Table 1. Clinical Profile and Serum Levels of Liver Markers and Their Ratios in Patients without Liver Cirrhosis

	Control	Hepatitis B	Hepatitis C	AIH	NASH	ALD	PBC	PSC	p value*
N	80	29	50	31	143	11	55	25	
Age (years)	38.0 (30.5, 50)	48.0 (36, 61)	61.0 (51, 68)	61.0 (50, 69)	46.5 (38, 60)	61.0 (57, 67.5)	63.0 (52.5, 71)	64.0 (46, 71)	0.021
Male (%)	54	45	44	17	57	100	11	44	<0.001
OCT (ng/mL)	18 (10, 29)	24 (8.5, 46)	39 (20, 85.5)	25 (9, 73)	51 (29.5, 89)	110 (48.5, 136)	50 (25, 85.5)	68 (18, 147)	<0.001
AST (U/L)	18 (16, 21)	25 (21, 30)	35 (25, 53)	24 (18.5, 35.5)	33 (24, 44)	33 (21.5, 51)	26 (23, 32.5)	28 (22, 32)	<0.001
ALT (U/L)	14 (12, 19)	24 (17, 33)	36 (22, 59)	25 (16, 46.5)	45 (30, 73.5)	33 (13.5, 48)	23 (18.5, 27)	26 (18, 41)	<0.001
OCT/AST	1.0 (0.6, 1.4)	0.8 (0.4, 1.4)	1.2 (0.8, 1.7)	1.1 (0.4, 1.7)	1.5 (1.1, 2.0)	2.3 (2.1, 2.7)	1.9 (1.0, 3.0)	2.3 (0.6, 4.0)	<0.001
OCT/ALT	1.1 (0.8, 1.6)	0.9 (0.4, 1.4)	1.1 (0.7, 1.7)	0.9 (0.5, 1.4)	1.3 (0.8, 1.6)	2.7 (2.0, 3.6)	2.2 (1.3, 3.1)	1.7 (0.7, 3.8)	<0.001
AST/ALT	1.2 (1.0, 1.4)	1.0 (0.9, 1.3)	1.0 (0.8, 1.3)	1.1 (0.8, 1.3)	0.7 (0.6, 0.9)	1.3 (0.9, 1.6)	1.2 (1.0, 1.5)	1.1 (1.0, 1.3)	<0.001
Plt ($\times 10^4/mm^3$)	ND	20.4 (16.6, 22.0)	17.6 (14.7, 20.1)	21.3 (15.2, 23.9)	21.5 (16.0, 24.8)	20.9 (16.5, 26.3)	21.8 (17.5, 26.2)	20.9 (18.7, 27.0)	0.012
Albumin (g/dL)	ND	4.3 (4.1, 4.7)	4.2 (4.0, 4.9)	4.0 (3.8, 4.4)	4.4 (4.1, 4.8)	3.9 (3.7, 4.3)	4.1 (4.0, 4.4)	4.1 (3.9, 4.5)	NS
T-Bil (mg/dL)	ND	0.5 (0.4, 0.8)	0.7 (0.5, 0.9)	0.7 (0.6, 1.1)	0.6 (0.5, 0.7)	0.6 (0.6, 0.7)	0.7 (0.5, 0.8)	0.9 (0.7, 1.0)	0.002
GGT (U/L)	ND	20 (14, 27)	28 (20, 44.5)	41 (18.5, 84)	58 (32, 119)	158 (38, 238)	42 (25, 102)	75 (53, 172)	<0.001

Data are expressed as median values, and 25th and 75th percentile (Q25, Q75).
*p values correspond of the eight groups. Kruskal-Wallis test for continuous factos or Pearson's chi-square for categorical variables were used. NS: not significant.
Q25: 25th percentile, Q75: 75th percentile, Hepatitis B: chronic hepatitis B, Hepatitis C: chronic hepatitis C, AIH: autoimmune hepatitis, NASH: non-alcoholic steatohepatitis, ALD: alcoholic liver disease, PBC: primary biliary cirrhosis, PSC: primary sclerosing cholangitis, ND: not done, OCT: ornithine carbamoyltransferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, OCT/AST: the ratio of OCT to AST, OCT/ALT: the ratio of OCT to ALT, Plt: platelet count, T-Bil: total billirubin, GGT: gamma-glutamyl transferase. Patients with hepatocellular carcinoma were excluded.

OCT subgroup (1.6 vs. 1.1, p<0.001); the same trend was noted for the OCT/ALT ratio (1.3 vs. 1.1, p<0.001).

Characteristics of the LC group

The characteristics of the LC group (n=96) are displayed in Table 2. Patients with hepatitis B (n=2) and AIH (n=2) were excluded from the analysis due to their small numbers. The median age of the LC patients was older than that of the non-LC patients, except for those with ALD and PSC.

The LC patients with NASH, ALD, PBC and PSC comprised the high-OCT subgroup, while those with hepatitis C formed the low-OCT subgroup. Interestingly, the OCT level that defined the high-OCT subgroup among the non-LC patients also identified the high-OCT subgroup among the LC patients, while the hepatitis C patients were classified into the low-OCT subgroup in both the LC and non-LC groups.

The differences in the OCT levels between the high-OCT subgroup (101.0 ng/mL) and the low-OCT subgroup (62.4 ng/mL) of the LC group were statistically significant (p= 0.019). The OCT/AST ratios were greater in the high-OCT subgroup of the LC group than in the low-OCT subgroup (2.2 vs. 1.3, p=0.005), and the same trend was observed for the OCT/ALT ratio (2.9 vs. 1.7, p=0.034). Among the patients in the high-OCT subgroup, those with PSC had significantly higher OCT levels than those with NASH (p< 0.001), ALD (p=0.006) or PBC (p=0.002). The OCT/AST and OCT/ALT ratios were also significantly higher among the patients with PSC than among those with NASH (p< 0.001 and p<0.001, respectively), ALD (p=0.002 and p= 0.008, respectively) or PBC (p<0.001 and p=0.013, respectively).

Comparison between the non-LC and LC groups

Patients with hepatitis B and AIH were also excluded

Table 2. Clinical Profile and Serum Levels of Liver Markers and Their Ratios in Patients with Liver Cirrhosis

	Control	Hepatitis B	Hepatitis C	AIH	NASH	ALD	PBC	PSC	p value*
N	80	2	10	2	39	15	13	15	
Age (years)	38.0 (30.5, 50)	50.0 (44, 56)	69.5 (67, 74)	78.0 (77, 79)	62.0 (57, 73.5)	60.0 (54, 69)	64.0 (60, 74)	54.0 (38, 75.5)	<0.001
Male (%)	54	100	70	0	51	87	15	67	0.003
OCT (ng/mL)	18 (10, 29)	57 (27, 87)	59 (52.5, 69)	66 (65.5, 66)	72 (47, 119)	122 (63.5, 169)	79 (46, 175.5)	309 (221, 487.5)	<0.001
AST (U/L)	18 (16, 21)	49 (36, 62)	41 (36, 58)	41 (38, 44)	36 (28.5, 53)	52 (38.5, 55)	46 (38, 56)	65 (35, 138)	<0.001
ALT (U/L)	14 (12, 19)	37 (23, 51)	35 (19, 40)	32 (20, 43)	32 (25.5, 53.5)	28 (22.5, 40.5)	33 (22, 34)	74 (41, 104)	<0.001
OCT/AST	1.0 (0.6, 1.4)	1.1 (0.6, 1.5)	1.3 (1.1, 1.7)	1.6 (1.5, 1.7)	2.2 (1.5, 3.1)	2.0 (1.5, 3.5)	2.0 (1.1, 3.1)	4.5 (2.9, 7.1)	<0.001
OCT/ALT	1.1 (0.8, 1.6)	1.5 (0.9, 2.1)	1.7 (1.5, 2.8)	2.0 (1.6, 2.4)	2.5 (1.4, 3.5)	3.0 (1.7, 5.7)	2.9 (2.0, 3.6)	5.0 (3.4, 6.8)	<0.001
AST/ALT	1.2 (1.0, 1.4)	1.4 (1.2, 1.6)	1.3 (1.1, 2.0)	1.5 (1.0, 1.9)	1.1 (0.9, 1.4)	1.4 (1.2, 2.1)	1.5 (1.0, 2.3)	1.2 (1.0, 1.4)	NS
Plt ($\times 10^4/mm^3$)	ND	2.8 (2.7, 2.8)	7.6 (5.8, 8.2)	6.0 (5.1, 6.8)	11.6 (7.5, 12.8)	9.0 (8.0, 11.1)	11.0 (7.4, 19.6)	18.2 (12.2, 24.6)	<0.001
Albumin (g/dL)	ND	3.2 (2.8, 3.5)	3.5 (2.4, 3.9)	3.4 (3.2, 3.6)	3.9 (3.3, 4.4)	3.3 (2.1, 3.6)	3.4 (2.3, 3.7)	3.3 (2.8, 4.1)	NS
T-Bil (mg/dL)	ND	2.6 (2.3, 2.9)	1.2 (0.9, 1.5)	2.0 (1.0, 3.0)	1.1 (0.8, 1.9)	1.2 (0.9, 1.9)	1.0 (0.8, 1.5)	4.9 (1.2, 7.5)	NS
GGT (U/L)	ND	63 (42, 83)	34 (24, 56)	34 (28, 39)	76 (45, 89)	151 (43.5, 246)	104 (45, 152)	239 (87.5, 271)	0.002

Data are expressed as median values, and 25th and 75th percentile (Q25, Q75).
*p values correspond of the eight groups. Kruskal-Wallis test for continuous factos or Pearson's chi-square for categorical variables were used. NS: not significant.
Q25: 25th percentile, Q75: 75th percentile, Hepatitis B: chronic hepatitis B, Hepatitis C: chronic hepatitis C, AIH: autoimmune hepatitis, NASH: non-alcoholic steatohepatitis, ALD: alcoholic liver disease, PBC: primary biliary cirrhosis, PSC: primary sclerosing cholangitis, ND: not done, OCT: ornithine carbamoyltransferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, OCT/AST: the ratio of OCT to AST, OCT/ALT: the ratio of OCT to ALT, Plt: platelet count, T-Bil: total billirubin, GGT: gamma-glutamyl transferase. Patients with hepatocellular carcinoma were excluded.

from the analysis due to the small number of such patients in the LC group.

Fig. 2 displays the OCT levels, OCT/AST ratios and OCT/ALT ratios for the patients with and without LC who had hepatitis C, NASH, ALD, PBC and PSC. The difference in the OCT levels between the non-LC and LC groups was 20 ng/mL among the patients with hepatitis C, 21 ng/mL among the patients with NASH, 12 ng/mL among the patients with ALD, 29 ng/mL among the patients with PBC and 241 ng/mL among the patients with PSC, with the difference being significant for each disease, except ALD ($p<0.001$). The OCT/AST ratios were higher in the LC patients than in the non-LC patients with each type of chronic liver disease, except for ALD, with significant differences for NASH ($p=0.017$) and PSC ($p=0.004$). The OCT/ALT ratios were also significantly higher among the LC patients than among the non-LC patients with hepatitis C ($p=0.011$), NASH ($p<0.001$) and PSC ($p=0.003$).

Concerning the transaminase levels, the differences in the AST levels between the non-LC and LC groups were significant among the patients with PBC ($p=0.001$) and PSC ($p<0.001$), whereas the ALT levels were similar in the non-LC and LC groups, with the exception of the LC patients with PSC ($p<0.001$). Among the NASH patients, the AST/ALT ratios exhibited a significant difference between those with and without LC ($p<0.001$), although there were no significant differences among the patients with the other types of chronic liver disease.

Markers for predicting LC

The differences in the OCT levels and OCT/AST and OCT/ALT ratios between the non-LC and LC groups were significant among the patients with hepatitis C, NASH, PBC and PSC; therefore, a ROC analysis of the patients with

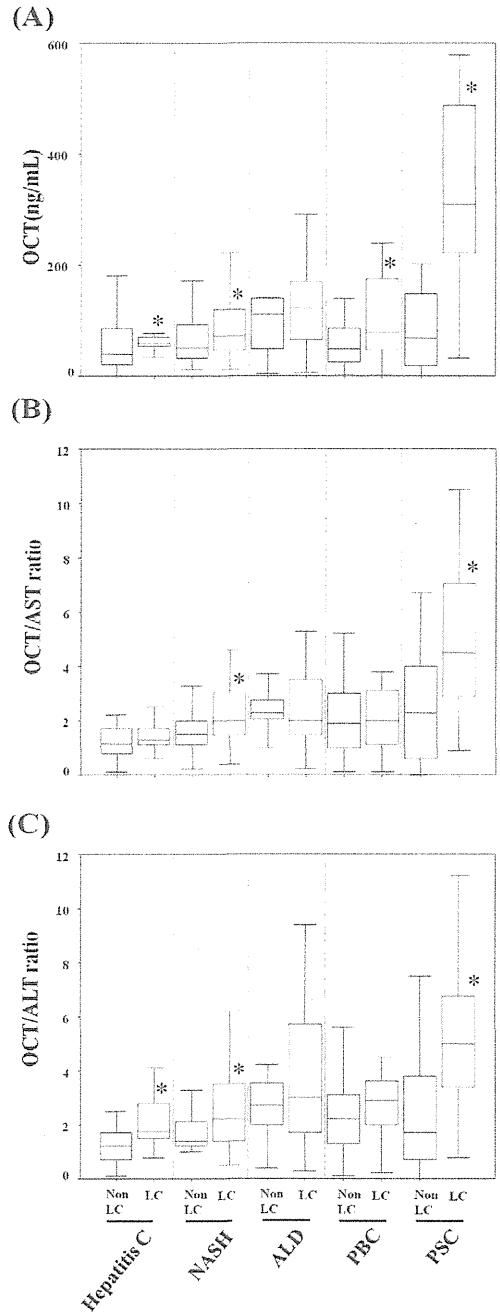

Figure 2. **Box plots for the serum markers in the patients with different chronic liver diseases among the liver cirrhosis (LC) group and the non-LC group. (A) Ornithine carbamoyltransferase (OCT), (B) Ratio of OCT to aspartate aminotransferase (AST) (OCT/AST ratio), (C) Ratio of OCT to alanine aminotransferase (ALT) (OCT/ALT ratio). The box plots present the median and interquartile ranges with outliers: white boxes, non-LC patients; grey boxes, LC patients. *p<0.05 versus the non-LC group according to the Mann-Whitney test. Hepatitis C: chronic hepatitis C, NASH: non-alcoholic steatohepatitis, ALD: alcoholic liver disease, PBC: primary biliary cirrhosis, PSC: primary sclerosing cholangitis**

these diseases was performed. The predictive value for LC of the OCT level, OCT/AST ratio, OCT/ALT ratio and AST/ALT ratio is shown in Fig. 3 and Table 3. Among these four indexes, the OCT/ALT ratio displayed the largest AUROC for predicting LC (0.75) in the patients with hepatitis C. When the cut-off value was set at 1.26, the OCT/ALT ratio had a sensitivity of 80.0% and a specificity of 60.0% for predicting LC. A similar pattern was observed in the patients with NASH, as the OCT/ALT ratio again had the largest AUROC for predicting LC (0.79). When the cut-off value was set at 1.51, the OCT/ALT ratio showed a sensitivity of 74.4% and a specificity of 75.0% for predicting LC. The OCT level had the largest AUROC (0.64) among the four indexes in the patients with PBC. In the patients with PSC, the OCT level also had the largest AUROC (0.91) for predicting LC among the four indexes. When the cut-off value was set at 96.5 ng/mL, the OCT level showed a sensitivity of 93.3% and a specificity of 68.0% for predicting LC.

Fig. 4 displays the results of the Spearman's rank correlation analysis of the relationships between the Plt count and the OCT level, OCT/AST ratio and OCT/ALT ratio among all patients with chronic liver disease. There were no significant correlations between these variables. The Plt count and OCT level also showed no significant correlations with LC within each disease category.

Discussion

We investigated the OCT levels in patients with various liver diseases and found that the OCT levels of non-LC patients with steatohepatitis (NASH and ALD) and cholestatic hepatitis (PBC and PSC) were much higher than those of non-LC patients with hepatitis B, hepatitis C or AIH. The non-LC patients with high OCT levels also had higher OCT/AST and OCT/ALT ratios than the patients with low OCT levels. Similar results were obtained in the patients with LC. A comparison of the OCT levels between the patients with hepatitis C, NASH, PBC and PSC showed that those in the LC group had higher levels than those in the non-LC group, and the difference was marked for PSC patients.

Therefore, the OCT levels varied depending on the etiology of liver disease, although they exhibited a similar pattern in the patients with and without LC, and the liver diseases associated with high OCT levels were also associated with high OCT/AST and OCT/ALT ratios. These findings suggest that more severe mitochondrial injury may occur in patients with NASH, ALD, PBC and PSC than in those with hepatitis B/C and AIH throughout the course of disease, as reflected by the pattern of OCT release. Mitochondrial damage due to oxidative stress has been reported to play a very important role in the pathogenesis of ALD and NASH (14). Interestingly, the OCT levels of the ALD patients were extremely high both in the presence and absence of LC; thus, the OCT level may not be a useful biomarker of fibrosis. Among the liver disease groups, the patients in the LC

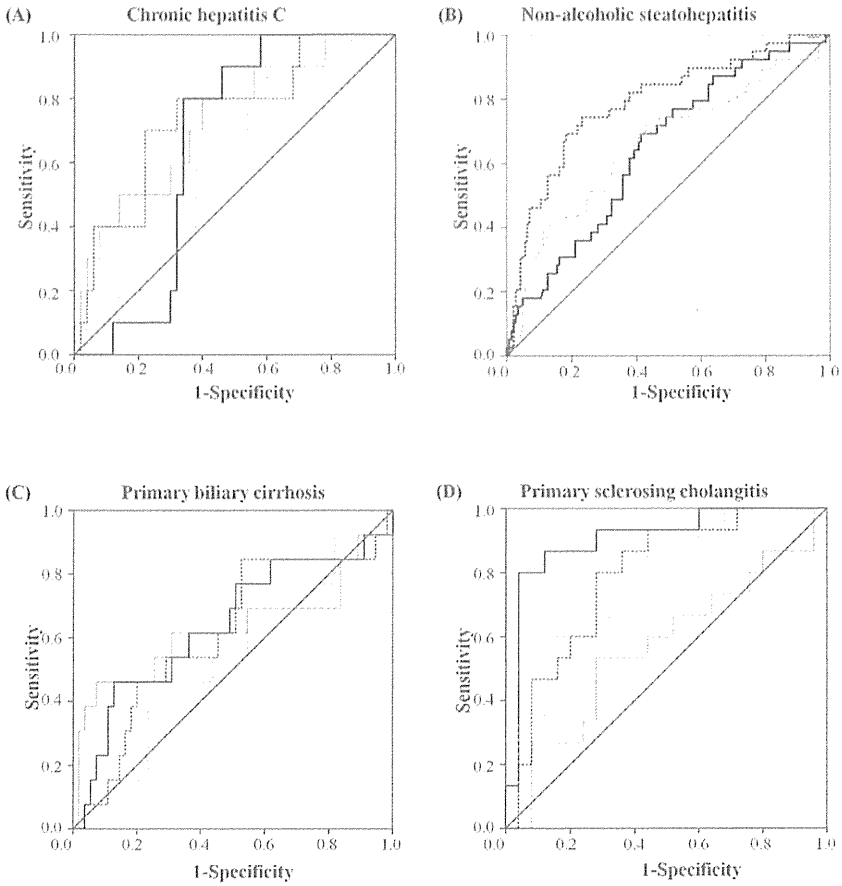

Figure 3. **Receiver operating characteristic curves for predicting liver cirrhosis. (——): ornithine carbamoyltransferase (OCT), (----): OCT/aspartate aminotransferase (AST) ratio, (----): OCT/alanine aminotransferase (ALT) ratio, (—·—): AST/ALT ratio, (—): reference line**

group with PSC had very high OCT levels, OCT/AST ratios and OCT/ALT ratios. It has been reported that OCT exhibits massive elevation in patients with obstructive jaundice (15-18), which may explain the extremely high OCT levels observed in PSC patients. The PSC patients evaluated in the present study included 13 subjects with a Child A status and two subjects with a Child B status; therefore, we were unable to analyze the OCT levels in the PSC patients based on stratification according to the Child-Pugh score. Further investigation is needed to elucidate the relationship between the OCT level and Child-Pugh score. In patients with PBC, only the small bile ducts are involved, and the OCT levels demonstrate marked differences between these two cholestatic liver diseases.

The AST/ALT ratio is a well-known biomarker for predicting LC. However, the ROC analysis of the OCT level, OCT/AST ratio, OCT/ALT ratio and AST/ALT ratio in the hepatitis C, NASH, PBC and PSC patients did not identify the AST/ALT ratio to be best predictor of the presence of LC in any of these disease categories. Instead, the OCT/ALT ratio, OCT/ALT ratio and OCT level were found to be the best predictors of the presence of LC in patients with hepatitis C, NASH and PSC, respectively. The comparison the OCT/AST, OCT/ALT and AST/ALT ratios between the non-LC and LC groups revealed that these three ratios were similar in both the low-OCT subgroups (patients with hepatitis B and hepatitis C). In the high-OCT subgroups (patients with NASH, ALD, PBC and PSC), however, the OCT/AST and OCT/ALT ratios were almost twice as high as the AST/ALT ratio. This finding suggests that the OCT/AST and OCT/ALT ratios were more sensitive markers of fibrosis in the high-OCT subgroup than in the low-OCT subgroup.

There were no significant correlations between the Plt count and OCT level in any of the chronic liver disease categories, suggesting that the OCT level is possibly a clinically useful independent serum biomarker of LC. It remains unclear why the OCT levels were higher in the LC patients than in the non-LC patients. One possibility is that even if the degree of hepatocyte necrosis is diminished in LC patients, the hepatocytes in patients with cirrhosis suffer from mitochondrial damage due to remodeling of the hepatic architecture.

The primary limitation of this study is the small number of patients with each type of chronic liver disease, because this was a prospective study and we only assessed new serum samples.

Table 3. Predictive Value of OCT, the OCT/AST Ratio, the OCT/ALT Ratio, and the AST/ALT Ratio for Liver Cirrhosis in Patients with HCV, NASH, PBC, and PSC

	AUROC	Cut-off Value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hepatitis C						
OCT (ng/mL)	0.66	22.5	100	28.0	21.7	98.0
OCT/AST	0.61	0.64	90.0	26.0	17.6	88.9
OCT/ALT	0.75	1.26	80.0	60.0	28.6	93.8
AST/ALT	0.73	1.16	60.0	68.0	27.3	89.6
NASH						
OCT (ng/mL)	0.65	46.9	74.4	50.0	28.7	87.7
OCT/AST	0.66	1.77	61.5	65.0	32.4	86.1
OCT/ALT	0.79	1.51	74.4	75.0	44.6	91.5
AST/ALT	0.71	0.90	79.7	51.6	78.9	85.3
PBC						
OCT (ng/mL)	0.64	71.5	53.8	65.5	26.9	85.7
OCT/AST	0.52	0.70	92.3	18.2	21.1	90.9
OCT/ALT	0.61	2.38	61.5	52.7	23.5	85.3
AST/ALT	0.63	1.44	53.8	72.7	31.8	87.0
PSC						
OCT (ng/mL)	0.91	96.5	93.3	68.0	63.6	94.4
OCT/AST	0.76	2.00	86.7	48.0	50.0	85.7
OCT/ALT	0.79	2.01	86.7	56.0	54.2	87.5
AST/ALT	0.57	1.12	66.7	48.0	43.5	70.6

AUROC: area under the receiver operating characteristic curve, PPV: positive predict value, NPV: negative predict value, Hepatitis C: chronic hepatitis C, NASH: non-alcoholic steatohepatitis, PBC: primary biliary cirrhosis, PSC: primary sclerosing cholangitis, OCT: ornithine carbamoyltransferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, OCT/AST: the ratio of OCT to AST, OCT/ALT: the ratio of OCT to ALT, AST/ALT: the ratio of AST to ALT

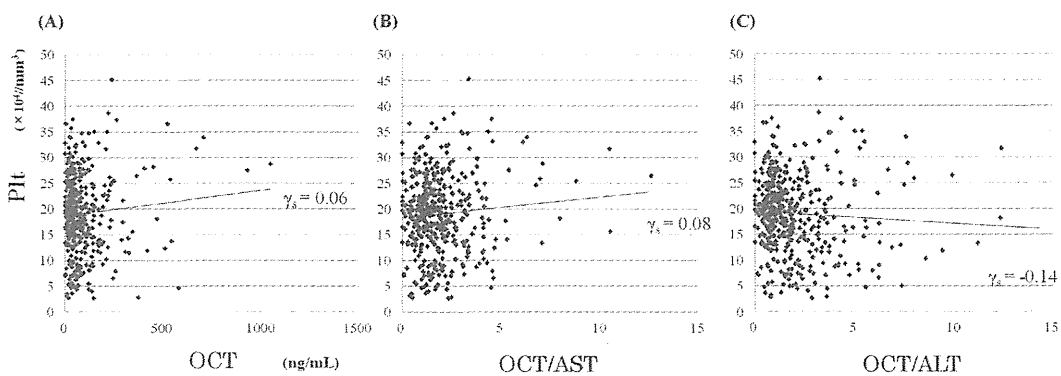

Figure 4. Spearman's rank correlation analysis of the relationship between the platelet (Plt) count and ornithine carbamoyltransferase (OCT) level (A), the ratio of OCT to aspartate aminotransferase (AST) (OCT/AST ratio: B) and the ratio of OCT to alanine aminotransferase (ALT) (OCT/ALT ratio: C) in each chronic liver disease

It has been reported that the OCT level is influenced by age (19). However, there were no correlations between the OCT levels and age among any of the patient groups, including all patients with chronic liver disease and the patients in each chronic liver disease category (including the controls), in the present study.

In conclusion, in the current study, the OCT levels were much higher in the patients with NASH, ALD, PBC and PSC than in those with hepatitis B, hepatitis C and AIH. A similar pattern was also observed with respect to the OCT/AST and OCT/ALT ratios. The role of OCT in each type of liver disease should be evaluated further, which may improve our understanding of the pathogenesis of these diseases. Both the serum OCT level and OCT/ALT ratio may be useful surrogate markers of LC. In particular, among PSC patients, the OCT level is a useful biomarker for LC. However, the significant differences observed in the OCT levels between the different disease categories in this study may also be a disadvantage of this marker. The accumulation of more data regarding the OCT levels in various chronic liver diseases would be useful for making the differential diagnosis between these diseases. The OCT level can

be measured using a simple, reliable and inexpensive test and is a highly liver-specific protein. Accordingly, it may be a useful marker in general practice. However, large-scale studies are needed to confirm the value of the serum OCT level as a marker of LC in patients with various liver diseases.

The authors state that they have no Conflict of Interest (COI).

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Original Article

Hepatocellular carcinoma based on cryptogenic liver disease: The most common non-viral hepatocellular carcinoma in patients aged over 80 years

Katsutoshi Tokushige,[1] Etsuko Hashimoto,[1] Yoshinori Horie,[2] Makiko Taniai[1] and Susumu Higuchi[3]

[1]*Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University,* [2]*Department of Internal Medicine, Sanno Hospital, International University of Health and Welfare, Tokyo, and* [3]*National Hospital Organization Kurihama Alcoholism Center, Kanagawa, Japan*

Aim: To clarify the clinical features of patients with hepatocellular carcinoma (HCC) with cryptogenic liver diseases, we analyzed the data from a nationwide survey in Japan.

Methods: The survey was conducted in 2009. The factors examined included age and underlying liver diseases: alcoholic liver disease (ALD; $n = 991$), non-alcoholic fatty liver disease ($n = 292$), modest alcohol intake (intake between 20 and 70 g/day, $n = 214$) and cryptogenic liver diseases ($n = 316$). We compared the clinical features of cryptogenic HCC among patient-age subgroups.

Results: HCC with ALD etiology was most common among the non-viral HCC patients under 80 years old; for those aged 80 years or older, cryptogenic HCC was the most common etiology. Among the cryptogenic HCC patients, the body mass index values and the prevalences of liver cirrhosis (LC) and diabetes mellitus (DM) were significantly lower in the 80 years or older group versus the 50–79 years group. In the 80 years or older group, 28% of the patients developed HCC without cirrhosis, obesity and DM.

Conclusion: In the HCC patients aged 80 years and over, the etiology of most of the non-viral HCC cases was classified as cryptogenic. In light of our finding that the prevalences of obesity, DM and LC in the 80 years or older group of cryptogenic HCC patients were significantly lower those in the younger patients, it is apparent that analyses of HCC cases must take age differences into account.

Key words: cryptogenic liver disease, diabetes mellitus, hepatocellular carcinoma, liver cirrhosis, old age

INTRODUCTION

PRIMARY LIVER CANCER is the fifth most common cancer worldwide, and the third most common cause of cancer mortality.[1–3] According to the most recent nationwide Japanese registry data, primary liver cancer ranked fourth for men and sixth for women as a cause of death from malignancy.[4] Several recent Japanese surveys of hepatocellular carcinoma (HCC) studies have shown that the underlying liver diseases for HCC have changed; the incidence of hepatitis C virus (HCV)-related HCC has gradually decreased to approximately 60–70%, whereas the incidence of HCC associated with non-viral chronic liver disease has gradually increased to approximately 15–25%.[5–8] Among the cases of non-viral HCC, alcoholic liver disease (ALD)-HCC was found to account for 43–51% of cases, followed by unknown etiology liver disease HCC (18–35%) and non-alcoholic fatty liver disease (NAFLD)-HCC (13–28%).[6–8]

Non-alcoholic fatty liver disease is usually defined by a daily alcohol consumption of less than 20 g in women and less than 30 g in men, because ALD can occur above these thresholds.[9,10] However, there is no clear consensus regarding the threshold alcohol consumption for defining NAFLD and non-alcoholic steatohepatitis (NASH), and because the definitions are not clear, it is difficult to summarize the etiological analyses of liver disease underlying non-viral HCC.

To clarify the etiology of HCC in Japanese patients with non-viral liver disease, we performed a nationwide

Correspondence: Dr Etsuko Hashimoto, Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. Email: drs-hashimoto@mti.biglobe.ne.jp

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survey of HCC patients in 2009.[8] We studied the clinical features of HCC patients with NAFLD, ALD (alcohol consumption, ≥70 g/day) and chronic liver disease of unknown etiology. We divided the cases of unknown etiology HCC into two subgroups: no alcohol intake group (alcohol consumption, <20 g/day) and modest alcohol intake group (alcohol consumption, 20–70 g/day).

We found that among the non-viral HCC cases, ALD-HCC was the most common etiology, and we observed that the patients in the ALD-HCC group were the youngest and showed the lowest percentage of females. The patients in the modest alcohol intake HCC group showed the same tendencies as the ALD-HCC patients regarding sex, body mass index (BMI), prevalence of lifestyle-related disease, and liver function. We reported that a modest intake of alcohol may have a more significant role in hepatic carcinogenesis than is presently thought.

In the present study, we focused on the clinical features and pathogenesis of HCC patients who reported consuming no alcohol and those who had cryptogenic HCC. In our experience, it is not rare that patients over 80 years old develop HCC in normal liver with no etiology (unpubl. data). To investigate the characteristics of cryptogenic HCC, we focused on age. First, we assessed the etiologies of non-viral HCC patients divided into 10-year age subgroups, and then we compared the clinical features of the cryptogenic HCC patients in the different age subgroups.

METHODS

IN 2009, WE conducted a nationwide survey of patients who received a diagnosis of HCC in Japan. We sent questionnaires to all of the hospitals in Japan that are approved by the Japanese Society of Gastroenterology, asking about the etiology of their HCC cases, and we sent case cards for ALD-HCC, NAFLD-HCC, modest alcohol intake HCC and cryptogenic HCC cases. We asked for data on all patients who were diagnosed with HCC between April 2006 and March 2009.

A total of 115 hospitals across the country responded to the questionnaire and provided case cards. These institutions are listed in Appendix I. The present retrospective study was conducted according to the Declaration of Helsinki (2000 version). We enrolled 14 530 patients with a clinical and/or histological diagnosis of HCC. Among these patients, 2299 (15.8%) were diagnosed as having non-hepatitis B virus (HBV), non-hepatitis C virus (HCV)-HCC and we analyzed their case cards.

All patients with non-viral HCC were shown to be negative for hepatitis B surface antigen and for anti-HCV antibody and/or HCV RNA by polymerase chain reaction analysis.

Among the 2299 non-viral HCC patients, we excluded the cases of 274 patients because their clinical data, such as the amount of alcohol intake and laboratory data, were not sufficient for the analysis (Fig. 1). We categorized each of the remaining 2025 non-viral HCC patients into one of five groups according to the etiology of their HCC: (i) ALD-HCC; (ii) NAFLD-HCC; (iii) modest alcohol intake HCC; (iv) cryptogenic HCC; and (v) miscellaneous disease.

Alcoholic liver disease (ALD-HCC group, $n = 991$) was diagnosed according to the modified criteria proposed by Takada *et al.*,[11] and the alcohol consumption in ALD was defined as habitual alcohol consumption over 70 g daily. The diagnosis of NAFLD ($n = 292$) was based on the following criteria: (i) detection of hepatic steatosis (or steatohepatitis) by liver biopsy or imaging; (ii) intake of less than 20–30 g of ethanol daily (as confirmed by the attending physician and family members in close contact with the patient); and (iii) the appropriate exclusion of other liver diseases.[8,12,13] "Modest intake of alcohol" ($n = 214$) was defined as unknown liver disease with alcohol consumption of 20–70 g/day. "Cryptogenic HCC" was defined as unknown liver disease without steatosis by imaging modalities or liver biopsy among patients

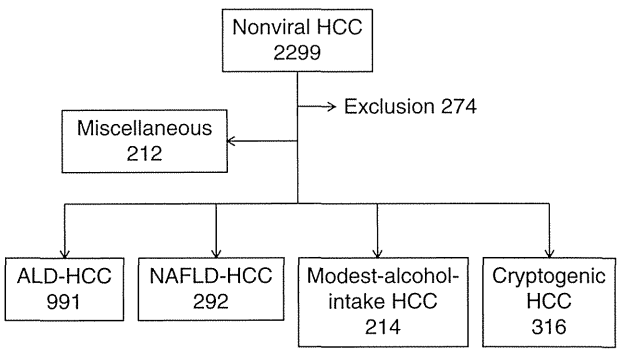

Figure 1 The cases of 2299 patients with non-viral HCC were collected by a national survey in Japan, and the cases of 274 patients were excluded because of incomplete clinical data. We analyzed the cases of the remaining 991 patients with ALD-HCC, 292 with NAFLD-HCC, 214 with modest-alcohol-intake HCC, and 316 with cryptogenic HCC; the underlying causes of the other 212 patients were classified as miscellaneous disease. ALD, alcoholic liver disease; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease.

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with alcohol consumption of less than 20 g/day. Many miscellaneous diseases were excluded, such as congestive disease, metabolic disease (e.g. Wilson's disease, hemochromatosis), primary biliary cirrhosis, autoimmune hepatitis and primary sclerosing cholangitis. The final diagnosis of HCC and the assessment of etiology were conducted at each participating institution.

Etiologies of non-viral HCC divided by 10-year age subgroups

To elucidate the etiological characteristics of the non-viral HCC cases, we investigated the deviations of the following four non-viral HCC groups: ALD-HCC, NAFLD-HCC, modest alcohol intake HCC and cryptogenic HCC. We divided these patients into five subgroups according to age in 10-year increments: less than 50, 50–59, 60–69, 70–79 and 80 years or older. We compared the number of patients and the percentages of these groups against the total population of non-viral HCC patients.

Characteristic features of cryptogenic HCC: comparison of age-dependent groups

We also divided the group of 316 cryptogenic HCC patients into three broader age subgroups and compared their clinical data: (i) less than 50 years old ($n = 7$); (ii) 50–79 years old ($n = 216$); and (iii) 80 years and over ($n = 93$).

Obesity is defined by the Japanese Obesity Association criteria as a BMI of more than 25 kg/m^2.[14] For the present patient population, the diagnosis of type II diabetes mellitus (DM) was based on the World Health Organization (WHO) criteria.[15] Dyslipidemia was diagnosed if the patient was currently on lipid-lowering medications, or if the patient had shown elevated serum levels of total cholesterol (>220 mg/dL) and/or triglycerides (>150 mg/dL) on at least three occasions. Hypertension was diagnosed if the patient was receiving antihypertensive therapy or had a recorded blood pressure of more than 140/90 mmHg on at least three occasions.

Liver cirrhosis (LC) was diagnosed on the basis of histological biopsy findings, laparoscopy or abdominal imaging (left lobe hypertrophy with splenomegaly, nodular changes in the liver surface) and laboratory findings (lower platelet count, albumin level and/or prolonged prothrombin time) compatible with LC. Clinical findings of esophageal varices, ascites and/or hepatic encephalopathy were also taken into account.

The following laboratory parameters were measured: albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), fasting blood sugar, hemoglobin Alc (HbAlc), platelet count, prothrombin time (PT), des-γ-carboxyprothrombin (DCP) and α-fetoprotein (AFP).

Statistical analysis

The statistical analyses were performed with SPSS version 13.0 J software (SPSS, Tokyo, Japan). Data are shown as the mean ± standard deviation (SD) or as percentages. The Mann–Whitney *U*-test or the χ^2-test were used to compare data between the 50–79 years and 80 years or older subgroups of the cryptogenic HCC patients. $P < 0.05$ was considered significant.

RESULTS

Etiologies of non-viral HCC in the 10-year age subgroups

THE DISTRIBUTION OF the patients with ALD-HCC, NAFLD-HCC, modest alcohol intake HCC and cryptogenic HCC divided by each 10-year age subgroup is shown in Figure 2(a). Among the patients under 70 years old, the number of ALD-HCC cases was markedly higher in each of the three under 70 years age groups compared to NAFLD-HCC, modest alcohol intake HCC and cryptogenic HCC. In contrast, among the patients aged 80 years or older, cryptogenic HCC was the most common etiology.

Among the patients with ALD-HCC, the age-grouped numbers of patients peaked at 60–69 years old, with a mean ± SD age of 67.1 ± 9.10 years, whereas in each of the groups of patients with NAFLD-HCC (71.6 ± 8.4 years), modest alcohol intake HCC (70.4 ± 9.0 years) and cryptogenic HCC (74.1 ± 10.2 years), the ages of the three groups peaked at 70–79 years old, respectively.

Figure 2(b) shows the percentages in the four non-viral HCC groups (ALD-HCC, NAFLD-HCC, modest alcohol intake HCC and cryptogenic HCC). Among the patients under 70 years old, ALD-HCC accounted for approximately 70% of the cases; among the patients 70 years old or older, this percentage was markedly decreased, and the percentage of NAFLD-HCC cases was slightly increased. Among the patients over 70 years old, the percentage of cryptogenic HCC cases was markedly increased.

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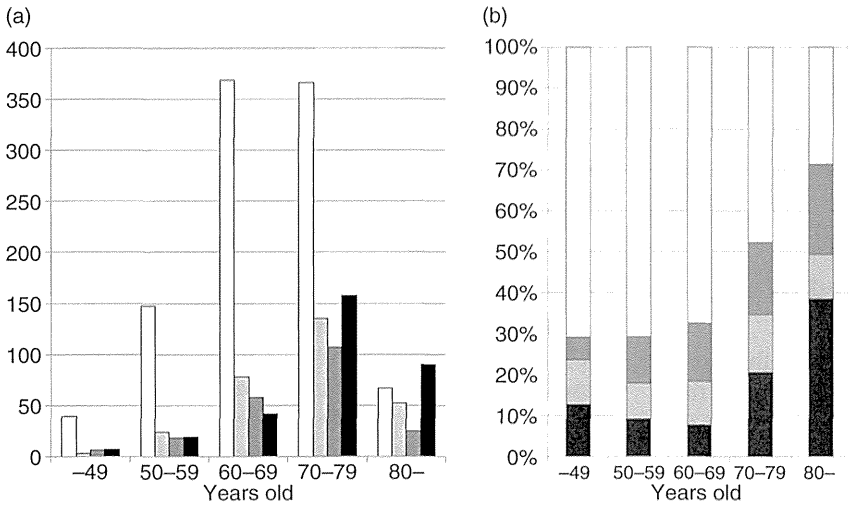

Figure 2 (a) Distribution of the patients in the four non-viral HCC groups (ALD-HCC, NAFLD-HCC, modest alcohol intake HCC and cryptogenic HCC). Among the patients under 70 years old, ALD-HCC was the most common etiology; among the patients aged 80 years or older, cryptogenic HCC was the most common etiology. (b) The percentages in the four non-viral HCC groups. □, ALD-HCC; ▩, NAFLD-HCC; ▨, modest alcohol intake HCC; ■, cryptogenic HCC. ALD, alcoholic liver disease; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease.

Cryptogenic HCC cases classified by age

Table 1 shows the clinical characteristics and HCC features among the three age groups of less than 50 years, 50–79 years and 80 years or older. As there were only seven patients in the less than 50 years group, we performed the statistical analysis between the 50–79 years group ($n = 216$) and the 80 years or older group ($n = 93$).

Table 1 Characteristic features among age-dependent groups in cryptogenic HCC

	(1) <50 years old ($n = 7$)	(2) 50–79 years old ($n = 216$)	(3) ≥80 years old ($n = 93$)	*P*-value* (2 vs 3)
Age (year)	36.0 ± 11.9	71.2 ± 6.8	84.0 ± 3.4	
Sex (female)	49%	54%	63%	NS
Obesity (BMI, >25 kg/m^2)	0%	42%	33%	NS
BMI (kg/m^2)	19.2 ± 3.3	24.5 ± 4.64	23.2 ± 3.9	0.037
DM	14%	45%	33%	0.048
Hypertension	0%	44%	53%	NS
Dyslipidemia	0%	14%	19%	NS
Liver cirrhosis	0%	62%	49%	0.048
Albumin (g/dL)	4.1 ± 0.4	3.5 ± 0.7	3.6 ± 0.7	NS
Total bilirubin (mg/dL)	1.0 ± 0.5	1.2 ± 1.2	1.2 ± 2.0	NS
AST (IU/L)	38 ± 15.9	62 ± 68.0	61 ± 55.1	NS
ALT (IU/L)	48 ± 27.2	41 ± 48.1	38 ± 35.5	NS
GGT (IU/L)	95 ± 109.3	155 ± 215.5	107 ± 110.8	NS
FBS (mg/dL)	110 ± 50.2	124 ± 52.1	119 ± 49.1	NS
HbAlc (%)	5.3 ± 0.4	6.0 ± 1.4	5.7 ± 0.9	NS
Platelet count (×10^4/mm^3)	22.7 ± 6.6	15.6 ± 8.9	17.1 ± 9.4	NS
PT (%)	98 ± 17.5	79 ± 16.8	85 ± 16.8	0.008
AFP (ng/mL)	11 779 ± 22 849	8586 ± 57 379	6903 ± 30 775	NS
DCP (ng/mL)	1460 ± 3373	16 550 ± 87 884	69 666 ± 45 294	NS
HCC size (mm)	66.2 ± 50.5	47.4 ± 37.8	56.3 ± 67.4	0.076
No. of HCC	5.5 ± 4.9	2.9 ± 3.3	2.4 ± 2.9	0.066

Expressed as the mean ± standard deviation.
**P*-value, comparison between 50–79 years age group and ≥80 years group.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DCP, des-γ-carboxyprothrombin; DM, diabetes mellitus; FBS, fasting blood sugar; GGT, γ-glutamyltransferase; HbAlc, hemoglobin Alc; HCC, hepatocellular carcinoma; NS, not significant; PT, prothrombin time.

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In terms of sex, the percentage of female patients was not significantly different between the two age groups. The BMI values of the 80 years or older group were significantly lower (23.2 ± 3.9 in the ≥80 years group vs 24.5 ± 4.6 in the 50–79 years group, $P = 0.037$). The prevalence of DM in the 80 years or older group was also significantly lower (33% in the ≥80 years group vs 45% in the 50–79 years group, $P = 0.048$).

The percentages of hypertension and dyslipidemia were not significantly different between the two age groups. The frequency of cirrhosis in the 80 years or older group was significantly lower (49% in the ≥80 years group vs 62% in the 50–79 years group, $P = 0.048$). In the 80 years or older group, 28% of the patients developed HCC without cirrhosis, obesity and DM.

The levels of serum albumin, total bilirubin, AST and ALT levels were similar between these two age groups. The serum GGT, fasting blood sugar and HbAlC levels were all slightly higher in the 50–79 years group, but the differences were not significant. The platelet count was slightly lower in the 50–79 years group. The percentage of prothrombin time in the 80 years or older group was significantly higher (mean PT%, 85% in the ≥80 years group vs 79% in the 50–79 years group, $P = 0.008$). The serum AFP and DCP levels were similar between the two groups. The maximum size of the HCC lesion in the 50–79 years group tended to be small, and the number of HCC tended to be larger.

We also investigated the clinical data of the patients with cryptogenic HCC as classified in the five age-dependent groups (<50, 50–59, 60–69, 70–79 and ≥80 years), shown in Table S1. The largest number of patients with cryptogenic HCC was in the 70–79 years group. The 50–59 years group of patients had clinical features similar to those of the less than 50 years group. The clinical features of the 60–69 years group were similar to those of the 70–79 years group. In the group of cryptogenic HCC patients aged 80 years or older, compared to the 70–79 years patients, the prevalences of LC and DM were significantly lower, the BMI values were significantly lower, and the PT values were significantly higher. There were no significant differences in clinical features between the 80 years or older group and the other age groups.

DISCUSSION

WE FOUND SEVERAL clinical characteristics of cryptogenic HCC that were related to age: (i) in the patients aged 80 years or older, cryptogenic HCC was the most common etiology among the non-viral HCC etiologies; and (ii) quite a few of the cases of HCC did not arise from obesity, DM or LC, especially in the 80 years or older group.

The etiology of cryptogenic HCC could include "burnt-out" NASH, occult HBV infection, HBV carriers with previous seroconversion to hepatitis B surface antigens and "burnt-out" autoimmune hepatitis. In the nationwide survey study used here, each hospital's gastrointestinal specialist conducted the final diagnosis of etiology. In the present study, we believe that the cases of cryptogenic HCC with obesity or DM did not have enough evidence of NASH, mild obesity or short history of DM. To exclude the possibility of including burnt-out NASH in the etiology of cryptogenic HCC, we compared the clinical features between the cryptogenic HCC patients with neither obesity nor DM and the cryptogenic HCC patients with obesity and/or DM. We found that except for the between-group differences in the prevalences of DM and hypertension and the difference in BMI, HbAlC and fasting blood sugar, no other clinical data were significantly different between these two groups (Table S2).

Both our and previous national surveys demonstrated that ALD is the most common disease among non-viral liver diseases in Japan.[5–7] However, according to the present study's detailed analysis, we found that the etiologies of HCC differed among the non-viral HCC patients by age: in the patients aged 80 years or older, cryptogenic HCC was the most common etiology of HCC.

It is well known that age and liver fibrosis are the most important risk factors for the development of HCC.[16,17] Obesity and DM also have been shown to be risk factors for HCC in both large cohort and experimental studies.[18,19] The increased risk of HCC associated with obesity and DM is probably due to two factors: the increased prevalence of NAFLD and the carcinogenic potential of obesity and DM. The most interesting finding of the present study was that the prevalences of obesity, DM and LC in the 80 years or older group of cryptogenic HCC patients were lower than those in the 50–79 years group. In this oldest group, 51% of the patients developed HCC without cirrhosis, and 28% developed HCC without cirrhosis, obesity and DM.

There were only seven patients in the cryptogenic HCC group under 50 years old. These patients had no risk factors for the development of HCC, such as LC and DM. Their HCC might have been associated with hepatoblastoma or genetic factors and occult HBV infection. Kato *et al.* reported that HBV genotype B may be associated with HCC in young (<50 years old)

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Taiwanese.[20] However, in the present study's survey, we did not assess the prevalence of the hepatitis B core antibody due to the survey format. The group under 50 years old was a rather special group. Because a nationwide survey was used to query multiple institutions, we did not obtain further details of these patients; further investigation is needed to examine this group.

In summary, our data suggested that in the elderly, especially in those 80 years or older, there is a possibility of HCC arising even in the absence of risk factors for HCC. This phenomenon may be associated with elderly individuals' decreased immune defenses against cancer, DNA damage and gene mutations.[21,22] Our results may have significant implications for the future, when there is expected to be a very large increase in the elderly population in Japan and around the world.

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APPENDIX I

A TOTAL OF 115 hospitals across the country responded to the questionnaire and provided case cards in the present study: Hakodate City Hospital, Harada Hospital, Oji General Hospital, Hokkaido P.W.F.A.C Engaru-Kosei General Hospital, Dohkohkai Hospital, Hirosaki University School of Medicine and

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Hospital, Akita University Hospital, Yamagata University Hospital, Fukushima Medical University Hospital, Gunma University Hospital, Saitama Medical University Hospital, Saitama City Hospital, Saitama Shakai Hoken Hospital, Aikawa Naika Hospital, Koga Red Cross Hospital, Chiba University Hospital, Kohnodai Hospital, National Center for Global Health and Medicine, Kameda Medical Center, Showa University Hospital, Kashiwa City Hospital, Hachioji Syokaki Hospital, Tokyo Women's Medical University Medical Center East, Tokyo Hospital, Showa General Hospital, Tokyo Metropolitan Geriatric Hospital, Toshiba Hospital, JR Tokyo General Hospital, Kyorin University Hospital, National Cancer Center Hospital, Tokyo Kosei Nenkin Hospital, Nippon Medical School Hospital, Toho University Omori Medical Center, EIJU General Hospital, Tokyo Medical and Dental University Hospital Faculty of Medicine, Tamananbu Hospital, Yokohama City University Hospital, Teikyo University Mizonokuchi Hospital, National Hospital Organization Sagamihara National Hospital, Kanto Rosai Hospital, Saiseikai Yokohamashi Nanbu Hospital, St Marianna University School of Medicine, Nippon Medical University Muasi Kosugi Hospital, Yokohama General Hospital, Fujisawa Shounandai Hospital, Showa University Fujigaoka Hospital, Yokosuka Kyosai Hospital, Niigata Prefecture Yoshida Hospital, Niigata University Medical and Dental Hospital, Niigata Medical Center Hospital, Prefecture Nagano Kiso Hospital, Yodakubo Hospital, University of Yamanashi Hospital, Aichi Saiseikai Hospital, Tokoname Municipal Hospital, Mie University Hospital, Aichi Medical University Hospital, Hamamatsu University of School of Medicine, University Hospital, IUHW Atami Hospital, Kikugawa General Hospital, Kyoto Prefectural Yosanoumi Hospital, National Hospital Organization Kyoto Medical Center, Aiseikai Yamashina Hospital, Japan Post Kyoto Teishin Hospital, Mitsubishi Kyoto Hospital, Osaka University Hospital, Iseikai Hospital, Kinki University Hospital, Osaka Rosai Hospital, Osaka Police Hospital, Osaka City University Hospital, National Hospital Organization Osaka Minami Medical Center, Matsushita Memorial Hospital, Kansai Medical University Takii Hospital, Kobe Asahi Hospital, Kinki Central Hospital of Mutual Aid Association of Public School Teachers, Ono Municipal Hospital, The Hospital of Hyogo College of Medicine, Okayama Saiseikai General Hospital, Kurashiki Central Hospital, Kawasaki Medical School Hospital, The Sakakibara Heart Institute of Okayama, Hiroshima University Hospital, Tokushima University Hospital, Tottori University Hospital, Shimane University Hospital, Matsue Seikyo General Hospital, Ehime University Hospital, Kubo Hospital, Kochi Health Sciences Center, Fukuoka University Hospital, Kurume University Hospital, Japanese Red Cross Fukuoka Hospital, Shinkoga Hospital, Nagasaki University Hospital, Mitsubishi Nagasaki Hospital, Kamigoto Hospital, Nagasaki Municipal Medical Center, Saga University Hospital, Oita University Hospital, Arita GI Hospital, Kumamoto University Hospital, University of Miyazaki Hospital, Kagoshima University Medical and Dental University, Kimotsuki-gun Medical Associated Hospital, Kagoshima City Hospital, Kirishima Medical Center, Heart Life Hospital, Juntendo University Hospital, Japan Self Defense Forces Hanshin Hospital, Yokote Municipal Hospital, Kawasaki City Tama Hospital, Saiseikai Kawaguchi General Hospital, Tokyo Women's Medical University Hospital, Nihon University Itabashi Hospital, and Saitama Cooperative Hospital

SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article at the publisher's website:

Table S1 Characteristic features among the five age-dependent groups of patients with cryptogenic hepatocellular carcinoma (HCC).

Table S2 Comparison between cryptogenic hepatocellular carcinoma (HCC) without obesity and diabetes mellitus (DM) and those with obesity and/or DM.

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Clinical Science

Prognostic role of Child-Pugh score 5 and 6 in hepatocellular carcinoma patients who underwent curative hepatic resection

Chisato Okajima, M.D.*, Shigeki Arii, M.D., Ph.D.*, Shinji Tanaka, M.D., Ph.D., F.A.C.S., Satoshi Matsumura, M.D., Ph.D., Daisuke Ban, M.D., Ph.D., Takanori Ochiai, M.D., Ph.D., Takumi Irie, M.D., Ph.D., Atsushi Kudo, M.D., Ph.D., Noriaki Nakamura, M.D., Ph.D., Minoru Tanabe, M.D., Ph.D.

Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo, Tokyo 113-8519, Japan

KEYWORDS:
Hepatocellular carcinoma;
Child-Pugh score;
Prognosis;
Milan criteria

Abstract

BACKGROUND: It is unclear whether Child-Pugh score discriminates a prognosis of the Child-Pugh A patients who underwent hepatic resection for hepatocellular carcinoma.

METHODS: Between April 2000 and March 2011, 361 patients with Child-Pugh A who underwent curative hepatectomy were divided into 2 groups: Child-Pugh score 5 points group (CPS5) and Child-Pugh score 6 points group (CPS6); both CPS5 (n = 274) and CPS6 (n = 87) groups were compared.

RESULTS: Overall survival rates (1/2/5 years of the CPS5 and CPS6 groups were 90.9%/82.5%/62.4% and 80.6%/68.0%/47.6%, respectively) and disease-free survival rates (67.6%/51.8%/30.1% and 36.9%/16.0%/5.9%, respectively) showed that the CPS5 group was significantly better than the CPS6 group. Multivariate analysis revealed that Child-Pugh score at overall survival (P = .0125) and disease-free survival (P = .0103) was a significant prognostic factor.

CONCLUSIONS: The overall survival and disease-free survival in Child-Pugh A showed quite a difference between the CPS5 and CPS6 groups. However, CPS5 and CPS6 may be a useful prognostic marker of hepatocellular carcinoma patients with hepatic resection.

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The prognosis of hepatocellular carcinoma (HCC) patients is affected mainly by the stage of the cancer and hepatic function. Curative treatments and preserved liver function are needed to attain better prognosis. Actually, HCC usually occurs in patients with chronic liver diseases and it is quite an important issue to maintain the hepatic functional reserve.[1–4] In this regard, liver transplantation may be the most effective treatment for HCC; however, the indication is limited to considerably early stage of the cancer and also donor shortage remains to be conquered. Under these circumstances, hepatic resection is a feasible treatment to be widely selected.[5–7]

Up to now, there have been many studies which have clarified the prognostic factors of HCC patients with

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* Corresponding authors. Tel.: +81-3-5803-5928; fax: +81-3-5803-0263.
E-mail addresses: okajima.msrg@tmd.ac.jp, sh-arii@hamamatsuh.ro-fuku.go.jp

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hepatic resection.[8–10] Among the pathologic factors of cancer, tumor number, tumor size, and vascular involvement are closely involved in the prognosis of HCC patients.[11–13] As to an estimation of the hepatic functional reserve, Child-Pugh (C-P) classification is widely used because of the simplicity and appropriateness.[14] Not a few studies reported that the grade of C-P classification is well correlated with the prognosis of the patients and is then assumed to be a valuable predictor of HCC patients. Indeed, Child-Pugh class A (C-P A) patients showed a better survival rate than Child-Pugh class B (C-P B) and C patients in general. However, C-P A is composed of scores 5 or 6. The former patient is thought to have a well-preserved liver function, while the latter may be partially disturbed in the liver function, suggesting that these scores might influence the prognosis of HCC patients with hepatic resection. However, it may not be always appropriate to estimate HCC patients with C-P A altogether by disregarding the scores 5 and 6.[15,16]

From this point of view, we attempted to make clear the prognostic significance of C-P A scores 5 and 6. This study will provide the evidence indicating that C-P scores 5 and 6 will clearly discriminate the prognosis of patients with C-P A who underwent curative hepatic resection for HCC.

Methods

Patients

Four hundred forty-five patients underwent initial hepatectomy for HCC at our institute between April 2000 and March 2011. Of these patients, C-P status was class A in 412 patients (92.6%), class B in 31 patients (6.9%), and class C in 2 patients (.4%).

Of the 412 patients classified as C-P A, 361 patients who underwent curative hepatectomy were evaluated. The remaining 51 patients were excluded. The breakdown is as follows: 20 patients underwent noncurative resection and 31 patients were unknown in their outcome including information about recurrence.

These 361 patients were divided into 2 groups: 274 patients were C-P score 5 points group (CPS5 group) and 87 patients were C-P score 6 points group (CPS6 group).

Methods

In this study, the CPS5 and CPS6 groups were compared in clinicopathologic characteristics, overall survival, disease-free survival, type of recurrence, and survival after recurrence, and prognostic factors were identified with univariate and multivariate analyses.

Clinicopathologic characteristics

The clinicopathologic variables which were compared between the CPS5 and CPS6 groups were age, sex, hepatitis virus markers, aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, platelet count, indocyanine green retention rate at 15 minutes (ICGR15), prothrombin time (%), serum albumin level, total bilirubin level, direct bilirubin level, preoperative alpha-fetoprotein level (AFP), protein induced by vitamin K absence or antagonists-II (PIVKAII), tumor size, number of tumors, pathologic portal vein involvement (pvp), pathologic hepatic vein involvement, pathologic TNM stage (TNM Staging System is defined by the Union Internationale Contre le Cancer), Milan criteria compatibility, and type of hepatectomy.

Overall survival and disease-free survival

The cumulative overall survival and disease-free survival for the CPS5 and CPS6 groups were estimated using Kaplan–Meier method and were statistically analyzed using log-rank test.

Prognostic factors

To identify prognostic factors for overall survival and disease-free survival, the statistical differences for the clinicopathologic variables were evaluated using univariate and multivariate analyses. Univariate analysis was performed using Kaplan–Meier method and log-rank test. Multivariate analysis was performed using the logistic regression analysis for the factors that were found to be significant by univariate analysis.

Types of recurrence and survival rates after recurrence

In the C-P A, the overall survival rates after recurrence and the types of recurrence classified by Milan criteria were calculated in relation to CPS5 and CPS6 groups.

Statistical analysis

The significant differences of clinicopathologic characteristics were assessed using chi-square test and Student *t* test. The cumulative overall survival and disease-free survival were generated using Kaplan–Meier method and were compared using log-rank test. Univariate analysis was performed using Kaplan–Meier curves and log-rank test, while multivariate analysis was performed using the logistic regression analysis. All statistical analysis in this study was performed with Stat View 5.0 (provided by the SAS Institute Inc). *P* value less than .05 was defined as statistically significant.

Results

Clinicopathologic characteristics

Table 1 summarizes the clinicopathologic characteristics of both the CPS5 and the CPS6 groups. Serum PIVKAII

Table 1 Clinicopathologic characteristics

Variable	Child-Pugh score 5 points (n = 274)	Child-Pugh score 6 points (n = 87)	P value
Age (years)*	66.92 ± 8.96	67.23 ± 8.80	.7786
Sex (male/female)†	215/59	62/25	.1659
Hepatitis†			
Hepatitis B virus antigen	49	15	.4662
Hepatitis C virus antibody	135	90	
Non-B/non-C hepatitis	90	23	
AST level (IU/L)*	49.47 ± 35.71	68.32 ± 49.60	.0001
ALT level (IU/L)*	47.04 ± 37.99	60.06 ± 56.58	.0148
Platelet count ($\times 10^4/\mu L$)*	15.95 ± 12.98	15.74 ± 10.84	.8951
ICGR15 (%)*	16.53 ± 9.74	22.75 ± 12.55	<.0001
Albumin level (g/dL)*	4.096 ± .3299	3.452 ± .3911	<.0001
Prothrombin time (%)*	88.17 ± 10.14	78.67 ± 11.42	<.0001
Total bilirubin level (mg/dL)*	.788 ± .2934	.916 ± .5076	.0039
Direct bilirubin level (mg/dL)*	.1167 ± .070	.2104 ± .3217	<.0001
AFP level (ng/mL)*	3,469 ± 28,356	4,062 ± 16,577	.8539
PIVKAII level (mAU/mL)*	3,225 ± 11,697	9,326 ± 39,675	.0248
Tumor number† 1	171 (62.4%)	54 (62.1%)	.9546
≧2	103 (37.6%)	33 (37.9%)	
Tumor size (cm)† ≦5	199 (72.6%)	53 (60.9%)	.0382
>5	75 (27.3%)	34 (39.1%)	
Pathologic portal vein involvement†			
0/1	241 (88.0%)	72 (82.8%)	.2135
2–4	33 (12.0%)	15 (17.2%)	
Pathological hepatic vein involvement†			
0	245 (89.4%)	78 (89.7%)	.9495
1–3	29 (10.6%)	9 (10.3%)	
Pathologic TMN stage†			
I	30 (10.9%)	8 (9.2%)	.7583
II	82 (29.9%)	31 (35.6%)	
III	112 (40.9%)	33 (37.9%)	
IVA	49 (17.9%)	14 (16.1%)	
IVB	1 (.4%)	1 (1.1%)	
Milan criteria†	160 (58.4%)	42 (48.3%)	.0977
Inclusion			
Exclusion	114 (41.6%)	45 (51.7%)	
Operative method†			
≦1 segmental resection	223 (81.4%)	69 (79.3%)	.6678
>2 segmental resection	51 (18.6%)	18 (20.7%)	

Portal vein involvement is classified according to the grade as follows: Vp0, invasion of (or tumor thrombus in) the portal vein undetected; Vp1, invasion of (or tumor thrombus in) distal to second-order branches (second-order branches not included) of the portal vein detected; Vp2, invasion of (or tumor thrombus in) second-order branches of the portal vein detected; Vp3, invasion of (or tumor thrombus in) first-order branches of the portal vein detected; Vp4, invasion of (or tumor thrombus in) the main trunk of the portal vein and/or contralateral portal vein branch to the primarily involved lobe detected.

Hepatic vein involvement is classified according to the grade as follows: Vv0, invasion of (or tumor thrombus in) the hepatic vein undetected; Vv1, invasion of (or tumor thrombus in) the peripheral branch of the hepatic vein detected; Vv2, invasion of (or tumor thrombus in) the right, middle, or left hepatic vein, the inferior right hepatic vein, or short hepatic vein detected; Vv3, invasion of (or tumor thrombus in) the inferior vena cava detected.

AFP = preoperative alpha-fetoprotein; ALT = alanine aminotransferase; AST = aspartate aminotransferase, ICGR15 (%) = indocyanine green retention rate at 15 minutes; non-B/non-C hepatitis = no hepatitis B virus antigen and hepatitis C virus antibody; PIVKAII = protein induced by vitamin K absence or antagonists-II; prevalence survival = surviving cases with recurrence.

*P value; Student t test.

†P value; chi-square test ($P \leqq .05$ was considered significant).

level and tumor size showed the statistical significance between the CPS5 and CPS6 groups among the tumor factors ($P = .0248$ and $P = .382$). On the other hand, except for the platelet count, most of the hepatic function including AST, ALT, ICGR15, prothrombin time (%), serum albumin level, total bilirubin level, and direct bilirubin level of the CPS5 group were significantly better than those of the CPS6 group.

Overall survival and disease-free survival

One hundred forty-three patients in C-P A died after hepatectomy during the observation period. The causes of death were cancer recurrence in 82 patients, liver failure including hospital death in 39, and other diseases in 22. The overall survival rates of the 361 patients at 1, 2, and 5 years were 89.2%, 79.4%, and 60.7%, respectively.

The cumulative overall survival curves and disease-free survival curves in the CPS5 and CPS6 groups are shown in Figs. 1 and 2, respectively. The overall survival rates of the CPS5 and CPS6 groups at 1, 2, and 5 years were 91.4%, 83.2%, 63.8% and 82.7%, 68.8%, 52.3%, respectively. The disease-free survival rates of the CPS5 and CPS6 groups at 1, 2, and 5 years were 68.6%, 51.8%, 30.7% and 43.3%, 25.5%, 12.3%, respectively .These showed that the CPS5 group was significantly better than the CPS6 group in overall survival and disease-free survival.

Prognostic factors

Univariate analysis showed that hepatitis C infection ($P = .0242$), AST ($P < .0001$), ALT ($P = .0405$), ICGR15 ($P = .0070$), C-P score ($P = .0007$), serum albumin level ($P = .0004$), direct bilirubin level ($P < .0001$), AFP ($P = .0009$), PIVKAII ($P < .0001$), tumor size ($P = .0035$), number of tumor ($P = .0003$), pvp ($P = .0002$), pathologic hepatic vein involvement ($P = .0138$), pathologic TNM stage ($P < .0001$), Milan criteria compatibility ($P < .0001$), and type of hepatectomy ($P = .0013$) were significant prognostic factors for overall survival, whereas hepatitis C infection ($P = .0104$), AST ($P < .0001$), ALT ($P < .0001$), ICGR15 ($P = .0007$), C-P score ($P < .0001$), serum albumin level ($P < .0015$), total bilirubin level ($P = .0262$), direct bilirubin level ($P = .0052$), AFP ($P < .0001$), PIVKAII ($P = .0045$), number of tumor ($P = .0001$), pvp ($P < .0001$), pathologic TNM stage ($P = .0001$), and Milan criteria compatibility ($P = .0230$) were significant prognostic variables for disease-free survival.

Multivariate analysis revealed that C-P score ($P = .0042$) and direct bilirubin level ($P = .0004$) were found to be significant prognostic factors for overall survival (Table 2), whereas C-P score ($P = .0131$), AFP ($P = .0225$), and ALT ($P = .0231$) were significant prognostic factors for disease-free survival (Table 3). Serum albumin level was excluded because it was included in C-P score.

Survival curves in relation to the presence and absence of recurrence

In this analysis, 33 patients who encountered from HCC-nonrelated death and hospital death were excluded. Among the remaining 328 patients classified as C-P A, 196 patients (59.8%) were recurred and 132 patients (40.2%) were recurrence-free. Of the 132 patients without recurrence, the CPS5 and CPS6 groups consisted of 116 patients (87.9%) and 16 patients (12.1%), respectively. Of the 196 patients with recurrence, on the other hand, the CPS5 and CPS6 groups consisted of 137 patients (69.9%) and 59 patients (30.1%), respectively. Fig. 3 indicated that the CPS6 group showed a higher cumulative recurrence rate than the CPS5 group. The survival curve in the patients without recurrence was significantly different between the CPS5 and CPS6 groups. However, the CPS5 group had a higher survival rate than the CPS6 group (survival rates in 1, 3, and 5 years were 89.6%, 88.3%, and 88.3% in the CPS5 group and 67.0%, 67.0%, and 50.2% in the CPS6 group, respectively) ($P = .0007$). But in the patients with recurrence, there were no significant differences between the CPS5 and CPS6 groups in the survival curves ($P = .2114$) (survival rates in 1, 3, and 5 years were 79.8%, 53.1%, and 29.0% in the CPS5 group and 72.4%, 48.0%, and 17.8% in the CPS6 group, respectively).

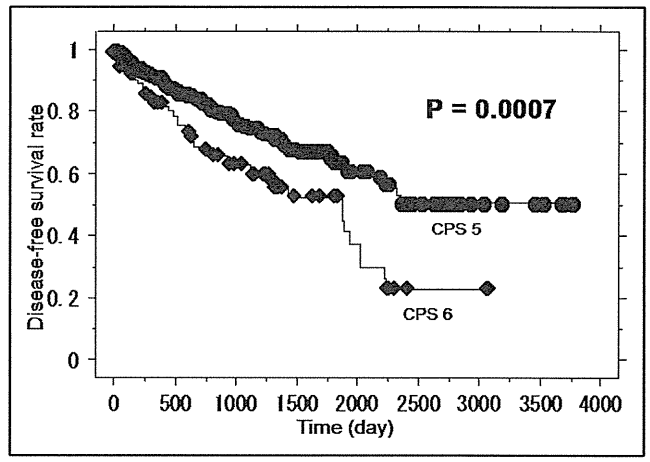

Figure 1 Cumulative overall survival curves of CPS5 group and CPS6 group after hepatectomy.

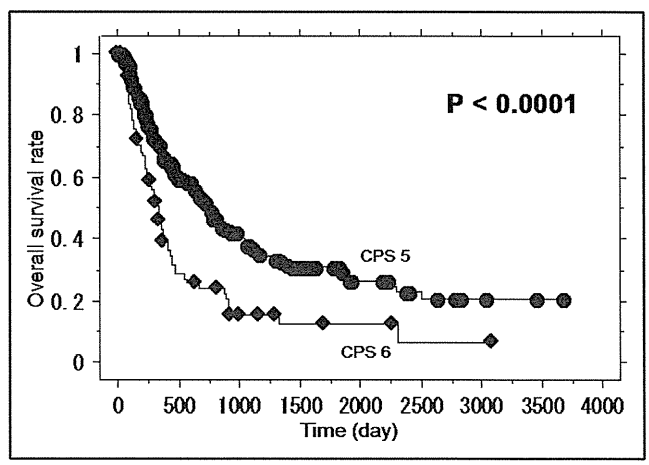

Figure 2 Cumulative recurrence-free survival curves of CPS5 group and CPS6 group after hepatectomy.

Types of recurrence and survival rates after recurrence in relation to Child-Pugh score

The types of initial recurrence were divided into 2 groups: 102 patients (52.0%) were within the Milan criteria and 94 patients (48%) beyond the Milan criteria. In the patients within the Milan criteria, the CPS5 and CPS6 groups consisted of 76 patients and 26 patients, respectively. Similarly, in those beyond Milan criteria, the CPS5and CPS6 groups consisted of 61 patients and 33 patients, respectively. No significant correlation was seen between C-P score and the recurrence pattern according to the Milan criteria. It was also found that survival rates after recurrence were not significantly different between the CPS5 and CPS6 groups irrespective of recurrence patterns (data not shown).

Comments

Many studies have shown that hepatic function plays an important role in the prognosis of HCC patients who underwent a curative hepatic resection. All studies among them have been focused to the some particular variables such as serum albumin level and serum bilirubin level.[4,17,18] In particular, not a few studies reported that preoperative serum albumin level was a valuable prognostic marker of HCC patients with hepatic resection. However, in the clinical setting, the hepatic functional reserve that decides the indication of hepatic resection is usually estimated with C–P classification, which is a widely used comprehensive variable in HCC patients accompanied by hepatic parenchymal diseases.[14] C-P classification is composed of A, B, and C, and hepatic resection for HCC is thought to be indicated mainly to C-P A patients and selected C-P B patients, because the patients with deteriorated liver function are at high risk of postoperative life-threatening liver failure.[18–20]

Thus, C-P A patients with HCC are main candidates for hepatic resection; however, C-P A patients are comprised from score 5 and 6, and the hepatic function in the latter group is thought to be considerably disturbed compared with the former patients. Nevertheless, no studies which are directed to the outcome of the patients with CPS5 and CPS6 have been conducted up to now. In this study, we clarified that the patients with CPS5 showed higher overall survival and disease-free survival rates than those with CPS6, although no significant differences were seen in the clinicopathologic factors regarding the tumors between the 2 groups. As to the hepatic function, however, many variables showed significant differences not only in the items including C-P classification, but also the other variables such as transaminases, ICG retention rate, and serum direct bilirubin level.

The main reason why these 2 groups showed significant difference in the survival was thought to be higher frequency of recurrence in the CPS6 group than the CPS5 group.[17] As shown in Fig. 2, recurrence-free survival rate of the CPS6 group was lower than that of the CPS5 group, suggesting higher frequency of recurrence in the CPS6 group than that of the CPS5 group. This was proved by cumulative intrahepatic recurrence rate as shown in Fig. 3. The intrahepatic recurrence is composed of either intrahepatic metastasis from the main tumors or metachronous multicentric carcinogenesis. The higher recurrence rate in the CPS6 group may be derived from the higher carcinogenic potential in the underlining hepatic parenchyma. Namely, patients in the CPS6 group may have higher potential of metachronous

Table 2 Multivariate analysis of risk factor for overall survival

Variable		Odds ratio (%)	95% CI	*P* value
Child-Pugh score	5 points/6 points	.130	.241–.767	.0042
HCV antibody	Negative/positive	.610	.363–1.025	.0617
AST	<60/≧60	1.048	.522–2.105	.8941
ALT	<60/≧60	.965	.478–1.947	.9198
ICGR15	<20/≧20	.868	.508–1.408	.6021
Direct bilirubin	<.2/≧.2	.358	.203–.634	.0004
AFP	<100/≧100	.598	.341–1.048	.0722
PIVKAII	<1,000/≧1,000	.746	.392–1.417	.3704
pvp	0–1/2–4	1.161	.554–2.432	.6919
pvv	0/1–3	1.552	.747–3.227	.2388
Tumor number	1/≧2	.830	.453–1.519	.5457
Tumor size (cm)	<5/≧5	1.053	.496–2.234	.8937
Stage	I–II/III–IV	.866	.457–1.634	.6601
Milan criteria	Inclusion/Exclusion	1.138	.531–2.437	.7403
Operating method	≦1 segmental resection/≧2 segmental resection	.527	.271–1.024	.0587

P ≦ .05 was considered significant

AFP = preoperative alpha-fetoprotein; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; HCV = hepatitis C virus; ICGR15 (%) = indocyanine green retention rate at 15 minutes, PIVKAII = protein induced by vitamin K absence or antagonists-II; pvp = pathologic portal vein involvement; pvv = pathologic hepatic vein involvement.

Table 3 Multivariate analysis of risk factor for recurrence-free survival

Variable		Odds ratio (%)	95% CI	*P* value
Child-Pugh score	5 points/6 points	2.109	1.170–3.801	.0131
HCV-Ab	Negative/positive	1.079	.664–1.784	.7723
AST	<60/≧60	1.239	.632–2.432	.5325
ALT	<60/≧60	2.213	1.115–4.391	.0231
ICGR15	<20/≧20	1.346	.813–2.229	.2480
Direct bilirubin	<.2/≧.2	1.039	.593–1.820	.8946
AFP	<100/≧100	1.914	1.096–3.342	.0225
PIVKAII	<1,000/≧1,000	.983	.544–1.774	.9533
Pvp	0–1/2–4	.953	.467–1.943	.8946
Tumor number	1/≧2	1.295	.735–2.280	.3711
Stage	I–II/III–IV	.890	.497–1.597	.6969
Milan criteria	Inclusion/Exclusion	1.458	.805–2.638	.2131

$P \leqq .05$ was considered significant.

AFP = preoperative alpha-fetoprotein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CI = confidence interval; HCV-Ab = hepatitis C virus antibody; ICGR15 (%) = indocyanine green retention rate at 15 minutes; PIVKAII = protein induced by vitamin K absence or antagonists-II; pvp = pathologic portal vein involvement.

multicentric carcinogenesis than those in the CPS5 group, because inflammatory and fibrogenic status, which is closely related to carcinogenic potential, is advanced in the former group compared with the latter group.[17,21,22] As shown in Tables 2 and 3, the factors which were thought to be related to the survival rates were quite similar with those of disease-free survival. Among the variables regarding hepatic functions related to overall survival and disease-free-survival, almost all factors showed significant differences between the CPS5 and CPS6 groups, and those of the CPS6 gave a negative impact on the overall survival and disease-free survival. On the other hand, the intrahepatic metastasis might not be markedly participated in the difference of the disease-free survival, because tumor characteristics such as tumor number, portal involvement, and tumor markers showed no significant differences between the 2 groups. Moreover, no apparent differences in the surgical procedures were noted as indicated by the extent of the area removed surgically.

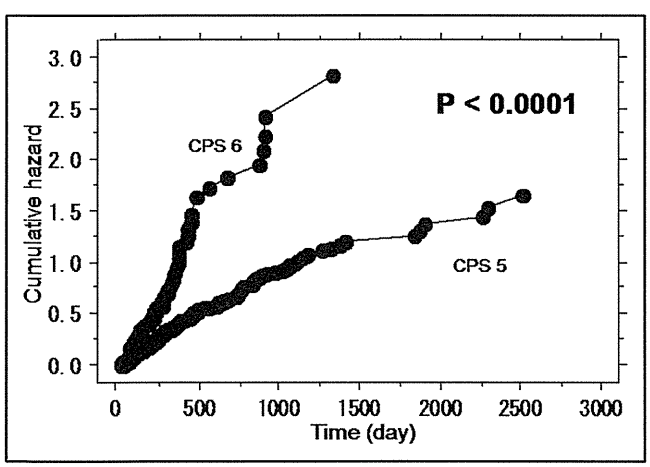

Figure 3 Cumulative intrahepatic recurrence rates of CPS5 group and CPS6 group after hepatectomy. (For interpretation of the references to color in this Figure, the reader is referred to the Web version of this article.)

The another reason for low survival rate in the CPS6 group is considered to be the higher frequency of death because of hepatic insufficiency in the CPS6 group than in the CPS5 group as indicated by the different survival rates between the CPS5 and CPS6 groups without the recurrence.

Multivariable analysis showed that C-P score was an independent predictive variable for both overall survival and disease-free survival and also revealed that serum direct bilirubin level and AFP value were significant factors in the overall survival and disease-free survival, respectively. Considering that direct bilirubin is a more specific marker for estimating hepatic parenchymal function among the variables which do not belong to C-P classification, it seems reasonable that direct bilirubin is a significant factor for survival. AFP as a predictive factor for disease-free survival is also explainable, because this marker is related to intrahepatic recurrence as reported by previous studies. ALT was also a predictive variable for disease-free survival. This result seems reasonable because ALT shows the state of parenchymal inflammation which is closely related to multicentric carcinogenesis.

One of the unique characteristic of HCC is that a variety of therapeutic strategies can be employed even to patients with recurrences, thereby leading to prolongation of survival. In other words, the prognosis of HCC patients is closely related to both the degree of recurrence and the therapeutic effect of recurrence, as well as initial treatment. Then, we analyzed the aggressiveness of initial recurrence classified by Milan criteria and the survival rates from the time of initial recurrence of the recurred patients. Consequently, we found that no significant difference in the recurrence pattern according to Milan criteria was seen between the CPS5 and CPS6 groups, although the CPS5 group showed a slightly higher ratio of recurrence within

Milan criteria than the CPS6 group. Taken together, these results showed that better survival rate in the CPS5 group was derived mainly from the low frequency of recurrence than the CPS6 group. Furthermore, the indifferent survival rates after recurrence between both groups suggested that a variety of treatments for recurrence of the CPS5 and CPS6 groups exerted comparable therapeutic effects, and may also say that the limitation in the therapeutic strategies was almost same among the C-P A patients even if C-P score was different.

In conclusion, overall survival and disease-free survival of CPS5 and CPS6 patients in C-P A was quite different and CPS may be a useful prognostic marker of HCC patients with hepatic resection.

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