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Viral serostatus and coexisting inflammatory activity affect metachronous carcinogenesis after hepatectomy for hepatocellular carcinoma. A further report

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Abstract: Little data are available regarding the effects of hepatitis virus serostatus and the severity of coexisting chronic inflammation on intrahepatic recurrence after hepatectomy for hepatocellular carcinoma (HCC). We investigated the extent to which these factors modified the prognosis of hepatectomized patients. A total of 274 patients treated in the period January 1981 to December 1996 were divided into three groups: anti-hepatitis C-positive (HCV; $n = 144$), hepatitis B surface antigen-positive and HCV antibody (Ab)-negative (HBsAg; $n = 106$), and HBsAg-negative and HCV Ab-negative (NBNC; $n = 20$). Positivity for HBV-related antibody in the HCV group was 76%. Histologic grading of inflammatory activity from coexisting hepatitis was determined according to Knodel's histological activity index (HAI) scoring system. Post-hepatectomy crude survival rates and disease-free survival (DFS) rates were compared, according to tumor characteristics, between the three groups. In the patients overall and also in the patients with a single nodular HCC, the HCV group had significantly higher HAI scores and preoperative serum aspartate aminotransaminase (AST) levels than the other two groups. When the patients were limited to those with a single nodular HCC, the crude survival was similar in the three groups with comparable tumor characteristics; however, the DFS was different (NBNC > HBsAg > HCV). When the patients were further limited to those with a single nodular HCC without microscopic extracapsular spread, in whom removal of the tumor was expected to be microscopically complete, the difference in the DFS became more marked. Irrespective of the viral serostatus, better crude and disease-free survivals were observed in the patients with lower AST levels (≦50 IU/l) than in those with higher AST levels (>50 IU/l). In contrast, there were no differences in survivals and HAI scores according to the presence or absence of HBV-related antibody in the HCV group. From our univariate analysis, we can conclude that the severity of virally induced inflammation, which was well correlated with viral serostatus, may be a factor that affects intrahepatic recurrence, which is more likely to originate from metachronous carcinogenesis. Prior co-infection of HBV in HCV patients may not be an adverse risk factor for intrahepatic recurrence.

Key words: hepatocellular carcinoma, hepatectomy, intrahepatic recurrence, metachronous carcinogenesis, viral serostatus, viral hepatitis

Introduction

In patients with hepatocellular carcinoma (HCC), at least three possible factors preclude long-term disease-free survival after hepatectomy: (1) Most patients with HCC in Japan have hepatitis virus-induced chronic liver disease.[1,2] This causes loss of hepatic reserve, and subsequently limits the extent of resection for HCC patients,[3,4] in whom there is frequent microscopic intrahepatic metastasis even at an early tumor stage. Poor hepatic reserve can also be a major obstacle to multidisciplinary treatments for post-hepatectomy recurrences. (2) Even small HCCs are often associated with extracapsular spread:[5,6] microscopic intra- or extracapsular spread was found in 46% of patients with a single nodular HCC of 3 cm or less. This increases the risk that undetected minute lesions will be left behind, even after an apparently complete resection. (3) Metachronous carcinogenesis frequently develops ever after complete resections.[7,8] This study examined the incidence of metachronous recurrence after hepatectomy for HCC with a single nodular growth, and the

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correlation of such recurrence with the patients' viral serologic status and the degree of virally induced inflammation.

Patients and methods

Serostatus of hepatitis virus

A total of 274 patients who underwent a hepatectomy for HCC during the period January 1981 to December 1996 were included in this study (Table 1). Of these, 106 patients were positive for hepatitis B virus surface antigen (HBsAg group). Eighteen of these 106 patients (17%) were also positive for hepatitis B virus envelope antigen (HBeAg). One hundred and forty-four patients were positive for anti-hepatitis C virus, determined by a second-generation enzyme-linked immunoassay (HCV group), and 20 patients were negative for both anti-HCV and HBsAg (NBNC group). The NBNC patients were treated after 1990, when it became possible to measure seromarkers for anti-HCV. Four patients were positive for both HBsAg and anti-HCV, but were excluded from the study because the patient number was too small to permit statistical analysis. The HCV and NBNC groups were subdivided according to whether the patients were positive for HBV-related antibody, including core antibody for HBV (HBcAb) and/or antibody for HBsAg (HBsAb). These antibodies were positive in 76% (109/144) and 75% (15/20) of the patients these groups respectively.

Age, sex, and liver function

The average age of the HBsAg group was about 10 years less than that of the other two groups (Table 1). Serum levels of liver enzymes, (asparate aminotransaminase [AST] and alanine aminotransaminase [ALT]) and the indocyanine green retention rate at 15 min after the injection (0.5 mg/kg) (ICGR15) were significantly lower in the HBsAg and NBNC groups than in the HCV group. Serum albumin was also lower in the HCV group than in the NBNC group. Patients in the NBNC group, but not those in the HCV group, who were negative for HBV-related antibody had lower serum liver enzyme concentrations than those positive for HBV-related antibody.

Histopathology and inflammatory activity of hepatitis

As a whole, the degree of histopathologic derangement was more marked in the HCV group than in the NBNC group: The proportion of patients with precirrhosis was lower, and conversely, the proportion of patients with chronic hepatitis was higher in the NBNC group, especially in the subset negative for HBV-related antibody (Table 1).

The inflammatory activity of coexisting hepatitis was graded according to the histologic activity index (HAI score) of Knodel et al.,[9] as follows. The scores for three features (degree of periportal hepatocellular necrosis and bridging, degree of intralobular degeneration and focal hepatocellular necrosis, and degree of portal in-

Table 1. Preoperative data by viral seromarkers

	n	Age (years)	Sex (M/F)	AST/ALT (U/l)	Bilirubin (mg/dl)	Albumin (g/dl)	ICGR15 (%)
HBsAg(+)	106	52.0 ± 9.7*	6.1:1	52 ± 36/47 ± 31*	0.8 ± 0.4	3.7 ± 0.4	14.9 ± 9.2*
HCVAb(+)	144	62.9 ± 6.3	4.8:1	70 ± 44/73 ± 43	0.9 ± 0.4	3.7 ± 0.4	18.9 ± 7.6
HBV-related Ab(+)	109	62.6 ± 5.9	4.3:1	71 ± 47/71 ± 44	0.9 ± 0.3	3.7 ± 0.4	18.9 ± 8.0
HBV-related Ab(−)	35	63.0 ± 6.7	6.0:1	66 ± 33/73 ± 42	0.9 ± 0.5	3.7 ± 0.4	18.8 ± 7.5
HBsAg(−), HCVAb(−) [NBNC]	20	63.1 ± 9.7	9.0:1	40 ± 26/38 ± 27*	0.8 ± 0.4	4.0 ± 0.3*	12.6 ± 5.2*
HBV-related Ab(+)	14	63.8 ± 6.7	6.0:1	47 ± 27/44 ± 30	0.7 ± 0.4	3.9 ± 0.4	12.9 ± 5.7
HBV-related Ab(−)	5	57.4 ± 13.3	5/0	21 ± 6/23 ± 9	0.9 ± 0.4	4.0 ± 0.2	11.2 ± 4.4

	Liver pathology				
	Normal	Chronic hepatitis	Precirrhosis	Liver cirrhosis	HAI score
HBsAg(+)	3/105 (3%)	22/105 (21%)	20/105 (19%)	60/105 (57%)	4.6 ± 2.2*
HCVAb(+)	1/144 (1%)	25/144 (17%)	42/144 (29%)	76/144 (53%)	6.1 ± 1.6
HBV-related Ab(+)	1/101 (1%)	19/101 (19%)	32/101 (32%)	49/101 (48%)	6.1 ± 1.6
HBV-related Ab(−)	0/35 (0%)	6/35 (17%)	9/35 (26%)	20/35 (57%)	5.9 ± 1.5
HBsAg(−), HCVAb(−) [NBNC]	1/20 (5%)	8/20 (40%)	3/20 (15%)	8/20 (40%)	4.3 ± 2.6*
HBV-related Ab(+)	1/14 (7%)	4/14 (29%)	3/14 (21%)	6/14 (43%)	4.0 ± 2.7
HBV-related Ab(−)	0/5 (0%)	3/5 (60%)	0/5 (0%)	2/5 (40%)	5.2 ± 2.5

* $P < 0.01$ versus HCVAb(+) group

ICGR15 (%), indocyanine green retention at 15 min after loading (0.5 mg/kg); HAI, histological activity index[9]

flammation) were totaled for each individual. The HAI score in each group was expressed as the average of the scores in three visual fields. The degree of histopathologic derangement was comparable in the HBsAg and HCV groups; however, the HAI score in the HCV group was significantly higher than that in the other two groups (Table 1). The presence or absence of HBV-related antibody did not influence the HAI score of the HCV and NCNB groups.

Follow-up and treatment of intrahepatic recurrences

All patients were followed in our and affiliated hospitals, based on the same follow-up protocol. This included ordinary liver function tests, measurements of serum alpha-feto protein and PIVKA-II, and ultrasound scans every 3 to 4 months. When an intrahepatic recurrence was detected as a single mass less than 2 cm in diameter by ultrasound, percutaneous ethanol injection (PEI)[10] or, currently, percutaneous microwave coagulation therapy (P-MCT)[11] was employed after ultrasound-guided biopsy confirmation of the hepatic lesion. Transcatheter hepatic arterial chemoembolization (TAE)[12] with Lipiodol was preferred for vascular-rich masses on spiral enhanced computed tomography (CT). If the recurrent mass(es) were 2 cm or more in size or they were multiple, TAE was chosen. If the regional therapeutic effects of TAE were evaluated as incomplete on the follow-up spiral enhanced CT (eg, development of a stained area or a defect of accumulated Lipiodol), the mass was treated repeatedly with TAE or supplemented with PEI, P-MCT, or open MCT.[13] In patients with good hepatic reserve and with a single recurrent mass situated favorably for resection, re-hepatectomy was performed. Upper gastrointestinal endoscopy was performed yearly. Esophageal varices with the red color sign were treated with prophylactic endoscopic sclerotherapy and/or variceal ligation. These protocols were applied equally to all patients.

Statistical analysis

All data values are expressed as means ± SD. Differences were analyzed using the two-tailed Student's *t*-test after one-way analysis of variance. Survival rates, estimated by the Kaplan-Meier method, were statistically compared using the log-rank test. Six HBsAg-positive and three anti-HCV positive patients, who did not survive hepatectomy, were excluded from the analysis of long-term survival rates. A P value of less than 0.05 was considered significant.

Results

Liver background and tumor stage in patients with a single nodular HCC

The HCCs were divided into two macroscopic types;[14] single nodular HCC without macroscopic extracapsular spread (eg, vascular invasion and/or intrahepatic metastasis), and HCC with extracapsular spread. The percentage of patients with a single nodular growth was significantly less in the HBsAg group than in the HCV group (27%; 27/100 vs 55%; 77/141, excluding the hospital deathes), although the percentages were comparable in the HCV group (55%) and NBNC group (60%, 12/20). Among the patients with a single nodular growth type without extracapsular spread, the HCV group had higher serum liver enzyme levels, higher HAI scores, and higher ICGR15 values than the other two groups (Table 2). Results of the other liver function tests (serum albumin and bilirubin) were comparable in the three groups. The mean tumor size and tumor stage, determined by the International Union against Cancer (UICC) TNM classification,[15] were also similar in the three groups (Table 2).

Table 2. Liver background and tumor stage in patients with a single nodular HCC

			NBNC
Growth type	HBsAg(+)	HCVAb(+)	HBsAg(−) and HCVAb(−)
Single nodular type, extracapsular spread (−)	$n = 27$	$n = 77$	$n = 12$
ALT/AST (U/l)	45 ± 32/45 ± 35*	67 ± 33/74 ± 42	40 ± 30/36 ± 25*
Bilirubin (mg/dl)	0.8 ± 0.5	0.9 ± 0.3	0.9 ± 0.4
Albumin (g/dl)	3.9 ± 0.5	3.7 ± 0.4	4.0 ± 0.3
ICGR15 (%)	15.4 ± 9.2**	19.4 ± 7.5	11.9 ± 3.3*
HAI score[9]	5.0 ± 2.3**	6.0 ± 1.6	3.7 ± 2.5*
Tumor size (cm)	4.9 ± 3.7	3.5 ± 1.8	4.6 ± 3.6
TNM I, II[15]	25/27 (93%)	71/77 (92%)	12/12 (100%)
III, IV[15]	2/27 (7%)	6/77 (8%)	0/12 (0%)

* $P < 0.01$, versus HCVAb(+) group; ** $P < 0.05$, versus HCVAb(+) group
HAI score, histological activity index score

Overall prognosis

The HBsAg group, with the highest incidence of macroscopic extracapsular spread, had the lowest crude 5-year survival rate among the three groups (22% for HBsAg vs 43% for HCV vs 70% for NBNC). Although the proportions of patients with single nodular growth and tumor stage I and II were comparable in the HCV and NBNC groups, the NBNC group had a better 5-year disease-free survival rate (45%) than the HCV group (11%).

Prognosis in patients with HCC with single nodular growth

There were 83 patients who had a single nodular HCC less than 3 cm in diameter. In 38 of these patients (46%) there was associated microscopic intra- or extracapsular portal invasion (vp) and/or intrahepatic metastatic foci (im), while the remaining 45 patients (54%) were negative for those factors. Although patients without vp and im achieved a crude survival rate of 62% at 5 years, the disease-free survival rate at 5 years was only 34%. In other words, 66% of these patients (100% minus 34%) in whom a risk of recurrence from latent, minute metastatic lesions was minimal, had intrahepatic recurrences originating most likely from metachronous carcinogenesis within the first 5 postoperative years.

There were no significant differences in crude survivals according to viral serostatus in the patients with a macroscopic single nodular growth (Fig. 1). However, the disease-free survival of the groups was NBNC > HBsAg > HCV. When a comparison was made among patients without vp and im, in whom removal of the tumor was expected to be microscopically complete, the difference in the disease-free survival between the HCV group and the other two groups became more prominent (Fig. 2).

Prognosis in patients with single nodular HCV-related HCCs with or without HBV-related antibody

Of the 70 patients with a single nodular HCC, who were positive for anti-HCV, 51 (73%) were positive for HBV-related antibody, and the remaining 19 (27%) were negative. Age, AST/ALT, ICGR15, HAI score, tumor size, and the incidence of vp and/or im were similar in the two subsets (Table 3). No significant differences were found in the crude and disease-free survivals between the two subsets (Fig. 3).

Prognosis by preoperative serum AST in patients with a single nodular HCC

The HCV and HBsAg groups were subdivided into two subsets according to preoperative serum AST levels, set

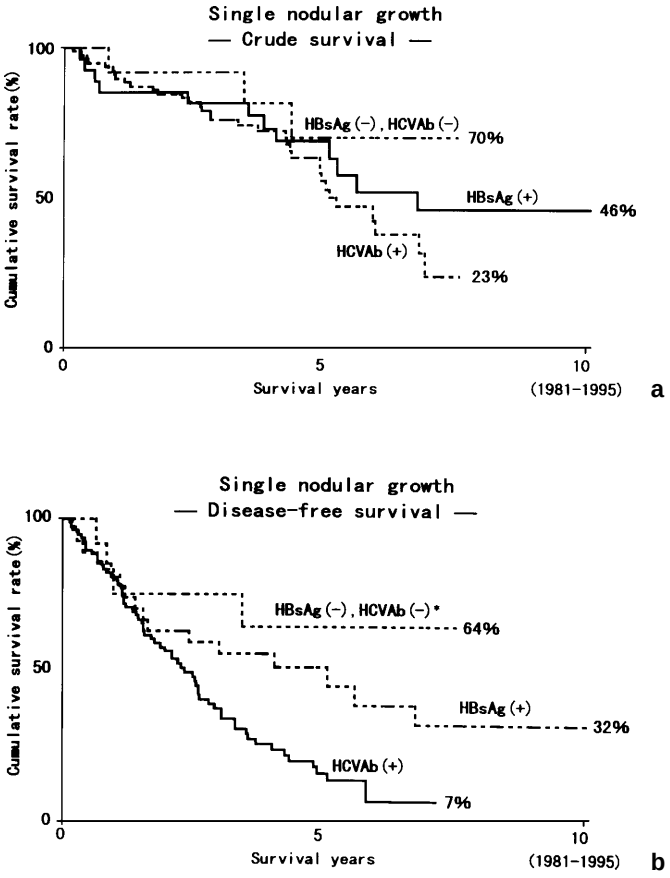
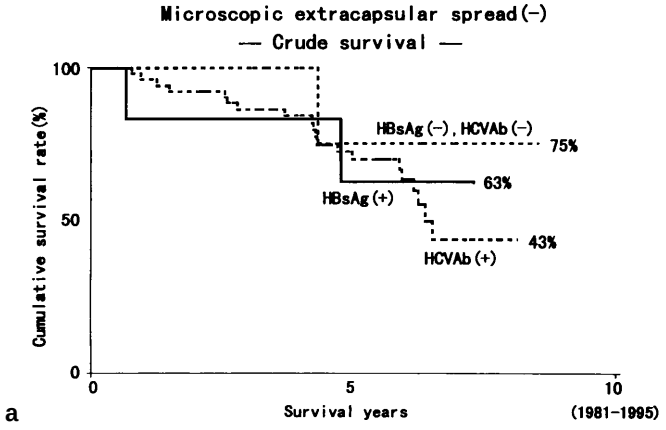


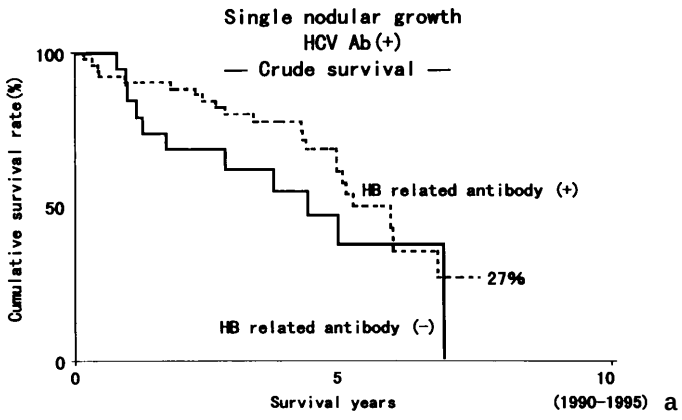
Fig. 1. a Crude survival and **b** disease-free survival rates according to viral serostatus in patients with a macroscopic single nodular hepatocellular carcinoma (HCC). $*P < 0.05$, Hepatic B surface artigen-negatric [*HBsAg(−)*], anti-hepatitis C virus antibody-negative [*HCVAb(−)*] vs *HCVAb(+)*

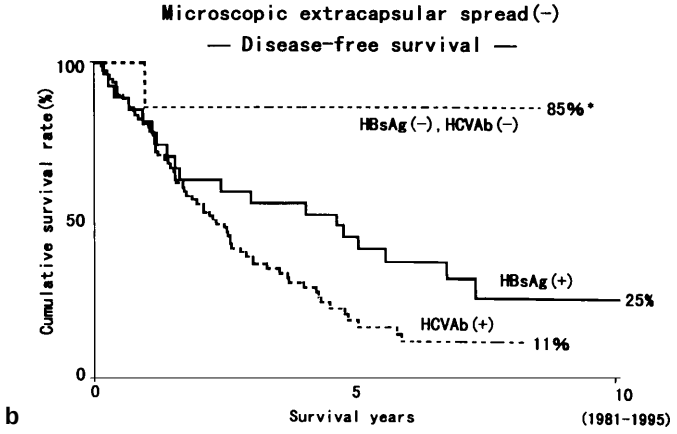
at double the normal upper limit (those with < 50 U/l and those with > 50 U/l). Sixty-two percent (16/26) of those in the HBsAg group, and 68% (50/74) of those in the HCV group had the higher serum AST concentration (Table 3). Age, ICGR15, HAI score, tumor size, and incidence of vp and/or im were comparable in the two subsets in each group. In the HBsAg group, the subset with the lower serum AST concentration had better crude and disease-free survivals than the subset with the higher serum AST (Fig. 4). A similar difference was also found between the two subsets in the HCV group (Fig. 5).

Discussion

Prognostic factors after hepatectomy have been extensively investigated, although most studies have focused on tumor factors per se, in terms of the macroscopic and microscopic features of the tumor,[6,16] and more recently,







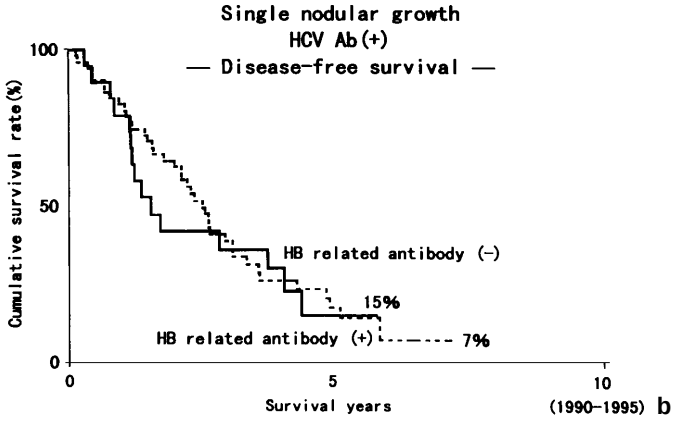


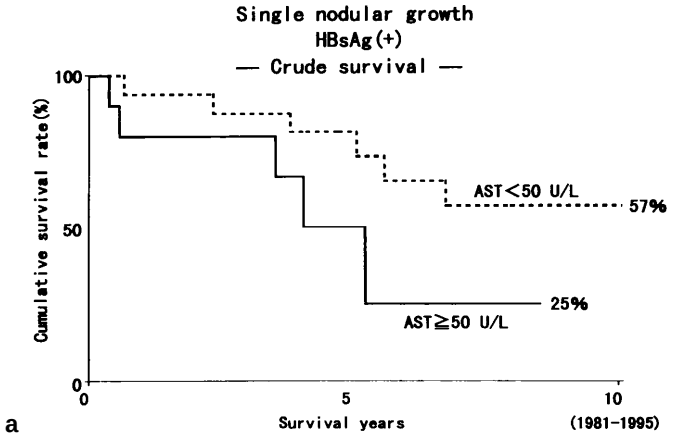
Fig. 2. **a** Crude survival and **b** disease-free survival rates according to viral serostatus in patients with a single nodular HCC without microscopic extracapsular invasion. $*P < 0.05$, *HBsAg(−)*, *HCVAb(−)* vs *HCVAb(+)*

Fig. 3. **a** Crude survival and **b** disease-free survival rates according to the presence or absence of hepatitis B virus (HBV)-related antibody in HCV antibody-positive patients with single nodular HCC

Table 3. Data for patients with a single nodular HCC according to subset

	n	Age (years)	AST/ALT (U/l)	HAI score	ICGR15 (%)	Tumor size (cm)	Histology vp-im (+) (%)
HCV Ab(+)							
HBV-related							
antibody (+)	51	63 ± 5	67 ± 32/73 ± 45	5.9 ± 1.7	20 ± 7.9	3.5 ± 2.1	27/51 (53%)
(−)	19	63 ± 5	65 ± 25/75 ± 33	6.1 ± 1.4	18 ± 6.9	3.7 ± 1.8	12/19 (63%)
HCV Ab(+)							
AST ≧ 50 (U/l)	50	64 ± 5	79 ± 32/92 ± 39	5.3 ± 2.1	17 ± 8.0	4.0 ± 2.6	13/24 (54%)
<50 (U/l)	24	63 ± 6	40 ± 12/36 ± 8	6.3 ± 1.2	21 ± 7.1	3.4 ± 1.6	28/50 (56%)
HBsAg(+)							
AST ≧ 50 (U/l)	16	53 ± 9	72 ± 28/80 ± 30	4.5 ± 2.4	15 ± 9.9	4.4 ± 2.8	9/16 (56%)
<50 (U/l)	10	53 ± 10	31 ± 17/28 ± 9	6.2 ± 1.3	18 ± 9.9	5.1 ± 4.3	5/10 (50%)

ICGR15, indocyanine green retention at 15 min after loading (0.5 mg/kg); HAI, histological activity index; vp, portal invasion; im, intrahepatic metastasis



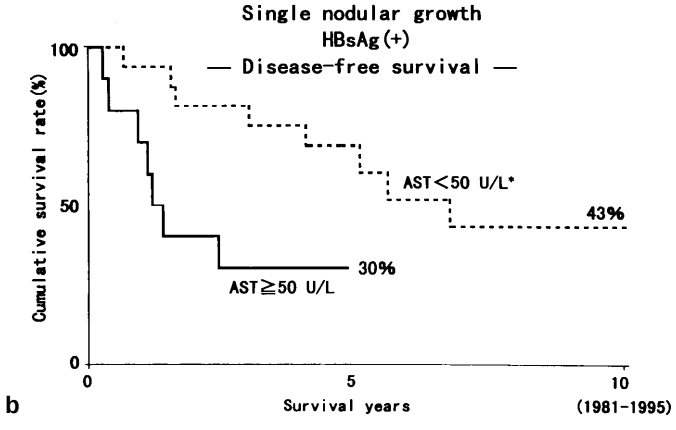
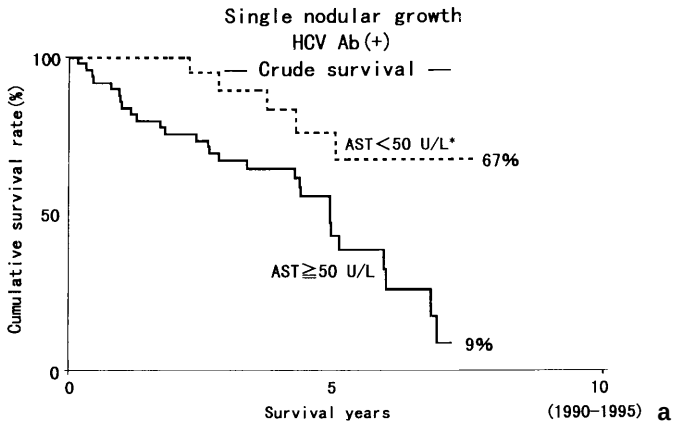


Fig. 4. **a** Crude survival and **b** disease-free survival rates according to preoperative serum aspartate aminotransferase (*AST*) level in HBsAg-positive patients with single nodular HCC. $*P < 0.05$, *AST < 50 U/l* vs *AST ≧ 50 U/l*



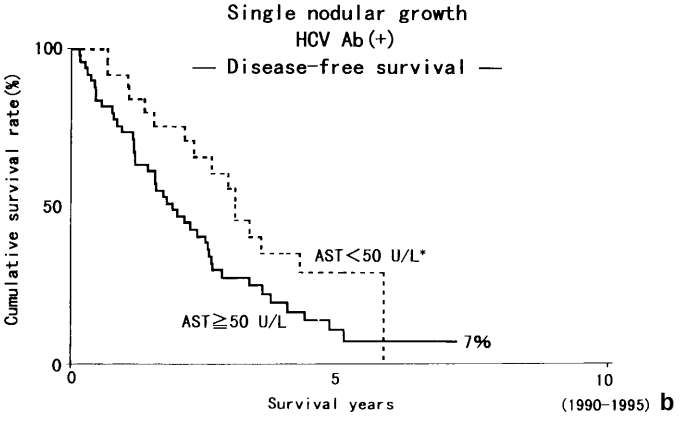


Fig. 5. **a** Crude survival and **b** disease-free survival rates according to preoperative serum AST level in HCV-antibody-positive patients with single nodular HCC. $*P < 0.05$, *AST < 50 U/l* vs *AST ≧ 50 U/l*

in terms of the biological characteristics of the tumor,[17–19] in addition to the surgical factors.[16] Current studies, however, indicate that intrahepatic recurrences after a hepatectomy may originate not only from minute intrahepatic metastatic lesions left behind but also from metachronous multicentric occurrences.[8] In fact, our data suggest that intrahepatic recurrence within 5 years posthepatectomy, which are highly indicative of multicentric tumor, developed in 66% of the patients who had a resection for a small HCC without evidence of microscopic extracapsular spread.

Strictly speaking, multicentric carcinogenesis should be defined based on the biomolecular evidence; genomically, a new clone that differs from the original clone. In the routine clinical setting this diagnosis has been made roughly on the basis of pathological differences between the original tumor and the recurrent one. Against this background, the intrahepatic recurrence in this current series could not always be determined to be multicentric in origin. Therefore, the term "metachronous" carcinogenesis would be better as a substitute for term "multicentric" carcinogenesis. Early intrahepatic recurrence within 6 months after hepatectomy may better be defined as synchronous carcinogenesis. Although early intrahepatic recurrence even after apparently complete resection for a single nodular HCC without extracapsular spread includes intrahepatic metastatic recurrence originating from the resected tumor, we tentatively defined all the recurrences as metachronous carcinogenesis.

The inflammatory activity of the coexisting chronic hepatitis[20,21] and the type of hepatitis virus[7,22] have recently been noted as possible prognostic factors. In fact, a high proliferative activity of the underlying liver parenchyma,[23] and the α-fetoprotein (AFP) value, which can serve as an indicator of hepatitis infection activity,

have been recognized as carcinogenic factors in cirrhotic patients[2] and in hepatectomized patients with cirrhosis.[24]

There have been conflicting reports, that a viral seromaker was not an independent variable that correlated with the later development of new hepatic malignancies after a hepatectomy,[21] and that the posthepatectomy prognosis of anti-HCV-positive HCC patients did not differ from that of patients who were HBsAg-positive,[22,25] although multicentricity in the resected specimens was significantly higher in the anti-HCV-positive patients.[21,25]

Among our patients overall, the HBsAg-positive patients, who more frequently had portal invasion and intrahepatic metastasis, had the worst prognosis. However, when prognosis was compared in the group of patients with a single nodular growth, the intrahepatic recurrence rate was clearly higher in the anti-HCV-positive patients. Furthermore, this difference was even more marked in the subset of patients without microscopic extracapsular spread, who were at a low risk of having residual, microscopic intrahepatic metastatic foci in the remnant liver. In other words, the hepatitis viral serostatus did not significantly affect the crude survival when the tumor was completely removed at the microscopic level, but clearly did affect the recurrence rate.

It is well known that the hepatic reserve is more compromised, and the inflammatory activity is higher in anti-HCV-positive patients than in HBsAg-positive or NBNC patients.[7] In this present study, the NBNC patients had the lowest levels of serum liver enzymes and the least histopathologic derangement. These host liver conditions were well correlated with the crude survival and intrahepatic recurrence rates. Furthermore, elevation of preoperative serum liver enzyme concentrations per se seemed to be a risk factor for intrahepatic recurrence, regardless of the type of viral infection. Thus, metachronous recurrence seems more likely to be correlated with persistence of inflammation per se, although there is interesting data that the core protein of hepatitis C virus is directly involved in the development of HCC,[26] similarly to findings of the integration of HBV DNA into the genomic DNA as a tumor initiator in HBV-related carcinogenesis.[27]

The present study also revealed that prior hepatitis B infection seemed unlikely to affect the risk of metachronous occurrence in anti-HCV-positive patients. Co-infection with HBV and HCV is common.[28] Demonstrable HBV-DNA sequences were found in 78% of HBsAg-negative and anti-HCV-positive cirrhotic patients.[29] In our present study, HBV-related antibody was positive in 73% of the anti-HCV-positive patients (51/70). There may be a reciprocal suppression of HCV replication by HBV;[30] however, the inflammatory activity and the serum ALT/AST concentrations were not different between anti-HCV-positive patients with and without HBV-related antibody. This suggests that the activity of coexisting hepatitis is influenced mainly by the presence or absence of HCV infection.[31] There is some controversy regarding whether anti-HBV positivity can be a risk factor for the development of HCC in HCV-related cirrhotic patients.[32–34] Although there is a study reporting that the combination of current HCV infection and prior HBV infection, compared with positivity for HCV alone, was more frequently associated with multicentric HCCs in the resected specimens,[35] our data, derived not only from cirrhotics but also from patients with chronic hepatitis, showed that the seropositivity of anti-HBV did not affect the incidence of later intrahepatic recurrences in the anti-HCV-positive patients who underwent a resection for a single nodular HCC.

Our multivariate analysis in another study (unpublished) demonstrated that the severity of coexisting hepatitis, determined by the HAI score, was an independent variable, along with the patient's age, ICG retention rate, and singularity and growth pattern of the HCC, which determined the duration of disease-free survival after complete resection for HCCs. Furthermore, the present univariate analysis revealed that the cumulative metachronous recurrence rate, 66% at 5 years posthepatectomy in the patients overall with a single nodular HCC, was significantly different according to viral serostatus, in correlation with the severity of the virally induced inflammation, and that prior coinfection with HBV did not increase the rate of metachronous recurrences.

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