Table 2. Univariate Analysis of Predictive Values (the Selected 14 *N*-Glycans) of Patient Survival (PS) and Disease-Free Survival (DFS)

		(n)	PS Hazard Ratio	PS *P*-value	DFS Hazard Ratio	DFS *P*-value
G2032	Low	206	1	0.9362	1	0.1054
	High	163	1.017		1.243	
G2890	Low	152	1	<0.0001	1	0.0001
	High	217	3.044		1.705	
G1793	Low	112	1	0.6829	1	0.2897
	High	257	1.095		1.168	
G1708	Low	145	1	0.0016	1	0.0043
	High	224	2.017		1.485	
G1870	Low	151	1	0.5552	1	0.4008
	High	218	1.132		1.122	
G1955	Low	113	1	0.4213	1	0.795
	High	256	1.2		1.038	
G3195	Low	206	1	<0.0001	1	0.0001
	High	163	3.238		1.662	
G3560	Low	246	1	<0.0001	1	<0.0001
	High	123	4.209		1.74	
G2114	Low	275	1	0.0056	1	0.1627
	High	94	1.776		1.232	
G1809	Low	238	1	0.0027	1	0.055
	High	131	1.824		1.306	
G3341	Low	188	1	<0.0001	1	0.0005
	High	181	3.185		1.592	
G1590	Low	167	1	0.0956	1	0.9102
	High	202	1.413		0.985	
G1362	Low	261	1	0.0399	1	0.0004
	High	108	1.526		1.634	
G3865	Low	192	1	<0.0001	1	0.0014
	High	177	3.145		1.532	

standard tests (χ^2, *t* test) where appropriate using StatView 5.0 for Windows (SAS Institute, Cary, NC). Significance was defined as $P < 0.05$.

Results

Profiling of Human Serum Glycoforms and ROC Analysis in HCC Patients and Normal Controls. *N*-glycan profiles of blood samples from our HCC cohort were obtained by MALDI-TOF MS analysis using the high-throughput features of the instrument. We thereby identified 67 *N*-glycans from which we selected molecules that showed statistical differences by ROC analysis between HCC and disease-free individuals (normal controls, NC) comprising living related liver transplantation donors. Glycans with an AUC value greater than 0.80 were selected for analysis (Table 1) and boxplots for these selected molecules (14 in total) are shown in Fig. 1. Clear differences in the distribution of these factors are evident between the NC and HCC patients. The cutoff values were determined using the maximum values for specificity plus sensitivity. G2890 was elevated more than a cutoff value in 305 (82.7%) of HCC patients and G3560 in 261 (70.7%).

Causes of Death. There were 115 deaths in total among our 369 HCC patient cohort (31.2%). The causes of death were as follows: HCC recurrence (n = 97; 84.3%), liver failure (n = 6; 5.2%), and other causes (n = 12; 10.4%).

Univariate Analysis and Multivariate Analysis of Overall Patient and Disease-Free Survival. The overall PS rates at 1, 3, and 5 years in our HCC cohort were 88.8%, 76.4%, and 67.6%, respectively. The DFS values for this groups at 1, 3, and 5 years were 64.0%, 35.5%, and 27.4%, respectively. The 14 serum N-glycans that were highly specific for HCC were evaluated for 3-year recurrence-free survival by ROC analysis to determine the cutoff values about these N-glycans. The patients were divided to two groups by these cutoff values. The PS and DFS measurements associated with the selected 14 selected *N*-glycans were evaluated by univariate analysis. The *P* values for the PS rates associated with G2890, G1708, G3195, G3560, G2114, G1809, G3341, G1362, and G3865 were all less than 0.05. The DFS *P* values for G2890, G1708, G3195, G3560, G3341, G1362, and G3865 were also less than 0.05 (Table 2). When clinical and tumor-associated factors were evaluated by univariate analysis, albumin, Child-Pugh classification,

Table 3. Univariate Analysis of Predictive Values (Clinical and Tumor Associated Factors) for Patient Survival (PS) and Disease-Free Survival (DFS)

		(n)	PS Hazard Ratio	PS *P*-value	DFS Hazard Ratio	DFS *P*-value
Sex	Male	301	1	0.7486	1	0.6535
	Female	68	0.913		0.943	
Age (years)	<=62	160	1	0.3272	1	0.6320
	62<	209	1.211		1.106	
HBV	Positive	176	1.259	0.1911	1.007	0.8093
	Negative	192	1		1	
HCV	Positive	119	1.291	0.2433	1.008	0.8183
	Negative	250	1		1	
Albumin (mg/dL)	<=4.05	147	2.128	<0.0001	1.626	0.0001
	4.05<	222	1		1	
Total bilirubin (mg/dL)	<=0.82	235	1	0.5831	1	0.5241
	0.82<	134	1.122		1.128	
ICGR15 (%)	<=16.7	223	1	0.1223	1	0.0106
	16.7<	146	1.349		1.375	
Child-Pugh	A	358	1	<0.0001	1	0.0374
	B	11	4.292		2.169	
Anatomical resection	Anatomical	282	1	0.8569	1	0.1435
	Nonanatomical	87	0.949		1.225	
AFP (ng/mL)	<=20	183	1	<0.0001	1	0.0008
	20<<=1000	115	2.395		1.449	
	1000<	71	4.433		1.870	
AFP-L3 (%)	<=15	255	1	<0.0001	1	0.0567
	15<	113	2.366		1.285	
PIVKA-II (mAU/mL)	<=40	109	1	<0.0001	1	0.0095
	40<<=1000	133	1.593		1.240	
	1000<	123	3.784		1.635	
Number	Single	235	1	<0.0001	1	<0.0001
	2,3	89	3.731		2.252	
	4<=	45	7.299		3.788	
Size (cm)	<=3	116	1	<0.0001	1	0.0086
	3<<=5	96	2.688		1.260	
	5<	157	4.049		1.570	
Differentiation	Well	17	1	0.0003	1	0.0002
	Moderately	190	2.568		2.990	
	Poorly	159	5.358		4.361	
Vp	Positive	94	4.630	<0.0001	2.156	<0.0001
	Negative	275	1		1	
Vv	Positive	35	5	<0.0001	1.969	0.0004
	Negative	334	1		1	
Macroscopic vascular invasion	Positive	48	6.135	<0.0001	1.961	<0.0001
	Negative	321	1		1	
Stage	1	26	1	<0.0001	1	<0.0001
	2	172	2.844		1.206	
	3	111	9.901		2.404	
	4A	60	15.625		3.106	
Noncancerous liver	Cirrhosis	120	1.199	0.3105	1.293	0.0398
	Noncirrhosis	249	1		1	

AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonism factor II; AFP-L3, lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; vp, microscopic tumor thrombus in the portal vein; vv, microscopic tumor thrombus in the hepatic vein; HBV, hepatitis B virus s antigen; HCV, anti-hepatitis C virus antibody; ICGR15, indocyanin green retention rate at 15 minutes.

AFP, AFP-L3 (lens culinaris agglutinin-reactive fraction of alpha-fetoprotein), PIVKA-II, tumor number, tumor size, differentiation, microscopic portal vein invasion, microscopic hepatic vein invasion, macroscopic vascular invasion, and stage were found to be significantly associated with the PS rate. When the same analysis was undertaken for the DFS rate by univariate analysis, albumin, indocyanin green retention rate at 15 minutes, Child-Pugh classification, AFP, PIVKA-II, tumor number, tumor size, differentiation, microscopic portal vein invasion, microscopic hepatic vein invasion, macroscopic vascular invasion, stage, and noncancerous liver were found to be significantly associated with this measure (Table 3).

The variable selection from 19 clinical and tumor-associated factors in Table 3 and the 14 serum

Table 4. Multivariate Analysis of Values That Is Predictive for Overall HCC Patient Survival

		P	Hazard Ratio	95% Confidence Interval	
ICGR15 (%)	16.7<	0.000209	2.435	1.5213	3.898
Child-Pugh	B	0.011136	3.007	1.2852	7.037
AFP (ng/mL)	20<<=1000	0.0003	2.558	1.5372	4.256
	1000<	0.000217	2.782	1.6177	4.786
Tumor number	2,3	0.011844	1.937	1.1575	3.241
	4<=	<0.0001	2.989	1.7693	5.049
Size (cm)	3<<=5	0.278625	1.483	0.7269	3.026
	5<	0.016071	2.237	1.1613	4.307
Vp	Positive	<0.0001	2.982	1.8446	4.822
C3560	>0.158	<0.0001	2.52	1.6191	3.923

ICGR15, indocyanin green retention rate at 15 minutes, AFP, alpha-fetoprotein; vp, microscopic tumor thrombus in the portal vein.

Table 5. Multivariate Analysis of Values That Are Predictive of Disease-Free Survival in HCC Patients

		P	Hazard Ratio	95% Confidence Interval	
ICGR15 (%)	16.7<	0.00334	1.519	1.149	2.008
AFP (ng/mL)	20<<=1000	0.04904	1.366	1.001	1.864
	1000<	0.01851	1.591	1.081	2.342
Tumor number	2,3	0.0072	1.551	1.126	2.135
	4<=	<0.0001	2.649	1.704	4.118
Differentiation	Moderately	0.01495	2.838	1.225	6.577
	Poor	0.00501	3.398	1.446	7.984
vp	Positive	0.01023	1.544	1.108	2.152
C2890	>1.12	0.01125	1.443	1.087	1.915

ICGR15, indocyanin green retention rate at 15 minutes, AFP, alpha-fetoprotein; vp, microscopic tumor thrombus in the portal vein.

N-glycans using the AIC was performed and the selected valuables were analyzed with PS and DFS by multivariate analysis. G3560 were found to be independent risk factors for PS (Table 4) and G2890 for DFS (Table 5).

The PS rates of HCC cases with low serum G3560 levels at 5 years were 80.5% and of high serum G3560 at 5 years were 40.4%. The DFS outcomes associated with low and high serum G2890 levels at 5 years were 21.3% and 35.1%, respectively (Fig. 2).

Relationship Between Clinical and Tumor-Associated Factors in HCC and Specific Glycans. Among the low and high G2890 HCC groups, there were significant differences found in a number of clinical and tumor-associated factors including albumin, Child-Pugh classification, AFP, PIVKA-II, tumor number, tumor size, microscopic portal vein invasion, microscopic hepatic vein invasion, macroscopic vascular invasion, and stage (Table 6). In comparing the low and high G3560 HCC patients, significant differences were found in albumin, Child-Pugh Classification, operative procedures, AFP, AFP-L3, PIVKA-II, tumor number, tumor size, differentiation profiles, microscopic portal vein invasion, microscopic hepatic vein invasion, macroscopic vascular invasion, and stage (Table 6).

Discussion

The *N*-glycan profiles of a large cohort of HCC patients were obtained in our current study by MALDI-TOF MS analysis and 67 of these molecules were thereby quantified. Of this group of factors, 14 *N*-glycans showed higher relative peaks in the HCC patients compared with normal controls and were

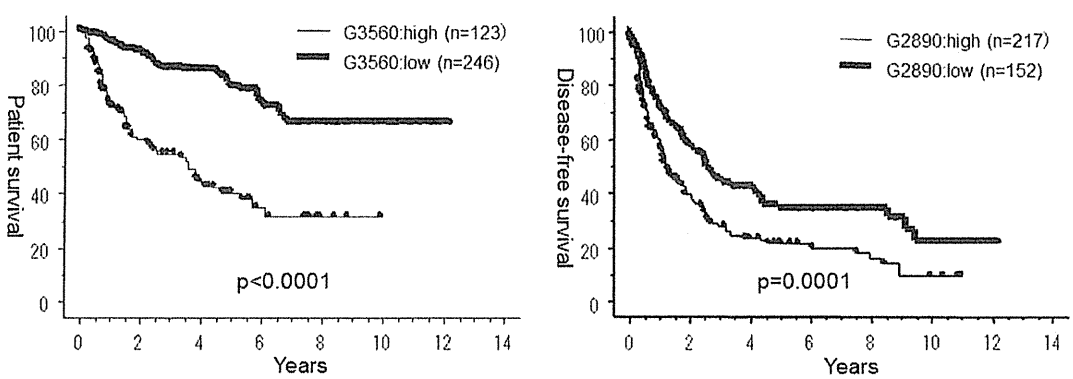

Fig. 2. The PS rates of HCC cases with low and high serum G3560 levels at 5 years were 80.5% and 40.4%, respectively. The DFS outcomes associated with low and high serum G2890 levels at 5 years were 21.3% and 35.1%, respectively.

Table 6. Correlation Between the G2890 and G3560 *N*-Glycans and Clinical and Tumor Associated Factors in HCC Cases

		G2890			G3560		
		High (n=217)	Low (n=152)	P	High (n=123)	Low (n=246)	P
Sex	Male	184	117	0.0767	105	196	0.2286
	Female	33	35		18	50	
Age	≦62	90	70	0.4433	49	111	0.393
	>62	127	82		74	135	
HBV	Positive	107	69	0.5254	59	117	0.9706
	Negative	110	83		64	129	
HCV	Positive	63	56	0.1425	32	87	0.0904
	Negative	154	96		91	159	
Albumin (mg/dL)	≦4.05	109	38	<0.0001	73	74	<0.0001
	>4.05	108	114		50	172	
Total bilirubin (mg/dL)	≦0.82	136	99	0.7088	82	153	0.4671
	>0.82	81	53		41	93	
ICGR15 (%)	≦16.7	125	98	0.2224	77	146	0.6246
	>16.7	92	54		46	100	
Child-Pugh	A	206	152	0.0034	115	243	0.008
	B	11	0		8	3	
Anatomical resection	Anatomical	172	110	0.1583	106	176	0.0028
	Nonanatomical	45	42		17	70	
AFP (ng/mL)	≦20	102	81	0.0461	52	131	<0.0001
	20< & ≦1000	64	51		30	85	
	>1000	51	20		41	30	
AFP-L3 (%)	≦15	143	112	0.1147	68	187	<0.0001
	>15	74	40		55	59	
PIVKA II (mAU/mL)	≦40	52	58	0.0001	22	88	<0.0001
	40< & ≦1000	74	60		33	101	
	>1000	91	34		68	57	
Number	Single	122	113	0.0009	68	167	<0.0001
	2, 3	60	29		27	62	
	≧4	35	10		28	17	
Size (cm)	≦3	48	68	<0.0001	15	101	<0.0001
	3< & ≦5	60	36		21	75	
	>5	109	48		87	70	
Differentiation	Well	12	8	0.0981	6	14	0.0003
	Moderately	102	88		46	144	
	Poorly	103	56		71	88	
vp	Positive	67	27	0.0065	49	45	<0.0001
	Negative	150	125		74	201	
vv	Positive	29	6	0.0043	24	11	<0.0001
	Negative	188	146		99	235	
Macroscopic vascular invasion	Positive	43	5	<0.0001	32	16	<0.0001
	Negative	174	147		91	230	
Stage	1	7	19	<0.0001	3	23	<0.0001
	2	88	84	45	127		
	3	71	40	35	76		
	4A	51	9	40	20		
Noncancerous liver	Cirrhosis	71	49	0.9876	35	85	0.2888
	Noncirrhosis	146	103		88	161	

AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonism factor II; AFP-L3, lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; vp, microscopic tumor thrombus in the portal vein; vv, microscopic tumor thrombus in the hepatic vein; HBV, hepatitis B virus s antigen; HCV, anti-hepatitis C virus antibody; ICGR15, indocyanin green retention rate at 15 minutes.

chosen for further analysis. These selected molecules were assessed for any correlation with surgical outcomes in the HCC cohort (i.e., prognosis and recurrence) by univariate and multivariate analysis. G3560 *N*-glycan was found to be a significant prognostic factor and G2890 *N*-glycan was found to be a significant recurrence factor for this disease. Moreover, G2890 and G3560 were found to strongly correlate with a number of well-known tumor-related prognostic and recurrent factors. These results show that quantitative glycoblotting based on whole serum *N*-glycan profiling is a potent screening approach for novel HCC biomarkers, and that the G3560 and G2890 *N*-glycans are promising biomarkers of the PS, DFS, and malignant behavior characteristics of HCC after hepatectomy.

Although glycans, once released from glycoproteins or glycopeptides, have been subjected to fluorescent labeling and purification for detection by high-performance liquid chromatography (HPLC) previously, this method is time-consuming and therefore not suited to clinical diagnosis. Our novel analytical method, which we refer to as glycoblotting, is far more rapid and accurate, as evidenced by the number of *N*-glycans detected in our current analysis. This chemoselective glycan enrichment technology known as glycoblotting was developed in our laboratory to purify oligosaccharides derived from glycoproteins in an effective and quantitative manner, thus enabling serum glycan profiling by way of a simpler method.[20] Our method is also applicable to the fully automated analysis of multiple samples simultaneously. It readily combines the isolation and labeling of oligosaccharides, which can then be subjected to conventional analytical methods including MS. We had already achieved high-speed quantitative and qualitative profiling of glycan expression patterns in biological materials using this technology. In our present study, we improved the method to allow quantitative analysis of high reproducibility and accuracy using a calibration curve of human serum standards. The analysis of the obtained 67 glycan profiles was performed using this new developed technology. The effectiveness of our method is evidenced by the identification of the G2890 and G3560 *N*-glycans as highly promising clinical markers of HCC associated with the PS, DFS, and tumor malignancy rates of these cancers.

It has been reported that AFP is the most significant tumor marker and independent predictor of prognosis for HCC,[26] even in patients who have received a hepatectomy.[27] Although high levels of AFP in cases of fully developed HCC, or in the serum of the host, are known to be associated with more aggressive behavior, and increased anaplasis,[28] AFP can also cause apoptosis in tumor cells.[29] Moreover, it has been suggested that AFP regulates the immune response and induces either stimulatory or inhibitory growth activity.[30] On the other hand, it is well known that AFP may increase in some patients with acute and chronic hepatitis without HCC,[31,32] and that the elevation of AFP correlates with inflammation of background disease and hepatocyte regeneration.[33] Hence, because the AFP profile does not always directly reflect the extent of tumor malignancy, the AFP levels do not influence patient survival and recurrence. On the other hand, AFP and many important tumor markers, such as carcinoembryonic antigen, carbohydrate antigen 125, and carbohydrate antigen 19-9, are glycoproteins, and this means that the glycan profiles in serum are altered by the onset of cancer. Indeed, the profiling of serum glycans has been performed previously as a screen for distinct potential glycan biomarkers of ovarian cancer and breast cancer.[18,19] Hence, we surmised that highly specific glycoprotein markers of HCC should be detected by monitoring the serum glycosylation profile in these patients. In glycan structure, both G2890 and G3560 are multiply branched (G2890 is tri-antennary and G3560 is tetra-antennary) glycans with a core fucose. In addition, both glycans have one nonsialylated branch, i.e., G2890 and G3560, are tri-antennary disialylated glycan, and tetra-antennary tri-sialylated glycan, respectively. The structure of G2890 and G3560 is quite different from the AFC-L3 (core fucosylated bi-antennary glycan) and CA19-9 (sialylated Lewis (a) antigen), which are well-known biomarkers related to HCC except for the core fucosylation.

There have been several previous studies of glycans in HCC. Kudo et al.[34] reported that *N*-glycan alterations are associated with drug resistance in HCC *in vitro*. In other reported clinical studies, only specific glycans have been assessed in relation to HCC. Vanhooren et al.[17] were the first to analyze the function of HCC-specific glycans, and reported that a triantennary glycan (NA-3Fb) correlated with the tumor stage and AFP levels in HCC patients. However, that study analyzed 44 patients with HCC but did not evaluate the relationship between the *N*-glycans and the clinical and pathological factors of this disease, the clinical course after hepatectomy, or prognosis and recurrence. In our current study, in contrast, we analyzed a far larger cohort than any other previous report, and evaluated a comprehensive panel of clinical and pathological parameters in relation to the *N*-glycan profile in HCC. Tang et al.[35] also described some HCC-specific glycans in their previous study that we did not find to be significant in our current analyses. This is likely due to the fact that the patient number in their study was smaller than ours, and the fact that the *N*-glycome profile in serum is gender- and age-dependent.[36] In this study, the mean age and the distribution of gender and infection of hepatitis B and C virus were the difference between NC and HCC patients. However, the selected 14 serum *N*-glycans were quantified by our MALDI-TOF MS analysis and compared with NC by ROC analysis. These were statistically different between HCC and NC with respect to the quantity. Because these 14 serum *N*-glycans of which the AUC values were greater than 0.80 were revealed to be specific for HCC, they had a high discriminating ability to differentiate HCC from NC. Further analyses are

required to determine whether G2890 and G3560 are elevated in patients with hepatitis B, hepatitis C, and/or cirrhosis without HCC.

The most important adverse prognostic factor for liver resection and transplantation in HCC has been found to be microscopic venous invasion.[5] However, microscopic portal invasion is not diagnosed preoperatively, and is revealed only by pathological examination. New biomarkers that are more strongly associated with prognosis and recurrence of HCC than AFP, AFP-L3, or PIVKA-II are therefore highly desirable. Our current data show that the *N*-glycans G2890 and G3560 correlate closely with well-known tumor-related prognostic and recurrent factors such as tumor number, size, microscopic portal vein invasion, microscopic hepatic vein invasion, differentiation, macroscopic vascular invasion, stage, AFP, AFP-L3, and PIVKA-II (Table 6). Moreover, when G2890 and G3560 were simultaneously included in multivariate analysis for PS and DFS with AFP, AFPL3 and PIVKA-II, *P*-values of G2890 and G3560 were lower than AFP, and AFPL3, and PIVKA-II were not selected as valuables by AIC. We demonstrate that these are novel independent prognostic factors for HCC that are related to the survival and recurrence of this disease and that show a lower *P*-value than other established tumor factors. Hence, we predict that G2890 and G3560 will prove to be markers that can preoperatively predict HCC tumor malignancy including microscopic portal vein invasion, and the PS and DFS rates more accurately and with more potency than the more well-known biomarkers.

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References

1. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer 2006;6:674-687.
2. Arii S, Yamaoka Y, Futagawa S, Inoue K, Kobayashi K, Kojiro M, et al. Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. HEPATOLOGY 2000;32:1224-1229.
3. Hasegawa K, Kokudo N, Imamura H, Matsuyama Y, Aoki T, Minagawa M, et al. Prognostic impact of anatomic resection for hepatocellular carcinoma. Ann Surg 2005;242:252-259.
4. Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Tahara M, Suzuki T, et al. Recurrence patterns after hepatectomy of hepatocellular carcinoma: implication of Milan criteria utilization. Ann Surg Oncol 2009;16:1560-1571.
5. Ikai I, Arii S, Kojiro M, Ichida T, Makuuchi M, Matsuyama Y, et al. Reevaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. Cancer 2004;101:796-802.
6. Shah SA, Cleary SP, Wei AC, Yang I, Taylor BR, Hemming AW, et al. Recurrence after liver resection for hepatocellular carcinoma: risk factors, treatment, and outcomes. Surgery 2007;141:330-339.
7. Imamura H, Matsuyama Y, Miyagawa Y, Ishida K, Shimada R, Miyagawa S, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. Br J Surg 1999;86:1032-1038.
8. Shimada M, Takenaka K, Fujiwara Y, Gion T, Kajiyama K, Maeda T, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein positive status as a new prognostic indicator after hepatic resection for hepatocellular carcinoma. Cancer 1996;78:2094-2100.
9. Shirabe K, Itoh S, Yoshizumi T, Soejima Y, Taketomi A, Aishima S, et al. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. J Surg Oncol 2007;95:235-240.
10. Esnaola NF, Lauwers GY, Mirza NQ, Nagorney DM, Doherty D, Ikai I, et al. Predictors of microvascular invasion in patients with hepatocellular carcinoma who are candidates for orthotopic liver transplantation. J Gastrointest Surg 2002;6:224-232; discussion 232.
11. Tamura S, Kato T, Berho M, Misiakos EP, O'Brien C, Reddy KR, et al. Impact of histological grade of hepatocellular carcinoma on the outcome of liver transplantation. Arch Surg 2001;136:25-30; discussion 31.
12. Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. Clin Gastroenterol Hepatol 2006;4:111-117.
13. Inoue S, Nakao A, Harada A, Nonami T, Takagi H. Clinical significance of abnormal prothrombin (DCP) in relation to postoperative survival and prognosis in patients with hepatocellular carcinoma. Am J Gastroenterol 1994;89:2222-2226.
14. Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol 2003;38:200-207.
15. Sumie S, Kuromatsu R, Okuda K, Ando E, Takata A, Fukushima N, et al. Microvascular invasion in patients with hepatocellular carcinoma and its predictable clinicopathological factors. Ann Surg Oncol 2008;15:1375-1382.
16. Kang P, Madera M Jr WRA, Goldman R, Mechref Y, Novotny MV. Glycomic alterations in the highly-abundant and lesser-abundant blood serum protein fractions for patients diagnosed with hepatocellular carcinoma. Int J Mass Spectrom 2011;305:185-198.
17. Vanhooren V, Liu XE, Franceschi C, Gao CF, Libert C, Contreras R, et al. N-glycan profiles as tools in diagnosis of hepatocellular carcinoma and prediction of healthy human ageing. Mech Ageing Dev 2009;130:92-97.
18. Kirmiz C, Li B, An HJ, Clowers BH, Chew HK, Lam KS, et al. A serum glycomics approach to breast cancer biomarkers. Mol Cell Proteomics 2007;6:43-55.
19. An HJ, Miyamoto S, Lancaster KS, Kirmiz C, Li B, Lam KS, et al. Profiling of glycans in serum for the discovery of potential biomarkers for ovarian cancer. J Proteome Res 2006;5:1626-1635.
20. Miura Y, Hato M, Shinohara Y, Kuramoto H, Furukawa J, Kurogochi M, et al. BlotGlycoABCTM, an integrated glycoblotting technique for rapid and large scale clinical glycomics. Mol Cell Proteomics 2008;7:370-377.
21. Nishimura S, Niikura K, Kurogochi M, Matsushita T, Fumoto M, Hinou H, et al. High-throughput protein glycomics: combined use of

chemoselective glycoblotting and MALDI-TOF/TOF mass spectrometry. Angew Chem Int Ed Engl 2004;44:91-96.
22. Furukawa J, Shinohara Y, Kuramoto H, Miura Y, Shimaoka H, Kurogochi M, et al. Comprehensive approach to structural and functional glycomics based on chemoselective glycoblotting and sequential tag conversion. Anal Chem 2008;80:1094-1101.
23. Ichida F, Tsuji T, Omata M, Ichida T, Inoue K, Kamimura T, et al. New Inuyama Classification; new criteria for histlogical assessment of chronic hepatitis. Int Hepatol Commun 1996;6:112-119.
24. The Liver Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. 3rd English ed. Tokyo, Japan: Kanehara & Co.
25. Akaike H. A new look at the statistical model identification. IEEE Trans Autom Control 1974;19:716-723.
26. Nomura F, Ohnishi K, Tanabe Y. Clinical features and prognosis of hepatocellular carcinoma with reference to serum alpha-fetoprotein levels. Analysis of 606 patients. Cancer 1989;64:1700-1707.
27. Hanazaki K, Kajikawa S, Koide N, Adachi W, Amano J. Prognostic factors after hepatic resection for hepatocellular carcinoma with hepatitis C viral infection: univariate and multivariate analysis. Am J Gastroenterol 2001;96:1243-1250.
28. Matsumoto Y, Suzuki T, Asada I, Ozawa K, Tobe T, Honjo I. Clinical classification of hepatoma in Japan according to serial changes in serum alpha-fetoprotein levels. Cancer 1982;49:354-360.
29. Yang X, Zhang Y, Zhang L, Mao J. Silencing alpha-fetoprotein expression induces growth arrest and apoptosis in human hepatocellular cancer cell. Cancer Lett 2008;271:281-293.
30. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. Exp Biol Med (Maywood) 2001;226:377-408.
31. Smith JB. Occurrence of alpha-fetoprotein in acute viral hepatitis. Int J Cancer 1971;8:421-424.
32. Silver HK, Gold P, Shuster J, Javitt NB, Freedman SO, Finlayson ND. Alpha(1)-fetoprotein in chronic liver disease. N Engl J Med 1974;291: 506-508.
33. Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R, Tomita K. Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. Oncology 2002;62(Suppl 1):57-63.
34. Kudo T, Nakagawa H, Takahashi M, Hamaguchi J, Kamiyama N, Yokoo H, et al. N-glycan alterations are associated with drug resistance in human hepatocellular carcinoma. Mol Cancer 2007;6:32.
35. Tang Z, Varghese RS, Bekesova S, Loffredo CA, Hamid MA, Kyselova Z, et al. Identification of N-glycan serum markers associated with hepatocellular carcinoma from mass spectrometry data. J Proteome Res 2010;9:104-112.
36. Ding N, Nie H, Sun X, Sun W, Qu Y, Liu X, et al. Human serum N-glycan profiles are age and sex dependent. Age Ageing 2011;40: 568-575.

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Original Article

Clinical usefulness of ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography for patients with primary liver cancer with special reference to rare histological types, hepatocellular carcinoma with sarcomatous change and combined hepatocellular and cholangiocarcinoma

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Aim: The role of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) in the diagnosis and staging of primary liver cancer has been demonstrated in several reports. However, no preoperative evaluations of sarcomatous hepatocellular carcinoma (HCC) and combined hepatocellular and cholangiocarcinoma (cHCC-CC) with FDG-PET have been reported so far.

Methods: Fifty-three HCC patients and three cHCC-CC patients who received liver resection or living-donor liver transplantation were enrolled in this study. All 56 patients had undergone preoperative FDG-PET, and a total of 67 HCC and three cHCC-CC were analyzed histologically. The relationship between clinicopathological features and the maximum standardized uptake value (SUVmax) of tumors were evaluated.

Results: The detection rate of HCC by FDG-PET was 43.3 %, and the sensitivity of FDG-PET for the detection of HCC was significantly associated with tumor differentiation, tumor size and microvascular invasion. All three cHCC-CC were detected by FDG-PET. The SUVmax values of the three sarcomatous HCC (SUVmax 14.1, 18.6 and 25.0) and the three cHCC-CC (SUVmax 9.9, 12.0 and 13.0) were higher than that of the poorly differentiated HCC (mean SUVmax 5.7 ± 2.3).

Conclusion: SUVmax may be a useful diagnostic tool for the preoperative evaluation of the aggressiveness of primary liver cancers such as sarcomatous HCC and cHCC-CC.

Key words: ^{18}F-fluorodeoxyglucose positron emission tomography, combined hepatocellular and cholangiocarcinoma, hepatocellular carcinoma, sarcomatous hepatocellular carcinoma

INTRODUCTION

POSITRON EMISSION TOMOGRAPHY (PET) using ^{18}F-fluorodeoxyglucose (FDG) has become standard procedure for the detection of a variety of malignant tumors.[1] It is considered a useful diagnostic tool for tumor characterization and assessing therapy response.[2] For hepatocellular carcinoma (HCC), however, several reports suggest that the sensitivity of FDG-PET (50–55%) is insufficient.[3,4] Because the enzymatic activity of well-differentiated HCC cells is similar to that of the surrounding normal liver, the accumulation of FDG in these tumors is low, and the role of FDG-PET imaging in the early detection of HCC is limited.[5] On the other hand, previous studies have demonstrated that FDG accumulation is increased in undifferentiated HCC, and recently, preoperative FDG-PET has been shown to be closely associated with tumor differentiation and prognosis in HCC patients.[6,7]

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The histological differentiation grade is an important prognostic factor for HCC.[8] Once cancer is established, HCC dedifferentiates to a more malignant histology in a multistep fashion, from well- and moderately to poorly differentiated tumors.[9] Although the prognosis of well-differentiated HCC is good following resection, poorly differentiated HCC have a poor prognosis due to a high rate of vascular invasion and metastasis.[10,11] The basic histological pattern of HCC is trabecular; however, a sarcomatous appearance has been sporadically reported as one of the histological features of HCC.[12] Approximately 1.8% of all resected HCC have a sarcomatous feature, usually associated with a very poor prognosis because of its rapid growth, low resectability and frequent recurrence after resection.[13,14]

Combined hepatocellular and cholangiocarcinoma (cHCC-CC) is a rare primary liver cancer that contains the histological features of both HCC and CC.[15] cHCC-CC has been reported to show frequent vascular invasion and lymph nodes metastasis, and has a poorer prognosis than HCC.[16,17] It is difficult for patients with cHCC-CC to get a correct preoperative diagnosis because of the lack of a sensitive diagnosis procedure.[18]

Although previous studies have shown that FDG-PET is useful for evaluating various liver tumors, there have been no reports regarding preoperative FDG uptake in resectable sarcomatous HCC and cHCC-CC. In the present study, we retrospectively investigated the feasibility of FDG-PET for the detection of different types of primary liver cancer including sarcomatous HCC and cHCC-CC.

METHODS

Patients

IN THIS STUDY, we retrospectively reviewed 53 HCC patients and three cHCC-CC patients who received liver resection (LR) or living-donor liver transplantation (LDLT) at Kyushu University Hospital between April 2010 and August 2011. There were 35 male and 21 female patients, and the mean age (± standard deviation [SD]) of the patients was 65 ± 12 years (range, 36–87). All 56 patients were diagnosed as having HCC or cHCC-CC by conventional radiologic imaging and FDG PET/computed tomography (CT). Thirteen patients with HCC in cirrhosis underwent LDLT, and the other 43 patients with HCC or cHCC-CC underwent LR. Among the HCC patients, 29 had a single lesion, and the other 24 had multiple lesions. Among the cHCC-CC patients, one had a single lesion and the other two had multiple lesions.

Patient follow up

After discharge, all patients were examined for recurrence by ultrasound and by tumor markers every 1–3 months. Dynamic CT was performed every 6 months. Patients with any sign of recurrence and/or inconclusive imaging studies underwent additional FDG PET/CT. All of the patients were followed up while they were alive.

FDG PET/CT

^{18}F-Fluorodeoxyglucose positron emission tomography studies were performed with Discovery ST Elite (GE Healthcare, Milwaukee, WI, USA) and Biograph mCT (Siemens AG, Erlangen, Germany) PET/CT scanners. All patients fasted for at least 4 h before FDG administration, and 185 MBq of FDG was i.v. administrated to each patient. Approximately 60 min after the FDG injection, whole-body PET images were acquired from thigh to head with 7–10 bed positions. The Discovery ST Elite scanner consists of a 16-slice multidetector CT and bismuth germanium oxide crystal. The unenhanced CT was performed first with the following parameters: 5-mm slice thickness, 120 kV, 30–250 mAs with auto mode (Smart mA). Then, PET images were obtained in 3-D mode for 3 min per bed position with a 3.27-mm slice thickness, at 70 cm field of view (FOV) in a 128 × 128 matrix. Based on the CT data, transmission maps were created and used for the attenuation correction of the PET images. The PET data were reconstructed using a 3-D ordered subset expectation maximization (3D-OSEM) algorithm (VUE Point Plus) with two iterations and 28 ordered subsets. A 6-mm post-filter of full-width at half maximum (FWHM) was applied. The Biograph mCT scanner is equipped with a 128-slice multidetector CT and lutetium crystal. The unenhanced CT was performed at 120 kV with automatic mAs adjustment (Care Dose 4D) and the slice thickness was 3 mm. The PET emission time was 2 min per bed position. The PET images were acquired with a 2-mm slice thickness, at 70 cm FOV in a 256 × 256 matrix. The concomitant CT data were used for attenuation correction. The PET data were reconstructed using a 3D-OSEM algorithm with two iterations and 21 subsets. Time of flight and point spread function techniques were also used for the image reconstruction (ultra-HD-PET). A 3-D Gaussian filter of 6-mm FWHM was applied. The PET images were qualitatively evaluated to assess whether the FDG uptake in the tumor was (PET positive status) or was not

(PET negative status) significantly higher than in the surrounding non-cancerous hepatic parenchyma.

Histopathological study

A total of 67 HCC and three cHCC-CC were evaluated histologically. Formalin-fixed specimens were embedded in paraffin. Deparaffinized 4-μm sections were stained with hematoxylin–eosin for microscopic evaluation. The histopathological definition of HCC and the criteria for cHCC-CC were based on the classification proposed by the World Health Organization. The cHCC-CC contain unequivocal hepatocellular and cholangiocellular components that are intimately admixed. The HCC displayed a trabecular pattern with little stroma, a pseudoglandular pattern with or without bile production, abundant eosinophilic cytoplasm, and immunoreactivity for Hep par 1. The CC was defined by a definite glandular pattern with fibrous stroma, low columnar cells with round vesicular nuclei, mucin production confirmed by Alcian blue, and immunoreactivity for cytokeratin 19 but not Hep par 1.

Statistical analysis

All statistical analyses were performed using the StatView ver. 5.0 software package. Continuous variables were compared using the Mann–Whitney *U*-test or Student's *t*-test. The χ^2-test was used for categorical variables. The differences were considered to be significant if $P < 0.05$.

RESULTS

Patients with HCC

PATIENT CHARACTERISTICS ARE summarized in Table 1(a). The mean age (± SD) was 66 ± 12 years (range, 36–87), and the sex ratio (M : F) was 32:21. Thirty-two patients (60.4%) were seropositive for hepatitis C virus, 11 for hepatitis B surface antigen (20.8%) and 10 (18.8%) had non-B/non-C etiologies. Twelve of the 53 patients had a serum α-fetoprotein (AFP) level of more than 100 ng/mL (median, 11.8; range, 1.6–994 600) and 24 patients had a serum des-γ-carboxy prothrombin (DCP) level above 100 mAU/mL (median, 81; range, 10–109 730). Twenty-nine patients with solitary tumors were divided into two groups: PET positive ($n = 16$) and PET negative ($n = 13$). Although there was no significant difference in serum AFP levels between the PET positive and negative groups (110.2 ± 196.9 and 132.9 ± 372.7 ng/mL, respectively), the PET positive group had higher serum

Table 1 Characteristics of patients with HCC and clinicopathological data of HCC

a. Characteristics of patients with HCC

Characteristic	No. of patients (%)
Total number of patients	53
Age (years)	
Mean (range)	66 (36–87)
Sex	
Male : female	32 (60.4):21 (39.6)
Etiology of liver disease	
Hepatitis B	11 (20.8)
Hepatitis C	32 (60.4)
Other	10 (18.8)
Child–Pugh classification	
A	40 (75.5)
B	6 (11.3)
C	7 (13.2)
Tumor stage (UICC)	
I	21 (39.6)
II	25 (47.2)
III	5 (9.4)
IV	2 (3.8)
Type of hepatic surgery	
Resection	40 (75.5)
Liver transplantation	13 (24.5)
Tumor number	
Solitary	29 (54.7)
Multiple	24 (45.3)
Preoperative serum AFP (ng/mL)	
Median (range)	11.8 (1.6–99 4600)
Preoperative serum DCP (mAU/mL)	
Median (range)	81 (10–109 730)

b. Clinicopathological data of HCC

Characteristic	No. of HCC (%)
Total number of nodules	67
Tumor differentiation	
Well	7 (10.4)
Moderately	47 (70.1)
Poorly	9 (13.4)
Undifferentiated	1 (1.5)
Moderately with sarcomatous change	1 (1.5)
Poorly with sarcomatous change	2 (3.0)
Tumor size (cm)	
Mean ± SD	3.4 ± 3.4
Microvascular invasion	16 (23.9)

AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; SD, standard deviation; UICC, Union for International Cancer Control.

Table 2 Association between PET status and clinicopathological data of HCC

Characteristic	PET negative ($n = 38$)	PET positive ($n = 29$)	*P*-value
Tumor differentiation (%)			<0.05
Well	7 (100)	0 (0)	
Moderately	31 (66)	16 (34)	
Poorly	0 (0)	9 (100)	
Undifferentiated	0 (0)	1 (100)	
Moderately with sarcomatous change	0 (0)	1 (100)	
Poorly with sarcomatous change	0 (0)	2 (100)	
Tumor size (cm)			
Mean ± SD	2.1 ± 1.5	5.1 ± 4.3	<0.05
Microvascular invasion (%)	4 (11)	12 (41)	<0.05

HCC, hepatocellular carcinoma; PET, positron emission tomography; SD, standard deviation; UICC, Union for International Cancer Control.

DCP levels than the PET negative group (529.6 ± 748.3 and 54.2 ± 50.7 mAU/mL, respectively; $P < 0.05$) (± SD). Using the modified Union for International Cancer Control staging system, we enrolled 21 (39.6%) stage I patients, 25 (47.2%) stage II patients, five (9.4%) stage III patients and two (3.8%) stage IV patients.

The characteristics of HCC are summarized in Table 1(b). The histological grades were well differentiated in seven HCC (10.4%), moderately differentiated in 47 (70.1%), poorly differentiated in nine (13.4%), undifferentiated in one (1.5%), moderately differentiated with sarcomatous change in one (1.5%) and poorly differentiated with sarcomatous change in two (3.0%). Mean tumor size (± SD) was 3.4 ± 3.4 cm, and microvascular invasion was observed in 16 HCC (23.9%). The detection rate of HCC by PET was 43.3%. The sensitivity of PET for the detection of HCC was significantly associated with tumor differentiation, tumor size and microvascular invasion (Table 2). None of the seven well-differentiated HCC were detected by PET. The mean maximum standardized uptake value (SUVmax) (± SD) was 4.7 ± 1.3 in moderately differentiated HCC with positive PET findings, 5.7 ± 2.3 in poorly differentiated HCC and 26.2 in undifferentiated HCC. One poorly differentiated HCC with a maximum diameter of 17.0 cm, direct invasion to the stomach, and lymph node and pulmonary metastases, had a high SUVmax of 11.3. Moderately differentiated HCC with sarcomatous change had a high SUVmax of 18.6, and poorly differentiated HCC with sarcomatous change also showed high FDG uptake (SUVmax 14.1 and 25.0) (Fig. 1). One poorly differentiated HCC with sarcomatous change had a high SUVmax of 14.1 despite the small size of the tumor (1.6 cm) and absence of microvascular invasion (Fig. 2). The patients with poorly differentiated HCC with sarcomatous change developed recurrences soon after surgery. One patient with an SUVmax of 14.1 had metastasis to the mediastinal lymph nodes 9 months after surgery, and another with an SUVmax of 25.0 developed intrahepatic metastasis 44 days after surgery.

Patients with cHCC-CC

Patient characteristics are summarized in Table 3. All three cHCC-CC were detected by PET and the SUVmax

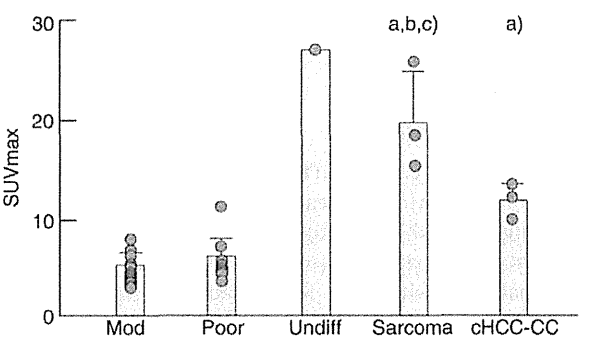

Figure 1 Maximum standardized uptake value (SUVmax) values of hepatocellular carcinoma (HCC) and combined hepatocellular and cholangiocarcinoma (cHCC-CC) with positive positron emission tomography (PET) findings. Undifferentiated HCC, moderately or poorly differentiated HCC with sarcomatous change, and cHCC-CC have high SUVmax values (>9.9), respectively. Data are expressed as mean ± standard deviation. (a) $P < 0.05$ vs mod; (b) $P < 0.05$ vs poor; (c) $P < 0.05$ vs cHCC-CC. Mod, moderately differentiated HCC; poor, poorly differentiated HCC; undiff, undifferentiated HCC; sarcoma, moderately or poorly differentiated HCC with sarcomatous change.

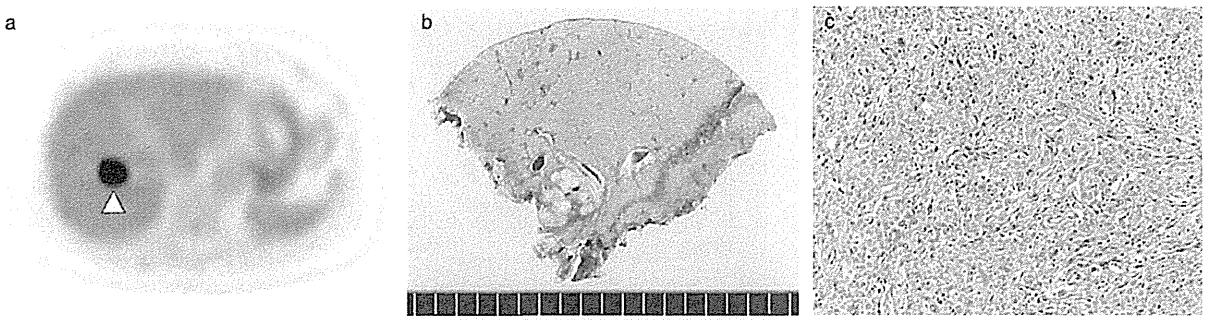

Figure 2 A 74-year-old female patient with poorly differentiated hepatocellular carcinoma (HCC) with sarcomatous change. (a) ^{18}F-Fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) image shows a liver mass with a maximum standardized uptake value (SUVmax) of 14.1 (arrow head). (b) Macroscopic image of the liver mass. (c) The liver tumor demonstrates histological features of poorly differentiated HCC with sarcomatous change (hematoxylin–eosin, original magnification ×100).

of cHCC-CC was 9.9, 12.0 and 13.0 (Fig. 1). One cHCC-CC had a high FDG uptake (SUVmax 12.0) despite the small size of the tumor (2.2 cm) and low levels of tumor markers (patient no. 1) (Fig. 3).

DISCUSSION

THE ROLE OF FDG PET/CT in the diagnosis and staging of HCC and other forms of liver cancer has been demonstrated in several reports.[6,7,19] However, preoperative evaluation of sarcomatous HCC and cHCC-CC with FDG PET/CT has not been reported so far. In the present study, we showed that sarcomatous HCC and cHCC-CC could be detected by PET/CT with high FDG uptake, and positive preoperative FDG uptake in HCC was significantly associated with tumor differentiation, tumor size and microvascular invasion.

Recently, several studies have shown that FDG-PET is useful for predicting tumor characterization, clinical outcome and prognosis in patients with HCC. Well-differentiated HCC regions were reported to show a tendency toward negativity by PET, whereas poorly differentiated types show increased FDG accumulation.[6,7] Our data also demonstrate that well-differentiated and some moderately differentiated HCC do not show FDG uptake exceeding that of the surrounding normal liver, whereas poorly differentiated and undifferentiated HCC have positive PET findings. There was no significant difference between the mean SUVmax of poorly differentiated HCC and that of moderately differentiated HCC with positive PET findings. On the other hand, the SUVmax of sarcomatous HCC were 18.6, 14.1 and 25.0, much higher than that of poorly differentiated HCC.

Table 3 Characteristics of patients with cHCC-CC

Characteristic	Patient no. 1	Patient no. 2	Patient no. 3
Age (years)/sex	78/M	54/M	47/M
Viral infection	HBsAg positive	Negative	HCVAb positive
Maximal tumor size (cm)	2.2	12.3	4.0
Microvascular invasion	Positive	Positive	Positive
Tumor stage (UICC)	II	IV	III
AFP (ng/mL)	4.3	16.4	18 286
DCP (mAU/mL)	20	45	231
CEA (ng/mL)	1.7	0.5	2.8
CA19-9 (U/mL)	7.4	76.6	31.9
Maximum SUV	12.0	9.9	13.0

CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; DCP, des-γ-carboxy prothrombin; HBsAg, hepatitis B surface antigen; HCVAb, anti-hepatitis C virus antibody; SUV, standardized uptake value; UICC, Union for International Cancer Control.

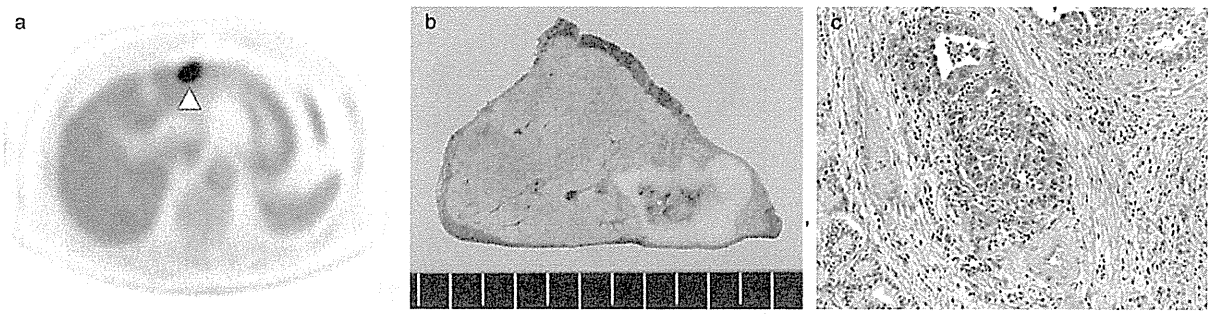

Figure 3 A 78-year-old male patient with combined hepatocellular and cholangiocarcinoma (cHCC-CC). (a) ^{18}F-Fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) image shows a liver mass with a maximum standardized uptake value (SUVmax) of 12.0 (arrow head). (b) Macroscopic image of the liver mass. (c) The liver tumor demonstrates histological features of cHCC-CC with microvascular invasion (hematoxylin–eosin, original magnification ×100).

Sarcomatous HCC is a rare histological variant of HCC.[13] Although the pathogenesis of sarcomatous HCC has not been clarified, the sarcomatous components are thought to be derived from a dedifferentiation or anaplasia, rather than from a combination of HCC and sarcoma.[13,20] Previous reports have suggested that anticancer therapy has an influence on the development of sarcomatous features in HCC, and the prognosis of patients with sarcomatous HCC is very poor due to frequent widespread metastases.[13,14,21] Although we performed curative resection for primary sarcomatous HCC, two of the three patients developed recurrences soon after surgery. Honda *et al.* reported that sarcomatous HCC appears as an irregularly demarcated intrahepatic mass with delayed or prolonged peripheral enhancement on CT.[22] However, it seemed to be difficult to make a correct preoperative diagnosis of sarcomatous changes by imaging or serological tumor markers. Our results show that FDG-PET may be a useful diagnostic tool for sarcomatous changes of HCC because the high FDG uptake of sarcomatous HCC seems to be related to its progression or aggressiveness.

In the present study, the SUVmax values of three cHCC-CC were higher than those of the poorly differentiated HCC. cHCC-CC is an uncommon subtype of primary liver cancer that contains elements of both HCC and CC.[15] Several studies have reported that the prognosis of patients with cHCC-CC was worse than that of patients with HCC because of frequent portal venous invasion and metastasis to lymph nodes and other organs.[16,17] Vascular invasion, tumor size and tumor stage were found to be prognostic factors for poor outcome in patients with cHCC-CC.[16,23] Moreover, recent studies have demonstrated that a large CC component in cHCC-CC and a high serum carbohydrate antigen 19-9 (CA19-9) level were also associated with poorer survival rates.[24,25] We demonstrated that one cHCC-CC showed high FDG uptake (SUVmax 12.0) despite the low CA19-9 level (7.4 U/mL) and small size of the tumor (2.2 cm) (patient no. 1). In addition, another cHCC-CC showed high FDG uptake (SUVmax 13.0) despite the small CC component in the tumor (1%) (patient no. 3) (data not shown). If the degree of FDG uptake in cHCC-CC also reflects the aggressiveness of the tumor like other malignant tumors, FDG-PET may become a useful diagnostic tool for the preoperative evaluation of cHCC-CC.

Our data show that the SUVmax of sarcomatous HCC and cHCC-CC are much higher than those of liver cancers reported to be associated with poor prognosis in previous studies. Seo *et al.* have demonstrated that high FDG uptake (SUVmax ≥5.0) was a predictive factor of postoperative early recurrence and poor survival in patients with HCC.[7] Riedl *et al.* have also reported that an SUVmax of 5.0 or greater was correlated with worse long-term prognosis after liver resection for colorectal metastases.[26]

In summary, our studies demonstrate that FDG-PET shows high FDG uptake in sarcomatous HCC and cHCC-CC that have been reported to be associated with poor prognosis after surgery. Therefore, FDG-PET may be an effective diagnostic tool for the non-invasive evaluation of the aggressiveness of primary liver cancer before surgical resection and liver transplantation. Further clinical studies are warranted.

REFERENCES

1 Rigo P, Paulus P, Kaschten BJ *et al.* Oncological application of positron emission tomography with fluorine-18 fluorodeoxyglucose. *Eur J Nucl Med* 1996; **23**: 1641–74.
2 Iglehart JK. The new era of medical imaging – progress and pitfalls. *N Engl J Med* 2006; **354**: 2822–8.
3 Trojan J, Schroeder O, Raedle J *et al.* Fluorine-18 FDG positron emission tomography for imaging of hepatocellular carcinoma. *Am J Gastroenterol* 1999; **94**: 3314–9.
4 Khan MA, Combs CS, Brunt EM *et al.* Positron emission tomography scanning in the evaluation of hepatocellular carcinoma. *J Hepatol* 2000; **32**: 792–7.
5 Torizuka T, Tamaki N, Inokuma T *et al.* In vivo assessment of glucose metabolism in hepatocellular carcinoma with FDG-PET. *J Nucl Med* 1995; **36**: 1811–7.
6 Hatano E, Ikai I, Higashi T *et al.* Preoperative positron emission tomography with fluorine-18-fluorodeoxyglucose is predictive of prognosis in patients with hepatocellular carcinoma after resection. *World J Surg* 2006; **30**: 1736–41.
7 Seo S, Hatano E, Higashi T *et al.* Fluorine-18-fluorodeoxyglucose positron emission tomography predicts tumor differentiation, P-glycoprotein expression, and outcome after resection in hepatocellular carcinoma. *Clin Cancer Res* 2007; **13**: 427–33.
8 Ng IO. Prognostic significance of pathological and biological factors in hepatocellular carcinoma. *J Gastroenterol Hepatol* 1998; **13**: 666–70.
9 Kudo M. Multistep human hepatocarcinogenesis: correlation of imaging with pathology. *J Gastroenterol* 2009; **44** (Suppl 19): 112–8.
10 Sato M, Watanabe Y, Lee T *et al.* Well-differentiated hepatocellular carcinoma: clinicopathological features and results of hepatic resection. *Am J Gastroenterol* 1995; **90**: 112–6.
11 Oishi K, Itamoto T, Amano H *et al.* Clinicopathologic features of poorly differentiated hepatocellular carcinoma. *J Surg Oncol* 2007; **95**: 311–6.
12 Ikebe T, Wakasa K, Sasaki M *et al.* Hepatocellular carcinoma with chondrosarcomatous variation: case report with immunohistochemical findings, and review of the literature. *J Hepatobiliary Pancreat Surg* 1998; **5**: 217–20.
13 Maeda T, Adachi E, Kajiyama M, Takenaka K, Sugimachi K, Tsuneyoshi M. Spindle cell hepatocellular carcinoma: a clinicopahtologic and immunohistochemical analysis of 15 cases. *Cancer* 1996; **77**: 51–7.
14 Tsujimoto M, Aozasa K, Nakajima Y, Kariya M. Hepatocellular carcinoma with sarcomatous proliferation showing an unusual and widespread metastasis. *Acta Pathol Jpn* 1984; **34**: 839–45.
15 Primary liver cancer in Japan. Clinicopathologic features and results of surgical treatment. Liver Cancer Study Group of Japan. *Ann Surg* 1990; **211**: 277–87.
16 Jarnagin WR, Weber S, Tickoo SK *et al.* Combined hepatocellular and cholangiocarcinoma: demographic, clinical, and prognostic factors. *Cancer* 2002; **94**: 2040–6.
17 Yano Y, Yamamoto J, Kosuge T *et al.* Combined hepatocellular and cholangiocarcinoma: a clinicopathologic study of 26 resected cases. *Jpn J Clin Oncol* 2003; **33**: 283–7.
18 Nishie A, Yoshimitsu K, Asayama Y *et al.* Detection of combined hepatocellular and cholangiocarcinomas on enhanced CT; comparison with histologic findings. *Am J Roentgenol* 2005; **184**: 1157–62.
19 Breitenstein S, Apestegui C, Clavien PA. Positron emission tomography (PET) for cholangiocarcinoma. *HPB* 2008; **10**: 120–1.
20 Kakizoe S, Kojiro M, Nakashima T. Hepatocellular carcinoma with sarcomatous change: clinicopathologic and immunohistochemical studies of 14 autopsy cases. *Cancer* 1987; **59**: 310–6.
21 Kojiro M, Sugihara S, Kakizoe S, Nakashima O, Kiyomatsu K. Hepatocellular carcinoma with sarcomatous change: a special reference to relationship with anticancer therapy. *Cancer Chemother Pharmacol* 1989; **23**: 4–8.
22 Honda H, Hayashi T, Yoshida K *et al.* Hepatocellular carcinoma with sarcomatous change: characteristic findings of two-phased incremental CT. *Abdom Imaging* 1996; **21**: 37–40.
23 Koh KC, Lee H, Choi MS *et al.* Clinicopathologic features and prognosis of combined hepatocellular cholangiocarcinoma. *Am J Surg* 2005; **189**: 120–5.
24 Aishima S, Kuroda Y, Asayama Y *et al.* Prognostic impact of cholangiocellular and sarcomatous components in combined hepatocellular and cholangiocarcinoma. *Hum Pathol* 2006; **47**: 283–91.
25 Kim KH, Lee SG, Park EH *et al.* Surgical treatments and prognosis of patients with combined hepatocellular carcinoma and cholangiocarcinoma. *Ann Surg Oncol* 2009; **16**: 623–9.
26 Riedl CC, Akhurst T, Larson S *et al.* 18F-FDG PET scanning correlates with tissue markers of poor prognosis and predicts mortality for patients after liver resection for colorectal metastases. *J Nucl Med* 2007; **48**: 771–5.

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Clinicopathological characteristics and prognostic factors in young patients after hepatectomy for hepatocellular carcinoma

Shingo Shimada*, Toshiya Kamiyama, Hideki Yokoo, Kenji Wakayama, Yosuke Tsuruga, Tatsuhiko Kakisaka, Hirofumi Kamachi and Akinobu Taketomi

Abstract

Background: The aim of this study was to analyze the clinicopathological characteristics and the prognostic factors for survival and recurrence of young patients who had undergone hepatectomy for hepatocellular carcinoma.

Methods: Between 1990 and 2010, 31 patients aged 40 years or younger (younger patient group) among 811 consecutive patients with hepatocellular carcinoma who had undergone primary hepatectomy were analyzed with regard to patient factors, including liver function, tumor factors and operative factors. The clinicopathological characteristics of the younger patients were compared with those of patients over the age of 40 (older patient group). Then the prognostic factors of the younger patients were analyzed. Continuous variables were expressed as the means ± standard deviation and compared using the χ^2 test for categorical variables. Overall survival and recurrence-free survival rates were determined by the Kaplan-Meier method and analyzed by the log-rank test. The Cox proportional hazards model was used for multivariate analysis.

Results: In the younger patients, the rates of HBs-antigen-positivity, high alpha-fetoprotein, portal invasion, intrahepatic metastasis, large tumors, low indocyanin green retention rate at 15 minutes, and anatomical resection were significantly higher than the same measures in the older patients. The five-year overall survival rate of the young patients was 49.6%. The prognostic factors of survival were HCV-antibody-positivity and low albumin status. Prognostic factors of recurrence were multiple tumors and the presence of portal invasion.

Conclusions: In younger patients, survival appeared to be primarily affected by liver function, while recurrence was affected by tumor factors. Young patients with hepatocellular carcinoma should be aggressively treated with hepatectomy due to their good pre-surgical liver function.

Keywords: Hepatocellular carcinoma, Young, Hepatectomy, Clinicopathological characteristics, Prognostic factors

Background

Liver cancers are malignant tumors and are the third leading cause of cancer-related death; they are responsible for approximately 700,000 deaths per year [1]. Hepatocellular carcinoma (HCC) has a poor prognosis and accounts for 70 to 85% of primary liver cancers [2]. Generally, there are few opportunities for discovery of malignant tumors in younger patients, and thus they tend to present with a highly advanced malignancy at the time of diagnosis; nonetheless, younger patients can expect long-term survival. The definition of what constitutes a "young patient" differs between studies [3-12]. HCC is fairly rare in younger individuals, with an occurrence rate of only 0.6 to 2.7% in those under 40 years of age, according to Japanese reports [12-14]. In Asia and Africa, which are areas with prevalent hepatitis B virus (HBV), the frequency of HCC is higher than in Japan [4,8,9,11,15]; however, there are still few reports on independent prognostic factors in young patients with HCC.

In this study, we examined the prognostic clinicopathological features, as well as the prognostic factors for

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survival and recurrence, in young patients with HCC who had undergone hepatectomy.

Methods

Between January 1990 and May 2010, 811 consecutive patients with HCC underwent primary liver resection at the Gastroenterological Surgery I unit of Hokkaido University Hospital in Sapporo, Japan. Of these patients, 31 patients (3.8%) were 40 years old or younger, while 780 patients (96.2%) were over 40 years of age. For group stratification, the former patients were defined as the younger patient group, and the latter as the older patient group. This study was approved by the Hokkaido University Hospital Voluntary Clinical Study Committee and was performed according to the Helsinki Declaration guidelines. The clinicopathological characteristics and surgical data of the patients are shown in Table 1.

The indications for hepatic resection and the type of operative procedures were usually determined based on the patients' liver function reserve, that is, according to the results of the indocyanin green retention test at 15 minutes (ICGR15) [16]. Anatomical resection was performed on patients in whom the ICGR15 was lower than 25%. Anatomical resection was defined as a resection in which the lesions were completely removed anatomically on the basis of Couinauds' classification (segmentectomy, sectionectomy, and hemihepatectomy or more). Non-anatomical partial but complete resection was achieved in other cases. In all patients, surgery was performed at R0 or R1. When R0 and R1 resections were performed, the resection surfaces were found to be histologically or macroscopically free of HCC, respectively. Follow-up studies after liver resection were conducted at three-month intervals, which included physical, serological (liver function test, serum alpha-fetoprotein (AFP) level, and serum protein induced by vitamin K absence-II (PIVKA-II)), and radiological examinations (ultrasound sonography (US) and contrast-enhanced computed tomography (CT) scan or contrast-enhanced magnetic resonance imaging (MRI)). Recurrence was diagnosed on the basis of the results of contrast-enhanced CT and elevation of serum levels of AFP and/or PIVKA-II. Extrahepatic metastasis (lung, lymph node, adrenal gland, brain and bone) was diagnosed by contrast-enhanced chest and abdominal CT, contrast-enhanced head MRI and bone scintigram. The median follow-up period was 111 months (range, 5 to 249 months).

Statistical analysis

Continuous variables were expressed as the means ± standard deviation and compared using the χ^2 test for categorical variables. Overall survival (OS) and recurrence-free survival (RFS) were determined by the Kaplan-Meier

Table 1 Clinicopathological characteristics

	Young (age ≤40 years) n = 31	Old (age >40 years) n = 780	*P*
Epidemiology			
Sex: Male/Female	24/7 (77%/23%)	644/136 (83%/17%)	*NS*
HBs-Ag positive	26 (84%)	321 (41%)	<0.0001
HCV-Ab positive	1 (3%)	310 (40%)	<0.0001
Biochemical Factors			
Albumin ≥4.0 g/l	17 (55%)	411 (53%)	*NS*
Total bilirubin ≥0.8 mg/dl	17 (55%)	379 (49%)	*NS*
ICGR15 ≥15	3 (10%)	360 (46%)	0.0001
AFP ≥200 ng/ml	16 (52%)	210 (27%)	0.0026
Tumor Factors			
Number of tumors: 1	20 (65%)	522 (67%)	*NS*
2 to 3	6 (19%)	183 (23%)	
≥4	5 (16%)	75 (10%)	
Maximum size of tumors: <2 cm	4 (12%)	83 (11%)	0.0074
≥2 cm, <5 cm	7 (23%)	395 (50%)	
≥5 cm	20 (65%)	303 (39%)	
Macroscopic classification: simple nodular type	10 (32%)	408 (52%)	*NS*
simple nodular type with extranodular grow	10 (32%)	222 (28%)	
confluent multinodular type	8 (26%)	122 (16%)	
infiltrative type	0 (0%)	6 (1%)	
others	3 (10%)	22 (3%)	
Distant metastasis positive	2 (6%)	18 (2%)	*NS*
Surgical Factors			
Anatomical resection	29 (94%)	525 (67%)	0.0021
Histological Factors			
Differentiation: well	3 (10%)	114 (15%)	*NS*
moderate	13 (42%)	430 (55%)	
poor	14 (45%)	209 (27%)	
others	1 (3%)	27 (3%)	
vp:vp0	14 (45%)	569 (73%)	0.0026
vp1	9 (29%)	125 (16%)	
vp2,3,4	8 (26%)	86 (11%)	
im	16 (52%)	264 (34%)	0.0413
cirrhosis	9 (29%)	287 (37%)	*NS*

AFP, alpha-fetoprotein; HBs-Ag, HBs-antigen; HCV-Ab, HCV-antibody; ICGR15, indocyanin green retention rate at 15 minutes; im, microscopic intrahepatic metastasis; NS, non-significant; vp0, no tumor thrombus in the portal vein; vpl, tumor thrombus distal to the second branches of the portal vein; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor thrombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

method and analyzed by the log-rank test. The Cox proportional hazards model was used for multivariate analysis. Significance was defined as a *P*-value of <0.05. Statistical analyses were performed using Stat View 5.0 for Windows (SAS Institute, Cary, NC, USA).

Results

Clinicopathological characteristics and operative variables

Patient factors

The ratio of males to females (24:7) in the younger patient group was not significantly different from that of the older patient group. Patients with HBV markers accounted for most of the virus-associated cases: HBs-antigen (HBs-Ag)-positive, 26/31 (total number in the younger group) vs. 321/780 (total number in the older group); 84% vs. 41%; *P* <0.0001. Patients who were hepatitis C virus (HCV)-antibody (HCV-Ab)-positive were significantly fewer in number, that is, 1/31 vs. 310/780 (3% vs. 40%; *P* <0.0001) in the younger group. Although serum albumin and total bilirubin levels were not significantly different between the groups, patients with ICGR15 ≥15 were 3/31 vs. 360/780 (10% vs. 46%; *P* = 0.0001).

Tumor factors

The younger group had significantly higher AFP levels compared to the older group (*P* = 0.0026). Although the number of tumors did not differ significantly between the younger and older patients, there were significantly more cases with a maximum tumor size of ≥5 cm in the younger group (*P* = 0.0072). The mean maximum tumor diameter in the younger group in this study was 8.6 ± 7.3 cm. Neither macroscopic type nor extrahepatic metastasis was significantly different between the groups.

Operative variables

The rate of anatomical resections in the younger patients was significantly higher than that in the older patients.

Pathological factors

There were significant differences between groups in terms of microscopic tumor thrombus in the portal vein (*P* = 0.0026) and microscopic intrahepatic metastasis (*P* = 0.0413) (Table 1).

Causes of death and recurrence

Among the total 811 patients, 390 (48.1%) died. The mortality rates were 17/31 (54.8%) in the younger patient group and 373/780 (47.8%) in the older patient group. The causes of death, which did not differ significantly between groups, were as follows: HCC recurrence (n = 301; 77.2%; 16 in the younger patients vs. 285 in the older patients), liver failure (n = 36; 9.2%; 0 in the younger vs. 36 in the older patients), and other causes (n = 53; 13.6%; 1 in the younger vs. 52 in the older patients). In addition, two patients in the older group died of operative complications prior to 1995. No patients in the younger group died of operative complications.

In the younger group, 22 patients experienced a recurrence (71.0%). There were 17 (77.3%) liver tumor recurrences, with a median recurrence time of six months (1 to 27). Lung metastases occurred in 11 (50.0%) cases, with a median recurrence time of 12 months (1 to 42); bone metastases in 7 (31.8%) cases, with a median recurrence time of 23 months (6 to 60); brain metastases in 6 (27.3%) cases, with a median recurrence time of 20 months (10 to 61); lymph node metastases in 3 (13.6%) cases, with a median recurrence time of 12 months (12 to 56); and adrenal gland metastases in 3 (13.6%) cases, with a median recurrence time of 10 months (5 to 50).

Cumulative rates of patient survival and recurrence-free survival

The five-year OS rate of all 811 patients was 57.1%. The five-year OS rate and median survival time (MST) of the younger group were 49.6% and 40 months, respectively, whereas those of the older group were 57.7% and 79 months, respectively (Figure 1). The median RFS time of all 811 patients was 23 months, while that of the younger patients was 6 months, and that of the older patients was 25 months (Figure 2). Neither OS nor RFS were significantly different between the younger and older groups, although recurrence tended to occur earlier in the younger patients.

Factors related to long-term survival and disease-free survival after primary hepatectomy in the younger patient group

Table 2 shows those factors that were found by univariate analysis to influence OS and RFS in the younger

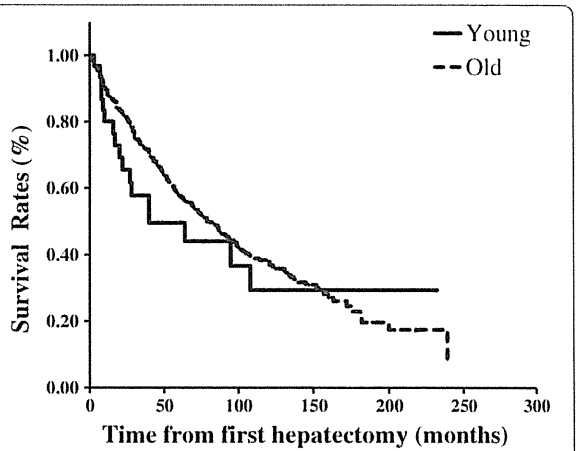

Figure 1 Overall survival curves of the younger and older patient groups after first hepatectomy.

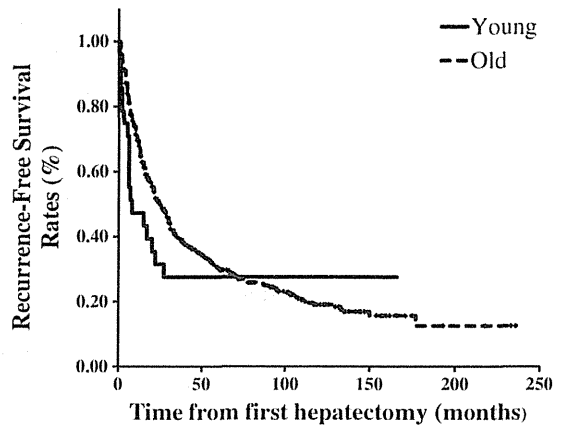

Figure 2 Recurrence-free survival curves of the younger and older patient groups after first hepatectomy.

Table 2 Univariate analyses of prognostic factors of survival and recurrence in the younger group

	Survival	Recurrence
	P	*P*
Epidemiology		
Sex: Male	*NS*	*NS*
HBs-Ag positive	*NS*	*NS*
HCV-Ab positive	0.0172	*NS*
Biochemical Factors		
Albumin <4.0 g/l	0.0088	*NS*
Total bilirubin ≥0.8 mg/dl	*NS*	*Ns*
ICGR15 ≥15	*NS*	*NS*
AFP ≥200 ng/ml	*NS*	*NS*
Tumor Factors		
Number of tumors: multiple	*NS*	0.0199
Maximum size of tumor: ≥5 cm	0.0034	0.0006
Macroscopic classification: except for simple nodular type	*NS*	*NS*
Distant metastasis positive	*NS*	-
Surgical Factors		
Non-anatomical resection	*NS*	*NS*
Histological Factors		
Differentiation: poor	*NS*	0.0395
vp2, 3, 4	0.0108	0.0020
im	0.0058	0.0053
cirrhosis	0.0446	*NS*

AFP, alpha-fetoprotein; HBs-Ag, HBs-antigen; HCV-Ab, HCV-antibody; ICGR15, indocyanin green retention rate at 15 minutes; im, microscopic intrahepatic metastasis; NS, non-significant; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor thrombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

group. The univariate analysis revealed that OS was significantly related to being HCV-Ab-positive, having a serum albumin level of <4.0 g/l and a maximum tumor size of ≥5 cm, the presence of tumor thrombus in the second and first branches and trunk or opposite side branch of the portal vein (vp2, 3, 4), microscopic intrahepatic metastasis, and histological liver cirrhosis of non-cancerous liver.

Univariate analysis showed that RFS was significantly related to multiple tumors, maximum tumor size of ≥5 cm, poor differentiation, the presence of tumor thrombus above vp2 and microscopic intrahepatic metastasis. Multivariate analysis showed HCV-Ab-positive status and serum albumin levels of <4.0 g/l to be independent predictive factors for OS, and multiple tumors and vp2, 3, 4 were independent predictive factors for RFS in the younger group of patients (Tables 3 and 4).

Discussion

In this study, the younger patients with HCC who underwent hepatectomy were more likely than the older patients to be HBV-positive, to have large tumors with portal invasion and to have high AFP, although they also retained better liver function than the older patients. Despite the significant difference in tumor progression, neither OS nor RFS were significantly different between the two groups, although recurrence tended to occur earlier in the younger patients. Multivariate analysis showed HCV-Ab-positive status and serum albumin levels of <4.0 g/l to be independent predictive factors for OS, and multiple tumors and vp2, 3, 4 were independent predictive factors for RFS in the younger patients. Therefore, young patients with hepatocellular carcinoma should be aggressively treated with hepatectomy due to their good pre-surgical liver function.

In the younger group of patients, HCV-Ab-positive status and low serum albumin levels were the liver-function-related factors that were found to be significantly unfavorable in terms of OS, while multiple tumors

Table 3 Multivariate analyses of prognostic factors of survival in the younger group

Risk factor	*P*-value	Hazard ratio	95% CI
HCV-Ab positive	0.0196	59.816	1.927 to 1856.714
Albumin <4.0 g/l	0.0296	6.665	1.207 to 36.813
Maximum size of tumor: ≥5 cm	*NS*	0.381	0.025 to 5.697
vp2, 3, 4	*NS*	2.313	0.420 to 12.738
im	*NS*	14.563	0.951 to 222.939
cirrhosis	*NS*	1.037	0.149 to 7.200

CI, confidence interval; HCV-Ab, HCV-antibody, im, microscopic intrahepatic metastasis; NS, non-signficant; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor rhombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

Table 4 Multivariate analyses of prognostic factors of recurrence in the younger group

Risk factor	*P*-value	Hazard ratio	95% CI
Number of tumor: multiple	0.0415	51.312	1.163 to 2264.565
Maximum size of tumor: ≥5 cm	*NS*	3.210	0.353 to 29.152
Differentiation: poor	*NS*	2.796	0.450 to 17.043
vp2, 3, 4	0.0253	13.517	1.380 to 132.442
im	*NS*	0.137	0.005 to 3.541

CI, confidence interval; im, microscopic intrahepatic metastasis; NS, non-significant; vp2, tumor thrombus in the second branches of the portal vein; vp3, tumor thrombus in the first branch of the portal vein; vp4, tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

and vp2, 3, 4 were the tumor-related factors that were significantly unfavorable in terms of RFS; moreover, these findings were obtained by both univariate and multivariate analyses. Although most of the younger patients had advanced tumors, no differences were found between the younger and older patients in terms of OS. These results indicate that aggressive and curative liver resection should be performed for young patients with HCC, because most young patients retain good presurgical liver function.

The definition of who should be classified as a "young patient" with HCC remains controversial. In the literature, the definition of a young patient with HCC has tended to be a patient aged 40 years or younger [4,8,10-12,14]. Cases of HCC in such patients are comparatively rare, for example, HCC occurs in only 0.6 to 2.7% of this age group in Japanese reports [12-14]. In other countries, the reported rates of HCC in this age range are as follows: 8.6% (40 years and younger) in Singapore [11], 10.9% (under 40 years) in Taiwan [8] and 6.5% (40 years and younger) in Hong Kong [4]. Thus most of the existing reports have been from Asia, and they show a difference in frequency among regions. There appear to be many young patients in Asia with HCC who are HBV-positive; HBV is an underlying disease of HCC in young patients, and many carriers live in Asia [17].

Many young patients with HCC have HBs-Ag, that is, up to 71.4 to 100% [3-5,7-11,14]. Meanwhile, cases of HCV-Ab-positivity plus HCC among younger patients are reported at rates of 0 to 10% [4,5,7-10,12,14], which is much lower than the range for older patients. Rates of Child-Pugh A are 69.1 to 92.3% among younger patients [4-6,8-12], which is higher than the range in older patients. It has been reported that histological hepatitis or cirrhosis of non-cancerous liver is significantly less common in younger hepatectomy patients than in older hepatectomy patients among cases with HCC [3,4,12]. Though HCC is generally found by medical examination or follow-up of liver function, in most young patients, HCC is found by symptoms such as pain and/or palpation of an abdominal mass [11,14,18,19]. Accordingly, members of the younger patient group in this study had larger tumors than the older patient group.

This study revealed that the rate of cases related to HBV was 93.5%, and the rate of HBs-Ag-positive cases was 87.0%. The MST of the younger group was 40 months, and the five-year OS rate was 49.6%. These results did not differ significantly from the previously reported MST and five-year OS rates of 27.8 to 52.5 months and 30.5 to 54.8%, respectively, among cases of liver resection for HCC across all ages [20,21]. Therefore, it appears likely that aggressive and curative liver resection contributes to prolonged prognosis.

In regard to tumor factors, several studies have reported that more young than old patients have high AFP levels, that is, the rates of cases in which AFP is equal to or exceeds a value of 400 ng/ml range from 52.6 to 82.0% [3,7,9-11,14], and rates for an AFP of ≥10,000 ng/ml range from 31.6 to 60.0% [3,10,11,14]. In addition, younger patients tend to have larger tumors than older patients, with the maximum diameter of tumors being 6.9 to 12.7 cm in younger patients [3,4,7,10,12,14]. Cases showing portal invasion count for 45.0 to 100% [10-12,14] of younger HCC patients. In the present study, the younger patient group had higher AFP levels and larger tumors, was more likely to have portal invasion and showed better liver function than the older group, as has been reported elsewhere [3,7,10-12,14]. It has also been reported that cases with high AFP levels have a poor prognosis due to a correlation between tumor size and AFP [22].

As regards prognostic factors, Chen *et al.* reported that hepatectomy was a significant favorable prognostic factor among HCC patients aged 40 years and younger [8]. As regards other prognostic factors, AFP [8,11], portal invasion [8,11] and reserved liver function [8,11,12] have been reported, although these remain controversial. In this study, prognostic factors related to OS were HCV-Ab-positive status and low serum albumin levels, and prognostic factors related to RFS were the number of tumors and vp2, 3, 4. It has been suggested that liver function preservation primarily influences survival, and tumor factors influence recurrence. Furthermore, while the time to recurrence in the younger patients was shorter than that in the older patients, the RFS of the younger group tended to overtake that of the older group in the long term. The recurrence rate was 71%, and the site of recurrence was almost always the liver. This rate was comparable to those of other reports, which ranged from 60.2 to 78.2% across all ages [20]. The results to date suggest that aggressive treatments, including re-hepatectomy for recurrence, contribute to an improvement in the long-term prognosis.

Moreover, in order to improve prognosis, we should take care to perform aggressive resections, and should also make note of cases with a background of potentially liver-affecting hepatitis B. Chuma *et al.* reported that the quantity of HBV-DNA and non-treatment for HBV were risk factors for a recurrence of HCC [23]. Li *et al.* reported that one-year and two-year RFS rates were 23.3% vs. 8.3%, and 2.3% vs. 0%, respectively, in a treatment group receiving lamivudine for HCC due to concurrent hepatitis B vs. a control group [24]. Therefore, viral treatments in combination with cancer treatments, including resection, are important to consider.

There have been few reports on liver transplantation for young patients with HCC. The reason for this lack of information is likely to be that younger patients have relatively larger tumors and, therefore, they tend to have tumors exceeding the Milan criteria. Ismail *et al.* reported that the outcomes of liver transplantation were better than those of liver resection among patients with HCC who were aged 2 to 27 years, namely, the OS rates were 72% vs. 40%, and the RFS rates were 91% vs. 30% [25]. It was also reported that primary liver transplantation for children with HCC without extrahepatic lesions has a good outcome, even if the tumors exceed the Milan criteria [26]. An accumulation of future cases is expected.

As noted above, many young HCC patients present with advanced tumors and unfavorable prognostic factors. In a study on 16 patients who received liver transplantation for HCC and who had low differentiation and vascular invasion beyond the Milan criteria, Saab *et al.* reported that those receiving sorafenib (n = 8) had one-year OS rates and RFS rates of 87.5% and 85.7%, versus 62.5% and 57.1% for the control group (n = 8) [27]. It is expected that supportive treatment with molecular target medicine after liver resection or transplantation could contribute to a prolonged prognosis.

Conclusions

In our younger patients with HCC, survival appeared to be mainly affected by liver function while recurrence was mainly affected by tumor factors. Young patients with HCC should be offered aggressive hepatectomy due to their relatively preserved liver function.

Abbreviations

AFP: Alpha-fetoprotein; CT: Computed tomography; HBV: Hepatitis B virus; HBs-Ag: HBs-antigen; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HCV-Ab: Hepatitis C virus-antibody; ICGR15: Indocyanin green retention test at 15 minutes; MRI: Magnetic resonance imaging; MST: Median survival time; OS: Overall survival; PIVKA-II: Protein induced by vitamin K absence-II; RFS: Recurrence-free survival; US: Ultrasound sonography; vp2: Tumor thrombus in the second branches of the portal vein; vp3: Tumor thrombus in the first branch of the portal vein; vp4: Tumor thrombus extension to the trunk or the opposite side branch of the portal vein.

Competing interests

All of the authors declare that they have no competing interests.

Authors' contributions

SS carried out the analysis of data and wrote the manuscript. TK and AT gave comments and revised the manuscript. HY, KW, YT, TK and HK made the database of patients. All authors read and approved the final manuscript.

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References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: **Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.** *Int J Cancer* 2010, **127**:2893–2917.
2. Ahmed F, Perz JF, Kwong S, Jamison PM, Friedman C, Bell BP: **National trends and disparities in the incidence of hepatocellular carcinoma,1998-2003.** *Prev Chronic Dis* 2008, **5**:A74.
3. Furuta T, Kanematsu T, Matsumata T, Shirabe K, Yamagata M, Utsunomiya T, Sugimachi K: **Clinicopathologic features of hepatocellular carcinoma in young patients.** *Cancer* 1990, **66**:2395–2398.
4. Lam CM, Chan AO, Ho P, Ng IO, Lo CM, Liu CL, Poon RT, Fan ST: **Different presentation of hepatitis B-related hepatocellular carcinoma in a cohort of 1863 young and old patients - implications for screening.** *Aliment Pharmacol Ther* 2004, **19**:771–777.
5. Sezaki H, Kobayashi M, Hosaka T, Someya T, Akuta N, Suzuki F, Tsubota A, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Matsuda M, Takagi K, Sato J, Kumada H: **Hepatocellular carcinoma in noncirrhotic young adult patients with chronic hepatitis B viral infection.** *J Gastroenterol* 2004, **39**:550–556.
6. Klein WM, Molmenti EP, Colombani PM, Grover DS, Schwarz KB, Boitnott J, Torbenson MS: **Primary liver carcinoma arising in people younger than 30 years.** *Am J Clin Pathol* 2005, **124**:512–518.
7. Kim JH, Choi MS, Lee H, Kim do Y, Lee JH, Koh KC, Yoo BC, Paik SW, Rhee JC: **Clinical features and prognosis of hepatocellular carcinoma in young patients from a hepatitis B-endemic area.** *J Gastroenterol Hepatol* 2006, **21**:588–594.
8. Chen CH, Chang TT, Cheng KS, Su WW, Yang SS, Lin HH, Wu SS, Lee CM, Changchien CS, Chen CJ, Sheu JC, Chen DS, Lu SN: **Do young hepatocellular carcinoma patients have worse prognosis? The paradox of age as a prognostic factor in the survival of hepatocellular carcinoma patients.** *Liver Int* 2006, **26**:766–773.
9. Cho SJ, Yoon JH, Hwang SS, Lee HS: **Do young hepatocellular carcinoma patients with relatively good liver function have poorer outcomes than elderly patients?** *J Gastroenterol Hepatol* 2007, **22**:1226–1231.
10. Yamazaki Y, Kakizaki S, Sohara N, Sato K, Takagi H, Arai H, Abe T, Katakai K, Kojima A, Matsuzaki Y, Mori M: **Hepatocellular carcinoma in young adults: the clinical characteristics, prognosis, and findings of a patient survival analysis.** *Dig Dis Sci* 2007, **52**:1103–1107.
11. Chang PE, Ong WC, Lui HF, Tan CK: **Is the prognosis of young patients with hepatocellular carcinoma poorer than the prognosis of older patients? A comparative analysis of clinical characteristics, prognostic features, and survival outcome.** *J Gastroenterol* 2008, **43**:881–888.
12. Takeishi K, Shirabe K, Muto J, Toshima T, Taketomi A, Maehara Y: **Clinicopathological features and outcomes of young patients with hepatocellular carcinoma after hepatectomy.** *World J Surg* 2011, **35**:1063–1071.
13. Tanioka H, Omagari K, Kato Y, Nakata K, Kusumoto Y, Mori I, Furukawa R, Tajima H, Koga M, Yano M, Kohno S: **Present status of hepatitis virus-associated hepatocellular carcinoma in Nagasaki Prefecture, Japan: a cross-sectional study of 1019 patients.** *J Infect Chemother* 2002, **8**:64–69.
14. Aramaki M, Kawano K, Sasaki A, Ohno T, Tahara K, Kai S, Iwashita Y, Kitano S: **Hepatocellular carcinoma in young adults.** *Hepatogastroenterology* 2005, **52**:1795–1797.
15. Kew MC: **Clinical, pathologic, and etiologic heterogeneity in hepatocellular carcinoma: evidence from southern Africa.** *Hepatology* 1981, **1**:366–369.