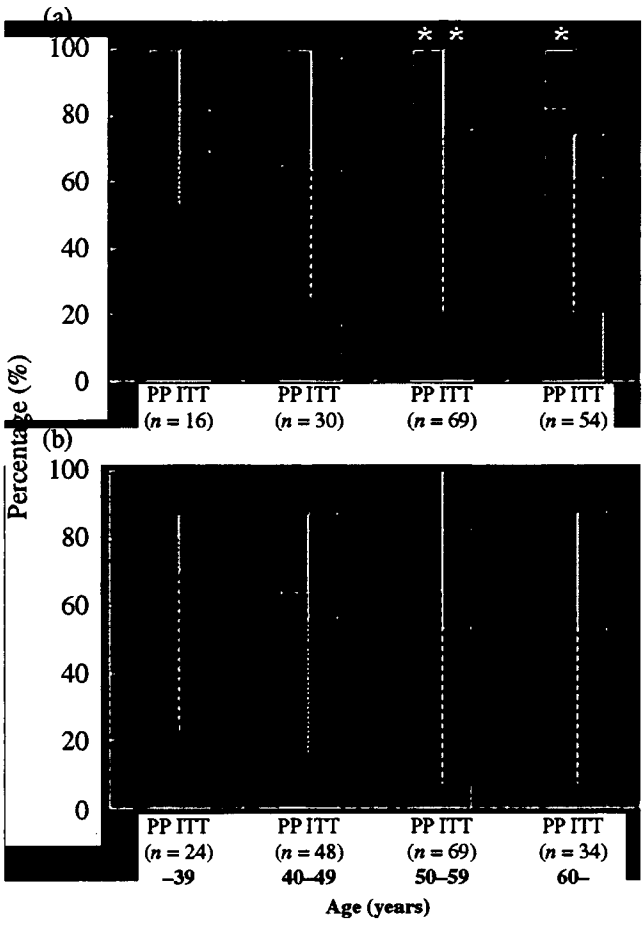

Figure 2 Virologic response to (a) combination therapy and (b) interferon (IFN) monotherapy according to age of patients with genotype 1 and a high viral load. Asterisks indicate significant differences *vs* the respective IFN monotherapy (*$P < 0.05$). (▥) Treatment discontinuation; (□) non-responder; (▩) relapse; (■) sustained virologic response. ITT, intention-to-treat analysis; PP, per-protocol analysis.

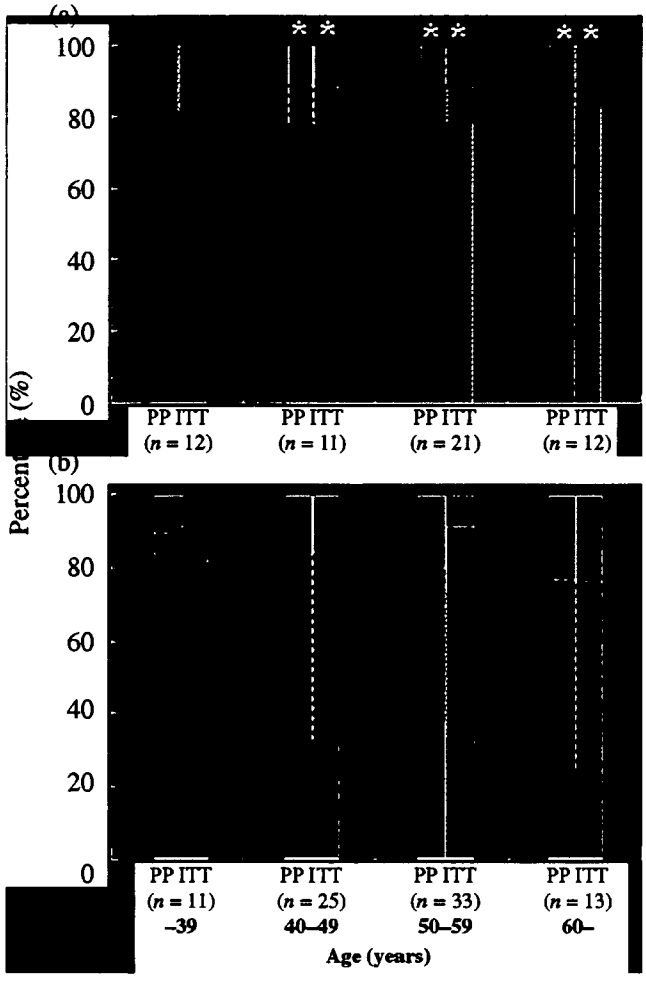

Figure 3 Virologic response to (a) combination therapy and (b) interferon (IFN) monotherapy according to age of patients with genotype 2 and high viral load. Asterisks indicate significant differences *vs* the respective IFN monotherapy (*$P < 0.05$). (▥) Treatment discontinuation; (□) non-responder; (▩) relapse; (■) sustained virologic response. ITT, intention-to-treat analysis; PP, per-protocol analysis.

Discussion

It is important to eradicate HCV by IFN to reduce the risk of hepatocellular carcinoma.[4,5] In addition, IFN reportedly reduces liver-related mortality in chronic hepatitis C patients aged >60 years.[16,17] However, these findings are based on studies of IFN monotherapy. The present study showed the effect of ribavirin and IFN in combination. Ribavirin has been used in combination with IFN to treat chronic hepatitis C, and this combination therapy has been reported to be more effective than IFN monotherapy for eradicating HCV.[7–10] However, ribavirin and IFN or pegylated IFN in combination produce a common adverse effect, that is, hemoglobin levels decrease in 20–36% of treated patients with chronic hepatitis C, necessitating dose reduction or discontinuation.[7,8,18,19]

It has been reported that there is no significant difference in the efficacy of IFN monotherapy between older and younger patients after standardization of their background clinical characteristics, suggesting that age itself does not influence the outcome of IFN monotherapy.[11,12] However, the efficacy and tolerability of combination therapy in the elderly patient has not been clarified. We therefore conducted a multi-institution study to evaluate the efficacy and tolerability of ribavirin plus IFN-α in older patients with chronic hepatitis C.

Multivariate analysis showed baseline viral load and genotype to be the only significant factors associated with SVR. Age was not associated with SVR. Many studies have shown baseline viral load and genotype to be significant factors associated with SVR.[8,19] Our results suggest that the SVR of patients aged ≥60 years is comparable to that of younger patients. Because the SVR differs according to genotype and viral load, we classified patients by genotype and compared the SVR rate for both combination therapy and IFN monotherapy. In patients aged ≥60 years, the SVR rate of combination therapy was significantly increased over that of IFN monotherapy (in patients with genotype 1 and a high

Table 5 Reasons for discontinuation of combination therapy

Patients aged < 60 years			Patients aged ≥ 60 years		
Reason	*n*	Weeks after starting treatment	Reason	*n*	Weeks after starting treatment
Cerebral hemorrhage	**1**	**4**	**Infarction in the retina**	**1**	**14**
Rash	5	1,1,5,22,25	Fatigue	4	4,12,12.14
Fatigue	5	6,12,20,20,21	Anemia	3	10,16, 22
Depression	2	4,10	Anorexia	2	1,19
Anorexia	2	21,23	Nervousness	1	2
Vomiting	1	2	Dizziness	1	6
Anemia	1	4	Vomiting	1	16
Worsening diabetes	1	16	Depression	1	18
Spontaneous pneumothorax	1	17			
Hypothyroidism	1	18			
Uterine cancer	1	20			
Thyroiditis	1	24			
Pancytopenia	1	37			

Bold, serious adverse effect.

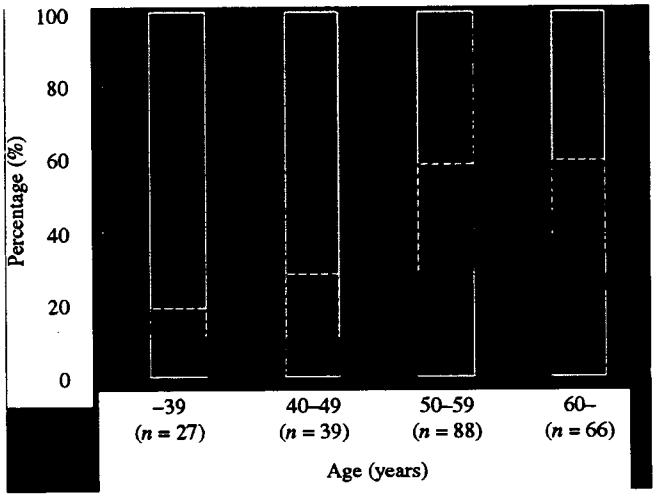

Figure 4 Ribavirin dose reduction and discontinuation rates according to age of patients ($n = 220$). (□) Completion: SVR 44.7% (51/114); (▨) dose reduction: SVR 36.5% (19/52); (■) treatment discontinuation: SVR 18.5% (10/54). SVR, sustained virologic response.

viral load by per-protocol analysis, 27.5% *vs* 6.7%, $P = 0.032$; in patients with genotype 2 and a high viral load by per-protocol analysis, 83.3% *vs* 23.1%, $P = 0.0048$; Figs 2,3). Moreover, the SVR rate among patients aged ≥60 years with HCV genotype 1 did not decrease with age. Neither did the SVR rate change with age for patients ≥60 years with genotype 2. Patients with genotype 2 achieved a high SVR rate of approximately 80% in all age categories. Adverse effects are thought to increase in elderly patients, but adverse effects necessitating discontinuation of IFN and ribavirin did not differ significantly between the older and younger patients (21.2% *vs* 14.9%). In addition, the severe adverse effects were not associated with age. These findings were similar to previously reported findings that there was no difference between young and elderly patients with respect to adverse effects.[11,12] The treatment discontinuation rate tended to be higher in combination therapy (14/66) than in monotherapy (4/47) among patients aged ≥60 years, but there was no significantly difference between the two groups. (Table 4). This is because there was a small number of patients in the monotherapy group. The reason for discontinuation of combination therapy in seven of 14 patients was ribavirin-related adverse effects such as general fatigue or anemia.

The ribavirin dose reduction and discontinuation rates increased with age, but the SVR rate did not differ significantly between patients with and without dose reduction who completed the treatment schedule (36.5% *vs* 44.7%). These findings are consistent with previously reported findings.[19] In patients aged ≥60 years with HCV genotype 1 and a high viral load, the SVR rate did not differ significantly between combination therapy and IFN monotherapy by intention-to-treat analysis, but it did differ significantly by per-protocol analysis. These findings indicate that rather than discontinuing treatment, we should continue as permitted by dose reduction of ribavirin. In groups 50–59 years and >60 years of age the rate of dose reduction and treatment discontinuation was similarly high. In contrast, in groups <50 years of age the rate was low. In the present study we focused on patients aged ≥60 years because 60 years is often used as a cut-off for older patients; if we had focused on patients ≥65 years the number of study patients would have decreased and the comparison would have been difficult. There were high dose-reduction and discontinuation rates in the patients aged ≥50 years, so we should consider dose modification for these patients in advance.

Careful monitoring and appropriate reduction of the ribavirin dose is required to circumvent the need for discontinuation in elderly patients.[20,21] Also, it will be necessary to be careful when treating elderly patients with other diseases commonly observed in this age group, such as diabetes or hypertension. The present study, however, was limited due to being a retrospective analysis and using of historical controls, therefore further prospective studies are needed.

In conclusion, combination therapy was shown to be of comparable efficacy for chronic hepatitis C between patients aged <60 years and those aged ≥60 years, although the rate of ribavirin

discontinuation was shown to be higher among the older patients than among the younger patients. The efficacy of combination therapy was shown to be greater than that of IFN monotherapy in older patients.

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Role of tumor markers in assessment of tumor progression and prediction of outcomes in patients with hepatocellular carcinoma

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The efficacies of tumor markers, alfa-fetoprotein (AFP), *Lens culinaris* agglutinin A-reactive fraction of alfa-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP) were evaluated for assessment of progression of hepatocellular carcinoma (HCC) and patient prognosis. The prevalence of elevated levels of each tumor marker increased with progression of tumor stage for all three markers among patients with HCC. Survival was poorer among patients with elevated levels of tumor markers than among those without elevated levels. Evaluation of tumor progression with tumor markers was based only on the results of laboratory tests. The tests are objective, simple to perform, and easy to repeat, and therefore, may be useful to supplement conventional tumor staging for the evaluation of tumor progression and prediction of patient outcome.

Key words: hepatocellular carcinoma, AFP, AFP-L3, DCP, progression, prognosis

Hepatocellular carcinoma (HCC) is a common cause of death in patients with chronic hepatitis and cirrhosis.[1,2] It is one of the most important malignancies in Japan; the incidence of HCC has increased over the last 30 years and has more than doubled in the last 10 years. HCC is currently the third leading cause of cancer-related death in Japan.[3]

Assessment of the progression of HCC is based on tumor morphology such as tumor size, number of tumors, and portal vein thrombosis. These factors are usually evaluated by imaging or pathologic examination. However, the sensitivity of imaging examination for evaluation of tumor progression varies and is related to the imaging modalities, the examiner's skill, and the imaging apparatuses. We often encounter discrepancies on the size or number of HCCs measured with different imaging modalities (Fig. 1). Another difficulty in the evaluation of tumor progression is discrepancy between imaging findings and pathologic results from the resected specimen, especially with respect to vascular invasion. It is not possible to evaluate microscopic vascular invasion by means of imaging studies, which likely leads to underestimation of the degree of vascular invasion. Moreover, it is often difficult to accurately evaluate tumor progression by imaging studies in cases of recurrent HCC. Patients who have undergone repeated treatments may have necrotized tumors from locoregional ablative therapy (LAT) such as radiofrequency ablation (RFA), or tumors that have retained lipiodol after transcatheter arterial chemoembolization (TACE).

Several tumor markers for HCC have been reported.[4–9] Of these, alfa-fetoprotein (AFP), *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP) are currently used in clinical practice in Japan. These markers were originally used for detection and diagnosis of HCC in routine clinical settings;[4–6] however, the efficacies of these tumor markers for this purpose are not satisfactory. AFP is the most common tumor marker for HCC, but there are many patients who show elevated AFP in the absence of HCC. AFP levels are elevated in up to 20% of patients with chronic hepatitis and in 20–60% of patients with cirrhosis, even in the absence of HCC.[10] In contrast,

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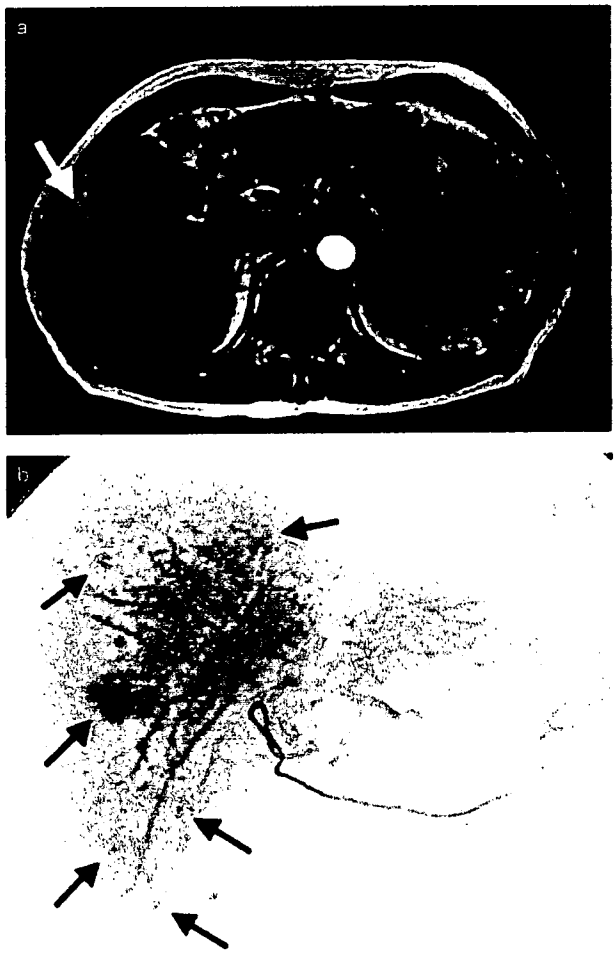

Figure 1 (a) Dynamic magnetic resonance imaging study of a 59-year old, male outpatient revealed a solitary tumor in the right lobe of the liver (white arrow). However, (b) digital subtraction arteriography performed after hospitalization showed multiple small hepatocellular carcinoma nodules throughout the right lobe (black arrows).

AFP-L3 has reportedly high specificity for HCC, but its sensitivity is low. Other investigators have reported that elevation of these tumor markers, especially of AFP-L3 and DCP, may be indicative of events related to tumor progression, such as invasion of the portal vein by HCC or an increase in the intratumoral arterial blood supply of HCC.[11–15] In a recent study, we showed that a combination of these tumor markers is reflective of progression of HCC and accurately predicts patient survival.[16] We found that tumor markers AFP-L3 and DCP reflect different features of tumor progression, and that the number of elevated tumor markers can be used to predict patient survival.

In this paper, we describe the role of tumor markers in the assessment of tumor progression and prediction of patient outcome.

MEASUREMENT OF TUMOR MARKERS AND CUT-OFF LEVELS

THE THREE TUMOR markers were measured routinely at the time of initial HCC diagnosis. The serum AFP level was determined by enzyme-linked immunosorbent assay with a commercially available kit (ELISA-AFP, International Reagents, Kobe, Japan). Serum AFP-L3 was measured by lectin-affinity electrophoresis coupled with antibody-affinity blotting (AFP Differentiation Kit I., Wako Pure Chemical Industries, Osaka, Japan) and was expressed as a percentage (AFP-L3 = AFP-L3 level/total AFP level × 100).[5,17] The serum DCP level was determined by means of sensitive enzyme immunoassay (Eitest PIVKA-II kit, Eisai Co., Tokyo, Japan) according to the manufacturer's instructions.[6,18,19] In our paper, values of 400 ng/mL, 15%, and 100 mAU/mL were used as cut-off values to establish elevation of AFP, AFP-L3, and DCP, respectively, according to previous reports.[20–22]

Tumor progression as shown by imaging findings was assessed on the basis of the TNM classification of the Liver Cancer Study Group of Japan.[23] In most cases, the maximum diameter of the tumor was determined with B-mode ultrasonography (US). Vascular invasion was assessed with dynamic computed tomography (CT) and angiography. Lymph node invasion and distant metastases were assessed with ultrasonographic, dynamic CT, and chest X-ray screenings. Bone scintigraphy or brain CT was performed if suggestive symptoms were present.

ELEVATION OF TUMOR MARKERS AND TUMOR PROGRESSION

THE PERCENTAGE OF patients with elevation of each tumor marker according to the progression of tumor stage is shown in Figure 2. The percentage of patients with elevated levels of AFP, AFP-L3, and DCP increased in parallel with increases in tumor stage. These findings indicate that levels of tumor markers of HCC increase with progression of the tumor and that mea-

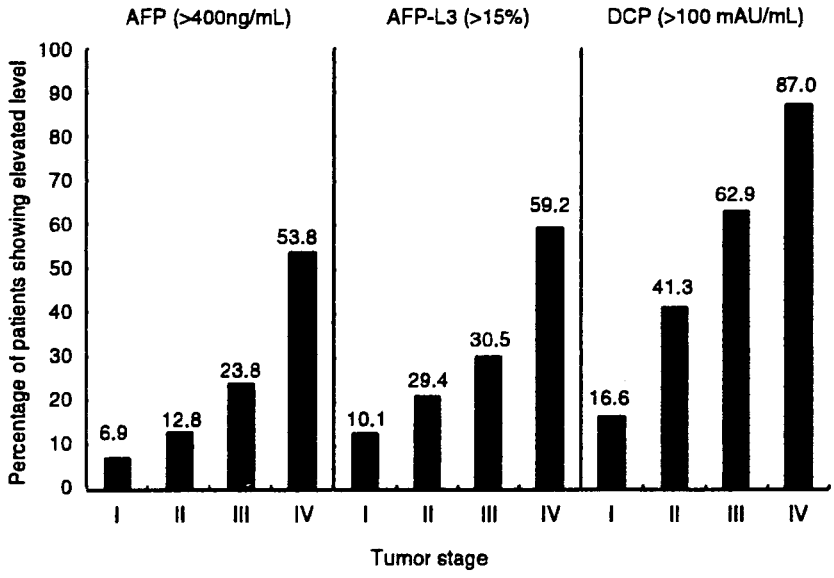

Figure 2 Percentage of patients with elevated level of tumor markers AFP, AFP-L3, and DCP. For all three tumor markers, the percentage of patients with elevated level gradually increased in parallel with the progression of tumor stage (I–IV). AFP, alfa-fetoprotein; AFP-L3, *Lens culinaris* agglutinin A-reactive fraction of alfa-fetoprotein; DCP, des-gamma-carboxy prothrombin.

surement of these markers would be useful also for evaluation of HCC progression.

ELEVATION OF TUMOR MARKERS AND PATIENT SURVIVAL

THE 1-, 3-, 5-, 8-, and 10-year survival rates of patients with HCC according to each tumor marker are shown in Table 1. For all three tumor markers, the rate of survival of patients with elevated tumor marker level is lower than that of patients without elevated level. The difference in survival was significant according to the elevation of AFP, AFP-L3, and DCP ($P < 0.0001$ for all three tumor markers).

USE OF TUMOR MARKERS TO MONITOR CLINICAL COURSES OF PATIENTS WITH HCC

EVALUATION OF TUMOR stage is often difficult in patients with recurrent HCC who have received repeated treatments for HCC, such as LAT including percutaneous ethanol injection (PEIT), percutaneous microwave thermocoagulation (PMCT), RFA, and TACE. US, CT, or magnetic resonance imaging studies usually reveal a mixture of necrotized tumor tissue due to previous LAT and tumors retaining lipiodol after previous TACE. Accurate assessment of tumor stage in these cases requires careful evaluation of imaging results with distinguishing viable tumors from treated tumors and identifying local recurrences (Figs 3,4). In addition, fre-

Table 1 Survival rates according to level of tumor markers at the time of initial diagnosis of hepatocellular carcinoma

	Survival rate (%)				
	1 year	3 years	5 years	8 years	10 years
AFP (≤400 ng/mL) ($n = 2076$)	88.6	64.8	45.3	20.4	14.1
AFP (>400 ng/mL) ($n = 524$)	62.6	40.7	25.7	0	0
AFP-L3 (≤15%) ($n = 1899$)	89.8	67.7	46.4	21.6	14.9
AFP-L3 (>15%) ($n = 721$)	66.2	38.5	27.9	11.1	0
DCP (≤100 mAU/mL) ($n = 1347$)	92.4	73.9	52.6	24.5	15.8
DCP (>100 mAU/mL) ($n = 1253$)	73.8	44.8	28.9	12.7	12.7

AFP, alfa-fetoprotein; AFP-L3, *Lens culinaris* agglutinin A-reactive alfa-fetoprotein; DCP, des-gamma-carboxy prothrombin.

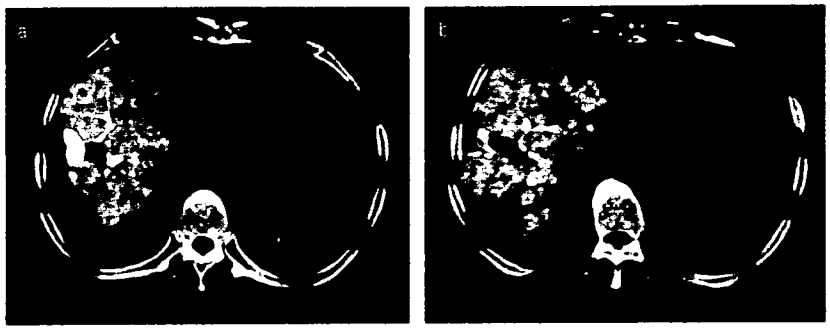

Figure 3 (a, b) Enhanced computed tomography images of recurrent HCC in a 63-year-old man. At the time of evaluation of recurrent HCC, many small, viable HCC nodules were mixed with tumor tissue necrotized by radiofrequency ablation and tumors retaining lipiodol after transcatheter arterial chemoembolization. Of the three tumor markers, only alfa-fetoprotein showed increased levels at the time of this imaging examination. This patient has survived for more than 4 years.

quent monitoring of patients by radiologic methods increases the exposure of patients to radiation.[24] In contrast, assessment of tumor progression with tumor markers can be done frequently with simple blood tests, and the results are not influenced by previous treatments. Monitoring of patients with HCC by means of imaging studies is necessary in the management of patients with HCC; however, monitoring these patients in combination with several tumor markers that can be done more easily and frequently would play a role as a supplemental tool for follow-up (Fig. 5).

LIMITATIONS OF TUMOR MARKERS

ONE IMPORTANT LIMITATION of the assessment of tumor progression with tumor markers is that it can not be used for planning of treatment or treatment itself. Treatment planning and treatment procedures always require imaging study, although data pertaining to tumor markers may provide additional information that would influence the choice of treatment options. Another disadvantage is that tumor marker level is influenced by drugs such as vitamin K and warfarin. Therefore, the results of tumor marker analyses must be interpreted carefully.

CONCLUSION

TUMOR MARKERS HAVE potential as modalities for assessment of the progression of HCC, in addition to their use for the detection and diagnosis of HCC. Although tumor markers can not replace the results of imaging or pathology studies, tumor markers are advantageous in terms of objectivity and simplicity. Evaluation of tumor progression with tumor markers may, therefore, be useful for global comparisons. In addition, tumor marker levels can be measured with stored serum samples, allowing comparison of tumor progression between patients at different times with the same standard. Although most currently available standards to evaluate tumor progression do not contain tumor markers, measurement of levels of those markers will provide additional important information for the management of patients with HCC.

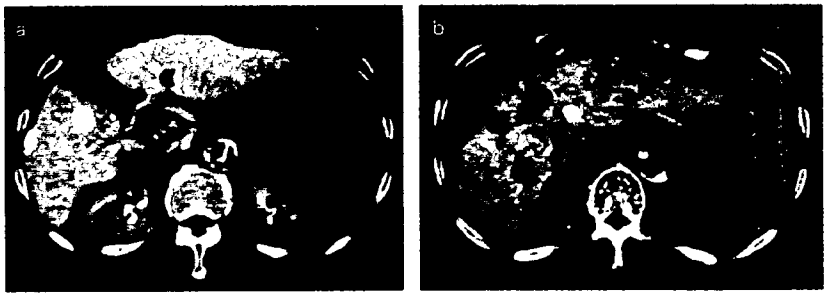

Figure 4 (a, b) Recurrent HCC in a 56-year-old man. Computed tomography images appear similar to those in Figure 3; however, all three tumor markers were elevated in this patient, and he died within 5 months of this imaging examination.

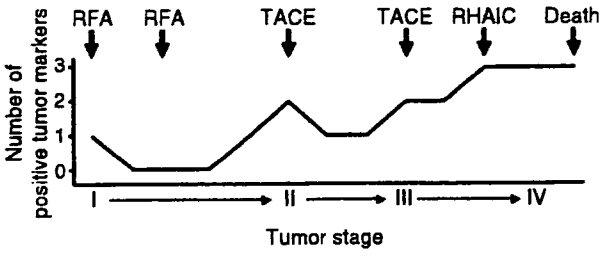

Figure 5 Changes in the number of elevated tumor markers during the clinical course of a patient with HCC. The number of elevated tumor markers increased with recurrence of HCC and tumor progression, and decreased with treatment. RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; RHAIC, repeated hepatic arterial infusion chemotherapy.

CONFLICT OF INTEREST

NO CONFLICT OF interest statement has been received from the authors.

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HEPATOLOGY

Characteristics and prognosis of patients in Japan with viral marker-negative hepatocellular carcinoma

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Abstract

Background and Aim: The characteristics and prognosis of patients with hepatitis virus marker-negative hepatocellular carcinoma (HCC) is not fully elucidated in Japan. We investigated the characteristics and prognosis of HCC patients in whom no markers for hepatitis virus infection were detected, in comparison with those of HCC patients with hepatitis virus infection.

Methods: Viral markers for hepatitis B and C virus (HBV and HCV) infection were measured in 1152 patients in whom initial HCC was diagnosed between 1991 and 2004. Patient characteristics, characteristics of HCC and survival were compared between patients in whom no marker was positive (viral marker-negative HCC) and those in whom chronic HBV or HCV infection was confirmed by viral markers (viral HCC).

Results: Overall, 119 patients (10.3%) were shown to have viral marker-negative HCC. Hepatocellular carcinoma was detected under surveillance in a significantly smaller percentage of patients with viral marker-negative HCC than of patients with viral HCC ($P < 0.0001$). The tumor was significantly larger ($P < 0.0001$) and vascular invasion was significantly more prevalent ($P = 0.0003$) in patients with viral marker-negative HCC than in those with viral HCC. The survival rate of patients with viral marker-negative HCC was significantly lower than that of patients with viral HCC ($P = 0.0378$).

Conclusion: The patients with HCC in whom hepatitis viral infection had not been confirmed tended not to be under surveillance, resulting in the detection of HCC at more advanced stage and with a poorer prognosis. Efforts to identify patients without hepatitis virus infection who should be under surveillance for HCC will be necessary in the future.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies, especially in southern and eastern Asia. Currently in Japan, HCC is the third-leading cause of death from cancer. The most important risk factor for development of HCC worldwide is chronic hepatitis caused by hepatitis B virus (HBV) or hepatitis C virus (HCV).[1–4] Most HCC develop in the presence of chronic hepatitis or cirrhosis, each of which occurs in Japan most often as a complication of chronic hepatitis B or C.[1,2] Currently, up to 80% of patients with HCC are infected with HCV.[5]

In Japan, absence of hepatitis virus infection has been confirmed in small number of patients with HCC.[5] In these patients, the characteristics and prognosis of HCC might differ from those in patients with chronic hepatitis virus infection. In the present study, we attempted to clarify the prevalence, characteristics and prognosis of HCC in patients without the detection of hepatitis virus infection in comparison with those of patients with hepatitis virus (HBV or HCV) infection.

Methods

A total of 1152 patients were diagnosed as having initial HCC (not recurrence) and treated at Ogaki Municipal Hospital between 1991 and 2004, these were 847 men and 305 women, with a mean age of 65.9 ± 9.1 years (range 29–93 years). Hepatocellular carcinoma was diagnosed on the basis of histologic examination of resected tumor tissue or biopsy specimens in 429 cases (37.2%). Diagnosis in the remaining 723 cases was based on clinical criteria:[6,7] pertinent clinical background (liver cirrhosis or chronic hepatitis) and typical imaging findings. Typical imaging features of HCC include a mosaic pattern with a halo on B-mode ultrasonographic images, hypervascularity on angiographic images and a high-density mass on arterial phase dynamic computed tomography (CT) images with a low-density mass on portal phase dynamic CT images obtained with a helical or multidetector row CT scanner. When findings typical of HCC were not obtained by means of dynamic CT or angiography, CT during hepatic arteriography and CT during arterial portography or superparamagnetic

iron oxide-enhanced T1- and T2-weighted magnetic resonance imaging were performed. In cases without typical imaging features, biopsy was performed to confirm the diagnosis of HCC.

Chronic HBV or HCV infection was tested at the time of HCC diagnosis. Hepatitis B virus infection was identified by positivity for serum HBV surface antigen. Hepatitis C virus infection was identified by positivity for serum HCV antibody and confirmed by positivity for serum HCV RNA. Patients were assigned to one of two groups according to the detection of hepatitis virus infection: patients with viral HCC in whom HBV or HCV infection was detected and patients with viral marker-negative HCC in whom HBV or HCV infection was not detected.

Surveillance status of each patient before diagnosis, remnant liver function at the time of HCC diagnosis, characteristics of HCC including maximum tumor size, number of tumors and vascular invasion, stage of HCC and patient survival were determined on the basis of clinical records. The Child–Pugh classification[8] was used as an indicator of remnant liver function. Vascular invasion was assessed by means of dynamic CT and angiography in cases in which pathologic evaluation had not been performed. Stage of HCC were evaluated according to the recently proposed CLIP[9] and JIS[10] scoring systems, and BCLC classification,[11] which incorporate both tumor extension and liver function factors. Patients were also classified into one of two groups according to whether they were under surveillance for liver tumor before the initial diagnosis of HCC: those in whom HCC was detected under surveillance (including surveillance at our center [$n = 464$] or under surveillance by a primary-care physician who referred them to us because liver tumor was suspected [$n = 401$]) and those not under surveillance until admission for HCC ($n = 287$).

Statistical analyses

Values are expressed as mean ± SD. Differences in distributions between groups were analyzed by χ^2 test. Differences in mean quantitative values were analyzed by Mann–Whitney *U*-test. The date of HCC diagnosis was defined as time zero for calculation of survival rates. Surviving patients and patients who died from a cause other than liver disease were censored. Patients who died from an HCC-related cause or liver failure were not censored. The Kaplan–Meier method[12] was used to calculate survival rates, and the log–rank test[13] was used to analyze differences in survival. The Cox proportional hazards model[14] was used for multivariate analysis of factors related to survival. The variables analyzed were patient age and sex, the presence or absence of surveillance before the diagnosis of HCC, Child–Pugh class (A *vs* B, C), tumor stage (stage I *vs* II, III, IV) and the presence or absence of hepatitis virus infection. Data analyses were performed with the JMP statistical software package, version 4.0 (Macintosh version; SAS Institute, Cary, NC, USA). All *P*-values were derived from two-tailed tests, and $P < 0.05$ was accepted as statistically significant.

The entire study was approved by the hospital ethics committee and carried out in compliance with the Helsinki Declaration.

Results

Hepatitis B virus or HCV infection was not detected in 119 of the 1152 patients (10.3%) with HCC. The numbers of cases of viral HCC and viral marker-negative HCC are shown per year in Fig. 1. The percentage of patients with viral marker-negative HCC was consistently around 10%, with some fluctuation. We found no particular increase or decrease in this percentage during the observation period.

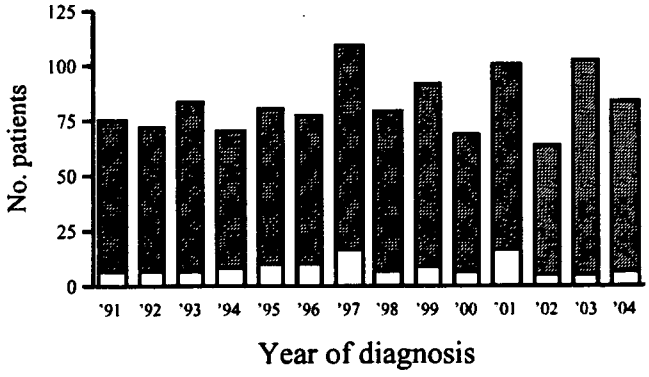

Figure 1 Number of patients in whom initial hepatocellular carcinoma (HCC; not recurrence) was diagnosed at our hospital per year (1991–2004). The annual incidence of viral marker-negative HCC was around 10% during the entire observation period. ■, viral HCC patients; □, viral marker-negative HCC patients.

Patient characteristics and characteristics of hepatocellular carcinoma with and without hepatitis virus infection

Characteristics of patients and HCC at the time of diagnosis are shown in Table 1 according to the detection or non-detection of HBV or HCV. Patients with viral marker-negative HCC were significantly older ($P = 0.0123$) and had a significantly more prevalent history of regular alcohol intake ($P = 0.0021$). In patients with viral marker-negative HCC, 21 patients had alcoholic cirrhosis and two had autoimmune hepatitis. The other 96 patients with viral marker-negative HCC did not have alcoholic cirrhosis, primary biliary cirrhosis, autoimmune hepatitis, or iron overload, and the definitive etiology was unknown.

No difference was found between the two groups in remnant liver function (Child–Pugh class). Maximum tumor size was significantly greater ($P < 0.0001$) and there was a significantly higher prevalence of suspected vascular invasion (by imaging diagnosis in some patients; $P = 0.0003$) in patients with viral marker-negative HCC than in patients with viral HCC, but there was no difference in the number of tumors. As for stage of patients, both CLIP scores ($P = 0.0348$) and JIS scores ($P = 0.0198$) were lower in patients with viral HCC. Also, the patients of earlier stage by BCLC classification were more prevalent in the group with viral HCC than in the group with viral marker-negative HCC ($P = 0.0023$). When we compared these factors between patients with HBV infection and those with HCV infection, we found no difference in all remnant liver function, characteristics of HCC or stage of HCC (data not shown).

A significantly smaller number of viral marker-negative HCC (*vs* viral HCC) patients had been under surveillance for HCC before the detection and diagnosis of HCC ($P < 0.0001$). The

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Table 1 Characteristics of hepatocellular carcinoma (HCC) in patients with and without hepatitis virus infection

	Viral HCC ($n = 1033$)	Viral marker-negative HCC ($n = 119$)	*P*-value
Age (years ± SD)	65.7 ± 9.1	67.7 ± 9.7	0.0123
Sex (M/F)	756 (73.2)/277 (26.8)	91 (76.5)/28 (23.5)	0.5095
Regular alcohol intake (yes/no)	274 (26.5)/759 (73.5)	48 (40.3)/71 (59.7)	0.0021
Surveillance before HCC (yes/no)	796 (77.1)/237 (22.9)[†]	69 (58.0)/50 (42.0)[‡]	<0.0001
Child–Pugh class (A/B/C)	532 (51.5)/385 (37.3)/116 (11.2)[§]	64 (53.8)/38 (31.9)/17 (14.3)[¶]	0.4096
Maximum tumor size (cm ± SD)	3.76 ± 3.01	5.96 ± 4.55	<0.0001
(≤2 cm/>2cm and ≤5cm/>5 cm)	369 (35.7)/386 (37.4)/278 (26.9)	29 (24.4)/29 (24.4)/61 (51.2)	<0.0001
Number of tumors (n ± SD)	2.26 ± 2.31	2.76 ± 3.92	0.9030
(single/multiple)	514 (49.8)/519 (50.2)	59 (49.6)/60 (50.4)	0.9706
Vascular invasion (absent/present)	836 (80.9)/197 (19.1)	79 (66.4)/40 (33.6)	0.0003
CLIP score	1.72 ± 1.59	2.04 ± 1.66	0.0348
BCLC classification (A/B/C/D)	565 (54.7)/209 (20.2)/165 (16.0)/94 (9.1)	49 (41.2)/21 (17.6)/35 (29.4)/14 (11.8)	0.0023
JIS score	2.00 ± 1.41	2.32 ± 1.41	0.0198
Treatment	226 (21.9)/231 (22.3)/343 (33.2)/	29 (24.4)/21 (17.7)/28 (23.5)/16 (13.4)/	0.0192
(surgery/LAT/TACE/others/none)	70 (6.8)/163 (15.8)	25 (21.0)	

[†]427 of 796 patients (53.6%) were outpatients under surveillance at our liver center; the other 369 (46.4%) were under surveillance of a primary-care physician.

[‡]37 of 69 patients (53.6%) were outpatients under surveillance at our liver center; the other 32 (46.4%) were under surveillance of a primary-care physician.

[§]Child–Pugh class A includes 256 patients without cirrhosis.

[¶]Child–Pugh class A includes 34 patients without cirrhosis.

Number of patients is shown unless otherwise indicated (percentage in parentheses).

BCLC, Barcelona Clinic Liver Cancer; CLIP, Cancer of the Liver Italian Program; JIS, Japan Integrated Staging; LAT, locoregional ablative therapies; TACE, transcatheter arterial chemoembolization.

percentage of patients in whom HCC was detected under surveillance is shown per observation period (i.e. 1991–1995, 1996–2000 and 2001–2004) in Fig. 2. The percentage of patients with viral HCC in whom HCC was diagnosed under surveillance increased significantly over time ($P = 0.0333$). In contrast, we found no increase over time in the percentage of patients with viral marker-negative HCC in whom HCC was diagnosed under surveillance ($P = 0.8603$). In patients under surveillance before the diagnosis of HCC, the percentage of patients under surveillance at our center is exactly the same (53.6%) between patients with viral HCC (427 of 796 patients) and those with viral marker-negative HCC (37 of 69 patients). For patients under surveillance at our center, all cirrhotic patients were followed up with ultrasonography every 3 months and, in addition, CT or dynamic magnetic resonance imaging was performed every 6 months in order to prevent the failure of HCC detection by ultrasonography. Regular monitoring of tumor markers (alpha-fetoprotein and des-gamma-carboxy prothrombin) was also performed. Patients without cirrhosis followed up with 6–12 months interval by ultrasonography and a measurement of tumor markers.[15] For patients under surveillance at a primary-care physician, the manner of surveillance simply depended on the physician. We did not supervise the care of these patients until they were referred to us.[15]

Characteristics of viral HCC and viral marker-negative HCC in patients with cirrhosis ($n = 862$) and in those without cirrhosis ($n = 290$) are shown in Table 2. Cirrhosis was evaluated clinically on the basis of laboratory data (serum albumin, serum bilirubin, prothrombin and platelet) and imaging findings (splenomegaly), except for 257 patients who were treated by surgery. Eighteen of 34 patients with viral marker-negative HCC without cirrhosis were

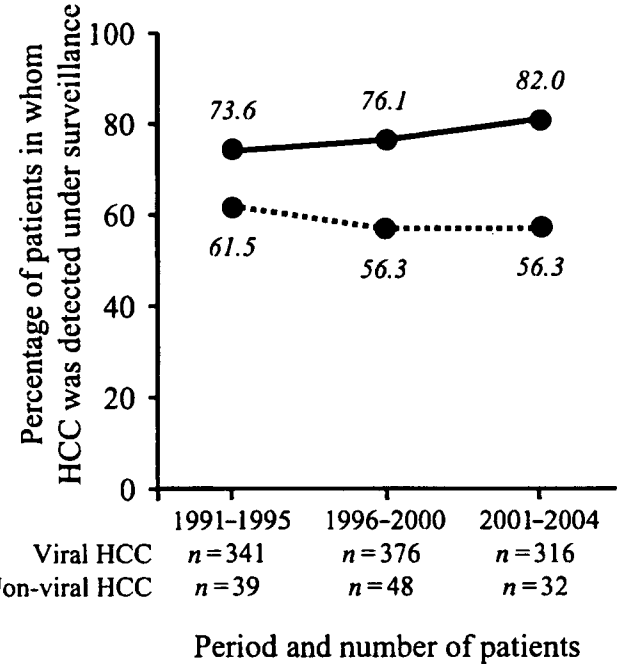

Figure 2 Changes in the percentage of patients in whom hepatocellular carcinoma (HCC) was detected under surveillance. There was a significant increase in the percentage among patients (—) with viral HCC ($n = 1033$, $P = 0.0333$). In contrast, there was no increase in the percentage among patients (·····) with viral marker-negative HCC ($n = 119$, $P = 0.8603$).

Table 2 Characteristics of hepatocellular carcinoma (HCC) in patients with and without hepatitis virus infection according to the presence or absence of cirrhosis.

Patients with cirrhosis	Viral HCC ($n = 777$)	Viral marker-negative HCC ($n = 85$)	*P*-value
Surveillance before HCC (yes/no)	591 (76.1)/186 (23.9)[1]	51 (60.0)/34 (40.0)[2]	0.0020
Maximum tumor size (cm ± SD)	3.66 ± 2.87	4.90 ± 4.21	0.1012
(≤2 cm/>2cm and ≤5cm/>5 cm)	276 (35.5)/290 (37.3)/211 (27.2)	25 (29.4)/23 (27.1)/37 (43.5)	0.0062
Vascular invasion (absent/present)	620 (79.8)/157 (20.2)	58 (68.2)/27 (31.8)	0.0198
Treatment (surgery/LAT/TAE/others/none)	119 (15.3)/187 (24.1)/279 (35.9)/ 48 (6.2)/144 (18.5)	17 (20.0)/17 (20.0)/24 (28.2)/8 (9.4)/19 (22.4)	0.3185
Patients without cirrhosis	**Viral HCC ($n = 256$)**	**Viral marker-negative HCC ($n = 34$)**	***P*-value**
Surveillance before HCC (yes/no)	205 (80.1)/51 (19.9)[3]	18 (52.9)/16 (47.1)[4]	0.0009
Maximal tumor size (cm ± SD)	4.06 ± 3.39	8.29 ± 4.44	<0.0001
(≤2 cm/>2cm and ≤5cm/>5 cm)	93 (36.3)/96 (37.5)/67 (26.2)	4 (11.8)/6 (17.6)/24 (70.6)	<0.0001
Vascular invasion (absent/present)	216 (84.4)/40 (15.6)	21 (61.8)/13 (38.2)	0.0030
Treatment (surgery/LAT/TAE/others/none)	107 (41.8)/44 (17.2)/64 (25.0)/ 22 (8.6)/19 (7.4)	12 (35.3)/4 (11.8)/4 (11.8)/8 (23.5)/6 (17.6)	0.0216

[1]314 of 591 (53.1%) patients were outpatients under surveillance at our liver center; the other 277 (46.9%) were under surveillance of a primary-care physician.
[2]33 of 51 patients (64.7%) were outpatients under surveillance at our liver center; the other 18 (35.3%) were under surveillance of a primary-care physician.
[3]113 of 205 patients (55.1%) were outpatients under surveillance at our liver center; the other 92 (44.9%) were under surveillance of a primary-care physician.
[4]2 of 18 patients (11.1%) were outpatients under surveillance at our liver center; the other 16 (88.9%) were under surveillance of a primary-care physician.
Number of patients is shown unless otherwise indicated (percentage in parenthesis).
LAT, locoregional ablative therapies; TACE, transcatheter arterial chemoembolization.

under surveillance. All these patients were receiving routine periodic ultrasonography examination of the liver with 1-year interval because of the liver damage (the elevation of serum alanine aminotransferase activity) of unknown etiology. Differences in maximum tumor size and the prevalence of suspected vascular invasion between viral HCC and viral marker-negative HCC were more marked in patients without cirrhosis than in those with cirrhosis.

Patient survival

The survival rate of patients with viral marker-negative HCC was significantly lower than that of patients with viral HCC ($P = 0.0378$; Fig. 3). By multivariate analysis, the surveillance before the diagnosis of HCC is a factor that affects patient survival independent of remnant liver function (Child–Pugh class) and of tumor progression (tumor stage of Liver Cancer Study Group of Japan[16]). In contrast, the presence or absence of hepatitis virus infection did not have an effect on patient survival in multivariate analysis (Table 3).

When survival was compared between patients with viral marker-negative HCC and those with viral HCC according to the presence or absence of cirrhosis, the difference in survival was significant among patients without cirrhosis ($P = 0.0016$), but not among patients with cirrhosis ($P = 0.2031$; Fig. 4). Among patients with viral HCC, the survival rate of those without cirrhosis was significantly higher than that of those with cirrhosis ($P < 0.0001$). In contrast, among patients with viral marker-negative HCC, there was no difference in survival between patients with and without cirrhosis ($P = 0.6205$; Fig. 5).

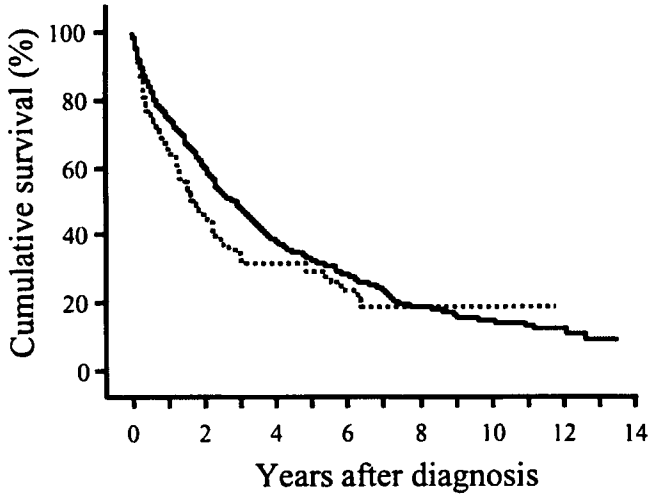

Figure 3 Survival of patients (—) with viral hepatocellular carcinoma (HCC; $n = 1033$) and patients (·····) with viral marker-negative HCC ($n = 119$). The survival rate of patients with viral HCC was higher than that of patients with viral marker-negative HCC ($P = 0.0378$).

Discussion

Nearly 90% of patients in Japan with HCC are chronically infected with HBV or HCV. Although the percentage is small, there is a subpopulation of Japanese patients with HCC in whom no

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Table 3 Multivariate analyses of factors associated with patient survival

Factor	Parameter estimate	Standard error	X	Risk ratio (95% confidence interval)	*P*-value
Age	0.0085	0.0048	3.17	1.0085 (0.9991–1.0179)	0.0749
Sex					
Male				1	
Female	−0.1390	0.0464	9.34	0.8702 (0.7936–0.9519)	0.0022
Surveillance before HCC					
No				1	
Yes	−0.0844	0.0424	3.98	0.9191 (0.8458–0.9988)	0.0459
Child–Pugh class					
A				1	
B	0.3500	0.0437	63.88	1.4190 (1.3025–1.5460)	<0.0001
C	0.6690	0.0567	115.79	1.9523 (1.7444–2.1790)	<0.0001
Tumor stage[†]					
Stage I				1	
Stage II	0.2722	0.0637	19.19	1.3128 (1.1605–1.4903)	<0.0001
Stage III	0.5559	0.0665	74.65	1.7435 (1.5326–1.9894)	<0.0001
Stage IV	1.1395	0.0707	282.81	3.1251 (2.7245–3.5956)	<0.0001
Hepatitis virus infection					
No				1	
Yes	0.0589	0.0611	0.90	1.0607 (0.9374–1.1916)	0.3424

[†]by Liver Cancer Study Group of Japan.
HCC, hepatocellular carcinoma.

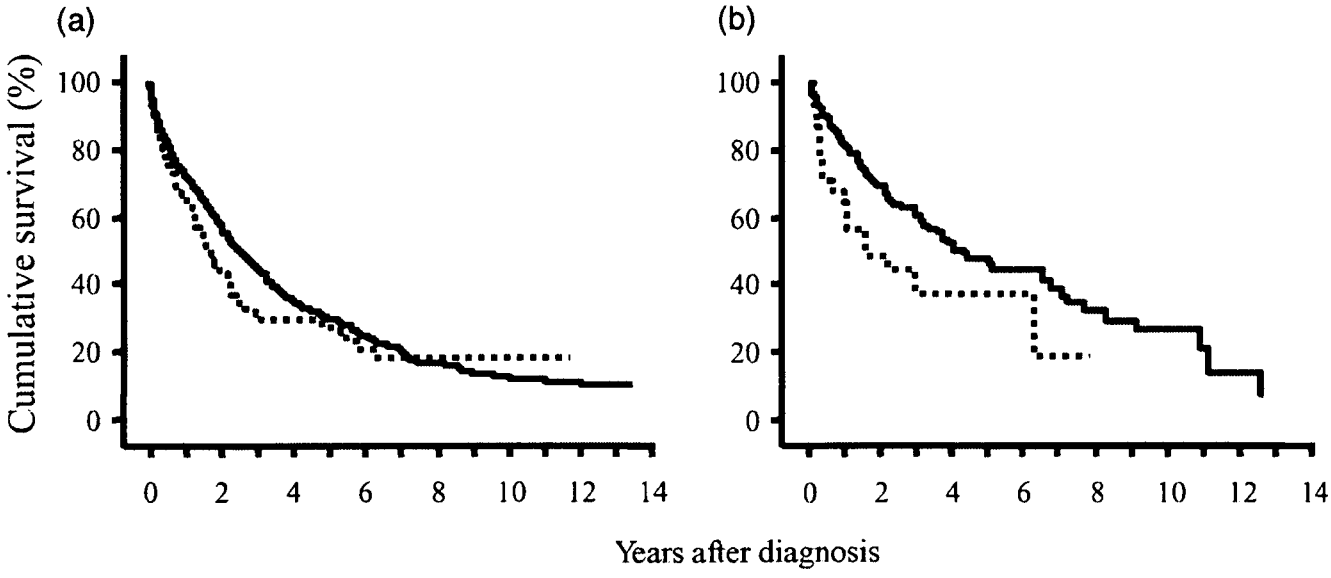

Figure 4 Survival rates of patients (—) with viral hepatocellular carcinoma (HCC) and patients (·····) with viral marker-negative HCC in relation to cirrhosis. (a) Survival of patients with viral HCC ($n = 777$) and patients with viral marker-negative HCC ($n = 85$), all with cirrhosis. No significant difference was observed between these two groups ($P = 0.2031$). (b) Survival of patients with viral HCC ($n = 256$) and patients with viral marker-negative HCC ($n = 34$), all without cirrhosis. Among these patients, the survival rate of patients with viral HCC was significantly higher than that of patients with viral marker-negative HCC ($P = 0.0166$).

hepatitis virus infection is found. The percentage is reportedly much higher in Western countries.[17–19]

There have been studies of the mechanism underlying development of HCC in patients with no apparent hepatitis virus infection. Few studies, however, have investigated in detail the characteristics and prognosis of patients with HCC in whom no hepatitis virus infection is detected. Some studies reported favorable survival of patients with viral marker-negative HCC, including preserved liver function and absence of multicentric carcinogenesis, in comparison with that of patients with viral

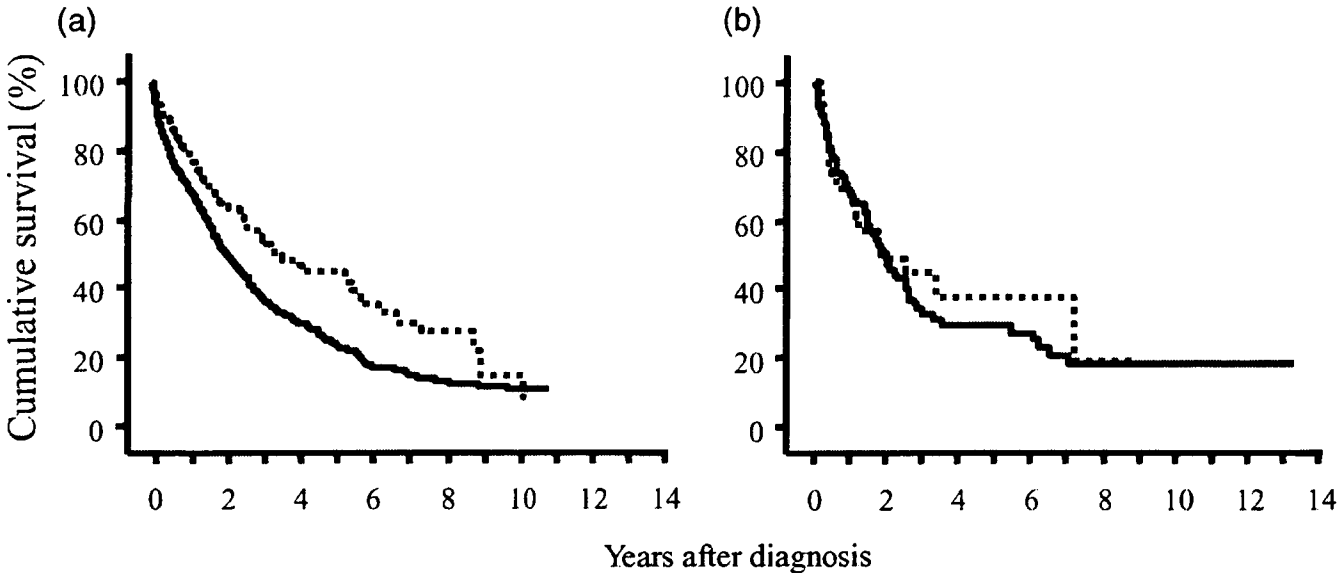

Figure 5 Survival rates of patients (—) with cirrhosis and patients (·····) without cirrhosis in relation to hepatitis virus infection. (a) Survival of patients with ($n = 777$) and without ($n = 256$) cirrhosis, all having viral hepatocellular carcinoma (HCC). The survival rate of patients without cirrhosis was significantly higher than that of patients with cirrhosis ($P < 0.0001$). (b) Survival of patients with ($n = 85$) and without ($n = 34$) cirrhosis, all having viral marker-negative HCC. No significant difference was observed between these two groups ($P = 0.6205$).

HCC.[20–23] These studies, however, focused on patients who were treated by hepatic resection and do not reflect the entire population of patients with viral marker-negative HCC.

In the present study, we analyzed the characteristics and prognosis of patients with HCC in whom no hepatitis virus was detected. The infection of hepatitis virus was evaluated by means of examinations that are used routine clinical settings: serum HBV surface antigen for HBV infection, and serum HCV antibody and HCV RNA for HCV infection. These examinations do not necessarily reflect the infection with HBV or HCV accurately due to their limitation of sensitivity, especially in case of HBV infection. Some studies have reported occult HBV infection in patients with viral marker-negative HCC.[24–27] In the present study, however, we did not investigate this occult infection. Hepatitis virus infection was usually examined by routine serologic and virologic analyses (positivity for serum HBV surface antigen, HCV antibody, or HCV RNA) and further analyses for occult viral infection was not performed in daily clinical settings. The patients in whom HBV or HCV was not detected with routine examination therefore were defined as those without hepatitis virus infection. The purpose of the present study was to investigate the characteristics and prognosis of the patients with HCC who were defined as not having hepatitis viral infection.

The results of our present study clearly show viral marker-negative HCC to be more advanced than viral HCC at the time of diagnosis: maximum tumor size was greater, the prevalence of vascular invasion was higher and survival rate was lower. In our previous studies showing improved survival of patients with HCC over the past few decades, the contribution of early detection of HCC to the improved patient prognosis and the importance of surveillance of patients at high risk for development of HCC were also shown.[15,28] Our present study shows a lower percentage of patients under surveillance before the detection and diagnosis of viral marker-negative HCC. In addition, among patients with viral marker-negative HCC, we observed no increase in the rate of surveillance over time, whereas, among patients with viral HCC, we observed a significant increase in the rate of surveillance. Increased awareness of the risk of HCC in patients with cirrhosis or chronic viral hepatitis could have contributed to the increase in the number of patients under surveillance. Awareness of the risk of developing HCC spread especially after the 1990s, when HCV was identified and many patients were admitted to the hospital for examination and treatment of hepatitis virus infection. In contrast, it is difficult to identify patients at high risk for development of HCC if no hepatitis virus infection is detected, especially when they do not have symptomatic liver disease such as cirrhosis. Early-stage HCC is usually asymptomatic, and early detection of HCC is difficult without periodic surveillance. Therefore, patients who are not under surveillance for HCC and who are admitted to the hospital after HCC becomes symptomatic usually have advanced-stage disease. This accounts for the significantly larger tumors and greater prevalence of vascular invasion in patients with viral marker-negative HCC than in those with viral HCC. The surveilled patients in groups with viral HCC and viral marker-negative HCC are the mixture of patients under surveillance at out liver center and those at a primary-care physician. The surveillance at a primary-care physician is likely to be less intensive than that at out liver center, and subsequently the survival rate of patients under surveillance at a primary-care physician is lower than that of patients under surveillance at our center.[15] However, the percentage of patients under surveillance at our center is exactly the same between in surveilled patients with viral HCC and in those with viral marker-negative HCC, and therefore the mixture of the two kinds of surveilled patients would not have

affected the comparison of patient survival between viral HCC and viral marker-negative HCC.

The advanced disease stage and decreased survival in association with viral marker-negative HCC versus viral HCC was marked among patients without clinically evaluated cirrhosis. We did not find a difference in survival between viral marker-negative patients with and without cirrhosis, but a significant difference was observed between these two subgroups among patients with viral HCC. The benefit of well-preserved remnant liver function did not contribute to survival of patients with viral marker-negative HCC without cirrhosis, because the HCC was advanced when it was found. Viral marker-negative HCC without cirrhosis is usually asymptomatic until the HCC progresses to an advanced stage. Although some of the patients were being followed up for abnormal liver function before the detection of HCC, most were not under surveillance for HCC. Indeed, only two of 18 patients with viral marker-negative HCC without cirrhosis were under surveillance at our liver center, whereas the other 16 patients were under surveillance of a primary-care physician. Such surveillance for HCC would typically be less intensive than at our liver center.[15]

There are several reported risk factors for the development of HCC, in addition to HBV or HCV infection. Heavy drinkers are reported to be at high risk for the development of HCC and should be under surveillance even in the absence of hepatitis virus infection.[29] Non-alcoholic steatohepatitis may be another risk factor for the development of HCC.[17,30–33] Detailed analysis of risk factors for development of HCC other than hepatitis viral infection is important to identify patients at high risk for development of HCC in the absence of the detection of hepatitis virus infection.

In conclusion, when HCC is diagnosed in patients in Japan without the detection of hepatitis virus infection, it is generally more advanced and has a poorer prognosis than that of patients with hepatitis virus infection. This could be due to the lower percentage of patients under surveillance for HCC before its detection and diagnosis. The increase in the number of patients under close surveillance has contributed to improved survival in cases of viral HCC. In contrast, it is difficult to identify patients without hepatitis virus infection that are at high risk for developing HCC. Further studies are needed to find a strategy for identifying patients that should be placed under close surveillance so that viral marker-negative HCC will be detected in the early stage. Such a strategy will be of even greater importance in other parts of the world where viral hepatitis is not a predominant cause of HCC.

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Impact of hepatitis B virus (HBV) X gene integration in liver tissue on hepatocellular carcinoma development in serologically HBV-negative chronic hepatitis C patients☆

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***Background/Aims*: We analyzed hepatitis B virus (HBV) X gene integration in hepatocytes of HBV-negative, chronic hepatitis C (CH-C) patients with mild fibrosis, and prospectively followed these patients for the development of hepatocellular carcinoma (HCC).**

***Methods*: The study included 39 HBV-negative CH-C patients with mild fibrosis. HBV-X integration was determined by Alu-PCR analysis of liver specimens obtained by fine-needle biopsy.**

***Results*: Integration of HBV-X gene sequence into liver genome occurred in 9 of the 39 patients. Six of the 39 patients developed HCC during the 12-year follow-up period. No significant difference was found in the incidence of HCC between patients with and without HBV-X integration. However, the two patients with HBV-X integration who developed HCC did not have cirrhosis at the time when HCC was diagnosed, whereas the four patients without HBV-X integration who developed HCC did have cirrhosis.**

***Conclusions*: Our findings suggest that HBV-X integration detected at the mild fibrosis stage might not indicate a high risk for HCC. HBV-X integration may be associated with HCC development in the absence of cirrhosis. However, we did not find evidence that HBV-X integration directly plays a role in hepatocarcinogenesis in CH-C patients. Further studies will be needed to clarify this point.**

***Keywords*: HBV-X integration; Chronic hepatitis C; Hepatocellular carcinoma**

1. Introduction

Chronic viral hepatitis is a leading cause of hepatocellular carcinoma (HCC) worldwide [1–4]. Occult hepatitis B virus (HBV) infection, characterized by the absence of circulating HBV surface antigen [HBsAg] but presence of the HBV genome in serum or liver tissue, has been identified in hepatitis C virus (HCV)-infected patients. HBV may affect the clinical course of chronic hepatitis C (CH-C) [5] and increase the risk of hepatocarcinogenesis [6]. Pollicino reported that both integrated and free HBV–DNA sequences were highly prevalent in the liver tissue of CH-C patients with HCC compared to CH-C patients without HCC [7].

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