of liver function prior to SR and for prediction of clinical outcomes for NBNC-HCC patients who undergo SR, although there are several reports regarding the clinical significance of preoperative APRI on survival in HBV-related HCC patients treated with SR (23,24).

The aims of the present study were to examine the relationship between preoperative FIB-4 index and background liver fibrosis in non-tumor parts obtained from extracted surgical specimens and to investigate whether the preoperative FIB-4 index can be a useful predictor for NBNC-HCC patients treated with SR.

Patients and methods

Patients. A total of 128 treatment-naïve NBNC-HCC patients received SR at our institution between June 2004 and June 2014 with curative intent. Curative surgery was defined as resection of all tumors detectable using imaging modalities. NBNC-HCC was defined as HCC negative for both HBsAg and HCVAb. Patients with severe alcoholic cirrhosis (n=7), a patient with autoimmune hepatitis (n=1) and patients with primary biliary cirrhosis (n=2) were excluded from the present study. A total of 118 NBNC-HCC patients were thus analyzed in the present study. A diagnosis of diabetes mellitus (DM) was based on past medical history or 75-g oral glucose tolerance test results (25). We examined predictive factors associated with overall survival (OS) and recurrence-free survival (RFS) in univariate and multivariate analyses.

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

Calculated scores. The APRI score was calculated using Wai's formula: (AST/upper limit of normal)/platelet count (expressed as platelets x 10^9/l) x 100 (26). The FIB-4 score was calculated using Sterling's formula as: [age (years) x AST (IU/l)/platelet count (x10^9/l) x ALT^{½} (IU/l)] (27).

HCC diagnosis. HCC was diagnosed using abdominal ultrasound and dynamic 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 (28). Arterial- and portal-phase dynamic CT images were obtained at ~30 and 120 sec, respectively, after the injection of the contrast material. HCC stage was determined using the Liver Cancer Study Group of Japan staging system (29). All HCC was confirmed pathologically except for two cases with complete necrosis due to preoperative transcatheter arterial chemoembolization (TACE).

Hepatectomy and surgical procedure. All surgical procedures were performed by one of four surgeons with at least 10 years experience of SR. Anatomical SR was defined as a resection in which tumors were completely removed anatomically on the basis of Couinaud's classification (segmentectomy, sectionectomy and hemihepatectomy, or extended hemihepatectomy). Non-anatomical partial SR was carried out as a limited resection or tumor enucleation. Anatomical SR was performed in 68 patients (57.6%) and non-anatomical SR was performed in 50 patients (42.4%) in the present study. Conventional open hepatectomy was performed in 95 patients (80.5%), and laparoscopic hepatectomy was performed in 23 patients (19.5%) in the present study.

Histological evaluation of the extracted liver specimens. All extracted liver specimens were reviewed by a single pathologist in our hospital. Background liver fibrosis was staged as F0 to F4: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and a few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. The degree of differentiation of HCC in each resected specimen was determined as well-differentiated HCC, moderately differentiated HCC, poorly differentiated HCC or combined type of HCC and cholangiocellular carcinoma (CCC) (30).

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 PIVKA-II 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. When HCC recurred, the most appropriate therapy for HCC recurrence was performed considering tumor status, liver function or performance status of the patients.

Statistical analysis. Data were analyzed using univariate and multivariate analyses. Continuous variables were compared between groups by the Mann-Whitney U test. Receiver operating characteristic (ROC) curve analysis was performed for calculating the area under the ROC (AUROC) for the FIB-4 index, APRI, AST to ALT ratio, serum albumin, total bilirubin and platelet count and selecting the optimal cut-off value that maximized the sum of sensitivity and specificity for liver cirrhosis (F4). Time to recurrence was defined as the interval between initial 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 <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 means ± standard deviation (SD). Values of $P<0.05$ were considered to indicate statistically significant differences.

Table I. Baseline characteristics of the patients with non-B and non-C hepatocellular carcinoma (n=118).

Variables	Data (N=118)
Age (years)	68.9±9.0
Gender, male/female	93/25
Body mass index (kg/m^2)	24.3±3.9
Diabetes mellitus, yes/no	53/65
HCC stage, I/II/III/IV	4/74/33/7
Maximum tumor size (cm)	5.7±3.2
Tumor number, single/multiple	75/43
AST (IU/l)	43.4±26.0
ALT (IU/l)	37.8±29.4
ALP (IU/l)	343.8±186.8
GGT (IU/l)	158.8±170.6
Serum albumin (g/dl)	4.0±0.5
Total bilirubin (mg/dl)	0.8±0.4
Prothrombin time (%)[a]	92.5±18.7
Platelets (x10^4/mm^3)	17.0±8.1
AFP (ng/ml)	1,713±11,917
DCP (mAU/ml)[b]	8,629±48,077
Histological findings (extracted surgical specimen)	
Background liver fibrosis, F4/3/2/1/0	39/20/22/14/23
Tumor differentiation, well/moderate/poor/combined/necrosis	11/69/33/3/2

Data are expressed as number or mean ± standard deviation. HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin. [a]Missing data, n=2; [b]missing data, n=2. Combined means combined type of HCC and cholangiocellular carcinoma. Necrosis means complete necrosis.

Results

Baseline characteristics. The baseline characteristics of the analyzed patients (n=118) are shown in Table I. The mean age was 68.9±9.0 years. The median observation period was 3.2 years (range, 0.1-10.1 years). There were 93 males and 25 females. The mean maximum tumor size was 5.7±3.2 cm. The mean body mass index (BMI) was 24.3±3.9 kg/m^2. As for the histological findings, in terms of the degree of liver fibrosis in the non-tumor portion, F4 was observed in 39 patients, F3 in 20, F2 in 22, F1 in 14 and F0 in 23, whereas in terms of HCC histology, well-differentiated HCC was observed in 11 patients, moderately differentiated HCC in 69, poorly differentiated HCC in 33, combined type of HCC and CCC in 3 and complete necrosis due to preoperative TACE in 2 patients.

Comparison of the area under receiver operating curves for serum markers for liver cirrhosis. We evaluated the correlation between serum markers including FIB-4 index, APRI, AST to ALT ratio, platelet count, serum albumin and total bilirubin and liver cirrhosis (F4). Receiver operating curves of the serum markers used for predicting liver cirrhosis are demonstrated in Fig. 1. FIB-4 index, APRI and platelet count exhibited reliable discriminative ability for predicting liver cirrhosis. Among these, the FIB-4 index yielded the highest AUROC with a level of 0.887 at an optimal cut-off value of 2.97 (sensitivity, 92.3%; specificity, 69.6%) (Table II). The FIB-4 index in patients with liver cirrhosis (F4, n=39) was significantly higher than that in the patients with non-liver cirrhosis (F0-3, n=79) (P<0.001, Mann-Whitney U test) (Fig. 2).

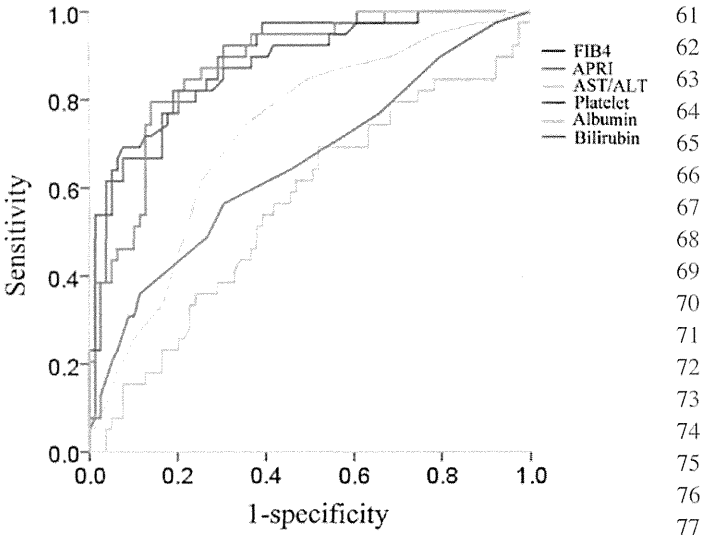

Figure 1. Correlation between serum markers including FIB-4 index, AST to platelet ratio index (APRI), AST to ALT ratio, platelet count, serum albumin and total bilirubin and liver cirrhosis (F4). FIB-4 index yielded the highest AUROC with a level of 0.887 at an optimal cut-off value of 2.97 (sensitivity, 92.3%; specificity, 69.6%). AST, aspartate aminotransferase; ALT, alanine aminotransferase; AUROC, area under the ROC; ROC, receiver operating characteristic.

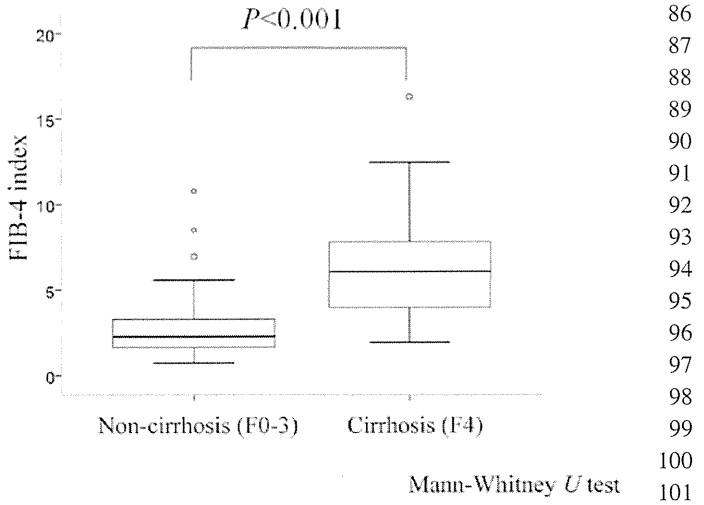

Figure 2. Box plots of the FIB-4 index between patients with non-cirrhosis (F0-3) and those with cirrhosis (F4). The FIB-4 index in patients with liver cirrhosis (n=39) was significantly higher than that in those with non-liver cirrhosis (n=79) (P<0.001, Mann-Whitney U test).

Cumulative OS and RFS rates for all patients. For all patients (n=118), the 1-, 3- and 5-year cumulative OS rates were 92.0,

Table II. Comparison of the area under the receiver operating curves (AUROCs) for FIB-4 index, APRI, AST to ALT ratio, platelet count, serum albumin and total bilirubin for liver cirrhosis.

	AUROC	95% CI	Standard error	P-value
FIB-4 index	0.887	0.828-0.947	0.030	<0.001
APRI	0.877	0.814-0.941	0.032	<0.001
AST to ALT ratio	0.559	0.446-0.671	0.057	0.302
Platelet count (/mm^3)	0.883	0.818-0.949	0.034	<0.001
Serum albumin (g/dl)	0.724	0.629-0.820	0.049	<0.001
Total bilirubin (mg/dl)	0.652	0.543-0.761	0.056	0.007

APRI, AST to platelet ratio index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CI, confidence interval; AUROCs, area under the ROC; ROC, receiver operating characteristic.

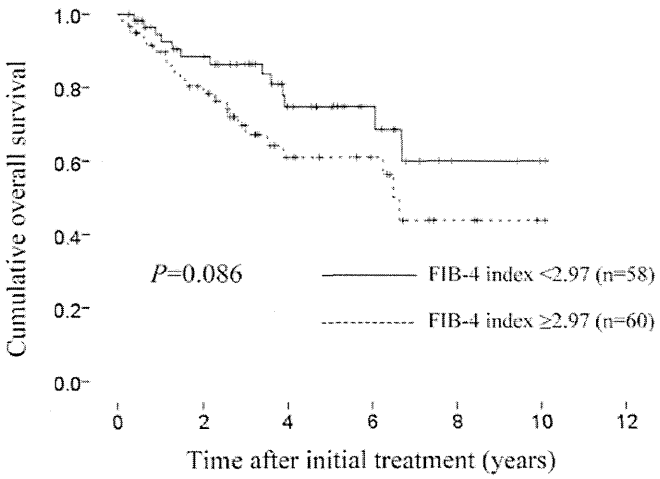

Figure 3. Cumulative overall survival (OS) rates according to FIB-4 index. The 1-, 3- and 5-year cumulative OS rates in patients with FIB-4 index >2.97 (n=60) were 89.7, 67.0 and 60.9%, respectively, and the corresponding cumulative OS rate in patients with FIB-4 index <2.97 (n=58) were 94.4, 86.3 and 74.7%, respectively (P=0.086).

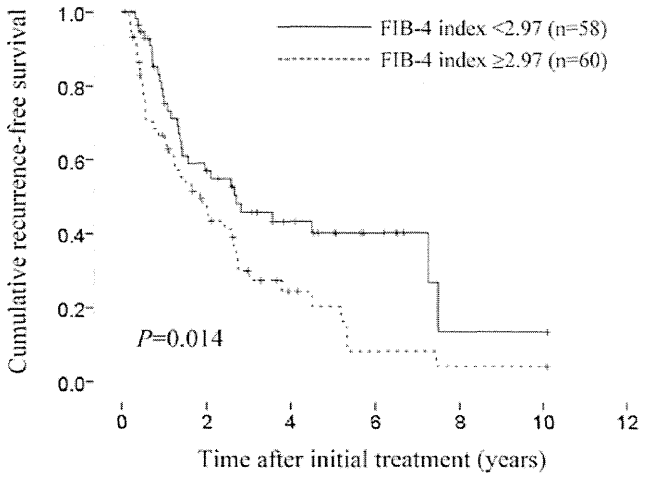

Figure 4. Cumulative recurrence-free survival (RFS) rates according to FIB-4 index. The 1-, 3- and 5-year cumulative RFS rates in patients with FIB-4 index >2.97 were 66.6, 29.8 and 20.2%, respectively, and the corresponding cumulative RFS rate in patients with FIB-4 index <2.97 were 75.2, 45.8 and 40.1%, respectively (P=0.014).

76.6 and 67.6%, respectively. The corresponding RFS rates were 71.9, 37.8 and 30.5%, respectively.

Cumulative OS and RFS rates according to FIB-4 index. The 1-, 3- and 5-year cumulative OS rates in patients with FIB-4 index >2.97 (optimal cut-off value) (n=60) were 89.7, 67.0 and 60.9%, respectively, and the corresponding cumulative OS rates in patients with FIB-4 index <2.97 (n=58) were 94.4, 86.3 and 74.7%, respectively (P=0.086) (Fig. 3). The 1-, 3- and 5-year cumulative RFS rates in patients with FIB-4 index >2.97 were 66.6, 29.8 and 20.2%, respectively, and the corresponding cumulative RFS rates in patients with FIB-4 index <2.97 were 75.2, 45.8 and 40.1%, respectively (P=0.014) (Fig. 4).

Univariate and multivariate analyses of factors contributing to OS. Univariate analysis identified the following factors as significantly associated with OS for all cases (n=118): tumor number (single or multiple) (P=0.006); alkaline phosphatase (ALP) >300 IU/l (P=0.037); serum albumin >4.0 g/dl (P=0.010); and AFP >40 ng/ml (P=0.048) (Table III). The hazard ratios (HRs) and 95% confidence intervals (CIs) calculated using multivariate analysis for the four factors with P-values of <0.05 in univariate analysis are detailed in Table III. Only the AFP value was found to be a significant predictor linked to OS in multivariate analysis (P=0.026).

Univariate and multivariate analyses of factors contributing to RFS. Univariate analysis identified the following factors as significantly associated with RFS for all cases (n=118): tumor number (single or multiple) (P<0.001); presence of microscopic vascular invasion (P=0.024); ALP >300 IU/l (P=0.002); γ-glutamyl transpeptidase (GGT) >100 IU/l (P=0.010); and FIB-4 index >2.97 (P=0.014) (Table IV). The HRs and 95% CIs calculated using multivariate analysis for the five factors with P-values of <0.05 in univariate analysis are detailed in Table IV. Tumor number (P=0.002) and FIB-4 index (P=0.044) were found to be significant prognostic factors linked to RFS.

Table III. Univariate and multivariate analysis of factors contributing to overall survival.

Variables	n	Univariate analysis	Multivariate analysis	
			Hazard ratio (95% CI)	P-value[a]
Gender, male vs. female	93/25	0.330		
Age (years), >70 vs. ≤70	58/60	0.802		
Tumor number, single vs. multiple	75/43	0.006	0.544 (0.271-1.092)	0.087
Maximum tumor size (cm), ≥4.5 vs. <4.5	59/59	0.386		
Microscopic vascular invasion, yes vs. no	52/66	0.100		
AST (IU/l), ≥40 vs. <40	53/65	0.561		
ALT (IU/l), ≥40 vs. <40	34/84	0.196		
ALP (IU/l), ≥300 vs. <300	56/62	0.037	0.514 (0.239-1.104)	0.088
GGT (IU/l), ≥100 vs. <100	58/60	0.370		
FIB-4 index ≥2.97, yes vs. no	60/58	0.086		
Serum albumin level (g/dl), ≥4.0 vs. <4.0	74/44	0.010	1.991 (0.986-4.022)	0.055
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	23/95	0.465		
Platelet count (x10^4/mm^3), ≥15 vs. <15	64/54	0.205		
Prothrombin time (%), ≥80 vs. <80[b]	98/18	0.051		
Diabetes mellitus, yes vs. no	53/65	0.150		
Body mass index (kg/m^2), ≥25 vs. <25	49/69	0.322		
Serum AFP (ng/ml), ≥40 vs. <40	31/87	0.048	0.427 (0.202-0.904)	0.026
DCP (mAU/ml), ≥200 vs. <200[c]	61/55	0.406		

CI, confidence interval; 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; [b]missing data, n=2; [c]missing data, n=2.

Table IV. Univariate and multivariate analysis of factors contributing to recurrence-free survival.

Variables	n	Univariate analysis	Multivariate analysis	
			Hazard ratio (95% CI)	P-value[a]
Gender, male vs. female	93/25	0.407		
Age (years), >70 vs. ≤70	58/60	0.396		
Tumor number, single vs. multiple	75/43	<0.001	0.468 (0.291-0.751)	0.002
Maximum tumor size (cm), >4.5 vs. ≤4.5	59/59	0.335		
Microscopic vascular invasion, yes vs. no	52/66	0.024	0.648 (0.406-1.032)	0.068
AST (IU/l), ≥40 vs. <40	53/65	0.035		
ALT (IU/l), ≥40 vs. <40	34/84	0.880		
ALP (IU/l), ≥300 vs. <300	56/62	0.002	0.827 (0.492-1.391)	0.474
GGT (IU/l), ≥100 vs. <100	58/60	0.010	0.680 (0.399-1.159)	0.156
FIB-4 index ≥2.97, yes vs. no	60/58	0.014	0.640 (0.390-0.988)	0.044
Serum albumin level (g/dl), ≥4.0 vs. <4.0	74/44	0.933		
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	23/95	0.817		
Platelet count (x10^4/mm^3), ≥15 vs. <15	64/54	0.063		
Prothrombin time (%), ≥80 vs. <80[b]	98/18	0.052		
Diabetes mellitus, yes vs. no	53/65	0.652		
Body mass index (kg/m^2), ≥25 vs. <25	49/69	0.916		
Serum AFP (ng/ml), ≥40 vs. <40	31/87	0.116		
DCP (mAU/ml), ≥200 vs. <200[c]	61/55	0.686		

CI, confidence interval; 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; [b]missing data, n=2; [c]missing data, n=2.

Causes of death. Thirty-five patients (29.7%) died during the follow-up period. The causes of death were HCC recurrence in 24 patients, liver failure in 6 patients and miscellaneous causes in 5 patients.

HCC recurrence. In the present study, 75 patients (63.6%) had HCC recurrences during the follow-up period. The patterns of HCC recurrence after initial treatment were: single HCC recurrence in the liver in 32 patients; multiple HCC recurrences in the liver in 28 patients; multiple HCC recurrences in the liver with lung metastases in three patients; multiple lung metastases in 5 patients; multiple HCC recurrences in the liver with lymph node metastases in 2 patients; multiple HCC recurrences in the liver with peritoneal dissemination in one patient; multiple HCC recurrences in the liver with bone metastases in one patient; multiple bone metastases in one patient; single HCC recurrence in the liver with duodenal invasion in one patient; and local tumor progression (recurrence in the SR site) in one patient. Treatment methods for the first HCC recurrence were: SR in 11 patients; RFA in 29 patients; TACE in 16 patients; systemic chemotherapy such as sorafenib in 7 patients; radiation therapy in 2 patients and no specific treatment in 10 patients.

In patients with a preoperative FIB-4 index >2.97 (n=60), HCC recurrence was found in 44 patients (73.3%), while in patients with a preoperative FIB-4 index <2.97 (n=58), HCC recurrence was observed in 38 patients (65.5%). Fifteen patients (25.0%) had late first confirmed HCC recurrence (>2 years after initial SR) in patients with a preoperative FIB-4 index >2.97, whereas 9 patients (15.5%) had late first confirmed HCC recurrence in patients with a preoperative FIB-4 index <2.97.

Discussion

To the best of our knowledge, this is the first reported study to investigate the relationship between preoperative FIB-4 index and clinical outcomes in NBNC-HCC patients treated with SR. Although the FIB-4 index has been demonstrated to be a useful noninvasive serum marker for predicting liver fibrosis in many previous studies, the effect of this marker on clinical outcomes in NBNC-HCC patients who undergo SR remains obscure (11-18). Hence, we conducted the current analyses.

In our results, the FIB-4 index yielded the highest AUROC with a level of 0.887 for cirrhosis and in the multivariate analysis, FIB-4 index was significantly associated with RFS although in terms of OS, FIB-4 index was not a significant predictor. These results revealed that the FIB-4 index has the highly discriminative ability for predicting liver cirrhosis and it could be a useful predictor for NBNC-HCC patients treated with SR. Liver biopsy, which has been considered as the 'golden standard' for defining liver fibrosis, carries some drawbacks: sampling error and inter-observer variability, which have raised questions on its value, whereas in our current analyses, we examined the effect of FIB-4 index on liver cirrhosis using non-tumor parts of extracted surgical specimens, which had sufficient amount of liver specimens for an exact assessment of extension of liver fibrosis (13,31). Thus, our current data are highly reliable. One possible reason that the FIB-4 index was not a significant predictor linked to OS in the present analysis is that HCC patients with extremely poor hepatic reserve were excluded from our candidates for SR.

In our baseline characteristics, the proportion of patients with DM was 44.9% (53/118). Wang *et al* performed a meta-analysis including a total of 25 cohort studies to investigate the relationship between DM and HCC development and reported that DM was associated with an increased incidence of HCC (HR=2.01, 95% CI=1.61-2.51) compared with individuals without DM, and it was also positively associated with HCC mortality (HR=1.56, 95% CI=1.30-1.87) (32). Our high proportion of diabetic patients may be associated with their results. On the other hand, the mean BMI in the present study was 24.3±3.9 kg/m^2 and the proportion of patients with BMI >30 kg/m^2 that indicated obesity was only 7.6% (9/118). Obesity and its related metabolic abnormalities, including chronic inflammatory condition, have been shown to increase the risk of HCC development (33). Although the reasons for these discrepancies are unclear, other carcinogenic factors than obesity may be closely associated with NBNC-HCC development in Japanese populations. Furthermore, it is of note that the proportion of patients with F0 or F1 that indicated minimal fibrosis was 31.4% (37/118) in the present study, although advanced fibrosis was found to be a significant risk factor of HCC development in many previous reports (1-7). The carcinogenic mechanisms between NBNC-HCC and virus-related HCC may be different.

In the multivariate analysis in terms of OS, AFP was an independent predictor (P=0.026) and serum albumin had marginal significance (P=0.055). Both tumor-related factors and liver function-related factors may be essential for the management of NBNC-HCC patients treated with SR. In that sense, branched-chain amino acid therapy for background liver disease may improve clinical outcomes (34). On the other hand, as for RFS, tumor number (P=0.002) and FIB-4 index (P=0.044) were found to be independent significant predictors and the presence of microvascular invasion had marginal significance (P=0.068) in the multivariate analysis. Particularly in patients with these risk factors, close observation for HCC recurrence after SR is needed. Furthermore, in our results, the proportion of late HCC recurrence in patients with FIB-4 index >2.97 was higher than that in patients with FIB-4 index <2.97 (25.0 vs. 15.5%). Several investigators have demonstrated that advanced fibrosis is related to late *de novo* HCC recurrence after SR in the remnant fibrotic liver (35,36). Our results were consistent with their reports.

The present study included several limitations. Firstly, this is a retrospective observational study. Secondly, the sample size in the present study was relatively small for statistical analyses. Thirdly, our study cohort included only Japanese HCC patients, who in general had lower BMI than populations in Western countries (37,38). Hence, caution should be exercised in interpreting our results and further larger prospective studies are necessary. However, our results demonstrated that the FIB-4 index had high discriminative ability for predicting liver cirrhosis and it could identify patients with a high risk of HCC recurrence in NBNC-HCC patients treated with SR.

In conclusion, the FIB-4 index is a useful serum marker for predicting liver cirrhosis and a useful predictor of clinical outcomes for NBNC-HCC patients who undergo SR.

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Review Article

Clinical significance of therapy using branched-chain amino acid granules in patients with liver cirrhosis and hepatocellular carcinoma

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The liver is the major organ for the metabolism of protein, fat and carbohydrate. A nutritional approach is required in the treatment of cirrhosis, which is frequently complicated with protein–energy malnutrition. Several advanced treatment approaches for hepatocellular carcinoma (HCC) have been established in the past decade. HCC is often complicated by cirrhosis, so treatment of the underlying liver diseases is also necessary to improve the prognosis. Branched-chain amino acid (BCAA) granules were developed originally for the treatment of hypoalbuminemia associated with decompensated cirrhosis. However, subsequent studies found various other pharmacological actions of this agent. We review the clinical significance of therapy using BCAA granules in patients receiving different treatment approaches for cirrhosis and HCC based on the published work as well as our own data.

Key words: branched-chain amino acid granules, hepatocellular carcinoma, liver cirrhosis, liver function, recurrence

INTRODUCTION

THE LIVER IS the major organ for the metabolism of protein, fat and carbohydrate.[1,2] Cirrhosis, which develops over a long period of time, is frequently complicated with protein–energy malnutrition (PEM).[1,2] Patients with cirrhosis-associated PEM starve even after a short period of fasting because of the increased energy consumption and decreased glycogen-storage capacity of the liver. The body consumes the endogenous fat as an energy substrate instead of carbohydrate. As a result, fasting hypoglycemia and postprandial hyperglycemia typically occur.[1–4] PEM affects the prognosis by increasing the risk of cirrhosis-associated events and deteriorating the patient's quality of life (QoL), so nutritional treatment is absolutely crucial.[1–3]

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The treatment of hepatocellular carcinoma (HCC) has improved appreciably in the past 20–30 years. The current treatment for HCC with established efficacy is: (i) hepatectomy/liver transplantation; (ii) transcatheter arterial chemoembolization (TACE); (iii) percutaneous radiofrequency ablation (RFA); (iv) percutaneous ethanol injection; (v) percutaneous microwave coagulation therapy; and (vi) molecular-targeted therapy (e.g. sorafenib).[5–9] The most suitable treatment should be selected for individual patients based on thorough evaluation of HCC stage (tumor factor) and hepatic functional reserve.[5–10] In general, HCC develops after cirrhosis associated with viral hepatitis or alcoholic liver disease, so treatment of the underlying liver diseases is no less important than HCC treatment.[5–9,11] Preserving adequate hepatic reserves is necessary after HCC recurrence, which is quite frequent no matter how successful the initial radical treatment for HCC.[12–16]

Branched-chain amino acid (BCAA) granules (Livact; Ajinomoto Pharma, Tokyo, Japan) contain L-valine, L-leucine, and L-isoleucine at a ratio of 1.2:2:1. L-Leucine induces albumin synthesis in hepatic cells via transcription factors such as mammalian target of rapamycin.[1–3,17] BCAA granules were developed originally for the treatment of hypoalbuminemia associated

Table 1 Pharmacological effects of branched-chain amino acid granules

1. Improvement of hypoalbuminemia
2. Improvement of liver cirrhosis-related complications
3. Improvement of insulin resistance
4. Improvement of oxidative stress
5. Improvement of fatty acid metabolism
6. Activation of immune function
7. Suppression of angiogenesis
8. Suppression of liver carcinogenesis

with decompensated cirrhosis. However, subsequent studies found various other pharmacological actions of this drug. Therapy using BCAA granules improves hypoalbuminemia.[16–19] In addition, such therapy also inhibits cirrhosis-related complications such as esophageal varices and ascites,[17,18,20] reduces insulin resistance[17,21,22] and oxidative stress,[17,23] improves fatty-acid metabolism,[17,24] stimulates the immune system,[17,25,26] and inhibits angiogenesis.[17,21,27] The most noteworthy pharmacological action of BCAA granules, however, is the inhibition of hepatic carcinogenesis (Table 1).[17,19,20,22,27–29] Based on the significant inhibition of hepatic carcinogenesis observed after therapy using BCAA granules in patients with liver cirrhosis with a body mass index of 25 kg/m^2 or more shown in a multicenter, randomized, placebo-controlled study (the Lotus Study), the 2010 guidelines for comprehensive treatment of hepatitis virus-related cirrhosis in Japanese patients recommend the use of BCAA granules to preserve liver function and inhibit hepatic carcinogenesis.[16–19,28,30] Conversely, the American Society for Parental and Enteral Nutrition (ASPEN) and the European Society for Clinical Nutrition and Metabolism recommend that BCAA supplementation be carried out only in cirrhotic patients with chronic hepatic encephalopathy that is refractory to pharmacotherapy.[31,32]

Here, we review the clinical significance of therapy using BCAA granules in different treatment approaches for cirrhosis and HCC (i.e. hepatectomy, liver transplantation, RFA, TACE and molecular-targeted agents) mainly based on the published work as well as our own data published between 1997 and 2013. We searched the published work in the PubMed database, and the search strategy was based on the following terms: "branched-chain amino acid", "liver cirrhosis", "liver function", "complication", "clinical outcome", "carcinogenesis", "hepatocellular carcinoma", "recurrence", "hepatectomy", "liver transplantation", "RFA", "TACE" and "molecular-targeted therapy".

Significance of cirrhosis treatment with BCAA granules

In cirrhotic patients, the plasma level of BCAA is positively correlated with the serum albumin level. Such a correlation is seen only in patients with chronic liver diseases such as cirrhosis. The albumin–BCAA correlation and the inability of cirrhotic patients to maintain an adequate plasma level of BCAA with diet alone serve as the theoretical rationale for the use of BCAA granules for the treatment of cirrhosis. In cirrhotic patients, BCAA uptake in skeletal muscle is increased for ammonia detoxification and energy production and, in turn, the plasma level of BCAA and albumin production decrease.[1–3]

Yatsuhashi *et al.* conducted a prospective multicenter study in 204 patients with decompensated cirrhosis and reported a mean increase in the serum albumin level of 0.2 g/dL after 6 months of treatment with BCAA granules as well as a significant increase in the serum albumin level in patients with intake of a poor diet (poor intake of energy).[33] Therapy using BCAA granules also significantly decreased the incidence of ascites even in patients with an unchanged serum albumin level because of qualitative improvement of the serum albumin level (specifically, an increase in the level of reduced albumin and decrease in the level of oxidized albumin).[33–35]

The importance of treatment compliance was suggested in a study conducted by Takaguchi *et al.*[36] That prospective, large-scale, multicenter, observational study in 2894 patients with decompensated cirrhosis reported that the incidence of cirrhosis-associated events was decreased significantly in patients with good adherence to BCAA treatment compared with those with poor adherence. The authors emphasized the importance of thorough instruction regarding medications to patients.[36]

The appropriate timing of the initiation of BCAA treatment is controversial. The approved indication of BCAA granules in Japan is for the treatment of decompensated cirrhosis in patients with a serum albumin level of 3.5 g/dL or less, and the Japanese Nutritional Study Group for Liver Cirrhosis has also recommended that BCAA granules should be administrated in cirrhotic patients with a serum albumin level of 3.5 g/dL or less, Fisher's ratio of 1.8 or less and/or BCAA : tyrosine ratio (BTR) of 3.5 or less.[37] Hence, therapy using BCAA granules is, in general, started when the serum

albumin level is 3.5 g/dL or less in clinical settings.[11,37] However, earlier initiation of BCAA treatment has been attempted in cirrhotic patients with a serum albumin level of 3.6 g/dL or more. Habu *et al.* classified their patients into four treatment arms based on their serum albumin level and the BTR.[38] The decrease in the serum albumin level was inhibited after therapy using BCAA granules even in patients with a serum albumin level of 3.6 g/dL or more if their BTR was 4 or less, so the authors highlighted the usefulness of early intervention with BCAA granules.[38,39] A prospective, multicenter study in Japanese patients with hepatitis C virus-related decompensated cirrhosis with a serum albumin level of 3.6 g/dL or more complicated with insulin resistance (BCAA Granules for patients with Hepatitis C virus-related Liver Cirrhosis and Insulin Resistance on the Effect of Reduction of Carcinogenic Risk in the Liver [BLOCK] study, Japan Liver Oncology Group [JLOG] 1004 Trial) is ongoing. If the superiority of therapy using BCAA granules is demonstrated in that study, BCAA granules will become available for a wider range of cirrhotic patients.

As mentioned above, BCAA granules can inhibit hepatic carcinogenesis.[17,19,20,22,27–29] Several reports have focused on the usefulness of BCAA granules for the inhibition of liver carcinogenesis through improvement of insulin resistance.[17,21,22] Insulin and insulin-like growth factor (IGF) can promote the growth of HCC.[40] Kawaguchi *et al.* reported that BCAA granules suppress liver carcinogenesis through amelioration of insulin resistance via: (i) BCAA activation of the insulin signaling cascade through upregulation of phosphatidylinositol 3-kinase with reduction of serum insulin levels; and (ii) inhibition of the IGF/IGF-1 receptor axis by suppressing the expressions of IGF-1, IGF-2 and IGF-1 receptor mRNA.[17,41] They also reported that the improvement of insulin resistance by BCAA granules may be related to the migration of HCC, suppression of angiogenesis and epithelial–mesenchymal transition of hepatocytes, and that BCAA granules may inhibit liver carcinogenesis (at least in part) by reduction of oxidative stress and strengthening of immune functions.[17]

There are several reports of the usefulness of BCAA supplementation on the QoL of patients with liver cirrhosis.[42,43] Kawamura *et al.* demonstrated that, in 453 patients with chronic liver disease, QoL decreased significantly according to the progression of disease as assessed by the scores from Short Form 36 ($P < 0.05$) and that the QoL of patients with chronic liver diseases was improved in the BCAA granules administration group ($n = 13$) compared with the control group ($n = 12$) after 6 months.[42] Hepatic encephalopathy (HE) is a major complication in patients with liver cirrhosis that is related to a poor prognosis and poor QoL.[44] Sleep disturbance may be associated with minimal HE.[45] Les *et al.* conducted a randomized study involving 116 patients who had experienced an episode of HE (58 patients in the BCAA group and 58 patients in the maltodextrin group) to examine the effect of BCAA: they reported that supplementation with BCAA improves minimal HE and muscle mass.[43] Tryptophan, which is a precursor of the neurotransmitter 5-hydroxytryptamine (which is related to sleep disturbance), may be regulated by BCAA supplementation.[46]

With the wide range of pharmacological actions, such as increasing the serum albumin level,[16–19] inhibiting cirrhosis complications/angiogenesis/hepatic carcinogenesis,[17–20,22,27–29] improving insulin resistance[17,21,22] and fatty-acid metabolism,[17,24] reducing oxidative stress,[17,23] and increasing stimulation of the immune system,[17,25,26] therapy using BCAA granules may be an indispensable treatment for cirrhosis.

Significance of BCAA granules in different approaches to HCC treatment

Hepatectomy

Along with liver transplantation, hepatectomy is a curative treatment approach for HCC.[6,8,9,47–49] According to guidelines set by the European Association for the Study of the Liver (EASL), hepatectomy is indicated in patients with a single tumor of 2 cm or less in diameter, performance status (PS) 0, Child–Pugh class A and no portal hypertension.[50] In Japan, however, hepatectomy is considered in patients with three or less tumors of less than 3 cm in diameter, no vascular invasion, Child–Pugh class A or B, and expected tolerance to surgery, or even in those with four or more tumors of more than 3 cm in diameter and vascular invasion if they are expected to tolerate surgery and the treatment may improve the prognosis.[51] Hepatectomy is considered the first-line initial treatment for resectable HCC because of generally good surgical outcomes and poor availability of brain-dead liver donors in Japan.[52,53]

In HCC patients in whom a large volume of liver has been removed and in those with concurrent cirrhosis, the hepatic functional reserve is expected to decrease after resection. In several studies, the serum albumin level has been identified as a contributing factor for the prolonged postoperative survival time in HCC patients.[13,54–57] Thus, nutritional treatment with BCAA granules would be an essential approach based on this

observation as well as the fact that BCAA therapy prevents perioperative complications.

Togo *et al.* reported, in their study in 43 HCC patients with advanced cirrhosis, that post-hepatectomy treatment with BCAA granules inhibited the progression of cirrhosis and improved the prognosis.[58] The usefulness of oral nutritional supplements to prevent post-hepatectomy hepatic failure[59] and the usefulness of BCAA granules to inhibit postoperative HCC recurrence[29] have also been reported. Ichikawa *et al.* reported, in their prospective study in 56 HCC patients aged 65 years or more, that post-hepatectomy HCC recurrence was suppressed significantly and that the postoperative clinical course was more favorable in the BCAA treatment group ($n = 26$) compared with the regular-diet group ($n = 30$).[29]

Treatment with BCAA granules has appreciable clinical significance in HCC patients (especially those with underlying advanced cirrhosis) in terms of preserving hepatic functional reserve, preventing perioperative complications and inhibiting postoperative recurrence.

Liver transplantation

As an important choice of HCC treatment in western countries,[8,60,61] liver transplantation is considered even in patients with decompensated cirrhosis of various causes.[62] Assuming that the Milan criteria are satisfied, living donor partial liver transplantation for the treatment of decompensated cirrhosis complicated by HCC has been covered by the national health insurance system in Japan since 2004.[63] As described above, living donor liver transplantation is the major choice of treatment because of the shortage of brain-dead donors in Japan.[8,60,61,63,64]

The usefulness of BCAA granules in patients who have undergone liver transplantation has been reported in two studies.[65,66] In a prospective randomized study in 56 Child–Pugh class A cirrhotic patients without major complications, Kawamura *et al.* reported that early intervention with BCAA granules significantly decreased cirrhosis-related complications and prolonged the time to liver transplantation.[65] In a retrospective study in 236 patients who underwent living donor liver transplantation, Shirabe *et al.* reported a significant decrease in post-transplantation septic complications in patients pretreated with BCAA granules.[66] Considering the global shortage of liver donors,[6–9] BCAA granules could be a promising treatment for subjects undergoing liver transplantation.

Percutaneous treatment

Since its introduction in Japan in 1999, RFA has rapidly gained popularity because of its excellent antitumor effect and low extent of invasiveness. Percutaneous RFA is the first-line percutaneous treatment for HCC.[5–9,11,14,67–72] EASL guidelines recommend percutaneous RFA for HCC of PS 0–2, Child–Pugh class A or B, and three or less unresectable tumors of 3 cm or less in diameter. In Japan, percutaneous RFA is, in general, indicated for patients of Child–Pugh class A or B and three or less unresectable tumors of 3 cm or less in diameter. Even in patients with unresectable tumors of 3 cm or more in diameter, percutaneous RFA in combination with TACE is recommended to expand the ablated area.[50,51,73]

Percutaneous RFA is less invasive than hepatectomy, but hepatic functional reserve may decrease after RFA in some patients.[74–76] The possible causes of a postoperative decrease in the serum albumin level include: (i) decreased albumin synthesis secondary to hepatocyte decrease; (ii) inhibition of albumin synthesis by inflammatory cytokines; and (iii) loss of protein due to inflammation at the ablation site.[74–76] We reported the association between the serum albumin level and survival of HCC patients treated with percutaneous RFA, so therapy using BCAA granules may be a useful treatment for RFA-treated HCC frequently complicated by cirrhosis.[11,67]

One of the disadvantages of percutaneous RFA is the high prevalence of recurrence of HCC.[6,8,9,15,48,67] We found the prevalence of HCC 5 years after RFA to be approximately 80% even in patients with a single HCC.[67] The regimen to prevent HCC after RFA includes antiviral therapy (interferon therapy for hepatitis C and nucleoside analog therapy for hepatitis B) and liver-support therapy to keep the hepatic enzymes at a low level.[67,77–83] BCAA granules with potential anticarcinogenic effects may also be useful for preventing HCC recurrence post-RFA.[11,27]

Yoshiji *et al.* focused on the inhibitory action of BCAA granules and an angiotensin-converting enzyme inhibitor (ACE-I) against angiogenesis, and evaluated the effect of these agents in preventing post-RFA recurrence of HCC in a prospective randomized study.[27] The post-RFA prevalence of HCC and levels of vascular endothelial growth factor were decreased significantly in the combined BCAA granules and ACE-I treatment group compared with the control group, suggesting a possible synergistic effect of the two drugs to inhibit HCC recurrence after RFA.[27] Our retrospective controlled study in 256 HCC patients with a serum albumin level of

3.5 g/dL or less treated with percutaneous RFA showed significantly higher overall and recurrence-free survival in patients treated with BCAA granules ($n = 115$) compared with those receiving a regular diet ($n = 141$).[11] The use of BCAA granules was identified as a contributing factor to prolonged survival in a multivariate analysis.[11] The mechanism of the inhibitory effect of BCAA granules against HCC recurrence after RFA needs to be verified in a large-scale prospective study. BCAA granules may inhibit HCC recurrence in patients who have undergone percutaneous RFA as well as in those who have undergone hepatectomy.[11,29]

TACE

Transcatheter arterial chemoembolization is a combination of local chemotherapy through feeding blood vessels and the use of embolizing material.[16,84–87] TACE is most frequently used for the treatment of HCC in Japan, where it was originally developed.[84,87–90] EASL guidelines recommend TACE for unresectable, Child–Pugh class A or B multiple HCC with no vascular invasion, whereas in Japan the therapy is recommended even for HCC with vascular invasion if it is Vp1 or Vp2.[50,51]

The factors affecting the survival of HCC patients treated with TACE are: (i) tumor stage; (ii) tumor markers; and (iii) hepatic functional reserve.[84] Preserving hepatic functional reserve is a critical issue in HCC patients who, in general, are treated repeatedly with TACE.[16,88–92] However, in some patients, hepatic functional reserve decreases after TACE because of complications such as post-TACE syndrome.[93]

The usefulness of BCAA granules or BCAA-enriched "snacks" for patients with unresectable HCC treated with TACE has been suggested in several studies.[16,91,92] In a randomized controlled trial (RCT) in 56 HCC patients treated with TACE, Takeshita *et al.* found that the post-TACE decrease in liver function was suppressed significantly in patients who received an enteral nutritional formula for hepatic failure given as a late-evening snack (LES) compared with the control group.[91,94] Our retrospective controlled study in 99 HCC patients treated with TACE showed that therapy using BCAA granules significantly inhibited the decrease in hepatic functional reserve at 3 months and 6 months compared with the regular diet group.[16] According to EASL guidelines, if HCC with Child–Pugh class B treated with TACE recurs as Child–Pugh class C, TACE is not indicated for the recurred HCC. The significance of therapy using BCAA granules is considerable in terms of permitting repeated TACE.

Molecular-targeted drugs (sorafenib)

There had long been a lack of evidence to support systemic chemotherapy for unresectable advanced HCC.[95] However, after the efficacy of a molecular-targeted drug, sorafenib, for unresectable advanced HCC was demonstrated in two RCT (SHARP trial and Asia–Pacific trial), the drug was approved for the treatment of unresectable advanced HCC in Japan in 2009.[96,97]

The action of sorafenib against tumor growth and angiogenesis is based on the inhibition of the activities of intracellular kinase and receptor tyrosine kinase.[96–106] The new era of systemic chemotherapy for unresectable advanced HCC was started with the introduction of sorafenib.[96–103,106] EASL guidelines recommend sorafenib for unresectable, advanced, Child–Pugh class A or B HCC with PS 0–2 and vascular invasion or distant metastasis.[50] According to Japanese guidelines, sorafenib is recommended for unresectable, advanced, Child–Pugh class A HCC with vascular invasion or distant metastasis as well as for patients intolerant to TACE or in whom the procedure is anatomically unsuitable.[51,104,105]

Several cases of adverse events associated with the use of sorafenib have been reported.[96–106] Patients should be monitored carefully for hepatic dysfunction during sorafenib therapy because decreased hepatic reserve caused by sorafenib may result in irreversible hepatic failure.[102] Even if hepatic failure is avoided, sorafenib treatment may have to be discontinued or the dose reduced.[102] Many HCC patients treated with sorafenib have concurrent cirrhosis.[96–106] Hence, intervention with BCAA granules has appreciable importance in terms of preserving hepatic functional reserve and ensuring continued sorafenib treatment.[107] Our previous study revealed that therapy using BCAA granules significantly inhibited the decrease in serum albumin level and prolonged the duration of sorafenib treatment and survival in patients with a serum albumin level of 3.5 g/dL or less compared with the regular diet group.[107] The synergistic effect of sorafenib and therapy using BCAA granules to inhibit angiogenesis may have contributed to the better prognosis.

There remains a lack of evidence to support the effect of nutritional intervention in patients with unresectable advanced HCC treated with sorafenib. However, therapy using BCAA granules should be considered as a treatment option.

CONCLUSION

WE DISCUSSED THE significance of the use of BCAA granules in the treatment of cirrhosis and

Table 2 Summary of current knowledge of branched-chain amino acid granules for hepatocellular carcinoma (HCC) therapy

1. Prolongation of survival due to the improvement of hypoalbuminemia after HCC therapy
2. Improvement of liver cirrhosis-related complications after HCC therapy
3. Suppression of septic complications due to the activation of immune function after HCC therapy
4. Possibility of suppression of HCC recurrence after HCC therapy

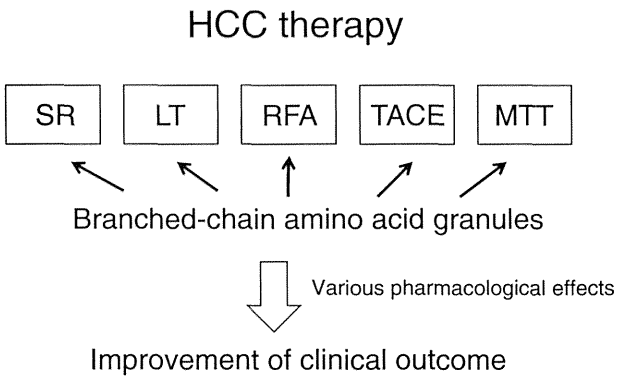

Figure 1 Schematic presentation of the effect of branched-chain amino acid granules for HCC therapy. HCC, hepatocellular carcinoma; LT, liver transplantation; MTT, molecular-targeted therapy; RFA, radiofrequency ablation; SR, surgical resection; TACE, transcatheter arterial chemoembolization.

HCC based on a review of the published work as well as our own data. With a variety of pharmacological actions, BCAA granules are a promising treatment for HCC. (Fig. 1) Summary of current knowledge of BCAA granules for HCC therapy is shown in Table 2.

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Research Paper

Sorafenib Therapy for BCLC Stage B/C Hepatocellular Carcinoma; Clinical Outcome and Safety in Aged Patients: A Multicenter Study in Japan

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Abstract

Background and aims: We aimed to compare clinical outcomes and safety after sorafenib therapy between patients with Barcelona Clinic Liver Cancer (BCLC) stage B or C hepatocellular carcinoma (HCC) aged ≥75 years (aged group, n=179) and those with BCLC stage B or C HCC aged <75 years (control group, n=279).

Patients and methods: We compared overall survival (OS), progression free survival (PFS), best treatment response and sorafenib related serious adverse events (SAEs) of grade 3 or more in the two groups. Furthermore, for reducing the selection bias, we compared clinical outcome of these two groups using propensity score matching analysis.

Results: The median OS and PFS intervals were 9.7 and 3.8 months in the aged group and 8.2 and 3.3 months in the control group (P=0.641 for OS and P=0.068 for PFS). Disease control rates were 49.2% (88/179) in the aged group and 49.1% (137/279) in the control group (P>0.999). Objective response rates were 15.1% (27/179) in the aged group and 14.3% (40/279) in the control group (P=0.892). Treatment related SAEs of grade 3 or more were observed in 51 patients (28.5%) in the aged group and in 69 patients (24.7%) in the control group (P=0.385). In the propensity score matched cohort (132 pairs), no significant difference in the two groups was observed in terms of OS (P=0.898) and PFS (P=0.407).

Conclusion: In BCLC stage B or C HCC patients treated with sorafenib, life expectancy, disease progression, treatment efficacy and SAEs are unaffected by age over 75 years.

Key words: Hepatocellular carcinoma, Sorafenib, Aged patients, Clinical outcome, Safety.

Introduction

Hepatocellular carcinoma (HCC) ranks fifth among the most prevalent deadly cancers in the world, and is the third most common cause of cancer related death. [1-4] The incidence of cancer has been reported to increase markedly with age, with >60% of all cancers developing in patients aged 65 years or more. [5] The risk of HCC development is known to be age dependent. [6] Thus, there will be an increasing

number of elderly HCC patients in the coming years owing to the increased longevity of the population. The current established HCC therapy includes surgical resection, liver transplantation, transcatheter arterial chemoembolization (TACE), ablative therapies such as radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI) and molecular targeted drug (sorafenib). [3]

Sorafenib, a multi-kinase inhibitor that blocks tumor growth and cell proliferation, was the first systemic chemotherapeutic drug found to improve the survival time of patients with advanced HCC both in the SHARP trial and the Asian Pacific trial. [7-9] Sorafenib has thus opened a novel era for the treatment of advanced HCC. In general, sorafenib therapy is indicated for patients with Barcelona Clinic Liver Cancer (BCLC) stage B HCC who are refractory to or had contraindications to locoregional therapies or for patients with BCLC stage C HCC. [7, 9]

As compared with younger patients, elderly patients generally have more comorbid diseases. Furthermore, in the management of elderly patients with advanced cancer, systemic chemotherapy is frequently either modified or withheld for fear of potential toxicities to chemotherapy. [10] To our knowledge, there have been few reports on clinical outcomes and safety in elderly HCC patients treated with sorafenib, although there have been several reports regarding patients treated with other therapies such as surgical resection, RFA, PEI and TACE. [11-32] Thus, there is urgent need for investigation of clinical outcomes and safety in elderly patients with HCC treated with sorafenib and this is a relevant topic for clinicians.

We have conducted a multicenter study of sorafenib therapy for HCC in Japanese Red Cross Liver Study Group. The aims of the present study were to evaluate clinical outcomes and safety after sorafenib therapy in BCLC stage B or C HCC patients aged ≥75 years as compared with BCLC stage B or C HCC patients aged <75 years. Furthermore, for reducing the selection bias, we compared clinical outcome of these two groups using propensity score matching analysis.

Patients and Methods

Patients

A total of 465 patients with unresectable HCC in the Japanese Red Cross Liver Study Group were treated with sorafenib between June 2008 and August 2013.

Our indications for sorafenib therapy were as follows: (1) Child-Pugh classification of A or B, (2) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, 1 or 2 (3) unresectable HCC determined by dynamic computed tomography (CT) scan and/or magnetic resonance imaging (MRI), (4) presence of extrahepatic metastases, (5) refractory to previous HCC therapies such as TACE and other locoregional therapies, (6) unsuitability for TACE for anatomical reasons, or (7) with vascular invasion such as portal vein tumor thrombus. However, in cases with Child-Pugh C in whom no other effective therapy could be adoptable, sorafenib was given according to decision by each attending physician after full explanation for sorafenib therapy. Of 465 patients, 7 patients had BCLC stage A or D. Thus, a total of 458 patients were analysed in the current analysis. They included 179 patients aged 75 years old or more (the aged group) and 279 patients aged less than 75 years old (the control group). We chose the cut off age of 75 years considering the aging population of HCC patients in our country. [12, 15-19, 29-32] In addition, in our country, patients aged ≥75 years are covered by a health insurance system which is different from that for patients aged <75 years. All patients analysed had at least one dose of sorafenib.

We compared the clinical outcomes and safety including overall survival (OS), progression-free survival (PFS), best response rate during follow-up period and SAEs of grade 3 or more between these two groups after sorafenib therapy. Prior to sorafenib therapy, written informed consent was obtained from all patients. The current study comprised a retrospective analysis of patient records and all treatments were conducted in an open-label manner. The ethics committees of all facilities that participated in this study approved the current study protocol and this study protocol complied with all of the provisions of the Declaration of Helsinki.

HCC diagnosis

HCC was diagnosed using abdominal ultrasound, 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 MRI. [33] In some patients, CT hepatic angiography (CTHA) and CT arterio-portography (CTAP) were performed to confirm HCC diagnosis. All patients were confirmed ineligible for surgery or locoregional therapies radiologically. In 90 patients (19.7%) out of 458 patients analysed in this study, percutaneous tumor biopsy was performed. They included Edmondson grade I (Ed. I) HCC in 29 patients, Ed. II HCC in 37, Ed. III HCC in 23 and mixed type HCC in one.

Sorafenib therapy

The recommended initial dose of sorafenib for HCC is 400 mg twice a day. [7-9] Nevertheless, ac-

cording to the phase-I study by Miller et al., patients with insufficient liver function or renal function (serum total bilirubin >1.5 mg/dL, serum albumin <2.5 g/dL and/or creatinine clearance <40 mg/mL) are recommended to receive a reduced dose of sorafenib. [34] Studies in several countries have reported SAEs in several individuals with advanced HCC given an initial dose of sorafenib of 800 mg/day, which led to treatment discontinuation. Moreover, in Japan, the proportion of the population with a body mass index (BMI) of ≥30 kg/m^2 has been reported to be lower than that in western countries and the recommended initial dose of sorafenib in elderly patients with advanced HCC is not well established because of paucity of available data. [35-37] Taking this information into consideration, the initial dose of sorafenib was determined according to factors such as body weight (BW), BMI, age, comorbid diseases, PS and liver function. Hence, the initial sorafenib dose in this study ranged from 200-800 mg/day. In cases of sorafenib related SAEs of grade 3 or more as defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), sorafenib dose was reduced by 200 to 400 mg/day at the discretion of attending physicians. Temporary treatment interruptions were also allowed. Sorafenib treatment continued until disease progression, unacceptable drug-related toxicity, or the patient's wish to discontinue treatment. Even if sorafenib therapy was discontinued, in patients who were potentially tolerable for other anti-cancer therapies, post-sorafenib therapies such as other systemic chemotherapies, TACE, transcathether arterial infusion chemotherapy and several ongoing clinical trials as second-line chemotherapy were considered.

Assessments of treatment efficacy and follow up

Best treatment efficacy of sorafenib during treatment was assessed in accordance with the modified Response Evaluation Criteria in Solid Tumors for Hepatocellular Carcinoma (mRECIST) criteria and/or tumor marker levels. [38] The treatment efficacy was classified as: (1) complete response (CR), (2) partial response (PR), (3) stable disease (SD) and progressive disease (PD). CR was defined as disappearance of any arterial enhancement within all target tumors. PR was defined as 30 % or greater decrease in tumor size as determined by evaluation of the sum of the diameters of the target tumors, whose size was estimated using unidirectional measurement. PD was defined as 20 % or greater increase in tumor size as determined by evaluation of the sum of the maximal dimensions of the target tumors. SD was defined as the absence of either PR or PD. [38] The objective response rate (ORR) was defined as the percentage of patients who had a best response rate of CR and PR. The disease control rate (DCR) was defined as the percentage of patients who had a best response rate of CR, PR and SD. Follow-up consisted of weekly or bi-weekly blood test analyses and physical examination at each visit.

Statistical analysis

Data were expressed as the median value (range) or the mean ± standard deviation (SD). Differences between the two groups were analyzed using the unpaired t test for continuous variables, and categorical variables were analyzed using Fisher's exact test. Data were analyzed using univariate and multivariate analysis. OS was defined as the interval between the date of sorafenib and the date of death from any cause or the last follow-up date. PFS was defined as the interval between the date of sorafenib and the date of disease progression or the last follow-up date. For analysis of OS, follow-up ended at the time of death from any cause, censoring the remaining patients at the last follow-up visit. For analysis of PFS, follow-up was terminated at the time of first radiologically confirmed tumor progression; other patients were censored at their last follow-up visit and at the time of death from any cause without tumor progression. The treatment duration of sorafenib was calculated from the date of treatment commencement until treatment termination or last follow-up date, including times of interruptions. The cumulative OS and PFS rates between the two groups were calculated using the Kaplan-Meier method, and tested using the log-rank test. The Cox proportional hazard model was used for multivariate analysis of factors with $P<0.1$ in univariate analysis. Values of $P<0.05$ were considered to be statistically significant.

Propensity score analysis

To overcome biases due to the different distribution of covariates between aged group and control group, a one-to-one match was created using propensity score analysis. [39] Clinical variables entered in the propensity model were gender, C-P score, cause of liver disease, BCLC stage, ECOG PS, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and initial dose of sorafenib. Subsequently, a one-to-one match between these two groups was obtained by using the nearest-neighbor matching method. [39]

Results

Clinical characteristics

The baseline clinical characteristics of the two groups (the elderly group [n=179] and the control group [n=279]) are shown in table 1. There were male

and Child-Pugh A predominance in both groups. There was a significantly lower positivity rate for hepatitis B surface antigen, poorer PS, lower BW, lower hemoglobin level, higher serum creatinine level and lower levels of hepatobiliary enzymes such as alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) in the aged group. In terms of previous therapies for HCC, TACE was most commonly performed procedure in both groups. Initial sorafenib dose of 800 mg/day was administered in 51 patients (28.5%) in the aged group and 132 patients (47.3%) in the control group (P<0.001), whereas initial dose of sorafenib based on BW tended to be lower in the aged group than in the control group (P=0.057). Regarding BCLC stage, no significant difference in the two groups was observed (P=0.921). Sixty six patients (36.9%) in the aged group and 129 patients (46.2%) in the control group had extrahepatic metastases with the most common sites being bone and lung.

Comparison of OS and PFS rates in the two groups

The median follow-up periods after sorafenib therapy were 7.5 months (range, 0.7-39.1 months) in the aged group and 7.6 months (range, 0.3-46.2 months) in the control group. The median OS intervals were 9.7 months (95% confidence interval [CI], 7.5-12.0 months) in the aged group and 8.2 months (95% CI, 6.9-9.6 months) in the control group (P=0.641). (Fig. 1) The median PFS intervals were 3.8 months (95% CI, 2.9-4.6 months) in the aged group and 3.3 months (95% CI, 3.0-3.6 months) in the control group (P=0.068). (Fig. 2)

Table 1. Baseline characteristics between the aged group and the control group.

Variables	Aged group (n=179)	Control group (n=279)	*P* value
Age (years)	79.4 ± 3.3	64.1 ± 7.6	<0.001[a]
Gender, male/female	136 / 43	233 / 46	0.053[b]
Body weight (kg)	56.6 ± 11.0	60.4 ± 12.0	0.001[a]
Child-Pugh A / B	152 / 27	222 / 57	0.174[b]
Causes of liver disease			
B/C/non B and non C/B and C	6 / 124 / 47 / 2	62 /140 / 74 / 3	<0.001[b]
BCLC stage B/C	63/116	100/179	0.921[b]
ECOG PS, 0/1/2	117/54/8	229/44/6	<0.001[b]
Portal vein invasion, yes/no	36/143	71/208	0.214[b]
Extrahepatic metastasis, yes/no	66/113	129/150	0.053[b]
Previous therapies for HCC, yes/no			
Transarterial chemoembolization	162/17	223/56	0.003[b]
Ablative therapies (RFA or PEI)	107/72	122/157	0.001[b]
Surgical resection	31/148	59/220	0.337[b]
AST (IU/L)	64.6 ± 57.1	70.3 ± 69.1	0.356[a]
ALT (IU/L)	43.9 ± 34.1	51.3 ± 43.6	0.054[a]
Total bilirubin (mg/dL)	0.92 ± 0.44	1.04 ± 1.87	0.386[a]
Albumin (g/dL)	3.48 ± 0.46	3.53 ± 0.53	0.309[a]
ALP (IU/L)[c]	434.1 ± 228.3	539.3 ± 445.9	0.004[a]
GGT (IU/L)[d]	103.7 ± 120.3	171.0 ± 194.8	<0.001[a]
LDH[e]	251.6 ± 75.9	254.8 ± 105.8	0.735[a]
Serum creatinine (mg/dL)[f]	0.97 ± 0.47	0.86 ± 0.64	0.036[a]
Prothrombin time (%)[g]	85.6 ± 19.4	86.8 ± 17.8	0.506[a]
Hemoglobin (g/dL)[h]	11.7 ± 1.9	12.4 ± 2.4	0.002[a]
Platelets ($\times 10^4$/mm^3)[i]	12.8 ± 5.7	13.5 ± 6.0	0.183[a]
AFP (ng/mL)[j]	14679 ± 111897	12459 ± 56756	0.773[a]
DCP (mAU/mL)[k]	21678 ± 142635	18709 ± 63674	0.796[a]
Initial dose of sorafenib (mg/day)			
800mg/600mg/400mg/200mg	51 / 0 / 120 / 8	132 / 2 / 134 / 11	<0.001[b]
Initial dose of sorafenib based on BW (mg/kg)	9.2 ± 3.9	9.9 ± 3.9	0.057[a]

Data are expressed as number or mean ± standard deviation. BCLC; Barcelona Clinic Liver Cancer, ECOG PS; Eastern Cooperative Oncology Group Performance Status, HCC; hepatocellular carcinoma, RFA; radiofrequency ablation, PEI; percutaneous ethanol injection, AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase, GGT; gamma glutamyl transpeptidase, LDH; lactose dehyrogenase, AFP; alpha-fetoprotein, DCP; des-γ-carboxy prothrombin, BW; body weight, [a] unpaired t test, [b] Fisher's exact test, [c] missing values, n=9, [d] missing values, n=8, [e] missing values, n=19, [f] missing values, n=1, [g] missing values, n=5, [h] missing values, n=1, [i] missing values, n=1, [j] missing values, n=9, [k] missing values, n=17