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Original Article

Effect of nucleoside analog use in patients with hepatitis B virus-related hepatocellular carcinoma

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Aim: To examine the effect of nucleoside analog (NA) therapy on clinical outcome in patients with hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) who underwent curative therapy.

Methods: A total of 131 patients with HBV-related HCC who underwent curative therapy were analyzed. They were divided into an NA group who received NA therapy ($n = 99$, group A) and a control group ($n = 32$, group B). Group A was further classified into two groups of patients who either received NA therapy before HCC therapy ($n = 34$, group Aa) or who received NA therapy with initial HCC therapy ($n = 65$, group Ab). Overall survival (OS) and recurrence-free survival (RFS) were compared in the three groups.

Results: The 1- and 3-year cumulative OS rates were both in group Aa, 100% and 88.0% in group Ab, and 100% and 75.7% in group B (overall significance, $P = 0.002$), respectively. The corresponding RFS rates were 93.1% and 36.0% in group Aa, 78.3% and 45.7% in group Ab, and 78.0% and 38.0% in group B (overall significance, $P = 0.734$), respectively. Multivariate analysis revealed that being part of group Aa ($P < 0.001$) or group Ab ($P < 0.001$) and having albumin levels of 4.0 g/dL or more ($P = 0.040$) were significantly associated with OS, while HCC stage ($P = 0.001$) and hepatitis B e-antigen positivity ($P < 0.001$) were independent predictors linked to RFS.

Conclusion: NA therapy in patients with HBV-related HCC may improve survival after curative therapy.

Key words: hepatitis B virus, hepatocellular carcinoma, nucleoside analog, overall survival, recurrence-free survival

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) ranks fifth among the most prevalent cancers in the world, and is the third most common cause of cancer-related death.[1] Thus, it is a major health problem worldwide. Most cases of HCC are attributable to chronic liver disease resulting from chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.[2] Chronic hepatitis B (CHB) is the leading cause of liver cirrhosis (LC), liver disease-related events and occurrence of HCC in Asian countries, although Japan has one of the lowest prevalence rates for CHB among Asian countries.[3,4]

Hepatocellular carcinoma often recurs even after curative therapy such as surgical resection (SR) or ablative therapy.[5–7] Its prognosis is generally poor because of a high post-treatment recurrence rate that characterizes HCC, and this is a major obstacle for improving prognosis in patients with HCC.[5–7]

Antiviral therapy, including nucleoside analogs (NA), for CHB is effective for suppressing HBV DNA levels, achieving sustained histological improvement and reducing the risk of liver-related complications and HCC.[8–13] In general, NA therapy for CHB is better tolerated than interferon therapy.[11,12,14] Recently, antiviral therapies for CHB using NA have become standard treatment modalities. In our country, lamivudine (LAM), adefovir dipivoxil (ADV) and entecavir (ETV) have been approved for clinical use in patients with HBV-related liver disease.[11] In patients with HBV-related HCC, NA

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therapy for background liver disease has also been recommended with the purpose of improving clinical outcome.[11,15–18]

Several factors including tumor size, HCC stage, tumor marker, severity of liver fibrosis, hepatitis B e-antigen (HBeAg) status and HBV viral load have been reported to affect clinical outcome in patients with HBV-related HCC after curative therapy.[19–24] However, to our knowledge, clinical studies on the efficacy of NA therapy in HBV-related HCC patients have been limited.[15–18,25–27] The aim of the current study was therefore to investigate the effect of NA therapy on clinical outcome in patients with HBV-related HCC who underwent curative therapy.

METHODS

Patients

A TOTAL OF 131 treatment-naïve HBV-related HCC patients received curative therapy at our institution between January 2001 and November 2012. All patients were positive for hepatitis B surface antigen (HBsAg) and negative for anti-HCV (HCVAb). Curative therapy was defined as therapy resulting in no apparent viable tumor on dynamic computed tomography (CT) performed within 1 month after initial treatment for HCC. As several investigators have used percutaneous ablative therapies (PAT) such as radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) to treat selected patients with resectable HCC with favorable clinical outcomes, we defined complete ablation of RFA or PEI as curative therapy as well as SR.[28–30] After diagnosis of HCC, the most appropriate therapeutic procedure was selected by discussions with surgeons and physicians, according to the tumor characteristics and underlying liver functional reserve of each patient.

First, 131 patients were classified into two groups: patients who received NA therapy (NA group [group A], $n = 99$) and patients who did not receive NA therapy (control group [group B], $n = 32$). In group A, 69 patients received ETV monotherapy, 18 received ADV add-on treatment converted from LAM monotherapy due to breakthrough hepatitis, five received ETV monotherapy switched from LAM monotherapy and seven received LAM monotherapy.[8–11,31,32] Group B patients did not receive NA therapy for the following reasons: (i) sustained low HBV viral load during the follow-up period ($n = 20$); (ii) informed consent for NA therapy was not obtained due to economic reasons ($n = 7$); and (iii) unknown reasons ($n = 5$). Second, we classified group A into two groups of patients who had either received NA therapy before initial treatment for HCC ($n = 34$, group Aa) or those in whom NA were administrated at the time of initial treatment for HCC ($n = 65$, group Ab). This is because in group Aa patients, it was not possible to exclude the possibility that reduction of necroinflammation in the liver already existed at the initial treatment for HCC. In group Aa patients, NA were administrated with the purpose of suppressing HBV viral load and alanine aminotransferase (ALT) elevation. However, HCC were detected by imaging studies after the initiation of NA therapy. Overall survival (OS) and recurrence-free survival (RFS) rates were compared in groups Aa, Ab and B.

Written informed consent was obtained from all patients prior to each therapy, and the study protocol complied with all of the provisions of the Declaration of Helsinki. This study was approved by the ethics committee of Osaka Red Cross Hospital, Japan, and the need for written informed consent in the current study was waived because the data were analyzed retrospectively and anonymously. The present study comprised a retrospective analysis of patient records registered in our database, and all treatments were conducted in an open-label manner.

HCC and LC diagnosis

Hepatocellular carcinoma was diagnosed using abdominal ultrasound and dynamic CT scans (hyperattenuation during the arterial phase in all or some parts of the tumor and hypoattenuation in the portal-venous phase) and/or magnetic resonance imaging (MRI), based mainly on the recommendations of the American Association for the Study of Liver Diseases.[33] Arterial- and portal-phase dynamic CT images were obtained at approximately 30 s and 120 s, respectively, after injection of the contrast material. HCC stage was determined using the Liver Cancer Study Group of Japan staging system.[34] HCC was confirmed pathologically only in patients who underwent surgery. LC was determined by specimens at surgery, imaging modalities, or portal hypertension such as esophageal varices and splenomegaly.

Serological studies

Hepatitis B surface antigen, HCVAb, HBeAg and hepatitis B e antibody (HBeAb) were detected using commercial enzyme immunoassay kits (Dainabot or Fujirebio, Tokyo, Japan). With regard to the titer of HBsAg, values between 0.1 cut-off index (COI) and 2000 COI were obtained using chemiluminescent enzyme immunoassays. Values of 1.0 COI or more were defined as HBsAg

positive. HBV DNA levels were quantified using the COBASR Amplicor HBV Monitor Test (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.6–7.6 log copies/mL, or the COBASR TaqMan HBV Test ver. 2.0 (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of over 2.1–9.0 log copies/mL.

Follow up

Follow up after each therapy consisted of periodic blood tests and monitoring of tumor markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), using chemiluminescent enzyme immunoassays (Lumipulse PIVKAII Eisai; Eisai, Tokyo, Japan). Dynamic CT scans and/or MRI were obtained every 2–4 months after each therapy. Chest CT, whole abdominal CT, brain MRI and bone scintigraphy were performed when extrahepatic HCC recurrence was suspected.

Statistical analysis

Data were analyzed using univariate and multivariate analyses. Continuous variables were compared using unpaired Student's *t*-tests, and categorical variables were compared using Fisher's exact tests. Time to recurrence was defined as the interval between each therapy and first confirmed recurrence. For analysis of RFS, follow up ended at the time of first recurrence; other patients were censored at their last follow-up visit or the time of death from any cause without recurrence. For analysis of OS, follow up ended at the time of death from any cause, and the remaining patients were censored at the last follow-up visit. The cumulative OS and RFS rates were calculated using the Kaplan–Meier method, and tested using the log-rank test. Factors with a *P*-value of less than 0.1 in univariate analysis were subjected to multivariate analysis using the Cox proportional hazards model. These statistical methods were used to estimate the interval from initial treatment. Data were analyzed using SPSS software (SPSS, Chicago, IL, USA) for Microsoft Windows. Data are expressed as means ± standard deviation (SD). Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Baseline characteristics

THE BASELINE CHARACTERISTICS of the patients in the three groups at initial treatment for HCC are shown in Table 1. The median observation periods were 3.5 years (range, 0.2–11.9) for group A (2.0 for group Aa and 4.9 for group Ab) and 4.0 years (range, 1.1–10.4) for group B.

In group Aa, SR was performed in 13 patients (38.2%), and PAT such as RFA and PEI in 21 patients (61.8%). The median interval between the initiation of NA therapy and initial treatment for HCC in group Aa was 1.5 years (range, 0.3–10.0). In group Ab, SR was performed in 21 patients (32.3%) and PAT in 44 patients (67.7%). In group B, SR was performed in 18 patients (56.3%) and PAT in 14 patients (43.7%). The difference in proportions of patients with 10^5 copies/mL or more HBV DNA pretreatment in each two groups reached significance. Similarly, differences in the proportions of patients with 10^4 copies/mL or more HBV DNA pretreatment or with 10^3 copies/mL or more HBV DNA pretreatment in each of the two groups also reached significance.[11] In terms of the proportion with LC presence, the difference in each of the two groups did not reach significance.

Cumulative OS and RFS rates

The 1-, 3- and 5-year cumulative OS rates were all 100% in group Aa, 100%, 88.0% and 85.9%, respectively, in group Ab, and 100%, 75.7% and 49.8%, respectively, in group B (Aa vs B, $P = 0.004$; Ab vs B, $P = 0.007$; Aa vs Ab, $P = 0.094$; overall significance, $P = 0.002$) (Fig. 1a). The corresponding RFS rates were 93.1%, 36.0% and 21.0%, respectively, in group Aa, 78.3%, 45.7% and 15.6%, respectively, in group Ab, and 78.0%, 38.0% and 19.6%, respectively, in group B (Aa vs B, $P = 0.540$; Ab vs B, $P = 0.607$; Aa vs Ab, $P = 0.650$; overall significance, $P = 0.734$) (Fig. 1b).

Univariate and multivariate analyses of factors contributing to OS

Univariate analysis identified the following factors as significantly associated with OS for all cases ($n = 131$): NA therapy ($P = 0.002$), HBeAg positivity ($P = 0.018$) and pretreatment serum albumin levels of 4.0 g/dL or more ($P = 0.047$) (Table 2). The hazard ratios (HR) and 95% confidence intervals (CI) calculated using multivariate analysis for the three factors with *P*-values of less than 0.1 in univariate analysis are detailed in Table 2. NA therapy ($P < 0.001$ for both group Aa and Ab) and serum albumin levels of 4.0 g/dL or more ($P = 0.040$) were revealed to be significant predictors linked to OS in the multivariate analysis.

Univariate and multivariate analyses of factors contributing to RFS

Univariate analysis identified the following factors as significantly associated with RFS for all cases ($n = 131$): HCC stage ($P = 0.011$), presence of LC ($P = 0.003$),

Table 1 Baseline characteristics at initial treatment in the three groups (group Aa, Ab and B)

Variables (at initial therapy)	NA group (Group A) ($n = 99$)	Group Aa ($n = 34$)	Group Ab ($n = 65$)	Group B ($n = 32$)	*P*-value (A vs B)	*P*-value (Aa vs B)	*P*-value (Ab vs B)	*P*-value (Aa vs Ab)
Age (years)	58.5 ± 11.4	63.1 ± 10.2	56.1 ± 11.2	60.7 ± 12.4	0.353*	0.387*	0.080*	0.003*
Sex, male/female	71/28	24/10	47/18	20/12	0.379**	0.603**	0.356**	>0.999**
HCC stage, I/II/III	28/50/21	12/17/5	16/33/16	7/16/9	0.642**	0.306**	0.921**	0.366**
Initial treatment for HCC								
Surgery/ablative therapy	34/65	13/21	21/44	18/14	0.037**	0.217**	0.029**	0.657**
Maximum tumor size (cm)	2.7 ± 1.8	2.4 ± 1.5	2.8 ± 1.9	3.2 ± 1.8	0.154*	0.051*	0.373*	0.221*
Tumor number, single/multiple	67/32	27/7	40/25	23/9	0.671**	0.570**	0.370**	0.112**
Liver cirrhosis, yes/no	58/41	20/14	38/27	15/17	0.307**	0.460**	0.386**	>0.999**
HBs antigen ≥500 COI, yes/no	56/43	25/9	31/34	5/27	<0.001**	<0.001**	0.003**	0.019**
HBV DNA								
$\geq 10^5$ copies/mL, yes/no	50/49	0/34	50/15	9/23	0.040**	0.001**	<0.001**	<0.001**
$\geq 10^4$ copies/mL, yes/no	58/41	0/34	58/7	12/20	0.043**	<0.001**	<0.001**	<0.001**
$\geq 10^3$ copies/mL, yes/no	67/32	2/32	65/0	16/16	0.092**	<0.001**	<0.001**	<0.001**
HBe antigen, positive/negative	28/71	6/28	22/43	4/28	0.097**	0.734**	0.029**	0.105**
AST (IU/L)	44.6 ± 24.4	34.5 ± 17.2	49.8 ± 26.1	40.5 ± 30.2	0.439*	0.326*	0.118*	0.003*
ALT (IU/L)	44.2 ± 30.1	27.7 ± 16.2	52.8 ± 31.1	40.0 ± 42.9	0.541*	0.126*	0.103*	<0.001*
Serum albumin (g/dL)	3.99 ± 0.48	4.08 ± 0.46	3.94 ± 0.49	4.18 ± 0.45	0.049*	0.388*	0.021*	0.160*
Total bilirubin (mg/dL)	0.98 ± 0.61	1.19 ± 0.81	0.87 ± 0.43	0.92 ± 0.56	0.598*	0.113*	0.643*	0.035*
Prothrombin time (%)	83.8 ± 15.7	82.3 ± 15.1	84.6 ± 16.0	93.4 ± 18.0	0.004*	0.009*	0.016*	0.501*
Platelets ($\times 10^4/mm^3$)	12.8 ± 7.4	12.0 ± 6.4	13.2 ± 7.9	12.9 ± 5.8	0.921*	0.548*	0.871*	0.461*
AFP (ng/mL)	1 313.8 ± 4 915.1 (21.2)	845.9 ± 3571.0 (6.8)	1 558.5 ± 5 499.1 (30.5)	1787.7 ± 8670.1 (17.7)	0.770*	0.571*	0.892*	0.439*
DCP (mAU/mL)	1 452.3 ± 10 496.0 (26)	349.1 ± 1779.7 (23)	2 029.4 ± 12 886.9 (29)	824.1 ± 1884.0 (109)	0.571*	0.297*	0.463*	0.305*

Data are expressed as number or mean ± standard deviation.
*Unpaired Student's *t*-tesm. **Fisher's exact test.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COI, cut off index; DCP; des-γ-carboxy prothrombin; HBs, hepatitis B surface; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NA, nucleoside analog.

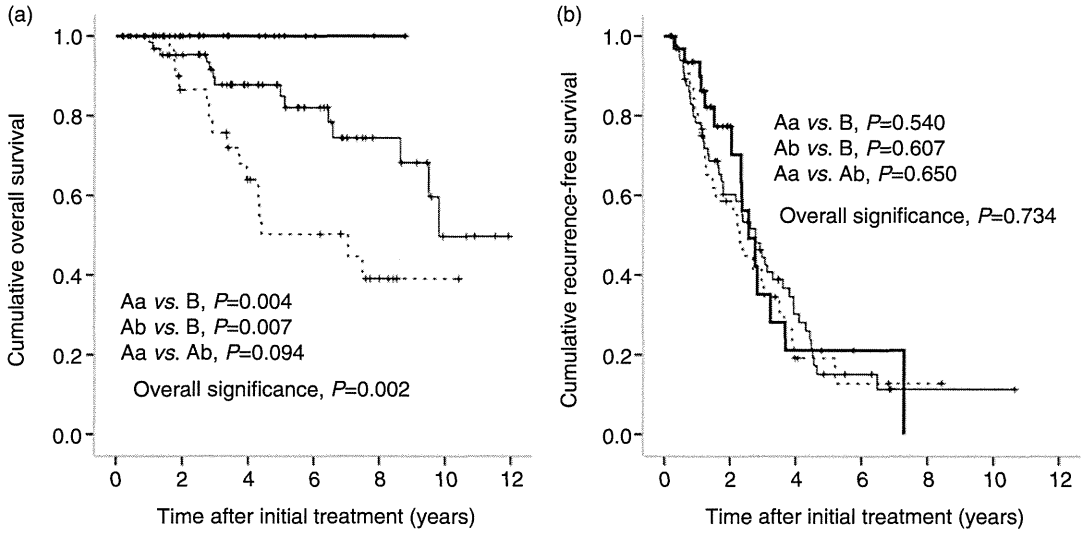

Figure 1 Cumulative overall survival (OS) and recurrence-free survival (RFS) in groups Aa ($n = 34$), Ab ($n = 65$) and B ($n = 32$). (a) The 1-, 3- and 5-year cumulative OS rates were all 100% in group Aa, 100%, 88.0% and 85.9%, respectively, in group Ab, and 100%, 75.7% and 49.8%, respectively, in group B (Aa vs B, $P = 0.004$; Ab vs B, $P = 0.007$; Aa vs Ab, $P = 0.094$; overall significance, $P = 0.002$) (b) The 1-, 3- and 5-year cumulative RFS rates were 93.1%, 36.0% and 21.0%, respectively, in group Aa, 78.3%, 45.7% and 15.6%, respectively, in group Ab, and 78.0%, 38.0% and 19.6%, respectively, in group B (Aa vs B, $P = 0.540$; Ab vs B, $P = 0.607$; Aa vs Ab, $P = 0.650$; overall significance, $P = 0.734$). ——, group Aa ($n = 34$); – – –, group Ab ($n = 65$); · · · ·, group B ($n = 32$).

HBeAg positivity ($P < 0.001$), serum albumin levels of 4.0 g/dL or more ($P = 0.003$) and platelet counts of $12 \times 10^4/mm^3$ or more ($P = 0.016$) (Table 2). The hazard ratios and 95% confidence intervals calculated using multivariate analysis for the seven factors with *P*-values of less than 0.1 in univariate analysis are detailed in Table 2. HCC stage ($P = 0.001$) and HBeAg positivity ($P < 0.001$) were found to be significant prognostic factors linked to RFS.

HBeAg seroconversion, HBeAg loss and HBsAg loss in the three groups

In group Aa, six patients were HBeAg positive upon initial treatment for HCC. In four of these patients (66.7%), HBeAg seroconversion was detected during the observation period. In one patient (16.7%), HBeAg loss without HBeAg seroconversion was recorded. In group Ab, 22 patients were HBeAg positive upon initial treatment for HCC. In nine of these patients (40.9%), HBeAg seroconversion was noted during the observation period. In one patient (3.6%), HBeAg loss without HBeAg seroconversion was observed. In group B, four patients had HBeAg positivity at initial treatment. In one patient (25.0%), HBeAg seroconversion was observed during the observation period. None of the patients in group Aa or Ab experienced HBsAg loss during the observation period, whereas in group B, four patients (12.5%) experienced spontaneous HBsAg loss during the observation period.

Effect of NA therapy on the reduction of HBV DNA viral load

In patients treated with ETV monotherapy ($n = 69$: 25 patients [73.5%] in group Aa and 44 [67.7%] in group Ab), 55 patients (79.7%) had achieved undetectable HBV DNA, 10 (14.5%) had achieved less than $10^{2.1}$ copies/mL HBV DNA and three (4.3%) had achieved less than $10^{2.6}$ copies/mL HBV DNA during the follow-up period. No viral breakthrough hepatitis as defined by 1 log increase from nadir with ALT elevation was observed during ETV monotherapy. In patients treated with ADV, add-on treatment was converted from LAM monotherapy because of breakthrough hepatitis ($n = 18$: five patients [14.7%] in group Aa and 13 [20.0%] in group Ab), and nine patients (50.0%) had achieved undetectable HBV DNA, three (16.7%) had achieved less than $10^{2.1}$ copies/mL HBV DNA and five (27.8%) had achieved less than $10^{2.6}$ copies/mL HBV DNA after conversion to ADV add-on treatment. In patients treated with ETV monotherapy switched from LAM monotherapy ($n = 5$: two patients [5.9%] in group Aa and three [4.6%] in group Ab), four patients (80.0%)

Table 2 Univariate and multivariate analysis contributing to overall survival (OS) and recurrence-free survival (RFS)

Variables (at initial treatment)	*n*	Univariate analysis (OS)	Multivariate analysis (OS)		Univariate analysis (RFS)	Multivariate analysis (RFS)	
		P-value*	HR (95% CI)	*P*-value**	*P*-value*	HR (95% CI)	*P*-value**
Sex (male vs female)	91/40	0.368			0.163		
Age (years) (≥60 vs <60)	70/61	0.573			0.676		
HCC stage (I or II vs III)	101/30	0.326			0.011	0.319 (0.163–0.626)	0.001
Maximum tumor size (cm) (≥2.5 vs <2.5)	64/67	0.625			0.610		
Tumor number (single vs multiple)	90/41	0.134			0.056	1.290 (0.712–2.338)	0.400
Liver cirrhosis (yes vs no)	73/58	0.262			0.003	0.729 (0.407–1.306)	0.288
HBs antigen titer ≥500 COI (yes/no)	61/70	0.624			0.962		
NA therapy (group Aa vs Ab vs B)	34/65/32	0.002	Aa: 4.292 (2.358–7.813) Ab: 3.584 (2.252–5.174) B: 1.000 (reference)	<0.001 <0.001	0.734		
HBe antigen (positive vs negative)	32/99	0.018	1.200 (0.726–1.983)	0.478	<0.001	0.353 (0.201–0.619)	<0.001
HBV DNA (≥10^5 copies/mL vs <10^5 copies/mL)	59/72	0.167			0.341		
AST (IU/L) (≥40 vs <40)	60/71	0.647			0.388		
ALT (IU/L) (≥40 vs <40)	54/77	0.980			0.918		
Serum albumin (g/dL) (≥4.0 vs <4.0)	84/47	0.047	0.636 (0.412–0.980)	0.040	0.003	1.344 (0.824–2.193)	0.237
Total bilirubin (mg/dL) (≥1.0 vs <1.0)	48/83	0.101			0.050	0.742 (0.446–1.235)	0.251
Platelet count (×10^4/μL) (≥12 vs <12)	64/67	0.432			0.016	1.114 (0.725–1.713)	0.623
Prothrombin time (%) (≥80 vs <80)	82/49	0.861			0.460		
Serum AFP (ng/mL) (≥20 vs <20)	65/66	0.649			0.861		
DCP (mAU/mL) (≥40 vs <40)	64/67	0.543			0.326		

*Log–rank test. **Cox proportional hazard model.

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; COI, cut off index; DCP, des-γ-carboxy prothrombin; HBs, hepatitis B surface; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; NA, nucleotide analog.

had achieved undetectable HBV DNA and one (20.0%) had achieved less than $10^{2.1}$ copies/mL HBV DNA during the follow-up period. In patients treated with LAM monotherapy ($n = 7$: two patients [5.9%] in group Aa and five [7.7%] in group Ab), two patients (28.6%) had achieved undetectable HBV DNA, one (14.3%) had achieved less than $10^{2.1}$ copies/mL HBV DNA and three (42.9%) had achieved less than $10^{2.6}$ copies/mL HBV DNA during the follow-up period except for one patient with poor response to LAM monotherapy.

Causes of death

Fourteen patients (14.1%) in group A (no patients in group Aa and 14 patients in group Ab) died during the follow-up period. The causes of death were HCC recurrence in eight patients, liver failure in four patients and miscellaneous causes in two patients. Fifteen patients in group B (46.9%) died during the follow-up period, and the causes of death were HCC recurrence in 11 patients and liver failure in four patients.

HCC recurrence

In the present study, 64 patients (64.6%) in group A (15 patients [44.1%] in group Aa and 49 [75.4%] in group Ab) and 25 patients in group B (78.1%) had HCC recurrence during the follow-up period. The patterns of HCC recurrence after initial treatment of group Aa were as follows: single HCC recurrence in the liver in eight patients and multiple HCC recurrences in the liver in seven patients. The patterns of HCC recurrence after initial treatment in group Ab were single HCC recurrence in the liver in 28 patients, multiple HCC recurrences in the liver in 18 patients, multiple HCC recurrences in the liver with lung metastases in one patient, multiple HCC recurrences in the liver with bone metastases in one patient and single lymph node metastasis in one patient. The patterns of HCC recurrence after initial treatment in group B were single HCC recurrence in the liver in 13 patients, multiple HCC recurrences in the liver in nine patients, multiple HCC recurrences in the liver with bone metastases in one patient, multiple HCC recurrences in the liver with lung metastases in one patient and single brain metastasis in one patient.

Treatment methods for the first HCC recurrence in group Aa were SR in one patient, PAT in 11 patients, transcatheter arterial chemoembolization (TACE) in two patients and no specific treatment in one patient. Treatment methods for the first HCC recurrence in group Ab were SR in two patients, PAT in 37 patients, TACE in seven patients, systemic chemotherapy in two patients and no specific treatment in one patient. Treatment methods for the first recurrence in group B were SR in three patients, PAT in 14 patients, TACE in three patients, radiation therapy in two patients and no specific treatment in three patients.

Subgroup analyses in patients with LC and without LC

Recent studies have demonstrated that the degree of liver fibrosis was closely associated with clinical outcome in patients with HBV-related HCC and it is considered as a major confounder for all liver-related complications. We therefore performed subgroup analyses according to the presence of LC. In patients with LC ($n = 73$), there were 58 patients in group A (20 in group Aa and 38 in group Ab) and 15 in group B. In patients without LC ($n = 58$), there were 41 patients in group A (14 in group Aa and 27 in group Ab) and 17 in group B. In terms of OS, there was a significant difference in the three groups in patients with LC (Aa vs B, $P = 0.003$; Ab vs B, $P = 0.010$; Aa vs Ab, $P = 0.093$; overall significance, $P = 0.002$), while the difference in the three groups did not reach significance in patients without LC (Aa vs B, $P = 0.254$; Ab vs B, $P = 0.164$; Aa vs Ab, $P = 0.673$; overall significance, $P = 0.257$) (Fig. 2). In terms of RFS, the difference in the three groups did not reach significance in patients with LC (Aa vs B, $P = 0.293$; Ab vs B, $P = 0.302$; Aa vs Ab, $P = 0.653$; overall significance, $P = 0.436$) and in patients without LC (Aa vs B, $P = 0.293$; Ab vs B, $P = 0.835$; Aa vs Ab, $P = 0.356$; overall significance, $P = 0.530$) (Fig. 3).

Subgroup analysis according to HBsAg titer

We also performed subgroup analyses according to HBsAg titer. In group Aa, there were 20 patients (58.8%) in whom HBsAg titer was more than 2000 COI. Such patients were observed in group Ab ($n = 26$; 40.0%) and group B ($n = 2$; 6.3%). In patients with a HBsAg titer of 500 COI or more ($n = 61$), there were 56 patients in group A (25 in group Aa and 31 in group Ab) and five in group B. In patients with a HBsAg titer of less than 500 COI ($n = 70$), there were 43 patients in group A (nine [36.8%] in group Aa and 34 [52.3%] in group Ab) and 27 (84.4%) in group B. In these 70 patients, the median value of HBsAg titer was 252.9 COI in group Aa, 296.5 COI in group Ab and 208.3 COI in group B. In terms of OS, there was a significant difference in the three groups in patients with a HBsAg titer of 500 COI or more (Aa vs B, $P = 0.034$; Ab vs B, $P = 0.065$; Aa vs Ab, $P = 0.193$; overall significance, $P = 0.033$) and in patients with a HBsAg titer of less than 500 COI (Aa vs B, $P = 0.035$; Ab

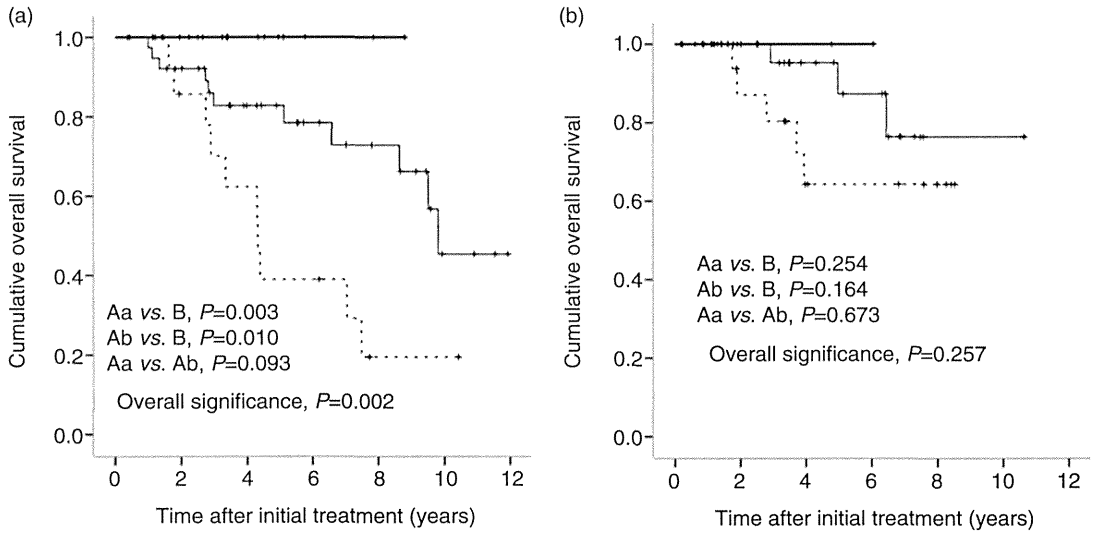

Figure 2 Subgroup analyses of patients with liver cirrhosis (LC) ($n = 73$: 20 in group Aa, 38 in group Ab and 15 in group B) and patients without LC ($n = 58$: 14 in group Aa, 27 in group Ab and 17 in group B) in terms of overall survival (OS). (a) In patients with LC, there was a significant difference in OS in the three groups (Aa vs B, $P = 0.003$; Ab vs B, $P = 0.010$; Aa vs Ab, $P = 0.093$; overall significance, $P = 0.002$). (b) In patients without LC, the OS difference in the three groups did not reach significance (Aa vs B, $P = 0.254$; Ab vs B, $P = 0.164$; Aa vs Ab, $P = 0.673$; overall significance, $P = 0.257$). (a) ——, group Aa ($n = 20$); – – –, group Ab ($n = 38$); · · · ·, group B ($n = 15$); (b) ——, group Aa ($n = 14$); – – –, group Ab ($n = 27$); · · · ·, group B ($n = 17$).

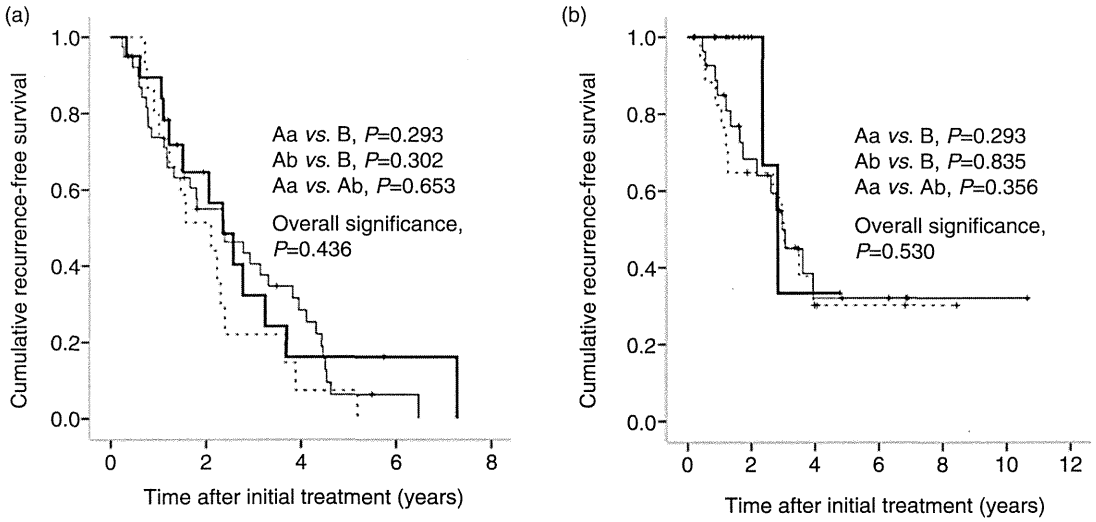

Figure 3 Subgroup analyses of patients with liver cirrhosis (LC) ($n = 73$: 20 in group Aa, 38 in group Ab and 15 in group B) and patients without LC ($n = 58$: 14 in group Aa, 27 in group Ab and 17 in group B) in terms of recurrence-free survival (RFS). (a) In patients with LC, the RFS difference in the three groups did not reach significance (Aa vs B, $P = 0.293$; Ab vs B, $P = 0.302$; Aa vs Ab, $P = 0.653$; overall significance, $P = 0.436$). (b) In patients without LC, the RFS difference in the three groups did not reach significance (Aa vs B, $P = 0.293$; Ab vs B, $P = 0.835$; Aa vs Ab, $P = 0.356$; overall significance, $P = 0.530$). (a) ——, group Aa ($n = 20$); – – –, group Ab ($n = 38$); · · · ·, group B ($n = 15$); (b) ——, group Aa ($n = 14$); – – –, group Ab ($n = 27$); · · · ·, group B ($n = 17$).

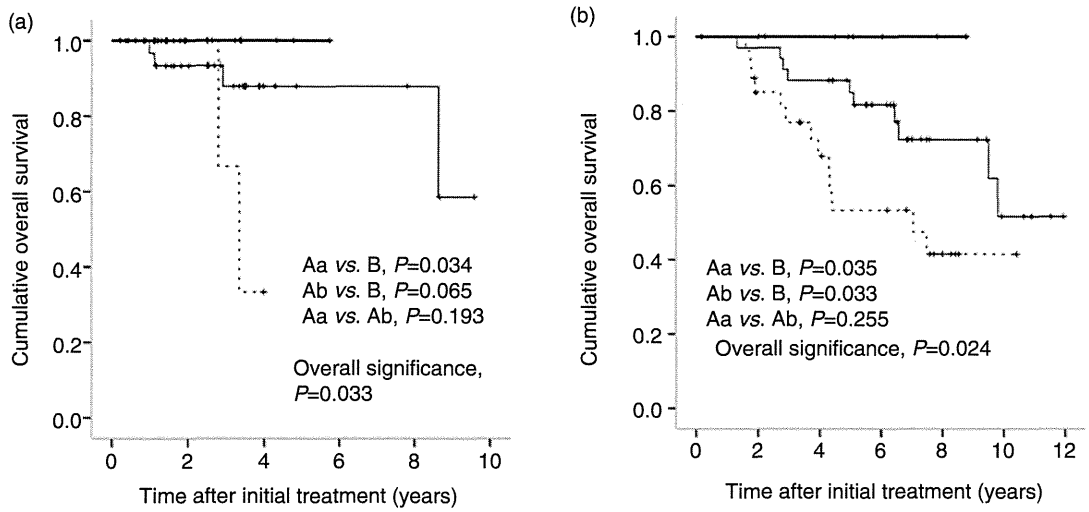

Figure 4 Subgroup analyses of patients with hepatitis B surface antigen (HBsAg) ≥500 cut-off index (COI) ($n = 61$: 25 in group Aa, 31 in group Ab and 5 in group B) and patients with <500 COI ($n = 70$: 9 in group Aa, 34 in group Ab and 27 in group B) in terms of overall survival (OS). (a) In patients with a HBsAg titer of ≥500 COI, there was a significant difference in the OS of the three groups (Aa vs B, $P = 0.034$; Ab vs B, $P = 0.065$; Aa vs Ab, $P = 0.193$; overall significance, $P = 0.033$). (b) In patients with a HBsAg titer of <500 COI, there was a significant difference in the OS of the three groups (Aa vs B, $P = 0.035$; Ab vs B, $P = 0.033$; Aa vs Ab, $P = 0.255$; overall significance, $P = 0.024$). (a) ——, Group Aa ($n = 25$); ——, Group Ab ($n = 31$); - - - -, Group B ($n = 5$); (b) ——, Group Aa ($n = 9$); ——, Group Ab ($n = 34$); - - - -, Group B ($n = 27$).

vs B, $P = 0.033$; Aa vs Ab, $P = 0.255$; overall significance, $P = 0.024$) (Fig. 4). However, in terms of RFS, the difference in the three groups did not reach significance in patients with a HBsAg titer of 500 COI or more (Aa vs B, $P = 0.917$; Ab vs B, $P = 0.766$; Aa vs Ab, $P = 0.200$; overall significance, $P = 0.455$) and in patients with a HBsAg titer of less than 500 COI (Aa vs B, $P = 0.896$; Ab vs B, $P = 0.375$; Aa vs Ab, $P = 0.510$; overall significance, $P = 0.624$) (Fig. 5).

Subgroup analysis according to HBV viral load

We conducted subgroup analyses according to HBV viral load. In patients with a HBV DNA count of 10^5 copies/mL or more ($n = 59$), there were 50 patients in group A (zero in group Aa and 50 in group Ab) and nine in group B. In patients with a HBV DNA count of less than 10^5 copies/mL ($n = 72$), there were 49 patients in group A (34 in group Aa and 15 in group Ab) and 23 in group B. In terms of OS, the difference reached significance in patients with a HBV DNA count of 10^5 copies/mL or more (Ab vs B, $P = 0.002$) and in patients with less than 10^5 copies/mL HBV DNA (Aa vs B, $P = 0.011$; Ab vs B, $P = 0.063$; Aa vs Ab, $P = 0.049$; overall significance, $P = 0.017$) (Fig. 6). In terms of RFS, the difference reached significance in patients with a HBV DNA count of 10^5 copies/mL or more (Ab vs B, $P = 0.012$), while in patients with HBV DNA of less than 10^5 copies/mL, the difference in the three groups did not reach significance (Aa vs B, $P = 0.862$; Ab vs B, $P = 0.713$; Aa vs Ab, $P = 0.496$; overall significance, $P = 0.823$) (Fig. 7).

DISCUSSION

TO THE BEST of our knowledge, clinical investigations of the effectiveness of NA therapy in HBV-related HCC patients have been limited, and most of these studies have used LAM in the treated cohort.[15–18,25–27] LAM-related drug-resistant viral mutations during the long course of its use are becoming one of the major adverse predictive factors in patients with HCC.[9] Although Wu *et al.* demonstrated in their large study that NA therapy after SR for HCC significantly reduced the rate of HCC recurrence as compared with the control group, their data lacked reliable laboratory data such as HBV DNA viral load and HBeAg positivity.[17] In the era of NA, the efficacy of NA on clinical outcome in patients with HBV-related HCC thus remains unclear. Hence, we conducted a current comparative study.

Our multivariate analysis identified that groups Aa and Ab were significant favorable predictors linked to

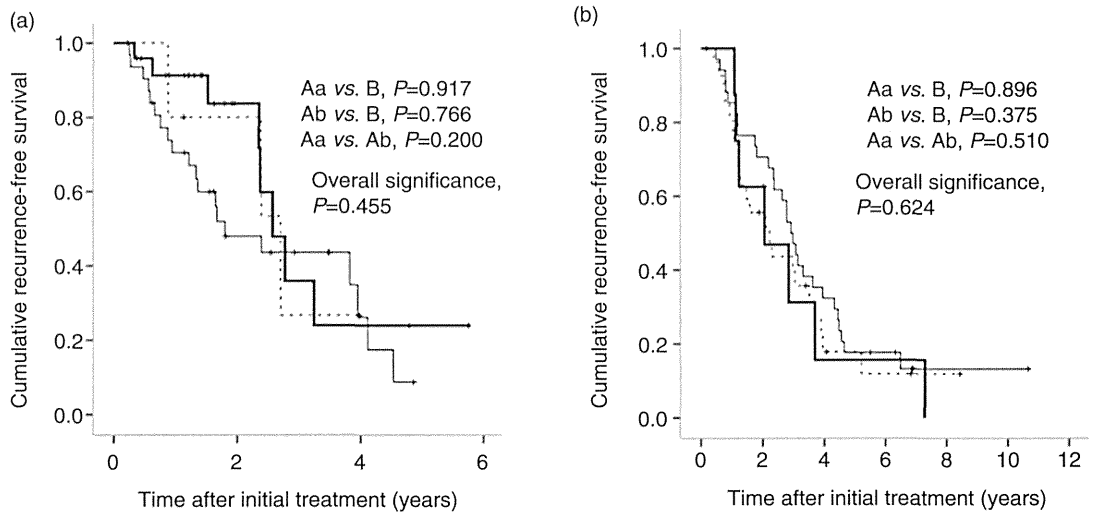

Figure 5 Subgroup analyses of patients with hepatitis B surface antigen (HBsAg) ≥500 COI ($n = 61$: 25 in group Aa, 31 in group Ab and five in group B) and patients with <500 COI ($n = 70$: 9 in group Aa, 34 in group Ab and 27 in group B) in terms of recurrence-free survival (RFS). (a) In patients with a HBsAg titer of ≥500 COI, the difference in the RFS of the three groups did not reach significance (Aa vs B, $P = 0.917$; Ab vs B, $P = 0.766$; Aa vs Ab, $P = 0.200$; overall significance, $P = 0.455$). (b) In patients with a HBsAg titer of <500 COI, the difference in the RFS of the three groups did not reach significance (Aa vs B, $P = 0.896$; Ab vs B, $P = 0.375$; Aa vs Ab, $P = 0.510$; overall significance, $P = 0.624$). (a) ——, group Aa ($n = 25$); ——, group Ab ($n = 31$); · · · ·, group B ($n = 5$); (b) ——, group Aa ($n = 9$); ——, group Ab ($n = 34$); · · · ·, group B ($n = 27$).

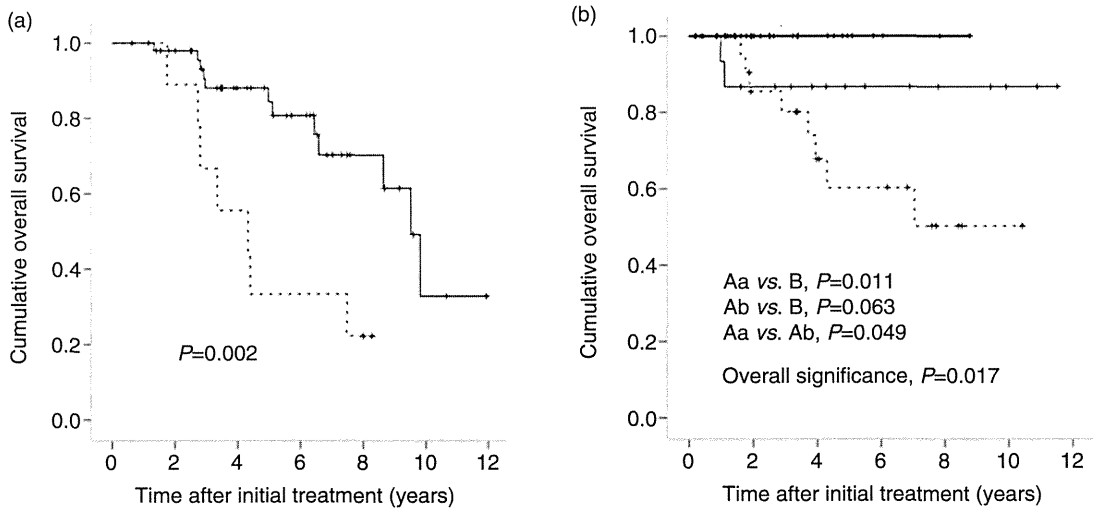

Figure 6 Subgroup analyses of patients with hepatitis B virus (HBV) DNA of $\geq 10^5$ copies/mL ($n = 59$: 50 in group Ab and 9 in group B) and patients with HBV DNA of $<10^5$ copies/mL ($n = 72$: 34 in group Aa, 15 in group Ab and 23 in group B) in terms of overall survival (OS). (a) In patients with HBV DNA of $\geq 10^5$ copies/mL, the OS difference reached significance (Ab vs B, $P = 0.002$). (b) In patients with HBV DNA of $<10^5$ copies/mL, the OS difference in the three groups reached significance (Aa vs B, $P = 0.011$; Ab vs B, $P = 0.063$; Aa vs Ab, $P = 0.049$; overall significance, $P = 0.017$). (a) ——, group Aa ($n = 0$); ——, group Ab ($n = 50$); · · · ·, group B ($n = 9$); (b) ——, group Aa ($n = 34$); ——, group Ab ($n = 15$); · · · ·, group B ($n = 23$).

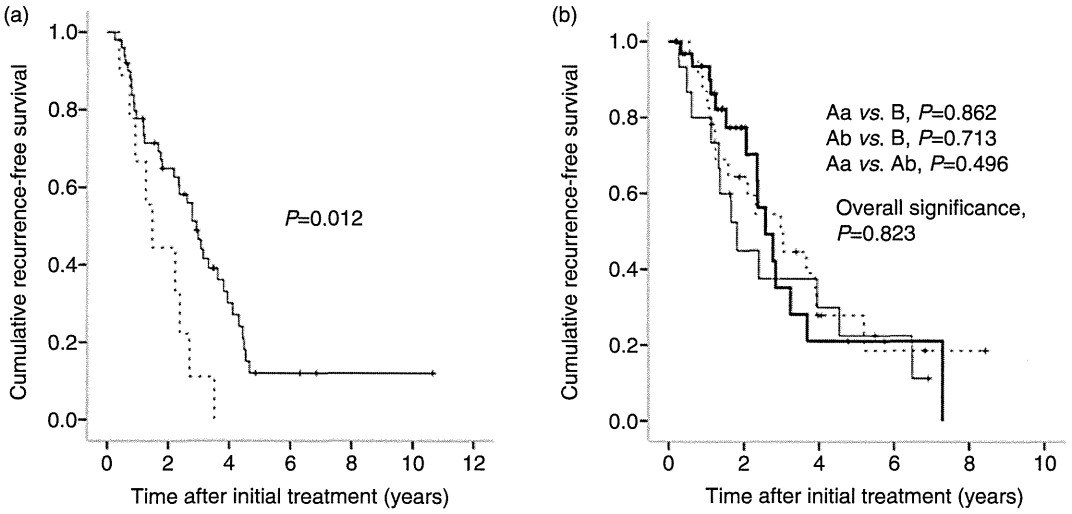

Figure 7 Subgroup analyses of patients with hepatitis B virus (HBV) DNA of $\geq 10^5$ copies/mL ($n = 59$: 50 in group Ab and nine in group B) and patients with HBV DNA of $<10^5$ copies/mL ($n = 72$: 34 in group Aa, 15 in group Ab and 23 in group B) in terms of recurrence-free survival (RFS). (a) In patients with HBV DNA of $\geq 10^5$ copies/mL, the RFS difference reached significance (Ab vs B, $P = 0.012$). (b) In patients with HBV DNA of $<10^5$ copies/mL, the RFS difference in the three groups did not reach significance (Aa vs B, $P = 0.862$; Ab vs B, $P = 0.713$; Aa vs Ab, $P = 0.496$; overall significance, $P = 0.823$). (a) ——, group Aa ($n = 0$); ——, group Ab ($n = 50$); · · · ·, group B ($n = 9$); (b) ——, group Aa ($n = 34$); ——, group Ab ($n = 15$); · · · ·, group B ($n = 23$).

OS, and most patients in group A had achieved less than $10^{2.6}$ copies/mL HBV DNA during the follow-up period. In addition, the proportion of death due to liver failure in group A tended to be lower than that in group B (four [4.0%] out of 99 patients in group A vs four [12.5%] out of 32 patients in group B, $P = 0.099$). These results suggest that maintaining viral suppression using NA and reducing the risk of hepatic events such as LC-related complications by NA therapy lead to improved survival after curative therapy.

In the current study, NA therapy was not a significant factor linked to RFS for all cases and for all subgroup analyses except in patients with HBV DNA of 10^5 copies/mL or more. These results suggested that factors other than NA therapy could significantly affect recurrence after curative therapy in patients with HBV-related HCC. Of note, none of the patients in groups Aa or Ab experienced HBsAg loss during the observation period, whereas in group B four patients experienced spontaneous HBsAg loss during the observation period, which indicated clinical remission in CHB. One possible reason for these is that the titer of HBsAg in group B was significantly lower than that in groups Aa or Ab. Indeed, all four patients who experienced spontaneous HBsAg loss had low HBsAg titer at initial treatment for HCC (48.0, 14.5, 9.4 and 2.2 COI, respectively),[35] and these results may partly explain the reason why in most of our analyses in terms of RFS, the difference among three groups did not reach significance.[36] However, in terms of the type of HCC recurrence, the proportion of first HCC recurrence with extrahepatic metastasis in group B was the highest among the three groups (no patients [0%] out of 15 patients with recurrence in group Aa, three patients [6.1%] out of 49 patients with recurrence in group Ab and three patients [12.0%] out of 25 patients with recurrence in group B). NA therapy may be thus associated with lowering the rate of occurrence of extrahepatic HCC recurrence after curative therapy, although further studies are needed to confirm these results.

Hepatitis B e-antigen positivity was a significant adverse predictor in terms of RFS in the multivariate analysis. Sun *et al.* demonstrated that HBeAg positivity was closely associated with a higher risk of early recurrence and poorer survival in patients after curative resection of small HBV-related HCC.[23] Our results were consistent with their reports. On the other hand, high HBV viral load was not an independent predictor linked to both OS and RFS in the present study, although several studies reported that high HBV viral load was a poor prognostic factor in patients with HBV-related HCC.[19–22,24] This may be due to the fact that most

patients in group A had sustained low HBV viral load by NA use during the observation period.

In subgroup analyses, in patients with LC there was an overall significant difference among three groups in terms of OS, while in patients without LC the difference in the three groups did not reach significance in terms of OS. Hosaka *et al.* reported that the treatment efficacy of ETV on prognosis in HBV-related liver disease was greater in patients with LC than patients without LC.[37] Although their study population included patients without HCC, our results were similar to their reports, indicating that in patients with HBV-related advanced liver fibrosis that can be associated with poorer clinical outcome, NA therapy is potentially more effective on prognosis than in patients without LC.

Serum albumin at levels of 4.0 g/dL or more was an independent predictor linked to OS in the present study. We previously demonstrated that lower serum albumin level was closely associated with poorer clinical outcomes in patients with HCV-related HCC.[38] In patients with HBV-related HCC with lower serum albumin level, branched-chain amino acid treatment also may be effective as well as in patients with HCV-related HCC for optimizing clinical outcomes.[38] Advanced HCC stage was an adverse prognostic factor linked to RFS in our study. Clinicians should thus be alert to not only HBV viral status and liver function but also to tumor-related factors for the management of patients with HBV-related HCC who undergo curative therapy.

In our study, HCC occurred in group Aa patients despite the fact that they were administrated NA.[36] The reasons for these are unclear, however, considering that the median interval between the initiation of NA therapy and initial treatment for HCC in group Aa was 1.5 years, microscopic tumor foci that were not detected during imaging studies may have already existed at the initiation of NA therapy in many of these patients.

Our study had several limitations. First, this study was a retrospective observational study with a relatively heterogeneous patient population. Second, in the current study, the baseline characteristics in the three groups were not well balanced for survival analysis, leading to bias. Third, patients in group A were treated with various kinds of NA. Hence, further prospective studies with well-balanced cohorts are needed in the future, although in the era of NA for CHB it is difficult to conduct randomized controlled studies due to ethical issues. However, our study results demonstrated that NA therapy for patients with HBV-related HCC improved survival after curative treatment.

In conclusion, NA therapy in patients with HBV-related HCC may improve prognosis after curative therapy.

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Comparison of transcatheter arterial chemoembolization and transcatheter arterial chemotherapy infusion for patients with intermediate-stage hepatocellular carcinoma

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Abstract. The aim of the present study was to compare clinical outcomes in patients with intermediate-stage hepatocellular carcinoma (HCC) who underwent the following treatments: transcatheter arterial chemoembolization (TACE) using an epirubicin-mitomycin-lipiodol (EML) emulsion at initial therapy (TACE group; n=145), and transcatheter chemotherapy infusion (TACI) using an EML emulsion at initial therapy (TACI group; n=81). Overall survival (OS) and treatment efficacy in the TACE and TACI groups were retrospectively compared. Prognostic factors associated with OS were examined using univariate and multivariate analyses. Treatment-related mortality was also calculated. The median observation periods were 1.8 years (range, 0.2-9.0 years) in the TACE group and 2.0 years (range, 0.2-8.7 years) in the TACI group. The median survival time and the 1-, 2-, 3- and 5-year cumulative OS rates were 2.68 years and 81.5, 63.4, 43.9 and 32.7%, respectively, in the TACE group, and 2.64 years and 85.0, 60.0, 43.2 and 26.0%, respectively, in the TACI group (P=0.691). The objective response rate was significantly higher in the TACE group compared to the TACI group (80.0 vs. 66.7%; P=0.009). Using multivariate analysis, the Child-Pugh classification (P=0.017), tumor number ≤5 (P=0.045) and des-γ-carboxy prothrombin level >100 mAU/ml (P=0.002) were found to be significant predictors linked to OS. In all subgroup analyses involving Child-Pugh classification, maximum tumor size and tumor distribution, the differences in the two groups did not reach statistical significance in terms of OS. Treatment mortality was 0% in the two groups. In conclusion, patients with intermediate-stage HCC had a comparable prognosis when treated with TACI or TACE.

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Introduction

Hepatocellular carcinoma (HCC) is a major health problem; it is the fifth most common type of cancer worldwide and the third most common cause of cancer-related mortality (1-3). The prognosis for untreated HCC is generally poor and the curative treatments for this disease consist of surgical resection, radiofrequency ablation and liver transplantation (1-3). Non-curative therapies for HCC include transcatheter arterial chemoembolization (TACE), transcatheter arterial chemotherapy infusion (TACI), continuous arterial chemoinfusion therapy, radioembolization, molecular targeting therapies such as sorafenib and radiation therapy (1-12).

TACE is a procedure whereby an embolic agent is injected into the tumor feeding artery to deprive it of its major nutrient source by means of embolization; this results in ischemic necrosis of the targeted tumor (11,12). The survival benefit of TACE for unresectable HCC was established in two randomized controlled trials (RCTs) and in one meta-analysis (13-15). Thus, TACE plays an important role in treating unresectable HCC. It is clearly defined as a first-line therapy with an improved 2-year survival rate as compared with conservative therapy (16).

The Barcelona Clinic Liver Cancer (BCLC) classification is regarded as one of the most reliable staging and treatment strategy staging systems for HCC as it considers liver function, tumor status and performance status (PS) (16,17). The BCLC intermediate stage (BCLC-B) includes Child-Pugh A and B patients with multifocal HCC, defined as >3 tumors of any size or 2-3 tumors with a maximal diameter >3 cm and a single HCC (>5 cm) (24,36). To be categorized as intermediate-stage HCC, patients should be asymptomatic and have extrahepatic spread or no vascular invasion. The BCLC classification indicates that these patients are optimal candidates for TACE (16,17).

In general, chemotherapeutic agents such as doxorubicin, epirubicin, cisplatin, mitomycin, 5-fluorouracil, zinostatin stimalamer and miriplatin are combined in TACE for the treatment of HCC; however, their treatment efficacy remains unclear (18). In cases where TACE was technically impossible due to anatomical reasons, a poor liver functional reserve or cessation of blood flow in the tumor feeding arteries recognized using lipiodol (iodine addition products of the ethyl esters of fatty acids obtained from poppy seed oil) chemolization alone, TACI was often performed in Japan. Indeed, Takayasu *et al* (11) reported in their large prospective Japanese nationwide study that out of 11,030 unresectable HCC patients who underwent transcatheter arterial therapies as an initial treatment, 2,523 patients (22.9%) were treated with TACI.

In our department, we have routinely performed TACE or TACI using an epirubicin-mitomycin-lipiodol (EML) emulsion for HCC when carrying out angiography (19,20). Epirubicin alone or in combination with other chemotherapeutic agents such as mitomycin has often been used in transcatheter arterial chemotherapy for HCC in Asian countries including Japan (18,21). However, to the best of our knowledge, whether TACE using an EML emulsion could benefit survival compared with TACI using an EML emulsion remains elusive. The aim of the present study was, therefore, to compare clinical outcomes between TACE and TACI, both using an EML emulsion, in patients with intermediate-stage HCC.

Materials and methods

Patients. We performed TACE therapy as an initial treatment in 148 treatment-naive patients diagnosed with intermediate-stage HCC in the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Japan, between January 2004 and December 2012. Of these patients, 145 were treated with TACE using an EML emulsion and 3 were treated with TACE using a miriplatin-lipiodol emulsion. During the same period, we performed TACI therapy as an initial treatment in 82 treatment-naive patients diagnosed with intermediate-stage HCC in our department. Of these patients, 81 were treated with TACI using an EML emulsion and 1 patient was treated with TACI using a miriplatin-lipiodol emulsion. Thus, a total of 226 patients with intermediate-stage HCC [the TACE group (n=145) and the TACI group (n=81)] were analyzed in the present study. Patients diagnosed with HCC rupture at initial therapy were not included in the present study since they were treated with transcatheter arterial embolization without chemolization alone. We compared the overall survival (OS) and the treatment efficacy in the two groups.

Written informed consent was obtained from all patients prior to each therapy, and the study protocol complied with all the provisions of the Declaration of Helsinki. The present study was approved by the Ethics Committee of Osaka Red Cross Hospital, Japan, and the need for written informed consent in the present study was waived since the data were analyzed retrospectively and anonymously. The present study comprised a retrospective analysis of patient records registered in our database and all treatments were conducted in an open-label manner.

HCC diagnosis. HCC was diagnosed using abdominal ultrasound and dynamic computed tomography (CT) scans (hyperattenuation during the arterial phase in all or some part of the tumor and hypoattenuation in the portal-venous phase) and/or magnetic resonance imaging (MRI), based mainly on the recommendations of the American Association for the Study of Liver Diseases (16). Arterial- and portal-phase dynamic CT images were obtained at ~30 and 120 sec, respectively, after the injection of the contrast material. When carrying out angiography, we also confirmed intermediate-stage HCC using CT during hepatic arteriography (CTHA) and arterial-portography (CTAP) (22,23).

TACE and TACI procedures. In our angiography room, a catheter was advanced to the superior mesenteric artery and CTAP was performed to investigate the site and the size of the HCCs. Furthermore, we confirmed the patency of the portal vein at post-mesenteric portography. Then, a catheter was advanced to the celiac artery and a micocatheter was advanced to the common hepatic artery or proper hepatic artery through a catheter. This approach was used to perform CTHA and digital subtraction angiography with the purpose of investigating tumor vascularity and identifying the feeding vessels. After the completion of these procedures, a microcatheter was advanced as close as possible to the feeding vessels of targeted tumors. This was followed by an intra-arterial infusion via the feeding arteries according to tumor size and liver function of an emulsion containing epirubicin (Farmorubicin; Pfizer) at a mean dose of 39.7±10.4 mg, mitomycin (Mitomycin C; Kyowa Hakko Kirin Company, Ltd., Tokyo, Japan) at a mean dose of 9.1±3.2 mg and lipiodol at a mean dose of 5.7±2.8 ml in the TACE group; in the TACI group, the emulsion contained epirubicin at a mean dose of 37.2±9.9 mg, mitomycin at a mean dose of 9.0±2.7 mg and lipiodol at a mean dose of 4.8±1.9 ml (19,20,24). For patients treated with TACE after the infusion of an EML emulsion, gelatin sponge particles were injected slowly into the feeding arteries to prevent reflux into untreated segments. The sites of injection of the embolizing agents were segmental or subsegmental in all patients treated with TACE. When patients had poor liver function, the dosages of the anticancer agents and lipiodol were reduced. The decision as to whether TACE or TACI was performed was mainly based on the recommendations of the attending physicians, who considered tumor-related factors, vascular anatomy and liver function. When selective catheterization of the tumor feeding arteries was technically impossible, TACI was performed. Additional embolization using gelatin sponge particles was not performed in patients when cessation of the blood flow in tumor feeding arteries was recognized using an infusion of an EML emulsion alone.

Assessment of treatment efficacy. Treatment efficacy was evaluated using CT findings within 2 months after the initial treatment. We regarded lipiodol accumulation in targeted tumors seen on CT scans as indicating necrosis. This was due to the fact that it had been previously reported in several studies that the lipiodol retention areas observed on CT corresponded to necrotic areas (25-27). Complete response (CR) was defined as the disappearance of all targeted tumors or 100% tumor necrosis, partial response (PR) was defined as a ≥50% reduc-

Table I. Baseline characteristics between the TACE group and the TACI group.

Variables	TACE group (n=145)	TACI group (n=81)	P-value
Age (years)	72.5±9.1	70.3±9.3	0.083[a]
Gender, male/female	94/51	57/24	0.462[b]
Maximum tumor size (cm)	5.4±3.0	3.5±2.0	<0.001[a]
Tumor number, >5/≤5	31/114	23/58	0.257[b]
Tumor distribution, bilobar/unilobar	56/89	49/32	0.002[b]
Child-Pugh classification, A/B	100/45	46/35	0.082[b]
Causes of liver disease B/C/non B non C/B and C	12/93/39/1	7/56/18/0	0.843[b]
Efficacy of initial treatment CR/PR/SD/PD	30/86/28/1	6/48/27/0	0.009[b]
AST (IU/l)	61.8±32.5	67.9±48.0	0.260[a]
ALT (IU/l)	48.9±34.3	54.7±48.5	0.294[a]
ALP (IU/l)	417.3±221.0	430.6±183.0	0.646[a]
GGT (IU/l)	140.1±202.1	120.8±138.4	0.446[a]
Serum albumin (g/dl)	3.63±0.52	3.61±0.56	0.793[a]
Total bilirubin (mg/dl)	1.07±0.85	1.19±0.73	0.273[a]
Prothrombin time (%)	84.2±18.2	78.2±16.5	0.015[a]
Platelets ($x10^4/mm^3$)	13.8±7.2	11.3±5.4	0.006[a]
AFP (ng/ml)	1310.0±3693.9	3692.5±16082.5	0.192[a]
DCP (mAU/ml)	9071.8±34906.2	5009.4±27603.3	0.339[a]

Data are expressed as number or the mean ± standard deviation. TACE, transcatheter arterial chemoembolization; TACI, transcatheter arterial chemotherapy infusion; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; [a]unpaired t-test; [b]Fisher's exact test.

tion in tumor size and/or necrosis, and progressive disease (PD) was defined as >25% tumor enlargement and/or the appearance of any new HCC tumors. Stable disease (SD) was defined as disease that did not qualify for classification as CR, PR or PD.

Follow-up. Follow-up after each therapy consisted of periodic blood tests and monitoring of tumor markers, including α-fetoprotein and des-γ-carboxy prothrombin (DCP). Dynamic CT scans and/or MRI were obtained every 2-4 months after each therapy. Chest CT, whole abdominal CT, brain MRI and bone scintigraphy were performed when extrahepatic HCC recurrence was suspected. When disease progression of the treated HCC lesions was observed after the initial therapy and/or new hepatic lesions were observed, the most appropriate therapies were performed if the liver functional reserve was adequate and if patients did not refuse such therapies. They included transcatheter arterial therapies in most cases. However, when the treated lesion was well controlled after the initial therapy and the new lesion appeared in the liver, percutaneous ablative therapies were also considered. In cases that were refractory to transcatheter arterial therapies or those involving extrahepatic metastases, molecular targeting therapy such as sorafenib was also considered.

Statistical analysis. Data were analyzed using univariate and multivariate analyses. Continuous variables were compared using the unpaired t-test and categorical variables were compared using Fisher's exact test. For analysis of OS, follow-up ended at the time of mortality from any cause, and the remaining patients were censored at the last follow-up visit. The cumulative OS rates were calculated using the Kaplan-Meier method, and tested using the log-rank test. Factors with a P-value <0.05 in univariate analysis were subjected to multivariate analysis using the Cox proportional hazards model. These statistical methods were used to estimate the interval from initial treatment. Data were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA) for Microsoft Windows. Data are expressed as the mean ± standard deviation. Values of P<0.05 were considered to indicate statistically significant differences.

Results

Baseline characteristics. The baseline characteristics of the patients in the two groups are shown in Table I. The median observation period was 1.8 years (range, 0.2-9.0 years) in the TACE group and 2.0 years (range, 0.2-8.7 years) in the TACI group. The mean age in the TACE group (72.5±9.1 years) tended to be higher (P=0.083) than that in the TACI group (70.3±9.3 years). Maximum tumor diameter was significantly larger (P<0.001) in the TACE group (5.4±3.0 cm) than in the TACI group (3.5±2.0 cm). The proportion of patients with

Table II. Univariate and multivariate analysis contributing to overall survival.

Variables	n	Univariate analysis	Multivariate analysis	
			Hazard ratio (95% CI)	P-value[a]
Gender, male vs. female	151/75	0.415		
Age (years), >70 vs. ≤70	123/103	0.113		
TACE vs. TACI	145/81	0.691		
Child-Pugh, A vs. B	146/80	<0.001	0.526 (0.310-0.892)	0.017
Tumor number, >5 vs. ≤5	54/172	0.011	0.631 (0.403-0.989)	0.045
Tumor distribution, bilobar vs. unilobar	105/121	0.003	1.125 (0.736-1.721)	0.586
Maximum tumor size, >4 cm vs. ≤4 cm	106/120	0.005	0.864 (0.583-1.279)	0.465
Objective response at initial therapy, yes/no	170/56	0.165		
AST (IU/l), >50 vs. ≤50	122/104	0.648		
ALT (IU/l), >40 vs. ≤40	116/110	0.158		
ALP (IU/l), >380 vs. ≤380	106/120	0.077		
GGT (IU/l), >80 vs. ≤80	101/125	0.381		
Serum albumin level (g/dl), ≥3.7 vs. <3.7	113/113	0.001	1.260 (0.795-1.996)	0.325
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	101/125	0.023	0.888 (0.603-1.307)	0.546
Platelet count (x10^4/mm^3), >12 vs. ≤12	110/116	0.651		
Prothrombin time (%), >80 vs. ≤80	125/101	0.044	0.935 (0.593-1.475)	0.772
Serum AFP (ng/ml), >100 vs. ≤100	80/146	0.053		
DCP (mAU/ml), >100 vs. ≤100	156/70	<0.001	0.483 (0.304-0.765)	0.002

CI, confidence interval; TACE, transcatheter arterial chemoembolization; TACI, transcatheter arterial chemotherapy infusion; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; [a]Cox proportional hazard model.

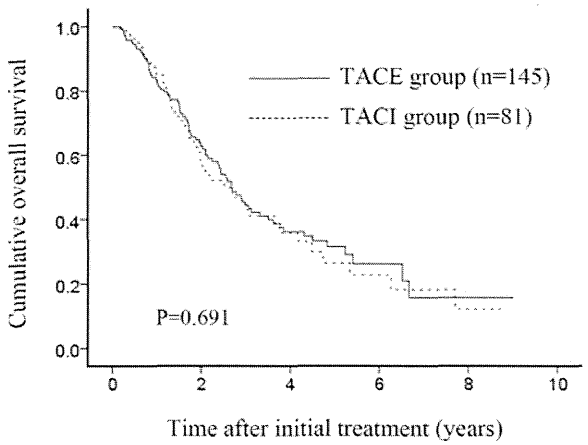

Figure 1. Median survival time (MST) and cumulative overall survival (OS) for patients in the TACE and TACI groups. The MST was 2.68 years in the TACE group and 2.64 years in the TACI group. The 1-, 2-, 3- and 5-year cumulative OS rates were 81.5, 63.4, 43.9 and 32.7%, respectively, in the TACE group, and 85.0, 60.0, 43.2 and 26.0%, respectively, in the TACI group (P=0.691).

bilobar disease was significantly lower (P=0.002) in the TACE group than in the TACI group. The prothrombin time (PT) and platelet count were significantly higher in the TACE group than in the TACI group. The proportion of patients with Child-Pugh class A disease was significantly higher in the TACE group than in the TACI group. These findings indicated that patients in the TACE group had a superior liver functional reserve to those in the TACI group.

Median survival time and cumulative OS rates. The median survival time (MST) and the 1-, 2-, 3- and 5-year cumulative OS rates were 2.68 years and 81.5, 63.4, 43.9 and 32.7%, respectively in the TACE group, and 2.64 years and 85.0, 60.0, 43.2 and 26.0%, respectively in the TACI group; there was no significant difference (P=0.691) in these parameters between the two groups (Fig. 1).

Treatment efficacy at initial treatment in the two groups. In the TACE group, a CR was achieved in 30 patients, a PR in 86 patients, SD in 28 patients and PD in one patient. Thus, the objective response rate (ORR) in the TACE group was 80.0% (116/145 patients). In the TACI group, a CR was achieved in 6 patients, a PR in 48 patients, SD in 27 patients; no patient had PD. Thus, the ORR in the TACI group was 66.7% (54/81 patients). In terms of treatment efficacy, the TACE group achieved significantly improved treatment efficacy relative to the TACI group (P=0.009).

Univariate and multivariate analyses of factors contributing to OS. Univariate analysis identified the following factors as being significantly associated with OS for all cases (n=226): the Child-Pugh classification (P<0.001); tumor number ≤5 (P=0.011); tumor distribution (P=0.003); a maximum tumor size

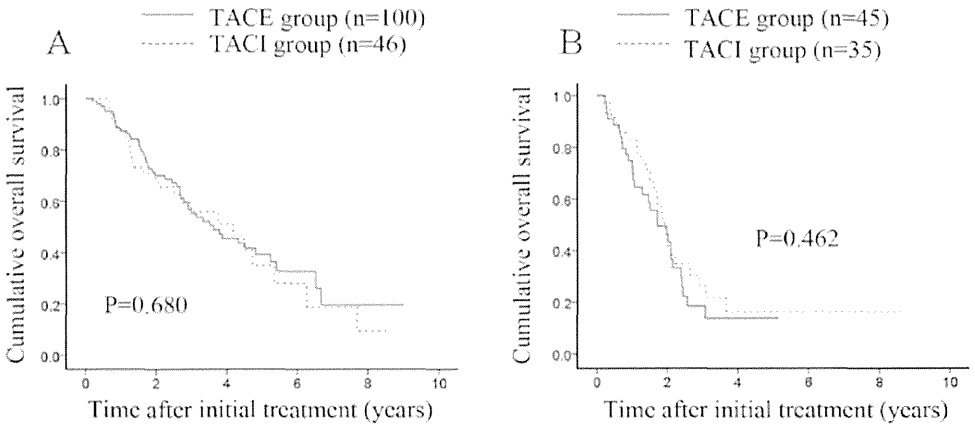

Figure 2. Subgroup analysis according to Child-Pugh classification. (A) There were 100 patients with Child-Pugh class A disease in the TACE group and 46 in the TACI group. The MST was 3.62 years for patients in the TACE group and 4.18 years for patients in the TACI group. The difference in the two groups did not reach significance in terms of OS (P=0.680). (B) There were 45 patients with Child-Pugh class B disease in the TACE group and 35 in the TACI group. The MST was 1.72 years for patients in the TACE group and 1.93 years for patients in the TACI group. The difference in the two groups did not reach significance in terms of OS (P=0.462).

≤4 cm (P=0.005); a serum albumin level ≥3.7 g/dl (P=0.001); a total bilirubin level ≥1.0 mg/dl, a PT >80% (P=0.044) and a DCP >100 mAU/ml (P<0.001) (Table II). The hazard ratios and 95% confidence intervals calculated using multivariate analysis for the eight factors with P-values of <0.05 in the univariate analysis are detailed in Table II. The Child-Pugh classification (P=0.017); tumor number ≤5 (P=0.045) and DCP >100 mAU/ml (P=0.002) were found to be significant predictors linked to OS in multivariate analysis.

Causes of mortality. Seventy-nine patients in the TACE group (54.5%) died during the follow-up period. The causes of mortality were HCC progression in 48 patients, liver failure in 23 patients and miscellaneous causes in 8 patients. Fifty patients in the TACI group (61.7%) died during the follow-up period and the causes of mortality were HCC progression in 28 patients, liver failure in 15 patients and miscellaneous causes in 7 patients.

Adverse events in the two groups. In both groups, symptoms associated with postembolization syndrome such as fever, appetite loss, abdominal pain and nausea were transient and were mostly resolved within 2 weeks after initial treatment (28). In the TACE group, serious adverse events (SAEs) were observed in 8 patients (5.5%). These 8 patients had one of the following SAEs: acute respiratory distress syndrome (ARDS), hepatic encephalopathy, cholangitis, hyponatremia, hyperbilirubinemia, aspiration pneumonia, liver abscess and refractory ascites. All SAEs were managed successfully, although in 1 patient who developed ARDS, management in the intensive care unit was required. Thus, TACE-related mortality was 0%. In the TACI group, SAEs were observed in 2 patients (2.5%) and included gastrointestinal bleeding and liver abscess in 1 patient each. These SAEs were successfully managed and TACI-related mortality was also 0%.

Subgroup analysis according to the Child-Pugh classification. Marginal significance was observed between the two groups in terms of the Child-Pugh classification (P=0.082), and we, therefore, performed subgroup analyses according to this classification. No significant difference (P=0.680) was observed between the two groups in terms of OS in patients with Child-Pugh class A disease [100 patients (69.0%) in the TACE group and 45 patients (55.6%) in the TACI group]; the MST was 3.62 years in the TACE group and 4.18 years in the TACI group (Fig. 2A). Similarly, no significant difference (P=0.462) was found between the two groups in terms of OS in patients with Child-Pugh class B disease [45 patients (31.0%) in the TACE group and 36 (44.4%) in the TACI group]; the MST was 1.72 years in the TACE group and 1.93 years in the TACI group (Fig. 2B).

Subgroup analysis according to maximum tumor size. The maximum tumor size was significantly larger in the TACE group than in the TACI group (P<0.001). Consequently, we performed subgroup analyses according to maximum tumor size. No significant difference (P=0.801) was observed between the two groups in terms of OS in patients with a maximum tumor size >4 cm [82 patients (56.6%) in the TACE group and 24 (29.6%) in the TACI group]; the MST was 2.15 years in the TACE group and 1.93 years in the TACI group (Fig. 3A). Similarly, no significant difference was found between the two groups in terms of OS (P=0.269) in patients with a maximum tumor size ≤4 cm [63 patients (43.4%) in the TACE group and 57 (70.4%) in the TACI group]; the MST was 3.51 years in the TACE group and 2.92 years in the TACI group (Fig. 2B).

Subgroup analysis according to tumor distribution. The proportion of patients with bilobar disease was significantly lower in the TACE group than in the TACI group (P=0.002). Hence, we performed subgroup analysis in terms of tumor distribution. There were 56 (38.6%) patients with bilobar disease in the TACE group and 49 (60.5%) in the TACI group. In terms of OS, there was no significant difference (P=0.289) between the two groups; the MST was 2.25 years in the TACE group and 2.23 years in the TACI group (Fig. 4A). There were 89 (61.4%) patients with unilobar disease in the TACE group and 32 (39.5%) in the TACI group. In terms of OS, there was no significant difference (P=0.564) between the two groups; the MST was 3.51 years in the TACE group and 3.09 years in the TACI group (Fig. 4B).