studies have shown that entecavir is more effective than lamivudine as prophylactic therapy for HBV-R.[15,16]

To our knowledge, there were few prospective long-term studies that have evaluated outcomes after prophylactic therapy in patients with HBsAg or preemptive therapy in patients without HBsAg. In particular, the endpoint of the nucleoside analogue therapy remains uncertain in patients with HBV-R. We performed this prospective study to elucidate the prevalence of HBV-R on regular screening and the characteristics of patients with HBV-R. We also evaluated the effectiveness of entecavir for HBV-R and assessed the risk of HBV reappearance after the end of entecavir treatment.

Patients and methods

Enrolled patients and management. In 2007, we organized a project team to perform a prospective study of HBV-R in patients with hematologic malignancy in Osaka City University Hospital. Before the start of rituximab-based chemotherapy or HSCT, HBsAg, antibodies against hepatitis B virus core antigen (anti-HBc), and antibodies against HBsAg (anti-HBs) in sera of the patients were tested by chemiluminescent enzyme immunoassay (CLEIA; Fujirebio Inc., Tokyo, Japan). Patients positive for one or more HBV serum marker were enrolled in the study. After enrollment, HBV-DNA was measured by a real-time polymerase chain reaction (real-time PCR)-based method (COBAS *TaqMan PCR*, Roche Diagnostics, Tokyo, Japan).[17] The quantified range of the real-time PCR assay was between 2.1 and 8.8 log copies/mL. Patients with hepatitis C virus, alcoholic liver disease, primary biliary cirrhosis, or autoimmune liver disease were excluded. HBV-DNA was regularly measured every month, from the start of chemotherapy or the time of HSCT to 1 year after the end of therapy. After monthly screening, HBV-DNA was measured once every 3 months. In patients in whom HBV-DNA was detected, HBV genotype was identified by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies to type-specific epitopes in the preS2-region (Institute of Immunology, Tokyo, Japan), as described elsewhere.[18] Prophylactic or preemptive treatment against HBV-R-associated hepatitis was given to patients with a serum HBV-DNA level exceeding 2.1 log copies/mL; such patients received 0.5 mg entecavir per day. Treatment with entecavir was discontinued after more than 6 months had elapsed from the disappearance of both HBsAg and HBV-DNA in serum. In the present study, HBV-R was defined as more than a 1-log increase in the serum HBV-DNA level as compared with the value at enrollment or as a serum HBV-DNA level higher than 2.1 log copies/mL.

Fifty-seven patients (23 women and 34 men) were enrolled from November 2007 to January 2013. The mean age was 60 years (range, 23–82). Eight patients were positive for both HBsAg and anti-HBc, 43 were positive for both anti-HBs and anti-HBc, and 6 were positive for only anti-HBc (Table 1). No patient had a history of HBV vaccination. The mean follow up was 16 months (range, 4–63). Seven patients died within less than 1 year because of progression of hematologic malignancy or infection without HBV.

Sequencing of HBV-DNA. In patients with HBV-R, the nucleotide sequences of HBV polymerase coding area were determined by the direct sequencing method after nested PCR amplification.[19] Briefly, HBV-DNA was extracted from 200 μL of serum and was amplified as two overlapping fragments, A (nucleotide [nt] 271–1941) and B (nt 1679–335), with the use of an Expand High Fidelity PCR System (Roche Diagnostics, Mannheim, Germany). To amplify fragment A, primers HBMF1 (5′-YCCTG CTGGTGGCTCCAGTTC-3′) and 1972R (5′-AAAGAATTCAG AAGGCAAAAAAGA-3′) were used for the first-round PCR, and primers HBMF2 (5′-GTCTAGACTCGTGGTGGACTTCTCTC-3′) and n1941R (5′ -CAGAAGCTCCAAATTCTTTATA-3′) were used for the second-round PCR. To amplify fragment B, primers 1653F (5′-CATAAGAGGACTCTTGGACT-3′) and HBMR2 (5′-AAGCCAXACARTGGGGGAAAGC-3′) were used for the first-round PCR, and primers 1679F (5′-AATGTCAACGACCG ACCTTG-3′) and 335R (5′-TGAYTGGAGRTTKGGGACT-3′) were used for the second-round PCR. Each PCR product was purified and sequenced directly by the dideoxy chain termination method, using a BigDye Terminator v1.1 Cycle Sequencing Kit and an ABI PRISM 3100 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Ethical considerations. This study protocol complied with the ethical guidelines of the Declaration of Helsinki 1975 (2008 revision) and was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (UMIN Clinical Trials Registry, UMIN000009491). Written informed consent was obtained from all enrolled patients.

Table 1 Clinical characteristics of the enrolled patients

	Age	Gender	Anti-HB marker	Disease	Treatment
HBsAg-positive					
n = 8	62 (53–79)	Male: 7	Anti-HBs positive: 7	ML: 7	CHOP-R: 6
		Female: 1	Anti-HBc positive: 8	Leukemia: 1	HSCT: 2
HBsAg-negative					
n = 49	60 (23–82)	Male: 27	Anti-HBs positive: 43	ML: 29	CHOP-R: 28
		Female: 22	Anti-HBc positive: 49	Leukemia: 14	HSCT: 19
				MDS: 6	R-Hyper CVAD: 2

CHOP-R, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, prednisolone, and rituximab; HSCT, hematopoietic stem-cell transplantation; MDS, myelodysplastic syndromes; ML, malignant lymphoma; R-Hyper CVAD, combination chemotherapy with cyclophosphamide, vincristine, doxorubicin, dexamethasone, and rituximab.

Results

Prophylactic therapy for patients with HBsAg. In the eight patients with HBsAg, prophylactic treatment with entecavir was started before cytotoxic therapy (Table 2). All eight patients were infected with HBV genotype C. In response to entecavir, the HBV-DNA load decreased to under 3 log copies/mL in all patients and fell to undetectable levels in all but one patient with HBeAg (case 32). Four of the eight patients died because of progression of hematologic malignancy or infection. Hepatic failure did not occur in any of the patients with HBsAg. Entecavir treatment has continuously prevented HBV-R in the other four patients.

Preemptive therapy for patients with HBV resolution. The clinical backgrounds of the 49 HBsAg-negative patients are shown in Table 1. At enrollment, HBV-DNA was not detected in patients without HBsAg. At the end of follow up, HBV-R has occurred in five (26%) of 19 patients who received HSCT and three (10%) of 30 patients who received rituximab-based chemotherapy. HBV-R occurred a median of 3 months (range, 2–10) after the end of rituximab-based chemotherapy. On the other hand, HBV-R occurred a median of 22 months (range: 9–36) after HSCT.

As compared with patients without HBV-R, anti-HBs titers at enrollment were slightly but not significantly lower in patients with HBV-R ($P = 0.085$). Among patients given rituximab-based chemotherapy, the anti-HBc titer was significantly higher in the presence of HBV-R ($P = 0.02$, Table 3). HBV-R occurred in one (17%) of six patients without anti-HBs. Reactivation occurred in six (26%) of 23 patients with anti-HBs titers below 50 mIU/mL, one (13%) of eight patients with anti-HBs titers between 50 and 200 mIU/mL, and none of 12 patients with anti-HBs titers exceeding 200 mIU/mL. During the screening period, anti-HBs titers gradually decreased in six patients with HBV-R. Anti-HBs titers became negative at the time of HBV-R in seven patients. Anti-HBs titers remained persistently positive in 36 patients without HBV-R.

Alanine aminotransferase (ALT) levels increased to more than five times the upper limit of normal in three of eight patients with HBV-R (Table 4). In one patient (case 4) who had received rituximab-based chemotherapy, the ALT level rose to 452 IU/L after entecavir treatment (Fig. 1). At that time, HBV-DNA decreased to below 2.1 log copies/mL. It was speculated that HBV-R was not directly related to ALT flare in this patient. Two other patients who underwent HSCT discontinued regular screening for HBV-DNA on their own initiative. Briefly, case 30 dropped out of regular screening 15 months after enrollment, and ALT levels rose to 362 IU/L with an increase in HBV viral load at month 22. Another patient (case 205) dropped out of the study 25 months after enrollment, and ALT levels elevated to 1642 IU/L with a concurrent increase in HBV viral load at month 36. Although HBV-R-related hepatitis occurred in these patients, treatment with entecavir fortunately prevented hepatic failure. With the exception of these two patients, preemptive therapy prevented hepatitis related to HBV-R. Treatments for hematologic diseases were completed without hepatic failure in all of the enrolled patients without HBsAg. One patient with HBV-R died of infection 43 months after HSCT. At the last follow up, HBV-DNA was not detected on real-time PCR. Among the seven survivors with HBV-R, four patients discontinued treatment with entecavir. After the withdrawal of entecavir, HBV-DNA was detected again in two patients without anti-HBs. One of the two patients required

Table 2 Baseline characteristics and outcomes of HBsAg-positive patients

No.	Gender	Age	Hematologic disease	Treatment	HBeAg	Anti-HBe (% inh)	HBV-DNA (log/mL)	ALT (IU/L)	Observation period (month)	Outcome
32	M	79	ML	CHOP-R	1600	—	8.5	78	26	Dead
66	M	63	ML	CHOP-R	—	100	ND	10	37	Alive
77	M	57	ML	CHOP-R	—	97	2.8	22	40	Alive
87	M	62	ML	HSCT	419	—	3.6	10	16	Dead
80	M	62	ML	CHOP-R	—	100	4	106	5	Dead
120	M	53	AML	HSCT	—	89	2.3	155	3	Dead
141	M	58	ML	CHOP-R	—	100	3.7	18	26	Alive
211	F	58	ML	CHOP-R	—	100	4	106	5	Alive

AML, acute myeloid leukemia; ML, malignant lymphoma; ND, data no available.

Table 3 Comparison between patients with or without HBV reactivation in the HBsAg-negative group

	All patients ($n = 49$)		Patients with HSCT ($n = 19$)		Patients with chemotherapy ($n = 30$)	
	With reactivation	Without reactivation	With reactivation	Without reactivation	With reactivation	Without reactivation
Age	55 (44–64)	64 (23–82)	55 (44–60)	49 (23–66)	60 (53–64)	67 (49–82)
Gender; M/F	2/6	21/20	2/3	8/6	3/0	13/14
Anti-HBs	35 ± 48	243 ± 366	41 ± 63	151 ± 210	25 ± 5	295 ± 420
Anti-HBc	77 ± 33	63 ± 38	80 ± 13	67 ± 36	99 ± 1*	69 ± 36*
Observation period	37 (24–63)	12 (4–61)	41 (32–52)	9 (4–55)	32 (24–63)	13 (4–61)

*$P = 0.02$, There were no differences in anti-HBs between the two groups.
Data were shown mean ± SD.

Table 4 Clinical characteristics of patients with HBV reactivation

No.	Gender	Age	Hematological Disease	Treatment	Anti-HBs/Anti-HBc at the enrollment	At the time of HBV reactivation (month)	HBV-DNA at reactivation (Log/mL)	HBsAg at or after reactivation (IU/mL)	ALT peak after reactivation (IU/L)	Outcome
4	M	53	ML	CHOP-R	19.6/98.4	2	5.4	1047	452	Alive
30	M	59	Chronic leukemia	HSCT	30.2/70	22†	6.6	2000	362	Death
37	M	60	ML	CHOP-R	28.5/97.9	10	3.6	negative	28	Alive
68	F	46	MDS	HSCT	ND/97.4	10	4.1	45.7	49	Alive
121	M	55	Acute leukemia	HSCT	151.7/71	22	2.8	negative	58	Alive
128	M	64	ML	R-Hyper CVAD	26.9/99.2	3	3.1	negative	45	Alive
150	F	60	MDS	HSCT	14/ND	9	5.4	63.4	22	Alive
205	M	44	MDS	HSCT	7.4/81.5	36†	5.4	145	1642	Alive

†Two patients with HSCT dropped out of regular screening for HBV-DNA 1 year after enrollment. In another patient who had received rituximab-based chemotherapy, ALT increased to 452 IU/L during entecavir treatment.
ALT flare occurred in three patients with HBV reactivation.

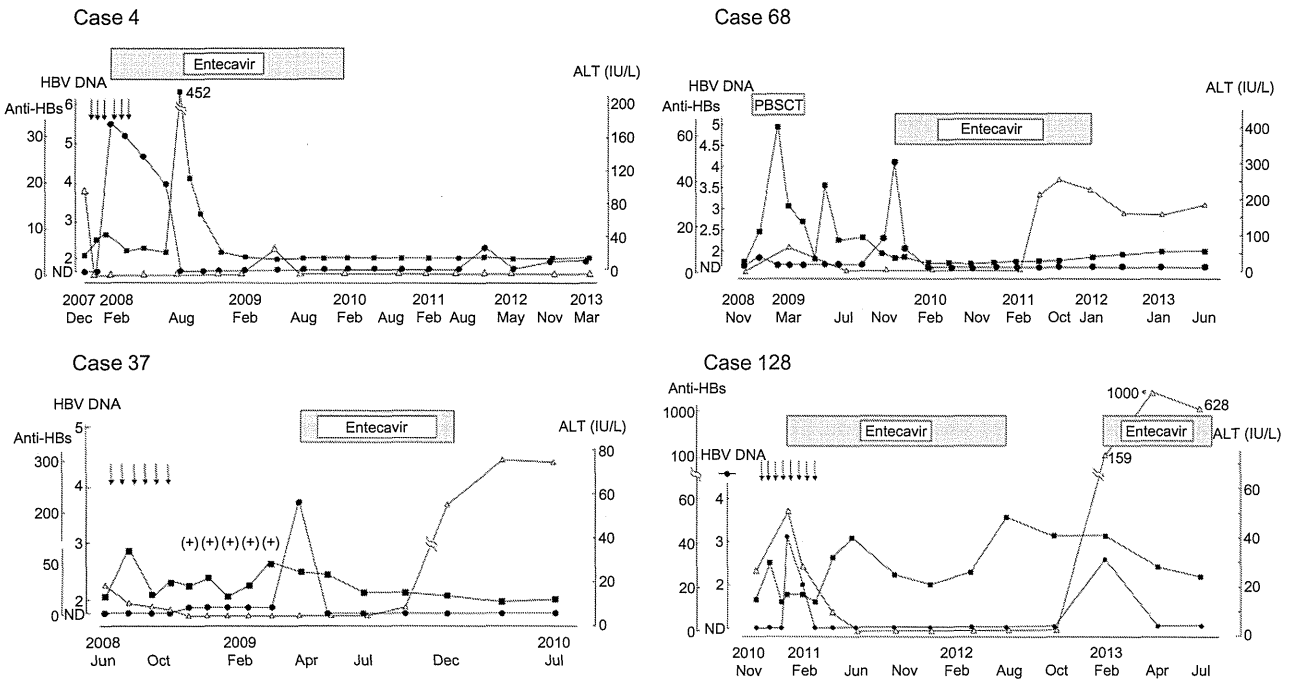

Figure 1 Clinical course of four patients with hepatitis B virus (HBV) reactivation in whom entecavir was withdrawn. After entecavir treatment, HBV-DNA was detected again in patients 4 and 128. On the other hand, HBV-DNA has not been detected in patients 37 and 68, in whom antibodies against HBsAg (anti-HBs) remains above 20 mIU/mL. CHOP-R: combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, prednisolone, and rituximab, PBSCT: peripheral blood stem-cell transplantation. ↓, CHOP-R; –●–, HBV-DNA; –△–, Anti-HBs; –■–, ALT (IU/L).

retreatment with entecavir. On the other hand, HBV-DNA has not been detected in two other patients who were persistently positive for anti-HBs (Fig. 1).

DNA sequence of reactivated HBV. All reactivated HBV was genotype C. Sequence analysis showed that reactivated HBV did not have mutations associated with resistance to nucleos(t)ide analogues in the reverse transcriptase region.

Four of eight reactivated HBVs had mutations in the "a" determinant region of the S gene region with amino acid replacement (Fig. 2). In detail, case 121 had two mutations: 113 threonine to serine and 143 serine to threonine. In case 128, two mutations were detected (129 glutamine to arginine and 130 glycine to asparagine), and anti-HBs was positive at HBV-R (Fig. 1, case 128). An amino acid replacement of 145 glycine to arginine was detected in cases 150 and 205. In both cases, anti-HBs were negative at the time of HBV-R. At the time of HBV-R, HBsAg was

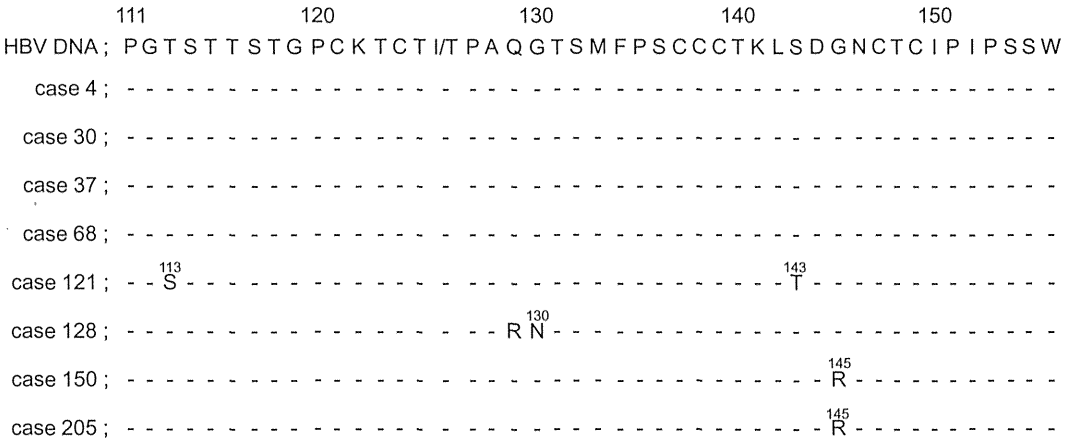

Figure 2 Alignment of amino acids codes from the 111th to 156th amino acids of hepatitis B (HB) surface antigen, the "a" determinant region. Comparison of the modified hepatitis B virus (HBV) ADR[20] and the eight reactivated HBV revealed several point mutations in "a" determinant region. Point mutations with amino acid replacement were detected in cases 121, 128, 150, and 205.

not detectable in two (cases 121 and 128) of four patients with HBV mutated in the "a" determinant region.

Discussion

In the present prospective study, the rates of HBV-R in patients with resolved HBV infection were 26% after HSCT and 10% after rituximab-based chemotherapy. Previous studies reported that HBV-R occurred in 12–20% of patients who had undergone HCST[6,7,21–23] and 4.1–17.9% of those who had received rituximab-based chemotherapy for malignant lymphoma.[4,24–26] The rate of HBV-R in our study is consistent with these previous finding. In retrospective studies of patients who underwent HSCT, HBV-R was defined as seroreversion in HBsAg-negative patients.[6,7,21] This is quite a difference from the present study, which used real-time PCR to measure HBV-DNA. During follow up, HBV-DNA was detected earlier than HBsAg. In addition, HBsAg did not turn positive in three of the eight patients with HBV-R. Two of the five patients in whom HBsAg was consistently negative had mutations in the S determinant region of HBV-DNA. Our data confirmed that detection of the viral genome was the most specific and sensitive screening tool for HBV-R, particularly as compared with serological tests. A recent large-scale prospective study using HBV-DNA test showed that HBV-R occurred in 17 (11.3%) of 150 HBV resolved patients who had received rituximab-based chemotherapy.[27]

In our patients with resolved HBV infection, HBV-R occurred within 1 year after the end of rituximab-based chemotherapy and more than 1 year after HSCT. Although HBV-R rarely occurs more than 3 years after HSCT,[28,29] the longest reported period to HBV-R after HSCT was 47 months.[23] In the two patients in the present study who discontinued HBV monitoring more than 15 months after enrollment, HBV-R-associated ALT flare occurred. These results might be useful for establishing follow-up periods for HBV-R according to treatment. Recently, careful monitoring for HBV-R has been broadly recommended for anti-HBc-positive patients who receive immunosuppressive or cytotoxic therapy. However, the incidence and timing of reactivation might differ according to the details of treatment, such as the drugs used or procedures performed. Cost–benefit analyses should be performed according to specific diseases and treatments to assess the value of screening for HBV-R.

Several studies have suggested that decreased levels or loss of anti-HBs is a predictor of HBV-R in anti-HBs-positive patients.[23,30] In our study, anti-HBs had become negative at the time of HBV-R in seven of eight patients. However, the other patient (case 128) was positive for anti-HBs at HBV-R. A case report has documented the development of fatal hepatitis in a patient with HBV-R who had a high titer of anti-HBs.[31] It is well known that HBV vaccination provides no protection against HBV with mutations in the HBsAg coding region (i.e. "escape mutant HBV"). Consequently, escape mutant HBV can increase in anti-HBs-positive patients. In our patient who was positive for anti-HBs at the time of HBV-R, two mutations in the "a" determinant region of the S gene were detected. Borentain *et al.* showed that reactivated HBV is associated with several mutations in the "a" determinant region of the S gene.[22] Interestingly, four reactivated HBVs in our study had mutations with amino acid replacement in "a" determinant region. This finding suggests that the mutated HBV might persist in some patients who have HBV-R without serum HBsAg and/or that such HBV might preferentially increase during immunosuppressive or cytotoxic therapy. Taken together, although patients with low anti-HBs titers might have an increased risk of HBV-R, assessment of anti-HBs alone without screening for HBV-DNA may fail to identify some patients at high risk for HBV-R.

Our study showed that prophylactic therapy in HBsAg-positive patients and preemptive therapy in HBV-resolved patients could prevent hepatic failure related to HBV-R associated with cytotoxic or immunosuppressive therapy for hematologic malignancies. Specifically, entecavir reduced HBV viral load in both patients with HBsAg and eight patients with HBV-R and maintained it below 2.1 log copies/mL for more than 6 months; the duration of entecavir treatment ranged from 3 to 35 months. The emergence of lamivudine-resistant HBV mutants has been reported in patients who received prophylactic treatment for HBV-R.[16,32] No entecavir-resistant mutants emerged in our study, suggesting that entecavir

might be better suited for patients who require longer periods of prophylactic or preemptive treatment.

In a recent randomized controlled study of HBV-resolved patients with lymphoma, prophylactic entecavir treatment before rituximab-based chemotherapy prevented HBV-R in all but one (2.4%) of 41.[26] As compared with preemptive treatment at the time of HBV-R, prophylactic treatment with entecavir more effectively prevented HBsAg reverse seroconversion. However, ALT levels increased to above 100 IU/mL in each patient who received prophylactic or preemptive treatment. Fatal hepatitis did not occur in that trial. Our study also showed that preemptive therapy prevented fatal hepatitis in patients with HBV-R who continued to undergo regular screening. Further studies are needed to establish whether prophylactic therapy should be started before cytotoxic or immunosuppressive treatment in all patients with resolved HBV infection.

Another important issue is whether entecavir treatment can be safely discontinued in patients with HBV-R. Fatal hepatic failure has been reported after the withdrawal of prophylactic lamivudine therapy in HBsAg-positive patients with HSCT.[33] In general, nucleot(s)ide analogue treatment should be continued in HBsAg-positive patients. However, there are no firm recommendations for patients who have HBV-R without HBsAg. We withdrew entecavir after more than 6 months after the disappearance of both HBV-DNA and HBsAg in four patients with HBV-R who had received preemptive therapy. After the withdrawal of entecavir, HBV-DNA was detectable in two patients without anti-HBs. On the other hand, HBV-R has not occurred in the other patients whose anti-HBs turned positive after preemptive therapy. Our findings suggest that entecavir can be safely discontinued in patients with HBV-R after anti-HBs has become consistently positive. To confirm our speculations, longer-term studies in larger groups of patients are necessary.

In conclusion, this prospective study confirmed that current recommendations for patients with HBsAg and those with resolved HBV infection can prevent fatal hepatitis related to HBV-R in patients who receive immunosuppressive or cytotoxic therapy. To improve cost–benefit ratios, future studies should attempt to find other reliable markers and to establish optimal screening periods for HBV-R according to specific diseases or treatments. Finally, we speculated that entecavir can be safely discontinued in patients with HBV-R who have acquired anti-HBs.

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A laboratory marker, FIB-4 index, as a predictor for long-term outcomes of hepatocellular carcinoma patients after curative hepatic resection

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Background. *Liver fibrosis is associated with the prognosis of patients with hepatocellular carcinoma (HCC) after treatment. The laboratory marker for liver fibrosis, the FIB-4 index, is reportedly correlated with the degree of liver fibrosis. We evaluated the predictive value of FIB-4 index on the recurrence and survival of HCC patients who underwent curative hepatectomy.*

Methods. *A total of 431 consecutive patients who underwent hepatectomy for primary, nonrecurrent HCC were analyzed. The FIB-4 index was calculated from the patient's age, serum alanine aminotransferase and aspartate aminotransferase levels, and platelet count at the time of HCC diagnosis. Postoperative recurrence and survival rates were compared according to tumor characteristics, tumor markers, Child-Pugh class, and the FIB-4 index.*

Results. *The pretreatment FIB-4 index was associated with recurrence and survival rates, independent of HCC progression or tumor marker levels in a multivariate analysis. Recurrence rates after hepatectomy were higher in patients with a FIB-4 index >3.25 versus ≤3.25 (5-year recurrence rates 69.6% vs 54.8%;* P = *.0049). Survival was also worse in patients with a FIB-4 index >3.25 than those with a FIB-4 index ≤3.25 (5-year survival rates 67.1% vs 72.2%;* P = *.0030).*

Conclusion. *The FIB-4 index is a predictive marker for long-term outcomes in patients with HCC treated with curative hepatic resection. (Surgery 2015;157:699-707.)*

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HEPATOCELLULAR CARCINOMA (HCC) is among the most prevalent cancers worldwide,[1] and its incidence is predicted to increase.[2,3] In Japan, HCC is the third and fifth most common cause of death from cancer in men and women, respectively.[4] Hepatectomy is usually a curative treatment for HCC that results in a better prognosis than other treatment modalities, such as percutaneous locoregional therapies, transcatheter arterial chemoembolization, or sorafenib. However, the outcome of patients treated with hepatectomy varies, despite its curative intent. This is partly owing to the high incidence of HCC recurrence, even after curative treatment.

Liver fibrosis is an important risk factor for the development of HCC.[5] Patients with progressive liver fibrosis or cirrhosis have a high likelihood of developing HCC. In addition, several previous studies have reported that liver fibrosis is also a risk factor for HCC recurrence after curative hepatectomy.[6-8]

Recently, several biomarkers of liver fibrosis calculated based on routine laboratory data have been described.[9-11] The FIB-4 index is a surrogate biomarker of liver fibrosis[9] that has been demonstrated to be correlated with liver fibrosis in patients with chronic liver diseases with various etiologies without HCC.[12-14] However, it remains unclear whether this laboratory marker of liver fibrosis can also serve as a biomarker that can predict outcomes in patients with HCC who undergo curative hepatectomy. In this study, we evaluated the ability of the FIB-4 index, a laboratory marker of liver fibrosis, to predict recurrence and survival rates in patients with HCC after hepatectomy with curative intent.

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METHODS

Patients. Between January 1995 and June 2013, 1,413 patients were diagnosed with primary, nonrecurrent HCC at Ogaki Municipal Hospital. Of these 1,413 patients, 431 underwent hepatectomy; we included all 431 consecutive patients in the present study. Decisions regarding individual treatment were made based on the treatment guidelines for HCC in Japan.[15] In all patients, HCC was resected as an anatomic hepatectomy with ample margins; enucleation of tumors without surgical margins was not performed. A diagnosis of HCC was confirmed by pathologic examination of the resected tissue.

After hepatectomy, all patients were followed for a median duration of 69.6 months (range, 8.1–213.6) with ultrasonography and CT or MRI performed every 3–6 months at our institution through the end of 2013. Regular monitoring of serum tumor markers (alpha-fetoprotein [AFP], *lens culinaris* agglutinin-reactive AFP [AFP-L3], and des-gamma-carboxy prothrombin [DCP]) was performed every 3 months. An increased tumor marker prompted additional imaging (usually CT or MRI) to check for HCC recurrence. If recurrence was confirmed, patients underwent treatment for recurrent HCC based on treatment guidelines.[15]

The entire study protocol was approved by the hospital's institutional review board and conducted in compliance with the Declaration of Helsinki. Written, informed consent was obtained from all patients.

Calculation of the FIB-4 index. The FIB-4 index was calculated based on laboratory data at the time of HCC diagnosis,[16] which was within 2 weeks of hepatectomy, as:

$$\frac{\text{AST [IU/L]} \times \text{age [years]}}{\text{platelet count [10}^9\text{/L]} \times \text{ALT[IU/L]}^{1/2}}$$

Measurement of tumor markers for HCC. Measurements of AFP, AFP-L3, and DCP were performed on serum samples collected at the time of HCC diagnosis. Serum AFP levels were determined by enzyme-linked immunosorbent assay using a commercially available kit (ELISA-AFP; International Reagents, Kobe, Japan). A cutoff of 20 ng/mL was used to define AFP positivity, as proposed by Oka et al.[16] Serum AFP-L3, expressed as a percentage of total AFP (AFP-L3/total AFP × 100), was measured by lectin-affinity electrophoresis followed by antibody-affinity blotting (AFP Differentiation Kit L; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The cutoff for AFP-L3 positivity was 10%, as proposed by Shimizu et al.[17] The serum DCP level was determined by a specific enzyme immunoassay (Eitest PIVKA-II kit; Eisai Laboratory, Tokyo, Japan) according to the manufacturer's instructions. The cutoff for DCP positivity was 40 mAU/mL, as proposed by Okuda et al.[18]

Statistical analysis. Differences in percentages between groups were analyzed using the Chi-square test. Differences in means of quantitative values were analyzed using the Mann–Whitney *U* test. Changes in quantitative values with the progression of liver fibrosis were analyzed with the Jonckheere–Terpstra test. The date of treatment (hepatectomy) was defined as time zero for calculations of recurrence and survival rates. For recurrence rates, patients in whom HCC recurred were noncensored, and patients with no HCC recurrence were censored. In the analysis of cancer-specific survival rates, patients who died from HCC-related causes were not censored, whereas all other patients were censored. In the analysis of overall survival, all patients who died were not censored; surviving patients were censored. The Kaplan–Meier method[19] was used to calculate recurrence and survival rates, and the log-rank test[20] was used to analyze differences in recurrence and survival.

The Cox proportional hazards model[21] was used for univariate and multivariate analysis of factors related to recurrence and survival. Variables analyzed included patient age and sex, Child-Pugh class (A/B), tumor size (≤2, >2 and ≤5, or >5 cm), number of tumors (single/multiple), portal vein invasion based on the preoperative imaging studies (absent/present), pretreatment serum AFP level (<20 or ≥20 ng/mL), pretreatment AFP-L3 percentage (<10 or ≥10%), pretreatment serum DCP level (<40 or ≥40 mAU/mL), degree of liver fibrosis of resected noncancerous tissue (F1-2, F3, or F4) according METAVIR liver fibrosis score,[22] and pretreatment FIB-4 index. Statistical analysis was performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC). All *P* values were derived from 2-tailed tests.

RESULTS

Patient characteristics and FIB-4 index. Table I summarizes the pretreatment characteristics of the study patients. This population was comprised of 335 males and 96 females with a mean age of 66.6 ± 9.2 years. HCC was detected during surveillance at our institution in 47.1% of patients, with 95.6% of patients in Child-Pugh class A.[23] Multiple tumors were present in 16.0% of patients at the diagnosis. Based on preoperative imaging studies,

Table I. Characteristics of the study patients (n = 431)

Characteristic	n *(%)**
Age (y)	
Mean ± SD	66.6 ± 9.2
Median (range)	68 (21–85)
Sex	
Female	96 (22.3)
Male	335 (77.7)
Surveillance status at the time of HCC diagnosis	
Our institution	203 (47.1)
Other	164 (38.1)
None†	64 (14.8)
Etiology	
HBV	81 (18.8)
HCV	270 (62.7)
HBV+HCV	4 (0.9)
Non-HBV, non-HCV	76 (17.6)
Child-Pugh class‡	
A	412 (95.6)
B	19 (4.4)
Albumin, g/dL (mean ± SD)	3.98 ± 0.45
Total bilirubin, mg/dL (mean ± SD)	0.75 ± 0.33
ICG 15-minute retention rate, % (mean ± SD)	14.8 ± 7.6
Prothrombin, % (mean ± SD)	92.4 ± 15.0
Platelet count, ×1,000/mL (mean ± SD)	149 ± 70
Tumor size (cm)	
Mean ± SD (range)	3.35 ± 2.54 (0.6–11.7)
≤2	167 (38.7)
>2 and ≤5	184 (42.7)
>5	80 (18.6)
No. of tumors (single/multiple)	
1	137 (84.0)
≥1	26 (16.0)
Portal vein invasion§	
Absent	404 (93.7)
Present	27 (6.3)
Extrahepatic metastasis	
Absent	431 (100)
Present	0
BCLC staging	
0	147 (34.1)
A	284 (65.9)
AFP (ng/mL)	
Median (range)	11.3 (0.8–69,300)
<20	251 (58.2)
≥20	180 (41.8)
AFP-L3 (%)	
Median (range)	0.5 (0–88.1)
<10	338 (78.4)
≥10	93 (21.6)
DCP (mAU/mL)	
Median (range)	40.0 (5–65,856)
<40	217 (50.3)
≥40	214 (49.7)
Liver fibrosis of noncancerous tissue‖	
F1-2	93 (21.6)
F3	114 (26.4)
F4	224 (52.0)
FIB-4 index	
Mean ± SD	4.15 ± 2.68
Median (range)	3.5 (0.3–19.3)
≤3.25	202 (46.7)
>3.25	229 (53.1)
Extent of hepatic resection	
Subsegmentectomy	145 (33.6)
Segmentectomy or lobectomy	286 (66.4)
Blood loss (mL)¶	
Mean ± SD	483 ± 604
Median (range)	330 (0–5,140)
Blood transfusion¶	40 (9.3)
Perioperative complications	33 (7.7)
Perioperative death	8 (1.9)

*Unless otherwise specified.
†Our institution, under surveillance at our institution before the detection of HCC; other, under surveillance by a family physician prior to the detection of HCC; none, not under surveillance and admitted to our institution with symptoms.
‡Child-Pugh class A includes patients without cirrhosis.
§Based on preoperative imaging studies.
‖According to METAVIR fibrosis score.
¶During hepatic resection.
AFP, Alpha-fetoprotein; *AFP-L3*, *lens culinaris* agglutinin-reactive AFP; *BCLC*, Barcelona-Clinic Liver Cancer; *DCP*, des-gamma-carboxy prothrombin; *HBV*, hepatitis B virus; *HCC*, hepatocellular carcinoma; *HCV*, hepatitis C virus; *ICG*, indocyanine green.

portal vein invasion was observed in 6.3% of patients. No extrahepatic metastasis was observed because hepatectomy was not considered as a treatment option in patients with extrahepatic metastasis according to Japanese guidelines.[15] Pretreatment AFP, AFP-L3, and DCP levels were above the specified cutoffs in 41.8%, 21.6%, and 49.7% of patients, respectively. The METAVIR fibrosis score based on histologic examination of the noncancerous liver tissue adjacent to the resected HCC was F1-2 in 21.6%, F3 in 26.4%, and F4 (ie, cirrhosis) in 48.0% of patients. The pretreatment FIB-4 index was 4.15 ± 2.68. The pretreatment FIB-4 index was 3.09 ± 1.94 in patients with F1-2, 3.74 ± 2.08 in F3, and 4.95 ± 3.08 in F4. There was a gradual increase in the FIB-4 index

as the severity of liver fibrosis increased ($P < .0001$). Median blood loss during hepatic resection was 330 mL (range, 0–5,140) and 40 patients (9.3%) received blood transfusion during surgery. Thirty-three patients (7.7%) experienced perioperative complications. Perioperative mortality was 1.9%. Preoperative FIB-4 index was not associated with perioperative morbidity or mortality (data not shown).

Factors associated with the HCC recurrence rate after hepatectomy. We investigated factors associated with the recurrence rate of HCC in patients with curative hepatectomy. Univariate analysis identified patient age, tumor size (>2 and ≤5 and 5 cm), multiple tumors, portal vein invasion, FIB-4 index, blood loss during hepatectomy, and blood transfusion during hepatectomy as factors significantly associated with recurrence after hepatic resection. According to the multivariate analysis, all of these factors except for patient age were independent factors associated with higher recurrence rates (Table II). When we compared the preoperative FIB-4 index between patients in whom HCC recurred (n = 252) and those in whom HCC did not recur (n = 179), the FIB-4 index was significantly greater in patients with recurrence of HCC (4.44 ± 2.70 in patients with recurrence vs 3.75 ± 2.61 in patients without recurrence; P = .0010; Supplementary Fig 1). When postoperative recurrence rates were compared using the FIB-4 index cutoff of 3.25, which indicated severe fibrosis based on a previous report,[12] the 5- and 10-year recurrence rates were 54.8% and 71.4%, respectively, in patients with a pretreatment FIB-4 index of ≤3.25, compared with 69.9% and 84.7%, respectively, in patients with a pretreatment FIB-4 index of >3.25. The recurrence rate was significantly higher in patients with a pretreatment FIB-4 index of >3.25 than those with a pretreatment FIB-4 index of ≤3.25 (P = .0049; Fig 1). When patients were categorized based in the FIB-4 index cutoff of 3.25, hazard ratio of FIB-4 index of >3.25 was 1.66 (95% CI, 1.28–2.17; P = .0002) on multivariate analysis. We further categorized patients with FIB-4 index of ≤3.25 into patients with FIB-4 index of ≤1.45 and those with FIB-4 index of 1.45–3.25 to analyze the influence the moderate fibrosis[12] on the recurrence after hepatectomy. The recurrence rate was lowest in patients with FIB-4 index of ≤1.45 and increased in those with FIB-4 index of 1.45–3.25, and >3.25, in this order (FIB-4 index ≤1.45 vs 1.45–3.25 [P = .1936]; FIB-4 index 1.45–3.25 vs > 3.25 [P = .0192]; and FIB-4 index ≤1.45 vs > 3.25 [P = .0206]; Supplementary Fig 2).

Factors associated with cancer-specific and overall survival rates after hepatectomy. During the follow-up period, 160 patients died and the remaining 271 patients survived. Among 160 patients who died, 134 patients died from HCC-related causes. Twenty-six patients who died from non–HCC-related causes were censored in the analysis of cancer-specific survival rates but were not censored in the analysis of overall survival rates. We investigated factors that were associated with cancer-specific survival in HCC patients after curative hepatectomy. Univariate analysis identified patient age, tumor size (>5 cm), multiple tumors, portal vein invasion, pretreatment AFP (≥20 ng/mL) and AFP-L3 (≥10%) levels, FIB-4 index, blood loss during hepatectomy, and blood transfusion during hepatectomy as factors that are significantly associated with the survival rate after hepatic resection. According to the multivariate analysis, all of these factors, except for pretreatment AFP and AFP-L3 levels, were also independent factors associated with lower survival rates (Supplementary Table). When we investigated factors that were associated with overall survival in HCC patients after curative hepatectomy, univariate analysis identified patient age, tumor size (>5 cm), multiple tumors, portal vein invasion, pretreatment AFP-L3 levels (≥10%), FIB-4 index, blood loss during hepatectomy, and blood transfusion during hepatectomy as factors significantly associated with the survival rate after hepatectomy. According to the multivariate analysis, all of these factors except for pretreatment AFP-L3 level were also independent factors associated with lower survival rates (Table III). When postoperative survival rates were compared using the FIB-4 index cutoff of 3.25, the 5- and 10-year survival rates were 72.2% and 55.2%, respectively, in patients with a pretreatment FIB-4 index of ≤3.25, compared with 67.1% and 33.0%, respectively, in patients with a pretreatment FIB-4 index of >3.25. The survival rate was significantly lower in patients with a pretreatment FIB-4 index of >3.25 than those with a pretreatment FIB-4 index of ≤3.25 (P = .0030; Fig 2). When patients were categorized based on the FIB-4 index cutoff of 3.25, the hazard ratio for an FIB-4 index of >3.25 was 1.72 (95% CI, 1.20–2.51; P = .0034) on multivariate analysis. We further categorized patients with an FIB-4 index of ≤3.25 into patients with an FIB-4 index of ≤1.45 and those with an FIB-4 index of 1.45–3.25. The survival rate was highest in patients with an FIB-4 index of ≤1.45 and decreased in those with an FIB-4 index of 1.45–3.25, and >3.25, in this order (FIB-4 index ≤1.45 vs

Table II. Univariate and multivariate analysis of factors associated with postoperative recurrence of HCC in patients treated with curative hepatectomy (n = 431)

Factor	*Univariate analysis*	*Multivariate analysis*	*Hazard ratio (95% CI)*
Age	0.0049	0.3518	1.01 (0.99–1.03)
Sex			
Male			
Female	0.6496	—	
Child-Pugh class*			
A			
B	0.4953	—	
Tumor size (cm)			
≤2			1
>2 and ≤5	0.0303	0.2710	1.23 (0.85–1.76)
>5	<0.0001	0.0022	2.27 (1.35–3.75)
No. of tumors			
1			1
≥1	0.0310	0.0109	1.72 (1.14–2.51)
Portal vein invasion†			
Absent			1
Present	<0.0001	0.0011	2.83 (1.55–4.93)
Pretreatment AFP (ng/mL)			
<20			
≥20	0.2982	—	
Pretreatment AFP-L3 (%)			
<10			
≥10	0.1169	—	
Pretreatment DCP (mAU/mL)			
<40			
≥40	0.1158	—	
Liver fibrosis of noncancerous tissue‡			
F1-2			
F3	0.4691	—	
F4	0.2850	—	
FIB-4 index	0.0030	<0.0001	1.14 (1.07–1.20)
Blood loss§	<0.0001	0.1463	1.00 (0.99–1.01)
Blood transfusion§			
No			
Yes	0.0384	0.3049	0.67 (0.31–1.41)
Perioperative complications			
No			
Yes	0.9366	—	
Extent of hepatic resection			
Subsegmentectomy			
Segmentectomy or lobectomy	0.2365	—	

*Child-Pugh class A includes patients without cirrhosis.
†Based on preoperative imaging studies.
‡According to METAVIR fibrosis score.
§During hepatic resection.
AFP, Alpha-fetoprotein; *AFP-L3*, *lens culinaris* agglutinin-reactive AFP; *DCP*, des-gamma-carboxy prothrombin; *HCC*, hepatocellular carcinoma.

1.45–3.25 [P = .3956]; FIB-4 index 1.45–3.25 vs > 3.25 [P = .0093]; FIB-4 index ≤1.45 vs > 3.25 [P = .0401]; Supplementary Fig 3).

DISCUSSION

Child-Pugh class that reflects the liver function is among the important factors that influence the prognosis for patients with HCC.[24,25] However, the percentage of patients with better liver function at diagnosis of initial HCC continues increase in Japan,[26] partly owing to improved surveillance.[27] Most HCC patients who underwent curative hepatectomy have Child-Pugh class A liver function. Therefore, it would be difficult to discriminate

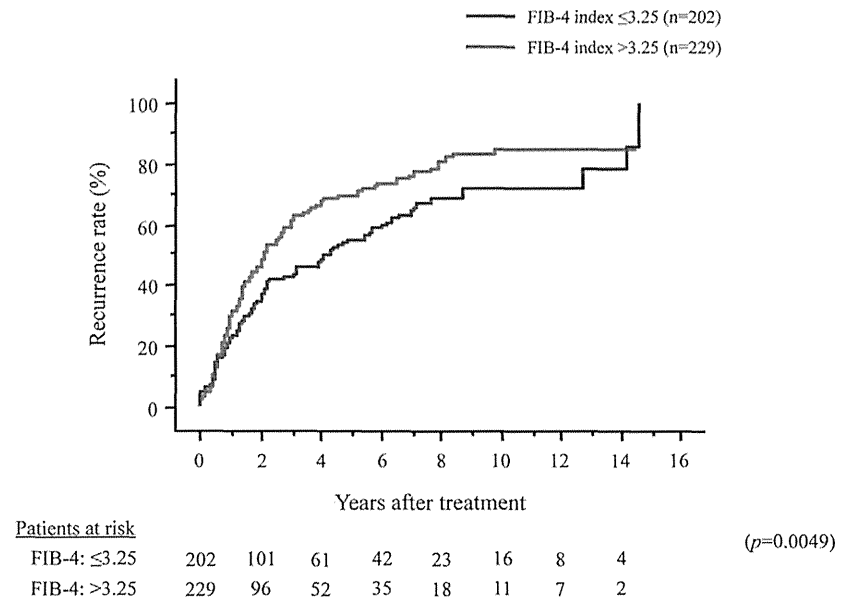

Fig 1. Recurrence rates after hepatectomy in patients with a pretreatment FIB-4 index of ≤3.25 (*blue line*) or >3.25 (*red line*). Recurrence rates were significantly higher in patients with a pretreatment FIB-4 index of >3.25 than those with a pretreatment FIB-4 index of ≤3.25 ($P = .0049$). (Color illustration of figure appears online.)

the prognosis of HCC patients treated with curative hepatectomy regarding liver function based on Child-Pugh class.

In the present study, we attempted to incorporate liver fibrosis as a factor of the background liver to predict the outcome of patients treated with curative hepatic resection, using a laboratory liver fibrosis marker, the FIB-4 index. The FIB-4 index was originally developed as a surrogate marker of liver fibrosis.[9] It has been found to be highly correlated with histologic evaluation of liver fibrosis in liver biopsy specimens.[12] The present study demonstrated that the FIB-4 index at the time of HCC diagnosis correlated with the degree of liver fibrosis in adjacent, noncancerous liver tissue. A previous study reported that a FIB-4 index cutoff of 3.25 showed an excellent discrimination between F0–F2 liver fibrosis and F3–F4 liver fibrosis.[12]

This study demonstrated that the FIB-4 index is strongly associated with recurrence and survival rates in patients with HCC who undergo hepatectomy with curative intent. Univariate and multivariate analyses identified the FIB-4 index as one of the strongest factors associated with higher recurrence and lower survival rates after hepatectomy, in addition to large tumor size (>5 cm) and portal vein invasion. Thus, the FIB-4 index is an independent predictor of prognosis in patients with HCC treated with hepatectomy with curative intent; it is unrelated to tumor factors, reflecting the risk of recurrence in the remnant liver. In contrast with the FIB-4 index, the degree of liver fibrosis was not associated with postoperative recurrence or survival rates in the present study. The reasons are unknown for this discrepancy between the FIB-4 index and the histologic liver fibrosis of the resected specimen observed in the present study, despite the significant increase in the FIB-4 index in association with the increase of fibrosis grade of the resected specimen. Because histologic assessment of liver fibrosis was based on noncancerous liver tissue adjacent to the resected HCC, it may have been influenced by the presence and growth of the liver tumor and might not always have reflected the degree of fibrosis in the entire liver. However, further studies are needed to clarify this discrepancy and determine whether the FIB-4 index or histologic grade of the resected specimen is a stronger indicator of recurrence and survival of HCC patients who underwent hepatectomy.

Pretreatment elevations of HCC tumor markers, especially AFP-L3 and DCP, have been reported to indicate a more aggressive potential in HCC tumors, which is associated with higher recurrence rates and lower survival rates.[28,29] In this study, however, we were unable to identify differences in recurrence or survival based on pretreatment elevations in tumor markers, except for a mild association between AFP-L3 elevation and decreased survival rates. This may be owing to our study's focus on patients who underwent hepatectomy

Table III. Univariate and multivariate analysis of factors associated with postoperative overall survival in HCC patients treated with curative hepatectomy (n = 431)

Factor	*Univariate analysis*	*Multivariate analysis*	*Hazard ratio (95% CI)*
Age	<0.0001	0.0908	1.02 (0.99–1.05)
Sex			
Male			
Female	0.4470	—	
Child-Pugh class*			
A			
B	0.9994	—	
Tumor size (cm)			
≤2			1
>2 and ≤5	0.0860	—	
>5	<0.0001	0.0004	3.18 (1.71–5.68)
No. of tumors			
1			1
≥1	0.0006	0.0324	1.75 (1.05–2.81)
Portal vein invasion†			
Absent			1
Present	<0.0001	0.0263	2.26 (1.11–4.38)
Pretreatment AFP (ng/mL)			
<20			
≥20	0.0503	—	
Pretreatment AFP-L3 (%)			
<10			1
≥10	0.0073	0.0415	1.63 (1.02–2.55)
Pretreatment DCP (mAU/mL)			
<40			
≥40	0.3553	—	
Liver fibrosis of noncancerous tissue‡			
F1-2			
F3	0.7061	—	
F4	0.2651	—	
FIB-4 index	0.0010	<0.0001	1.21 (1.13–1.29)
Blood loss§	<0.0001	0.7366	1.00 (0.99–1.01)
Blood transfusion§			
No			
Yes	0.0254	0.4576	1.47 (0.52–3.84)
Perioperative complications			
No			
Yes	0.4011	—	
Extent of hepatic resection			
Subsegmentectomy			
Segmentectomy or lobectomy	0.6050	—	

*Child-Pugh class A includes patients without cirrhosis.
†Based on preoperative imaging studies.
‡According to METAVIR fibrosis score.
§During hepatic resection.
AFP, Alpha-fetoprotein; *AFP-L3*, *lens culinaris* agglutinin-reactive AFP; *DCP*, des-gamma-carboxy prothrombin; *HCC*, hepatocellular carcinoma.

with curative intent, which excludes patients who underwent nonoperative management or no treatment. Hepatectomy may have a more significant impact on recurrence or survival than the aggressiveness in HCC tumors as reflected by a pretreatment elevation of tumor markers.

A comparison of the recurrence curve between patients with a pretreatment FIB-4 index of >3.25 and those with a pretreatment FIB-4 index of ≤3.25 (Fig 1) revealed that the difference in recurrence rates became marked at 1–2 years after hepatectomy. Previous studies reported that liver fibrosis and the FIB-4 index were associated with hepatocarcinogenesis,[5,30] which may account for the relation between the FIB-4 index and late recurrence after hepatectomy. Also, the difference

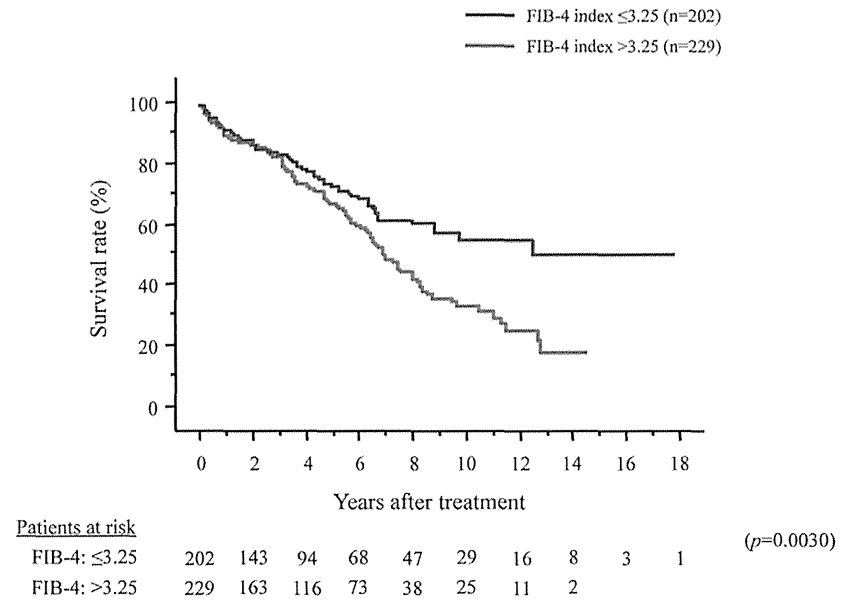

Fig 2. Overall survival rates after hepatectomy in patients with a pretreatment FIB-4 index of ≤3.25 (*blue line*) or >3.25 (*red line*). Overall survival rates were significantly lower in patients with a pretreatment FIB-4 index of >3.25 than those with a pretreatment FIB-4 index of ≤3.25 (P = .0030). (Color illustration of figure appears online.)

in survival rates between patients with a pretreatment FIB-4 index of >3.25 and those with a pretreatment FIB-4 index of ≤3.25 became marked 3 years after hepatectomy. Thus, the impact of the FIB-4 index that reflects the degree of fibrosis of the remnant liver seems to be stronger when predicting long-term outcomes. A previous study also reported that the degree of liver fibrosis was an important factor associated with recurrence and survival of HCC patients after 10 years of disease-free survival after curative hepatectomy.[8]

There are several limitations to this study. All patients were ethnically Japanese and the predominant etiology of liver fibrosis was hepatitis C virus infection. To generalize our findings, these results should be evaluated in other ethnicities with a different distribution of liver fibrosis etiology. In addition, the proportion of study patients with portal vein invasion was lower than the proportion in HCC patients from the Japanese general population,[31] likely owing to the high rate of early detection of HCC under surveillance at our liver center in the present study.[27] Furthermore, because none of our patients were treated with liver transplantation, we have no data on whether the FIB-4 index reflects the degree of fibrosis in the explanted liver and how this might influence recurrence or survival.

In conclusion, our examination of 431 consecutive patients treated with hepatectomy with curative intent revealed that the pretreatment FIB-4 index was associated with recurrence and survival after hepatectomy; patients with a higher pretreatment FIB-4 index had higher recurrence and lower survival rates. Further studies are needed in other populations to confirm whether the FIB-4 index is a clinically useful biomarker for predicting long-term outcomes in patients with HCC who undergo curative hepatectomy.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.surg.2014.10.022.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Accurate and rapid identification of feeding arteries with multidetector-row angiography-assisted computed tomography for transarterial chemoembolization for hepatocellular carcinoma

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Abstract

Background Transarterial chemoembolization (TACE) is an important treatment modality for hepatocellular carcinoma (HCC). Accurate identification of feeding arteries and catheterization are necessary for achieving treatment efficacy, especially with selective TACE. However, this often requires multiple imaging studies. We evaluated the utility of a newly developed apparatus that combines multidetector-row computed tomography (MDCT) and angiography (angio-MDCT) to facilitate TACE for treatment of HCC.

Methods A total of 73 patients who underwent selective TACE with angio-MDCT were compared with 57 patients who had undergone selective TACE with single-row computed tomography assisted by angiography (angio-CT) in terms of the number of imaging studies needed to complete TACE.

Results The mean number of digital subtraction arteriography (DSA) and CT studies required for characterization of feeding arteries before embolization was 3.53 (range 1–8) and 5.16 (range 2–11), respectively, with single-row angio-CT, and 1.67 (range 1–5) and 2.90 (range 1–5), respectively, with angio-MDCT. Fewer studies were needed in patients who underwent TACE with angio-MDCT ($p < 0.0001$ for both DSA and CT). Whereas single-row angio-CT failed to identify extrahepatic feeders in three patients (37.5 %), all extrahepatic feeders could be identified with angio-MDCT.

Conclusions Angio-MDCT facilitates rapid and accurate identification of feeding arteries in patients undergoing TACE through the three-dimensional image analyses by the reconstruction with the workstation.

Keywords Hepatocellular carcinoma · Transarterial chemoembolization · Angiography · Multidetector-row computed tomography · Three-dimensional vascular image

Introduction

Transarterial chemoembolization (TACE) is an important treatment modality for hepatocellular carcinoma (HCC), which is one of the most prevalent cancers worldwide [1]. TACE contributes to the prolonged survival of patients with HCC in whom curative treatment such as hepatic resection or radiofrequency ablation is contraindicated [2].

For effective TACE, accurate identification, confirmation, and catheterization of HCC feeding arteries are important to obtain acceptable therapeutic efficacy and avoid misembolization. However, identification of feeding arteries based on two-dimensional (2D) digital subtraction angiography (DSA) is sometimes difficult; it is sometimes associated with misidentification. Therefore, repeated computed tomography (CT) is often required before embolization to confirm that the selected artery does indeed feed the HCC.

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A combined CT and angiography (angio-CT) system was originally developed in 1996 [3, 4], and has been used by several liver centers in Japan. This integrated CT and angiography apparatus allows immediate and repeated CT examinations during angiography or TACE. Several previous studies have reported the utility of a system consisting of a C-arm cone-beam CT unit with a flat-panel-detector digital angiography unit for TACE [5–8]. However, it has the disadvantage of low contrast resolution and a smaller field of view [5, 9]. We previously reported the advantages of using angio-CT in the treatment of patients with HCC undergoing TACE [10]. Angio-CT improved the detection of feeding arteries, including extrahepatic feeding vessels. Accurate identification and confirmation of these feeding arteries resulted in higher survival rates [10]. However, this method required multiple DSA and CT examinations to identify and confirm the feeding artery, which is associated with higher radiation exposure and contrast medium use.

With the development of multidetector-row CT (MDCT) scanners, temporal and spatial resolutions have improved, making possible the detection of small HCCs and feeding arteries present in the hepatic parenchyma surrounding HCCs and rapid generation of maximum intensity projection, volume-rendering (VR), and multiplanar reconstruction images [11]. The combined use of MDCT and angiography (angio-MDCT) improves the visualization of minute feeding arteries and makes possible the construction of three-dimensional (3D) vascular images, thus facilitating rapid and accurate identification of feeding arteries. We have used the single-row angio-CT system during TACE procedures to treat HCC at our institution since July 1997, and have used the angio-MDCT system since December 2012. In the present study, we evaluated the usefulness of this angio-MDCT system in TACE for patients with HCC.

Materials and methods

Study patients and analyses

Individual decisions regarding treatment of HCC were made by the treating physicians and were based on patient preferences; they were principally based on the Japanese HCC treatment guidelines [12]. Initially, patients were assessed for eligibility for hepatectomy. Those who declined or were deemed not eligible for hepatectomy were considered for locoregional ablative therapy, including radiofrequency ablation or percutaneous ethanol injection. Patients with no extrahepatic spread or portal vein thrombosis who were not eligible for either hepatectomy or locoregional ablative therapy were offered TACE. Between December 2012 and November 2013, 106 patients with a diagnosis of HCC underwent TACE using the angio-MDCT system. Among these 106 patients, 33 patients underwent TACE of the entire liver or TACE of the right or left hepatic lobe because of the multiple HCCs in the liver. The remaining 73 patients who underwent selective TACE with the identification of feeding arteries were analyzed in this study. As a historical control, we analyzed 57 patients who had undergone selective TACE between December 2011 and November 2012 out of 99 patients who during that period had undergone TACE using the older single-row angio-CT technology.

Among these 130 patients, we compared the parameters associated with achieving successful catheterization and selective embolization of HCC feeding arteries in the medical records for TACE. The number of DSA and CT examinations needed to complete the TACE session was investigated, and the time required for the TACE session was calculated on the basis of the time of entry to and exit from the operating room. The entire protocol was approved by the hospital institutional review board and was conducted in compliance with the Declaration of Helsinki. Written informed consent to use their medical records was obtained from all study participants.

The combined angio-CT system

The original angio-CT system was introduced at our institution in July 1997. The combined system [3, 4] consists of single-row helical CT (X-vision Real; Toshiba Medical Systems, Tokyo, Japan) and angiography units in which DSA equipment and a C-arm (Angiorex, BLA-800A; Toshiba Medical Systems) are arranged in a linear configuration to form a common couch. This facilitates rapid transportation of the patient from one unit to the other for CT without risking dislodgment of the catheter.

A new angio-CT system using MDCT (angio-MDCT) was introduced at our institution in December 2012. It consists of 64-multidetector-row CT (Aquilion CX; Toshiba Medical Systems) and angiography units with DSA equipment and a C-arm (Infinix Celeve-I INFX 8000C; Toshiba Medical Systems).

Construction of 3D angiographic images to identify HCC feeding arteries with angio-MDCT

After the placement of the sheath via the femoral artery, the tip of a pig-tail catheter was advanced into the aorta to the level of the carina. Dynamic contrast-enhanced CT aortography was performed with the injection of 90 mL of nonionic contrast medium at a 1:2 dilution (Iopamiron 300; Bayer Yakuhin, Osaka, Japan) at a rate of 9 mL/s. CT images were obtained 8 s after the initiation of contrast

medium injection, from the diaphragm to the kidney at 120 kVp with automatic tube current modulation, 0.838 pitch, 0.5 s per rotation, and a 0.5 mm × 64 data acquisition system. After data acquisition, axial images were reconstructed with a slice thickness and slice interval of 1.0 and 0.5 mm, respectively. VR images were produced using a workstation (SYNAPSE VINCENT; FUJIFILM Medical, Tokyo, Japan) by one of the experienced radiological technologists (K.T., T.G., or H.I.) while two of the operators (H.T., T.T., T.I., or R.H.) were performing further examinations, including DSA, CT during arterial portography, and CT during hepatic arteriography (CTHA). To construct 3D vascular images, first the bony structures were removed from the baseline 3D CT images (Fig. S1a, b), followed by the removal of the abdominal organs (Fig. S1c). After the extraction of 3D vascular images, the bony structures were added (Fig. S1d) to facilitate the determination of the level at which distal arteries arose from proximal arteries on the X-ray monitor.

Target feeding arteries were first identified on the basis of the axial CT images (Fig. 1a, b), and were followed backward to the main artery (Fig. 1c–f). Irrelevant arteries that branched from the target artery were removed from the image (Fig. 1g). The visualized feeding arteries were finally combined in 3D vascular images and emphasized with color coding on the VR images for the operator to clearly recognize them in the operating room (Fig. 1h).

Characterization of feeding arteries and TACE

Prior to TACE, a baseline examination with DSA from the common or proper hepatic artery, CT during arterial portography, and CTHA was performed in all patients for accurate evaluation of the spread of HCC in the liver and the vascularity [13, 14]. On the basis of the baseline study findings, the feeding artery was identified. In 57 patients who underwent TACE with single-row angio-CT, feeding arteries were identified on baseline DSA and 2D CTHA images. In 73 patients who underwent TACE with angio-MDCT, feeding arteries were identified on baseline DSA and 3D vascular images constructed with CT aortography data. The origin of the target artery was sometimes detected by changing the orientation of the 3D vascular image (Fig. S2). The completion of TACE was confirmed by the deposition of iodized oil in the lesion covering the entire tumor visualized by unenhanced CT immediately after TACE. If there was inadequate iodized oil deposition in the lesion on CT, another feeding artery supplying the part of

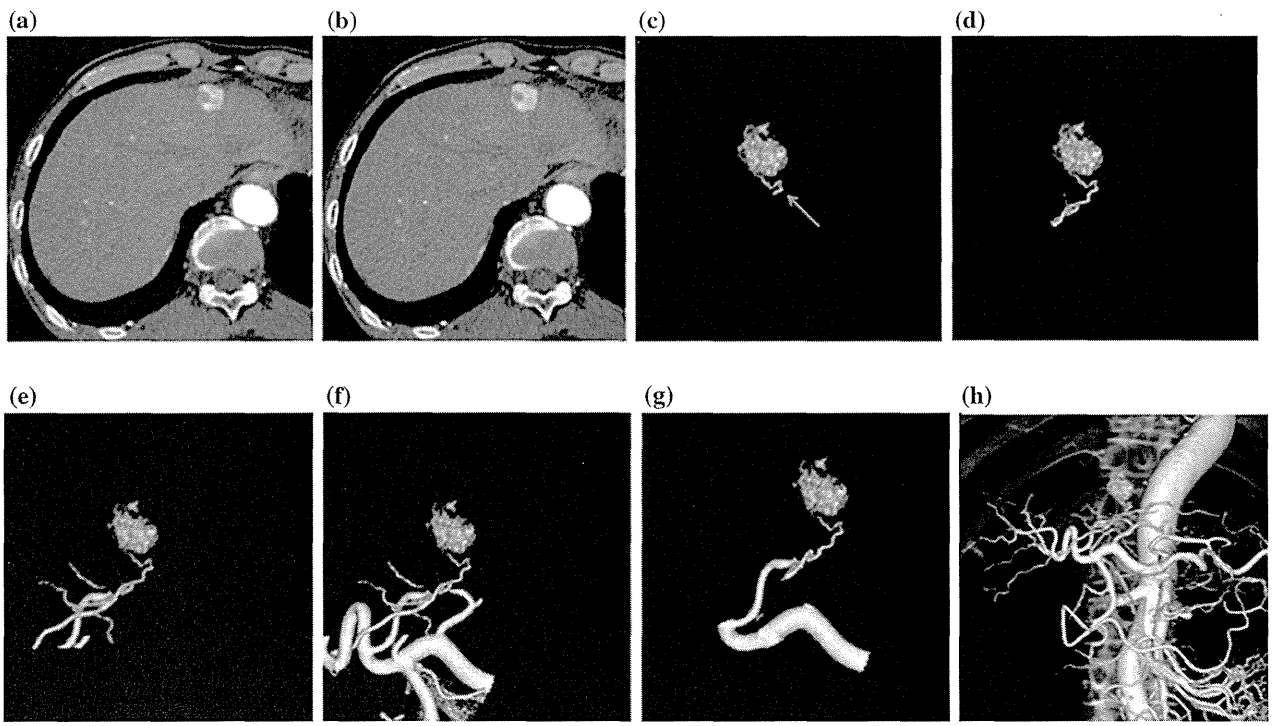

Fig. 1 Identification of target feeding arteries on three-dimensional (3D) vascular images. The target feeding artery was first identified on the axial computed tomography (CT) images (**a, b**). It was then traced backward to its origin (**c–f**; the *red arrow* indicates a feeding artery adjacent to the hepatocellular carcinoma). Irrelevant arteries branching from the target artery were then removed from the image (**g**). The feeding artery was finally combined with the 3D vascular image and color coded to facilitate recognition on the volume-rendering image in the operating room (**h**)

the lesion without deposition was sought. Dynamic magnetic resonance imaging was performed after TACE to confirm the disappearance of the arterial blood supply within the target HCC.

Statistical analysis

Numerical data are expressed as the mean ± the standard deviation. Differences in the proportions of patients between groups were analyzed using the chi square test. Differences in quantitative values were analyzed using the Mann–Whitney *U* test. The JMP statistical software package, version 4.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses. All p values were derived from two-tailed tests, and $p < 0.05$ was considered to indicate statistical significance.

Results

Patients characteristics

Table 1 shows the baseline characteristics of patients treated by TACE with single-row angio-CT ($n = 57$) and angio-MDCT ($n = 73$). No differences in patient age and sex were found between the groups. Hepatitis C virus infection was the predominant cause of HCC in both groups. The proportion of patients with replaced right or left hepatic arteries and the proportion of patients with an extrahepatic feeding artery were similar in the two groups. There was no difference in the number of embolized arteries between the two groups.

Number of DSA and CT examinations for the characterization of HCC feeding arteries to complete the TACE session

A 3D vascular image based on CT aortography was constructed within 10 min in all patients who underwent TACE using angio-MDCT. Figure 2 compares the number of DSA and CT examinations needed to complete the TACE session. The mean number of DSA and CT studies required for characterization of feeding arteries before embolization was 3.53 (range 1–8) and 5.16 (range 2–11), respectively, with single-row angio-CT, and 1.67 (range 1–5) and 2.90 (range 1–5), respectively, with angio-MDCT. The number of DSA examinations and the number of CT examinations were both significantly lower in patients who underwent TACE using angio-MDCT ($p < 0.0001$ for both).

In some cases, an HCC feeding artery originating from a main proximal artery that was distant to the tumor was easily identified using 3D angio-MDCT vascular images (Fig. S3). In other cases, the origin of the target feeding artery was confirmed by changing the orientation of the 3D vascular images and adjusting the orientation of the C-arm X-ray monitor view to that of the 3D vascular image in order to facilitate catheterization into the target branch (Fig. S4).

The median time needed to complete the TACE session divided by the number of target feeding arteries was 77.0 min (range 29.0–297.0 min) in patients who underwent single-row angio-CT and 60.0 min (range 24.0–124.0 min) in patients who underwent angio-MDCT. The time tended to be shorter in patients who underwent

Table 1 Characteristics of the study patients ($n = 130$)

Characteristic	TACE with single-row angio-CT ($n = 57$)	TACE with angio-MDCT ($n = 73$)
Age (years) (median, range)	70 (52–84)	71 (41–84)
Sex (female/male)	21 (36.8 %)/36 (63.2 %)	19 (26.0 %)/54 (74.0 %)
Cause of HCC (HBV/HCV/non-HBV, non-HCV)	8 (14.0 %)/43 (75.5 %)/6 (10.5 %)	7 (9.6 %)/54 (74.0 %)/12 (16.4 %)
Replaced right hepatic artery (present/absent)	7 (12.3 %)/50 (87.7 %)	8 (11.0 %)/65 (89.0 %)
Replaced left hepatic artery (present/absent)	2 (3.5 %)/55 (96.5 %)	3 (4.1 %)/70 (95.9 %)
Number of target feeding arteries (median, range)	1 (1–4)	2 (1–4)
Extrahepatic blood supply	8 (14.0 %)	10 (13.7 %)

A replaced right hepatic artery arose from the superior mesenteric artery in 14 patients and from the celiac axis in one patient. A replaced left hepatic artery arose from the left gastric artery in four patients and from the gastroduodenal artery in one patient. Extrahepatic blood supply was from the right inferior phrenic artery in ten patients, the right internal thoracic artery in three patients, the right adrenal artery in two patients, the gastroepiploic artery in one patient, and an artery branching directly from the abdominal aorta in two patients. There was no statistical significance for all comparisons ($p > 0.05$ for all comparisons)

angio-CT computed tomography and angiography, *angio-MDCT* multidetector-row computed tomography and angiography, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *TACE* transarterial chemoembolization

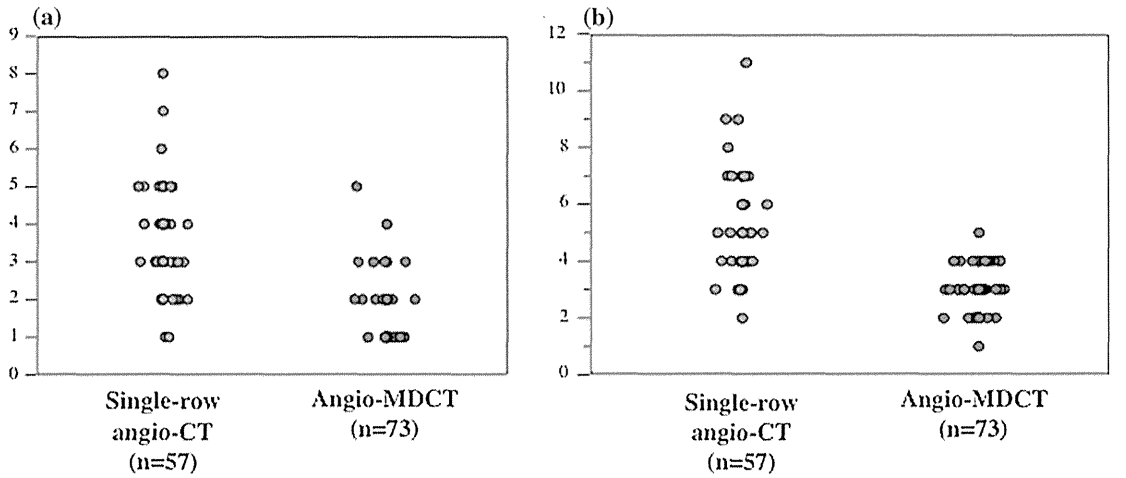

Fig. 2 The number of digital subtraction angiography (DSA) (**a**) and computed tomography (CT) (**b**) studies needed to complete the transarterial chemoembolization (TACE) session. The number of DSA examinations and the number of CT examinations were significantly lower when TACE was performed using a combined 64-multidetector-row CT and angiography (angio-MDCT) system than when a single-row CT system was used ($p < 0.0001$, respectively). *angio-CT* CT and angiography

TACE using angio-MDCT, although the difference was not significant statistically ($p = 0.0672$).

Detection and identification of extrahepatic blood supply to HCCs

Extrahepatic blood supply was observed in eight of 57 patients (14.0 %) in the single-row angio-CT group and in ten of 73 patients (13.7 %) in the angio-MDCT group. Whereas the identification of an extrahepatic blood supply, catheterization, and chemoembolization were unsuccessful in three of eight patients (37.5 %) in the single-row angio-CT group, TACE of an extrahepatic feeding artery was successful in all the patients in the angio-MDCT group ($p = 0.0339$; Fig. 3). In three patients, the extrahepatic feeding artery was the right internal thoracic artery, which arises from the right subclavian artery. The origin of this extrahepatic feeding artery was outside the scanned area during the CT-aortography study and was not depicted as an extrahepatic feeder.

Discussion

In the present study, we evaluated the ability of angio-MDCT to facilitate accurate identification of feeding arteries required for selective TACE, compared with single-row angio-CT in terms of the number of DSA and CT examinations needed to identify and confirm feeding arteries before chemoembolization. The objective evaluation of the facilitation of TACE is difficult. The time required for TACE strongly depends on the degree of difficulty involved in catheterizing the target artery, as well as on the identification of feeding arteries. Therefore, comparing the duration of TACE will not correctly reflect the advantageous effect of angio-MDCT.

A previous report reviewed the advantages of CT aortography with angio-MDCT in TACE for HCC. However, this report did not contain objective data demonstrating the advantages of this procedure [15]. Our present study attempted to verify this advantage using objective data. The present study clearly demonstrates the advantage of angio-MDCT over single-row angio-CT for TACE by the smaller number of DSA and CT examinations needed to complete the TACE session.

Identification of extrahepatic feeding arteries such as the inferior phrenic, cystic, right renal capsular, or right adrenal arteries is important for the completeness and efficacy of TACE [16–19]. In the angio-MDCT group, there were no patients in whom identification of extrahepatic feeding arteries was unsuccessful. Therefore, abdominal 3D vascular images based on angio-MDCT allowed rapid visualization of both hepatic and extrahepatic feeding arteries simultaneously, without the need for large volumes of contrast medium.

With the administration of an intravenous bolus of contrast medium, 3D vascular images can be constructed from dynamic CT. However, it is often difficult to follow the aorta continuously to the distal feeding artery because the feeding artery and the surrounding hepatic parenchyma may have similar Hounsfield units [15]. In addition, comparing axial CT aortography and CTHA images helps to identify HCC tumors or parts of tumors with an extrahepatic blood supply (Fig. 3a, b). Therefore, CT