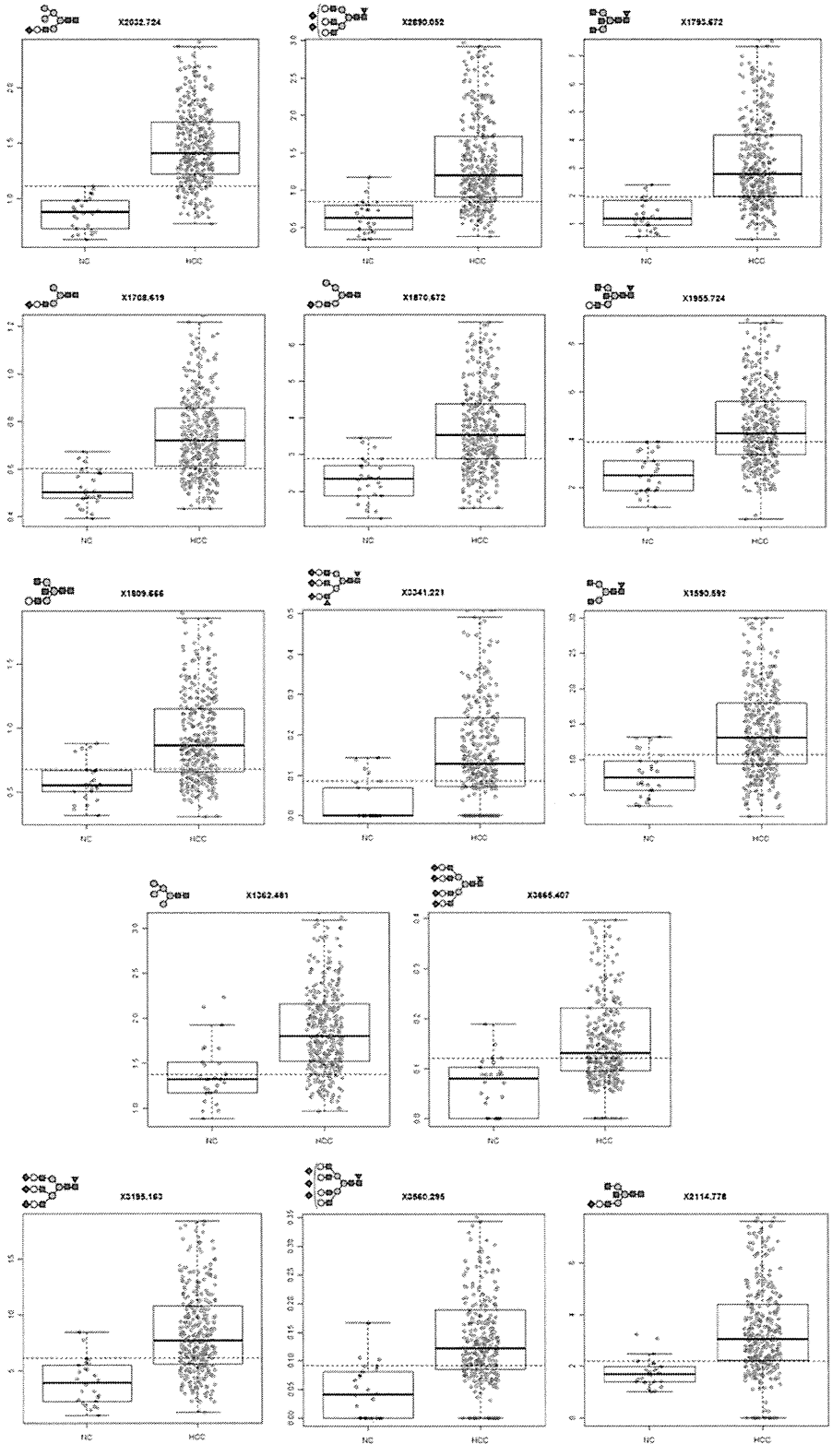

Fig. 1. Boxplots of the disease-free individuals (NC) and HCC patients for the selected 14 *N*-glycans. The dotted lines in the graphs represent the cutoff values determined in this analysis. These graphs were drawn using R v. 2.12.1.

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

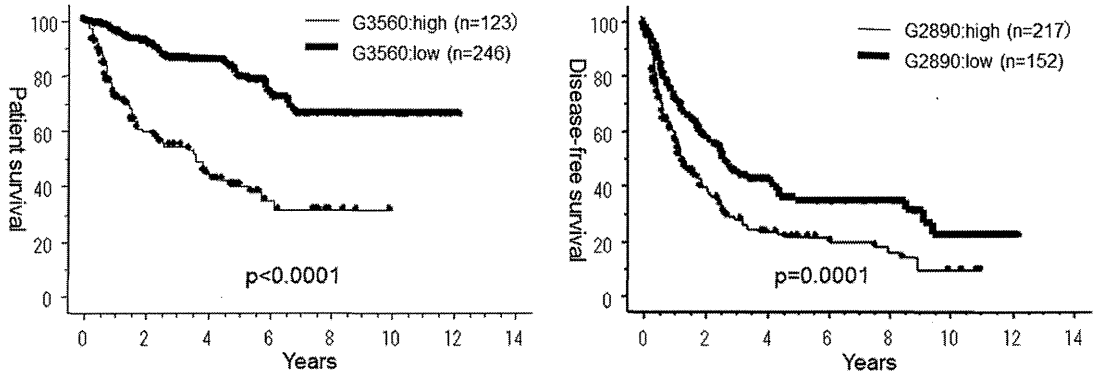

Fig. 2. The PS rates of HCC cases with low and high serum G3560 levels at 5 years were 80.5% and 40.4%, respectively. The DFS outcomes associated with low and high serum G2890 levels at 5 years were 21.3% and 35.1%, respectively.

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

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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

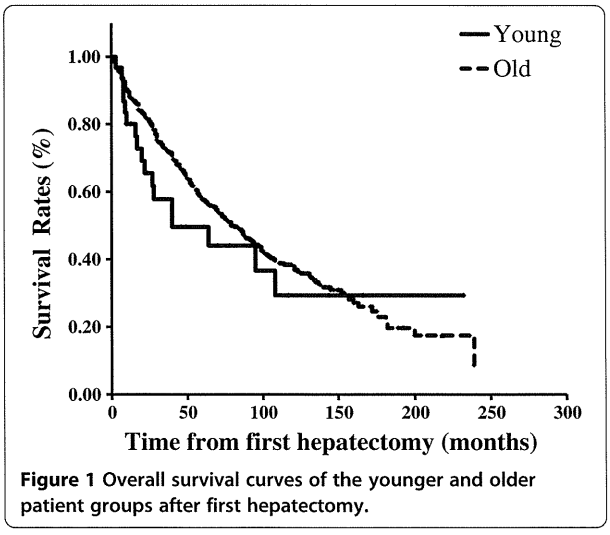

Figure 1 Overall survival curves of the younger and older patient groups after first hepatectomy.

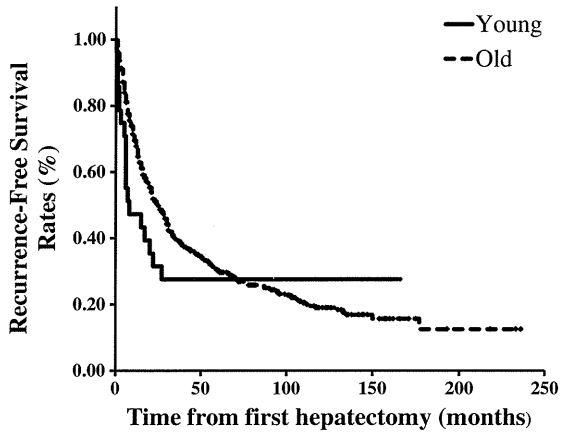

Figure 2 Recurrence-free survival curves of the younger and older patient groups after first hepatectomy.

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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CASE REPORT

Portal vein stenosis after pancreatectomy following neoadjuvant chemoradiation therapy for pancreatic cancer

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Abstract

Extrahepatic portal vein (PV) stenosis has various causes, such as tumor encasement, pancreatitis and as a post-surgical complication. With regard to post-pancreaticoduodenectomy, intraoperative radiation therapy with/without PV resection is reported to be associated with PV stenosis. However, there has been no report of PV stenosis after pancreatectomy following neoadjuvant chemoradiation therapy (NACRT). Here we report the cases of three patients with PV stenosis after pancreatectomy and PV resection following gemcitabine-based NACRT for pancreatic cancer and their successful treatment with stent placement. We have performed NACRT in 18 patients with borderline resectable pancreatic cancer since 2005. Of the 15 patients who completed NACRT, nine had undergone pancreatectomy. Combined portal resection was performed in eight of the nine patients. We report here three patients with PV stenosis, and thus the ratio of post-operative PV stenosis in patients with PV resection following NACRT is 37.5% in this series. We encountered no case of PV stenosis among 22 patients operated with PV resection for pancreatobiliary cancer without NACRT during the same period. A relationship between PV stenosis and NACRT is suspected, but further investigation is required to determine whether NACRT has relevance to PV stenosis.

Key words: Pancreatic cancer; Portal vein stenosis; Neoadjuvant chemoradiation therapy; Pancreatectomy; Expandable metallic stent

Core tip: Intraoperative radiation therapy for pancreatic cancer with/without portal vein (PV) resection is reported to be associated with PV stenosis. However, there has been no report of PV stenosis after pancreatectomy following neoadjuvant chemoradiation therapy (NACRT). Here we report the cases of three patients with PV stenosis after pancreatectomy and PV resection following gemcitabine-based NACRT for pancreatic cancer and their successful treatment with stent placement. We have performed pancreatectomy with PV resection following NACRT in 8 patients with borderline resectable pancreatic cancer since 2005. The ratio of post-operative PV stenosis is 37.5% in this series.

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INTRODUCTION

Extrahepatic portal vein (PV) stenosis can occur due to

tumor encasement[1], pancreatitis[2] and as a post-surgical complication-especially post-liver transplantation[3]. With regard to post-pancreaticoduodenectomy (PD), intraoperative radiation therapy (IORT) with/without PV resection is reported to be associated with PV stenosis[4-6]. The incidence rate for PV stenosis is reported to be 11% to 23%[5,6].

Portal hypertension secondary to PV stenosis causes gastrointestinal bleeding from gastroesophageal or jejunal varices, and refractory ascites[1]. Gastrointestinal bleeding is the most serious life-threatening complication. Refractory ascites is not fatal but affects the patient's quality of life.

Here we provide the case reports of three patients with PV stenosis after pancreatectomy and PV resection following neoadjuvant chemoradiation therapy (NACRT) for pancreatic cancer, and we discuss the relationship between PV stenosis and NACRT and the indications for stent placement. To the best of our knowledge, PV stenosis after pancreatectomy following NACRT has not been described in the literature.

CASE REPORT

Case 1

A 54-year-old man underwent PD and PV resection for pancreatic cancer following NACRT. The protocol consisted of external-beam radiotherapy to the pancreatic bed and regional lymphatics for a total dose of 50.4 Gy in 28 fractions. Concomitant chemotherapy consisted of gemcitabine at a dose of 150 mg/m^2 once weekly. The reconstruction between the PV and the superior mesenteric vein (SMV) was end-to-end anastomosis using a continuous running suture of 6-0 prolene. The splenic vein was not reconstructed.

Refractory ascites and malnutrition were recognized at 9 postoperative months (POMs). Computed tomography (CT) showed short segmental stenosis of the PV in the region of the anastomosis, severe ascites, and liver atrophy (Figure 1). Intraoperative portography through the catheter via the ileocolic vein and balloon dilation were performed but were not sufficiently effective because the region had elastic stenosis. Therefore, an expandable metallic stent (EMS; 1 cm diameter, 3 cm length) was inserted into the stenotic region. The portal venous pressure decreased from 14.5 to 8.6 cm H_2O, and the pressure gradient of 6.5 cm H_2O across the PV stenosis disappeared. Anticoagulant therapy was initiated immediately after stent placement. Heparin was administered at a dose of 10000 IU/d by intravenous infusion for 3 d initially, and then oral warfarin was administered. The warfarin was switched to aspirin 3 mo later. After the stent placement, a follow-up CT showed that the patient's ascites decreased and his liver atrophy improved (Figure 2). Stent patency is maintained at present, 5 years after the placement.

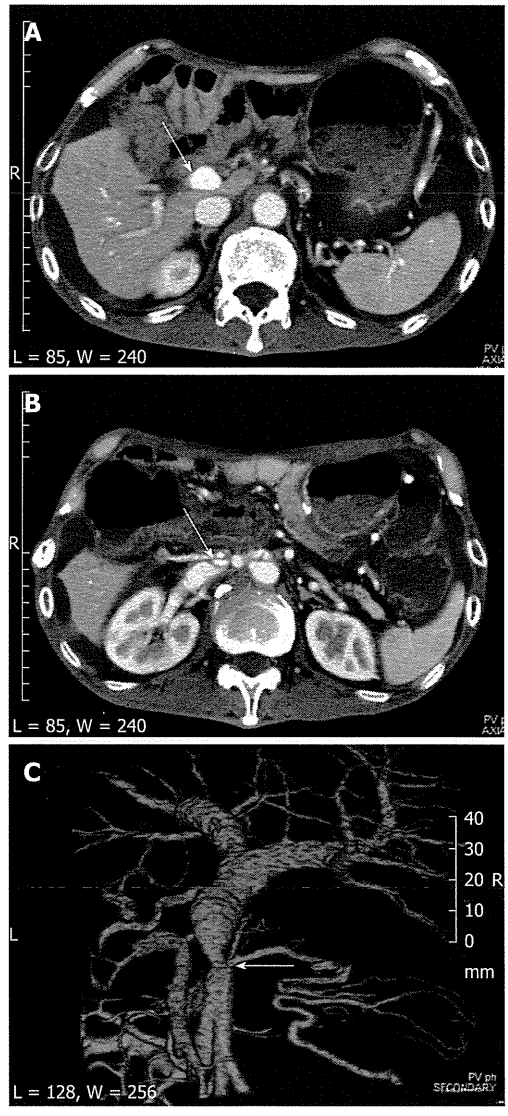

Figure 1 Computed tomography showed short segmental stenosis of the portal vein in the region of the anastomosis, severe ascites, and liver atrophy. A, B: Computed tomography (CT) scan shows severe ascites and liver atrophy (arrow) (A), and stenosis of the portal vein (arrow) behind the superior mesenteric artery (B); C: The image of the 3D reconstruction of the portal vein shows short segmental stenosis in the region of the anastomosis (arrow).

Case 2

A 44-year-old man underwent distal pancreatectomy and PV resection simultaneously with splenectomy and total gastrectomy for pancreatic cancer following NACRT using the same protocol as that described for Case 1. The PV was preoperatively occluded by tumor thrombus, and a cavernous transformation was identified. The reconstruction between the PV and the SMV was end-to-end anastomosis using the same procedure as that described for Case 1. Refractory diarrhea, ascites and malnutrition were recognized at 5 POMs. Initially, malabsorption was suspected, and thus total parenteral nutrition support was initiated. There was no improvement in symptoms after the improvement in nutrition status. CT showed short segmental stenosis of the PV in the region of the anastomosis, collateral circulation through the cavernous transformation of the pancreatic head, severe ascites, and thickness of the intestinal wall (Figure 3).

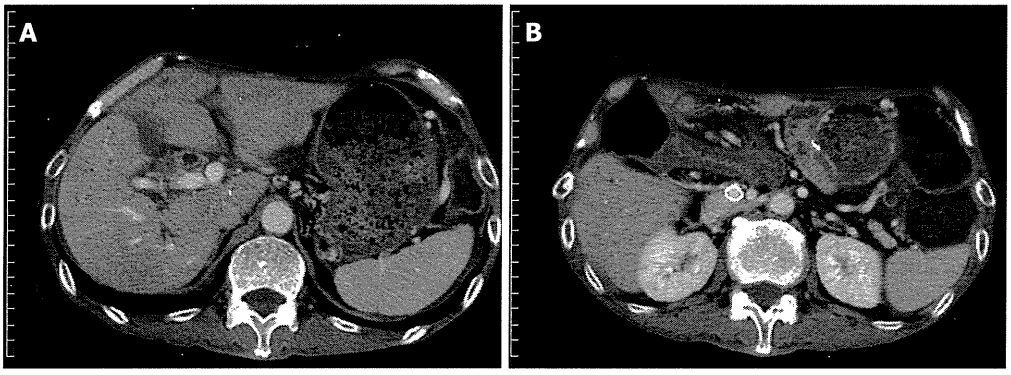

Figure 2 Computed tomography scan 3 mo after the expandable metallic stent placement. A: Shows that the ascites decreased and the liver atrophy improved; B: The stent placed in the portal vein remained patent.

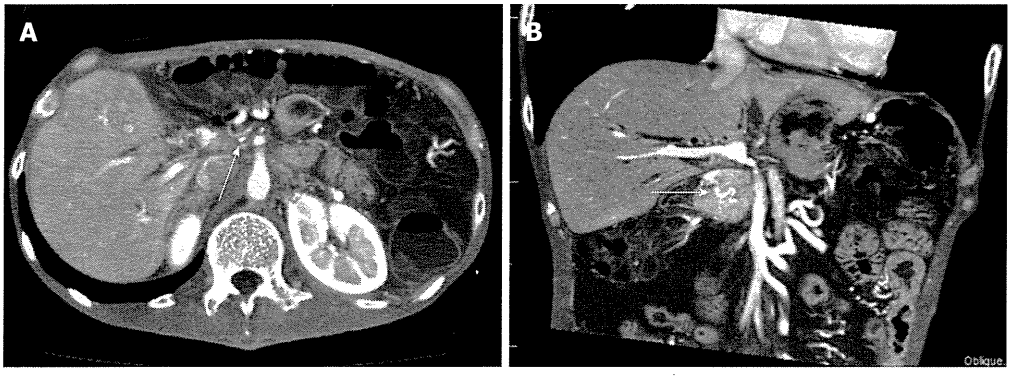

Figure 3 Computed tomography showed short segmental stenosis of the portal vein in the region of the anastomosis, collateral circulation through the cavernous transformation of the pancreatic head, severe ascites, and thickness of the intestinal wall. A: Computed tomography scan showing severe portal vein stenosis (arrow) in the region of the anastomosis; B: Multiplanar reconstruction revealed the collateral circulation through the cavernous transformation of the pancreatic head (arrow), severe ascites and thickness of the intestinal wall.

We suspected portal hypertension secondary to PV stenosis, even though the portal venous flow seemed to be sustained by the collateral circulation. Percutaneous transhepatic direct portography was performed (Figure 4A). The portal venous pressure was 31.0 cm H_2O, and the pressure gradient across the PV stenosis was 21.0 cm H_2O. An EMS (1 cm diameter, 4 cm length) was inserted into the stenotic region (Figure 4B). The portal venous pressure decreased to 17.0 cm H_2O, and the pressure gradient decreased to 2.0 cm H_2O. Anticoagulant therapy was performed as that described for Case 1 After a stent placement, the patient's diarrhea and ascites improved. Stent patency is maintained at present, 1.5 years after the placement.

Case 3

A 68-year-old woman underwent PD and PV resection for pancreatic cancer following NACRT using the same protocol as that described for Case 1. The reconstruction between the PV and the SMV was end-to-end anastomosis using the same procedure as that described for Case 1. Malnutrition and ascites were recognized at 5 POMs. Total parenteral nutrition support and repeated drainage of ascites were performed, but there was no improvement of the ascites. Cytology of the ascites showed no evidence of malignancy. CT showed short segmental stenosis of the PV in the region of the anastomosis. Percutaneous transhepatic direct portography was performed (Figure 4C). The portal venous pressure was 24.5 cm H_2O, and the pressure gradient across the PV stenosis was 12.0 cm H_2O. An EMS (1 cm diameter, 3 cm length) was inserted into the stenotic region (Figure 4D). The portal venous pressure decreased to 11.5 cm H_2O, and the pressure gradient disappeared. Anticoagulant therapy was performed as that described for Case 1. After the stent placement, the patient's ascites improved. Stent patency is maintained at present, 4 mo after the placement.

DISCUSSION

Despite considerable research, the prognosis for pancreatic cancer remains poor. For all stages combined, the 1- and 5-year relative survival rates are 25% and 6%, respectively[7]. Complete surgical resection is the only therapy to afford a chance of cure[8], but patients with borderline resectable pancreatic cancer are at high risk of having positive surgical margins due to vascular involvement[9]. NACRT for borderline resectable pancreatic cancer is expected to increase the margin-negative resection rate and improve survival[10].

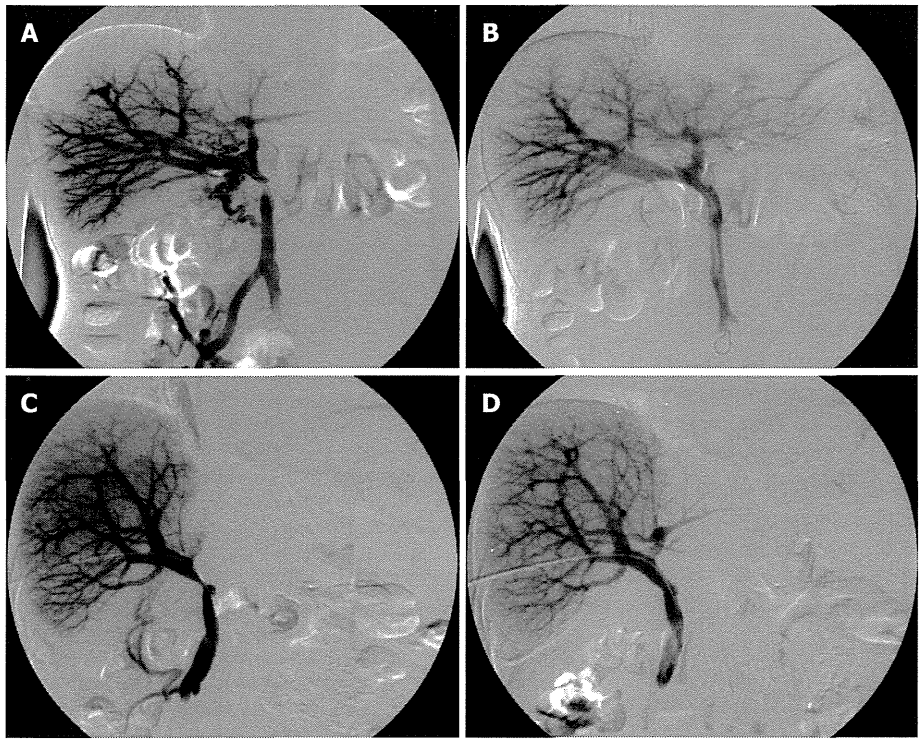

Figure 4 Percutaneous transhepatic direct portography showing short segmental stenosis of the portal vein in the region of the anastomosis. A: Collateral circulation of the pancreatic head; B: After the expandable metallic stent (EMS) placement, the stenosis was improved, and the collateral circulation disappeared; C: The blood flow of the umbilical portion of the left portal vein was unclear; D: The stenosis was improved, and the blood flow of the umbilical portion became clear after the EMS placement.

Table 1 The clinical characteristics of three patients

Pt. No.	Age (yr)	Sex	Operative procedure	Symptoms	Months before onset	Procedure of stent placement	Pressure gradient (cmH_2O)		Improvement in symptoms
							Before	After	
1	54	M	PD	Ascites, malnutrition	9	Intraoperative, via ileocolic vein	6.5	0	Yes
2	44	M	DP	Ascites, malnutrition, diarrhea	5	Percutaneous transhepatic	21.0	2.0	Yes
3	68	F	PD	Ascites, malnutrition	5	Percutaneous transhepatic	12.0	0	Yes

PD: Pancreaticoduodenectomy; DP: Distal pancreatectom; M: Male; F: Female.

We have performed gemcitabine-based NACRT in 18 patients with borderline resectable pancreatic cancer since 2005. Of 15 patients who completed the NACRT, nine had pancreatectomy. Combined portal resection was performed in 8 of the 9 patients. We report here the cases of three patients with PV stenosis (Table 1), and thus the ratio of post-operative PV stenosis in patients with PV resection following NACRT increases to 37.5% in our patient series.

There was no case of PV stenosis among 22 patients who underwent PV resection for pancreatobiliary cancer without NACRT in the same period in our department. The incidence of PV obstruction after PD with PV resection has been reported as 1.5%[11] and 25%[12]. The incidence of PV stenosis after PD with PV resection is rarely reported. Leach *et al*[12] reported the incidence 18% (5 of 29 patients), but the 18% includes 14 patients who received IORT. Mitsunaga *et al*[5] suggested that the mechanism of the development of extrahepatic PV occlusion after IORT is associated with the periportal changes induced by IORT and periportal fibrosis. We did not find any reports of PV stenosis after pancreatectomy following NACRT for pancreatic cancer because most papers about NACRT for pancreatic cancer report only perioperative complications[13,14]. However, it seems possible that the periportal changes are induced by neoadjuvant radiation, similar to those induced by IORT, and they increase the risk of the development of PV stenosis.

The first choice of treatment for PV stenosis after liver transplantation is balloon dilation[15]. The indication of stent placement is limited to elastic stenosis and recurrent stenosis. Case 1 had an elastic stenosis, and thus the EMS placement was done after the venoplasty. However, we placed the stents in Cases 2 and 3 before the

venoplasty. Placing a stent for benign stenosis before a venoplasty is controversial. Takaki *et al*[16] speculated that stent placement is essential for the treatment of early anastomotic stenosis because such stenosis is caused by reactive edema or technical problems, and balloon angioplasty fails to dilate the vessel due to recoil. The onset of the stenosis in the three cases presented here was also early (5-9 POMs). Considering the poor prognosis of pancreatic cancer, early improvement of a patient's symptoms and quality of life is important.

In Case 1, we placed the stent *via* ileocolic vein because massive ascites interfered with transhepatic puncture. However, in Cases 2 and 3, we could safely performed percutaneous transhepatic stent placement after draining the ascites.

In conclusion, we have reported the cases of three patients with PV stenosis after pancreatectomy and PV resection following NACRT for pancreatic cancer. A relationship between PV stenosis and NACRT is suspected, but further investigation is required to determine whether NACRT has relevance to PV stenosis.

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