Clinical implication of the preoperative GSA index in ^{99m}Tc-GSA scintigraphy in hepatitis C virus-related hepatocellular carcinoma

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Abstract. We aimed to examine the relationship between the preoperative GSA index [uptake ratio of the liver to the liver plus heart at 15 min (LHL15) to uptake ratio of the heart at 15 min to that at 3 min (HH15) ratio] calculated from ^{99m}Tc-labeled diethylene triamine pentaacetate-galactosyl human serum albumin (^{99m}Tc-GSA) scintigraphy and background liver fibrosis and to investigate whether the GSA index can be a useful predictor in hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) patients treated with surgical resection (SR). A total of 213 HCV-related HCC patients were analyzed. Receiver operating characteristic (ROC) curve analysis was performed for calculating the area under the ROC (AUROC) for nine noninvasive parameters including GSA index, indocyanine green retention at 15 min, aspartate aminotransferase (AST) to platelet ratio index, FIB-4 index, AST to alanine aminotransferase ratio, serum albumin, total bilirubin, platelet count and prothrombin time for cirrhosis. We also examined predictive factors associated with overall survival (OS) and recurrence-free survival (RFS) after SR in univariate and multivariate analyses. There were 153 males and 60 females with the mean age of 69.9 years. The median observation periods were 2.8 years. The mean maximum tumor size was 4.1 cm. HH15 ranged from 0.452 to 0.897. LHL15 ranged from 0.669 to 0.982. The mean value of the GSA index was 1.41. Among the nine parameters, the GSA index yielded the highest AUROC for cirrhosis with a level of 0.786 at an optimal cut-off value of 1.37 (sensitivity, 65.9%; specificity, 79.0%). In multivariate analyses, the GSA index was an independent predictor (P<0.001) linked to RFS and it had a marginal significance in terms of OS (P=0.074). In conclusion, the preoperative GSA index can be a useful predictor in HCV-related HCC patients treated with SR.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related death (1-3). In Japan, most HCC cases are due to chronic hepatitis C virus (HCV) infection (3). Curative therapies for HCC consist of liver transplantation, surgical resection (SR) and radiofrequency ablation (RFA) (1-3). The clinical outcome of HCC patients undergoing these therapies has improved substantially in recent years due to treatment advances. However, HCC often recurs even after curative therapies, leading to high mortality, and the pattern of HCC recurrence is frequently ectopic as well as local. The identification of predictive factors and effective management of HCC recurrence are essential for improving survival, even after curative treatment (1-5).

^{99m}Tc-labeled diethylene triamine pentaacetate-galactosyl human serum albumin (^{99m}Tc-GSA) is a radiopharmaceutical that binds specifically to the hepatic asialoglycoprotein receptor (ASGP-R). Expression of ASGP-R has been reported to be decreased in patients with chronic liver damage and thus it has been widely used to assess liver functional reserve in various pathological and pharmacological states (6-8). In clinical field practice, receptor index (uptake ratio of the liver to the liver plus heart at 15 min; LHL15) and blood clearance index (uptake ratio of the heart at 15 min to that at 3 min; HH15) characteristics are frequently used for this purpose (6,7,9,10). On the other hand, indocyanine green retention at 15 min (ICG15) is an easy and convenient method for obtaining parameters to determine the appropriate and safe extent of liver resection (11). However, in patients with jaundice or when a porto-systemic shunt is present, the results of ICG15 are not reliable. In addition, discrepancies between ICG clearance and the extent of liver fibrosis are occasionally noted in such cases (8,12). ICG mainly reflected hepatic blood flow, while GSA was associated with both the amount of functional hepatocytes and blood flow (6,7,9-11).

Recently, Yoshizumi *et al* demonstrated the clinical significance of blood appearance corrected hepatic uptake ratio (LHL15 to HH15 ratio; GSA index) as an index of liver functional reserve in patients treated with living donor liver

transplantation (13). However, to the best of our knowledge, there have been no reports regarding GSA index on clinical outcome in HCV-related HCC patients treated with SR. Furthermore, although there has been a substantial drive to noninvasive assessment of liver fibrosis particularly for the grading of severity of chronic hepatitis C (CHC), the relationship between GSA index and the extent of liver fibrosis in patients with CHC is unclear. The aims of the present analysis were thus to examine the relationship between preoperative GSA index calculated from ^{99m}Tc-GSA scintigraphy and background liver fibrosis in non-tumor parts obtained from extracted surgical specimens and to investigate whether the preoperative GSA index can be a useful predictor in HCV-related HCC patients treated with SR.

Patients and methods

Patients. Between March 2004 and April 2014, a total of 213 treatment-naïve HCV-related HCC patients in whom preoperative ^{99m}Tc-GSA scintigraphy was performed received SR at our institution with curative intent and they were thus analyzed. Curative surgery was defined as resection of all tumors detectable using imaging modalities. HCV-related HCC was defined as HCC positive for HCV antibody and negative for hepatitis B surface antigen. A diagnosis of diabetes mellitus was based on past medical history or 75-g oral glucose tolerance test results (14). We examined predictive factors associated with overall survival (OS) and recurrence-free survival (RFS) after SR 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.

^{99m}Tc-GSA scintigraphy and calculated scores. Three milligrams of Tc-GSA (185 MBq; Nihon Medi-Physics, Nishinomiya, Japan) was injected as a bolus into an antecubital vein. Dynamic imaging was performed in the supine position under a gamma camera with a large-field-of view. Digital images were acquired at a rate of 30 sec/frame. Static anterior abdominal images were obtained at 5, 10, 15, 20, 25 and 30 min after injection of Tc-GSA (15). LHL15 was calculated by dividing the radioactivity of the region of interest (ROI) of the liver by the radioactivity of the ROI of the liver and the heart 15 min after injection, and HH15 was calculated by dividing the radioactivity of the ROI of the heart 15 min after injection by that 3 min after injection (6). LHL15 to HH15 ratio (GSA index) was also calculated.

The aspartate aminotransferase (AST) to platelet ratio index (APRI) score was calculated using Wai's formula: (AST/upper limit of normal)/platelet count (expressed as platelets x 10^9/l) x 100 (16). The FIB-4 index was calculated using Sterling's formula as: age (years) x AST (IU/l)/platelet count (x10^9/l) x alanine aminotransferase (ALT) (IU/l)^{½} (17).

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 (18). 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 (19). All HCC was confirmed pathologically except for 19 cases with complete necrosis due to the 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 are 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 100 patients (46.9%) and non-anatomical SR was performed in 113 patients (53.1%) in the present study. Conventional open hepatectomy was performed in 166 patients (77.9%) and laparoscopic hepatectomy was performed in 47 patients (22.1%) in the present study.

Histological evaluation of extracted liver specimens. All extracted liver specimens were reviewed by a single pathologist in our hospital. Background liver fibrosis was staged as F0-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) (20).

Follow-up. Follow-up after each therapy consisted of periodic blood tests and monitoring of tumor markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), using chemiluminescent enzyme immunoassays (Lumipulse PIVKAII Eisai, Eisai, Tokyo, Japan). Dynamic CT scans and/or MRI were obtained every 2-4 months after each therapy. Chest CT, whole abdominal CT, brain MRI, and bone scintigraphy were performed when extrahepatic HCC recurrence was suspected. When HCC recurred, the most appropriate therapy for HCC recurrence was performed considering tumor status, liver function or performance status of 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 GSA index, ICG15, APRI, FIB-4 index, AST to ALT ratio, serum albumin, total bilirubin, platelet count and prothrombin time (PT) selecting the optimal cut-off value that maximized the sum of sensitivity and specificity for cirrhosis (F4). Time to recurrence was defined as the interval between initial therapy

Table I. Baseline characteristics of the patients with HCV-related hepatocellular carcinoma (N=213).

Variables	N=213
Age (years)	69.9±7.9
Gender, male/female	153/60
Body mass index (kg/m^2)	22.8±3.6
Diabetes mellitus, yes/no	47/166
HCC stage, I/II/III/IV	18/113/63/19
Maximum tumor size (cm)	4.1±2.3
Tumor number, single/multiple	123/90
AST (IU/l)	61.0±36.7
ALT (IU/l)	56.0±41.6
ALP (IU/l)	346.6±151.9
GGT (IU/l)	97.0±110.8
LHL15	0.898±0.058
HH15	0.657±0.094
GSA index	1.41±0.28
Serum albumin (g/dl)	3.8±0.5
Total bilirubin (mg/dl)	0.9±0.5
Prothrombin time (%)[a]	88.6±14.8
Platelets (x10^4/mm^3)	12.8±5.7
AFP (ng/ml)	2,180±11,580
DCP (mAU/ml)[b]	3,484±14,865
Histological findings (extracted surgical specimen)	
Background liver fibrosis, F4/3/2/1/0	132/34/19/27/1
Tumor-differentiation Well/moderate/poor/combined/necrosis	19/100/73/2/19

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; LHL15, uptake ratio of the liver to the liver plus heart at 15 min; HH15, uptake ratio of the heart at 15 min to that at 3 min; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; [a]missing data, n=1; [b]missing data, n=3. Combined means the combined type of HCC and cholangiocellular carcinoma. Necrosis means complete necrosis.

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 a statistically significant result.

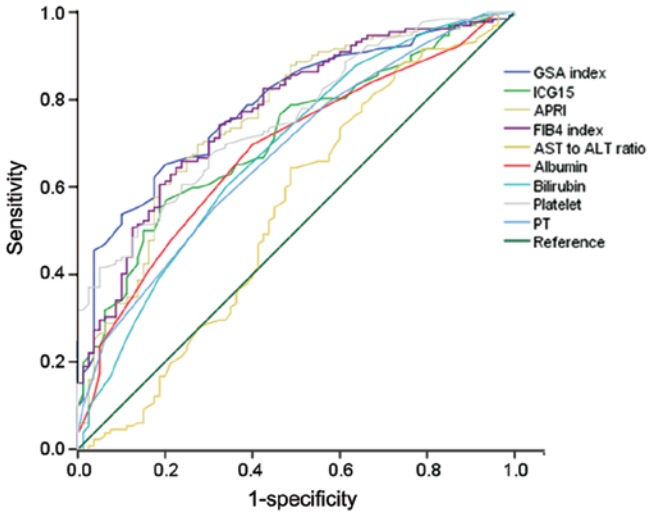

Figure 1. Correlation between GSA index and serum markers including indocyanine green retention at 15 min (ICG15), FIB-4 index, aspartate aminotransferase (AST) to platelet ratio index (APRI), AST to alanine aminotransferase (ALT) ratio, platelet count, serum albumin, total bilirubin and prothrombin time (PT) and cirrhosis (F4). GSA index yielded the highest AUROC with a level of 0.786 at an optimal cut-off value of 1.37 (sensitivity, 65.9%; specificity, 79.0%).

Results

Baseline characteristics. The baseline characteristics of the analyzed subjects (n=213) are shown in Table I. There were 153 males and 60 females with the mean (± SD) age of 69.9±7.9 years. The median observation periods were 2.8 years (range, 0.1-10.5 years). The mean maximum tumor size was 4.1±2.3 cm. HH15 ranged from 0.452 to 0.897. LHL15 ranged from 0.669 to 0.982. Thus, the mean value of the GSA index was 1.41±0.28. As for histological findings, in terms of the degree of liver fibrosis in the non-tumor portion, F4 was observed in 132 patients, F3 in 34, F2 in 19, F1 in 27 and F0 in 1, whereas in terms of HCC histology, well-differentiated HCC was observed in 19 patients, moderately differentiated HCC in 100, poorly differentiated HCC in 73, combined type of HCC and CCC in 2 and complete necrosis due to preoperative TACE in 19.

Comparison of area under receiver operating curves for GSA index and serum markers for cirrhosis. We evaluated the correlation between the GSA index and serum markers including ICG15, FIB-4 index, APRI, AST to ALT ratio, platelet count, serum albumin, total bilirubin and PT and cirrhosis (F4). Receiver operating curves of the serum markers used for predicting cirrhosis are demonstrated in Fig. 1. GSA index, ICG15, FIB-4 index, APRI and platelet count exhibited reliable discriminative ability for predicting cirrhosis. Among these, the GSA index yielded the highest AUROC with a level of 0.786 at an optimal cut-off value of 1.37 (sensitivity, 65.9%; specificity, 79.0%) (Table II). The GSA index in patients with cirrhosis (F4, n=132) was significantly lower than that in those with non-cirrhosis (F0-3, n=81) (P<0.001, Mann-Whitney U test) (Fig. 2). Between patients with F0 or 1 (n=28) and F4 (P<0.001), F2 (n=19) and F4 (P<0.001), F3 (n=34) and F4 (P<0.001), F0 or 1 and F2 (P=0.005) and F0 or 1 and F3

Table II. Comparison of the area under receiver operating curves (AUROCs) for the GSA index, ICG15, APRI, FIB-4 index, AST to ALT ratio, serum albumin, total bilirubin, platelet count and prothrombin time for cirrhosis.

Variables	AUROC	95% CI	P-value
GSA index	0.786	0.724-0.847	<0.001
ICG15	0.713	0.644-0.782	<0.001
APRI	0.761	0.693-0.828	<0.001
FIB-4 index	0.771	0.706-0.835	<0.001
AST to ALT ratio	0.542	0.458-0.627	0.304
Serum albumin	0.683	0.610-0.755	<0.001
Bilirubin	0.681	0.606-0.756	<0.001
Platelet	0.755	0.692-0.819	<0.001
Prothrombin time	0.676	0.603-0.749	<0.001

CI, confidence interval; ICG15, indocyanine green retention at 15 min; APRI, aspartate aminotransferase (AST) to platelet ratio index; ALT, alanine aminotransferase.

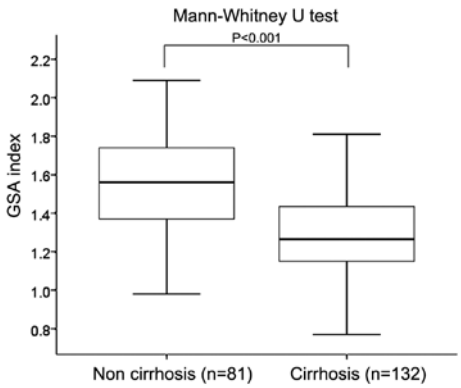

Figure 2. Box plots of the GSA index between patients with non-cirrhosis (F0-3) and those with cirrhosis (F4). The GSA index in patients with cirrhosis (n=132) was significantly lower than that in those with non-liver cirrhosis (n=81) (P<0.001, Mann-Whitney U test).

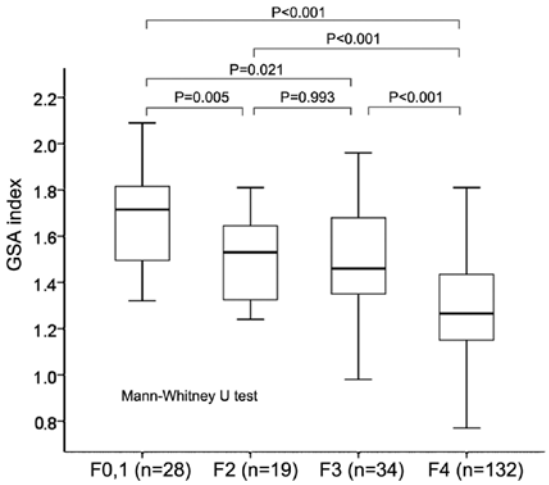

Figure 3. Box plots of the GSA index between patients with various stages of liver fibrosis [F0 or 1 (n=28), F2 (n=19), F3 (n=34) and F4 (n=132)]. GSA index had well discriminative ability between the various stages of liver fibrosis except for the relationship between F2 and F3.

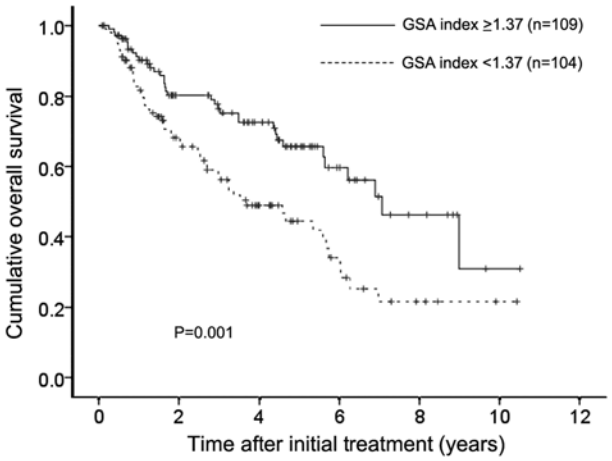

Figure 4. Cumulative overall survival (OS) rates according to the GSA index. The 1-, 3- and 5-year cumulative OS rates in patients with GSA index ≥1.37 (n=109) were 91.3, 76.5 and 65.6%, respectively, and the corresponding cumulative OS rates in patients with GSA index <1.37 (n=104) were 82.8, 57.6 and 44.5%, respectively (P=0.001).

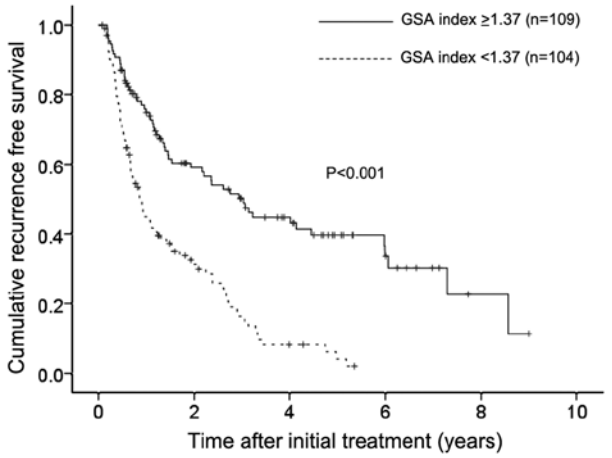

Figure 5. Cumulative recurrence-free survival (RFS) rates according to the GSA index. The 1-, 3- and 5-year cumulative RFS rates in patients with GSA index ≥1.37 (n=109) were 74.9, 50.2 and 39.7%, respectively, and the corresponding cumulative RFS rates in patients with GSA index <1.37 were 44.9, 16.3 and 4.1%, respectively (P<0.001).

(P=0.021), significant differences were observed in terms of the GSA index (Fig. 3).

Cumulative OS and RFS rates according to GSA index. The 1-, 3- and 5-year cumulative OS rates in patients with GSA index ≥1.37 (optimal cut-off value) (n=109) were 91.3, 76.5 and 65.6%, respectively, and the corresponding cumulative OS rates in patients with GSA index <1.37 (n=104) were 82.8, 57.6 and 44.5%, respectively (P=0.001) (Fig. 4). The 1-, 3- and 5-year cumulative RFS rates in patients with GSA index ≥1.37 were 74.9, 50.2 and 39.7%, respectively, and the corresponding cumulative RFS rates in patients with GSA index <1.37 were 44.9, 16.3 and 4.1%, respectively (P<0.001) (Fig. 5).

Univariate and multivariate analyses of factors contributing to OS. Univariate analysis identified the following factors

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	153/60	0.639		
Age (years), ≥70 vs. <70	119/94	0.296		
Tumor number, single vs. multiple	123/90	0.001	0.715 (0.452-1.131)	0.152
Maximum tumor size (cm), ≥3.5 vs. <3.5	109/104	0.010	0.906 (0.554-1.483)	0.696
Microscopic vascular invasion, yes vs. no	72/141	0.020	0.660 (0.414-1.052)	0.081
AST (IU/l), ≥50 vs. <50	109/104	0.025	0.824 (0.523-1.300)	0.406
ALT (IU/l), ≥50 vs. <50	91/122	0.459		
ALP (IU/l), ≥320 vs. <320	109/104	0.008	0.906 (0.566-1.452)	0.683
GGT (IU/l), ≥70 vs. <70	104/109	0.482		
GSA index ≥1.37, yes vs. no	109/104	0.001	1.594 (0.957-2.658)	0.074
Serum albumin level (g/dl), ≥3.9 vs. <3.9	112/101	0.005	1.642 (0.996-2.705)	0.052
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	67/146	0.002	0.647 (0.409-1.024)	0.063
Platelet count ($x10^4/mm^3$), ≥12 vs. <12	105/108	0.475		
Prothrombin time (%), ≥88 vs. <88[b]	105/107	0.113		
Diabetes mellitus, yes vs. no	47/166	0.475		
Body mass index (kg/m^2), ≥23 vs. <23	99/114	0.562		
Serum AFP (ng/ml), ≥100 vs. <100	62/151	<0.001	0.623 (0.385-1.007)	0.053
DCP (mAU/ml), ≥100 vs. <100[c]	129/81	0.001	0.451 (0.259-0.788)	0.005

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=1; [c]missing data, n=3.

as significantly associated with OS for all cases (n=213): tumor number (P=0.001); maximum tumor size ≥3.5 cm (P=0.010); microscopic vascular invasion (MVI) (P=0.020); AST ≥50 IU/l (P=0.025); alkaline phosphatase (ALP) ≥320 IU/l (P=0.008); GSA index ≥1.37 (P=0.001); serum albumin ≥3.9 g/dl (P=0.005); total bilirubin ≥1.0 mg/dl (P=0.002); AFP ≥100 ng/ml (P<0.001); and DCP ≥100 mAU/ml (P=0.001) (Table III). The hazard ratios (HRs) and 95% confidence intervals (CIs) calculated using multivariate analysis for the 10 factors with P<0.05 in univariate analysis are detailed in Table III. Only the DCP value was found to be a significant predictor linked to OS in the multivariate analysis (P=0.005).

Univariate and multivariate analyses of factors contributing to RFS. Univariate analysis identified the following factors as significantly associated with RFS for all cases: tumor number (P<0.001); MVI (P=0.002); AST ≥50 IU/l (P=0.010); ALP ≥320 IU/l (P=0.008); GSA index ≥1.37 (P<0.001); serum albumin ≥3.9 g/dl (P=0.016); total bilirubin ≥1.0 mg/dl (P=0.001); and PT ≥88% (P=0.023) (Table IV). The HRs and 95% CIs calculated using multivariate analysis for the eight factors with P<0.05 in univariate analysis are detailed in Table IV. Tumor number (P=0.002), MVI (P=0.002), ALP ≥320 IU/l (P=0.039) and GSA index (P<0.001) were found to be significant prognostic factors linked to RFS.

Causes of death. In patients with preoperative GSA index ≥1.37 (n=109), 35 patients (32.1%) died during the follow-up period. The causes of death were HCC recurrence in 26 patients, liver failure in 6 patients and miscellaneous causes in 3 patients, while in patients with preoperative GSA index <1.37 (n=104), 54 patients (51.9%) died during the follow-up period. The causes of death were HCC recurrence in 34 patients, liver failure in 10 patients and miscellaneous causes in 10 patients.

HCC recurrence. In patients with preoperative GSA index ≥1.37, 59 patients (54.1%) had HCC recurrences during the follow-up period. Nineteen patients (17.4%) had late first confirmed HCC recurrence (≥2 years after initial SR). The patterns of HCC recurrence after initial treatment were: single HCC recurrence in the liver in 23 patients; multiple HCC recurrences in the liver in 27 patients; multiple HCC recurrences in the liver with lung metastases in 2 patients; multiple bone metastases in 2 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 recurrence in the liver with right atrium 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 7 patients; RFA in 23 patients; percutaneous ethanol injection (PEI) in one patient; TACE in 19 patients; systemic chemotherapy such as sorafenib in 2 patients; radiation therapy in 2 patients and no specific treatment in 5 patients.

In patients with preoperative GSA index <1.37, 87 patients (83.7%) had HCC recurrences during the follow-up period. Twenty patients (19.2%) had late first confirmed HCC

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

Variables	n	Univariate analysis	Multivariate analysis	
			Hazard ratio (95% CI)	P-value[a]
Gender, male vs. female	153/60	0.733		
Age (years), ≥70 vs. <70	119/94	0.560		
Tumor number, single vs. multiple	123/90	<0.001	0.581 (0.410-0.823)	0.002
Maximum tumor size (cm), ≥3.5 vs. <3.5	109/104	0.251		
Microscopic vascular invasion, yes vs. no	72/141	0.002	0.567 (0.395-0.815)	0.002
AST (IU/l), ≥50 vs. <50	109/104	0.010	0.945 (0.663-1.345)	0.753
ALT (IU/l), ≥50 vs. <50	91/122	0.141		
ALP (IU/l), ≥320 vs. <320	109/104	<0.001	0.683 (0.475-0.982)	0.039
GGT (IU/l), ≥70 vs. <70	104/109	0.483		
GSA index ≥1.37, yes vs. no	109/104	<0.001	2.379 (1.594-3.550)	<0.001
Serum albumin level (g/dl), ≥3.9 vs. <3.9	112/101	0.016	1.056 (0.730-1.529)	0.771
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	67/146	0.001	0.840 (0.587-1.203)	0.342
Platelet count ($x10^4/mm^3$), ≥12 vs. <12	105/108	0.050		
Prothrombin time (%), ≥88 vs. <88[b]	105/107	0.023	1.064 (0.751-1.508)	0.727
Diabetes mellitus, yes vs. no	47/166	0.664		
Body mass index (kg/m^2), ≥23 vs. <23	99/114	0.662		
Serum AFP (ng/ml), ≥100 vs. <100	62/151	0.201		
DCP (mAU/ml), ≥100 vs. <100[c]	129/81	0.118		

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=1; [c]missing data, n=3.

recurrence (≥2 years after initial SR). The patterns of HCC recurrence after initial treatment were: single HCC recurrence in the liver in 37 patients; multiple HCC recurrences in the liver in 43 patients; multiple HCC recurrences in the liver with lung metastases in 3 patients; multiple bone metastases in one patient; multiple lung metastases in one patient; multiple HCC recurrences in the liver with lymph node metastases 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 3 patients; RFA in 38 patients; PEI in 2 patients; TACE in 29 patients; systemic chemotherapy such as sorafenib in 4 patients; radiation therapy in one patient and no specific treatment in 10 patients.

Discussion

To the best of our knowledge, this is the first reported study to examine the relationship between preoperative GSA index calculated from ^{99m}Tc-GSA scintigraphy and liver fibrosis and clinical outcomes in HCV-related HCC patients treated with SR. Although several noninvasive serum markers such as ICG15, FIB-4 index and APRI are associated with clinical outcomes in HCV-related HCC patients, no reports have assessed the impact of preoperative GSA index on clinical outcomes in HCV-related HCC patients treated with SR (21-24). Hence, we conducted the current analysis.

In the present study, the GSA index yielded the highest AUROC for cirrhosis and in multivariate analyses, GSA index was an independent predictor (P<0.001) linked to RFS and it had a marginal significance in terms of OS (P=0.074). Our results suggest that the preoperative GSA index well reflects hepatic functional reserve and is a useful predictor of clinical outcomes in HCV-related HCC patients treated with SR. Yoshizumi *et al* demonstrated that the 6-month survival probability was improved in the group with a GSA index ≥1.3 in patients who underwent liver transplantation, whereas our optimal cut-off value of the GSA index according to ROC analysis was 1.37 (13). Our results were consistent with their results. As mentioned earlier, ICG mainly reflected hepatic blood flow, while GSA was related to the amount of functional hepatocytes as well as blood flow. As shown in our results, the GSA index can reflect the liver fibrosis more accurately than ICG15.

On the other hand, FIB-4 index and APRI exhibited highly discriminative ability for predicting cirrhosis in our analysis. Several investigators demonstrated that FIB-4 and ARPI are useful noninvasive serum markers for predicting liver fibrosis in patients with CHC (25-28). In addition, a recent meta-analysis regarding diagnostic accuracy of FIB-4 and APRI in patients with chronic hepatitis B infection showed that the mean AUROCs of FIB-4 and APRI for predicting cirrhosis were 0.78 and 0.72, while our data of FIB-4 and APRI were 0.771 and 0.761. Although the causes of liver diseases were different between their data and ours, our results were similar to their reports (29).

In our analysis, as demonstrated in Fig. 3, the GSA index had well discriminative ability between various stages of liver fibrosis except for the relationship between F2 and F3. The

reason why the GSA index did not show well discriminative ability between patients with F2 and F3 is unclear, however, the small sample size in patients with F2 (n=19) may be attributed to our current results.

Liver biopsy, which has been considered as the 'golden standard' for assessing the extent of liver fibrosis, carries some drawbacks: sampling error and interobserver variability, which have raised questions on its value, whereas in our present analyses, we investigated the impact of the preoperative GSA index on cirrhosis using non-tumor parts of extracted surgical specimens, which had sufficient amount of liver specimens for exact evaluation of the degree of liver fibrosis (30-32). Thus, our data are highly reliable and this is a major strength of the present study.

The presence of MVI was a significant factor linked to RFS and it had a tendency toward poorer OS in our multivariate analyses. Postoperative factors as well as preoperative factors may be essential for predicting survival. Indeed, Lim *et al* reported that MVI is a better predictor of HCC recurrence and OS after SR for HCC (33). On the other hand, it is of interest that a higher ALP value was significantly linked to higher HCC recurrence in multivariate analysis. Cumulative evidence derived from Asian populations with HCC revealed that a higher ALP level was associated with poor outcomes, which is in line with the present study results (34).

We acknowledge several limitations to the present study. First, the present study was a retrospective observational study with heterogeneous HCC patients with various HCC stages. Second, postoperative therapy such as interferon was not included in our analysis, leading to bias. Third, subjects in whom ^{99m}Tc-GSA scintigraphy prior to surgery was not performed were excluded from our analysis (data not shown) and whether ^{99m}Tc-GSA scintigraphy was performed or not before SR mainly depends on the decision of attending surgeons in our hospital, also leading to bias. Thus, a well characterized study will be needed in the future. However, the present study results demonstrated that the preoperative GSA index well reflected the extent of liver fibrosis and it is closely associated with clinical outcomes in patients with HCV-related HCC treated with SR.

In conclusion, the preoperative GSA index calculated from ^{99m}Tc-GSA scintigraphy can be a useful predictor for patients with HCV-related HCC treated with SR.

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