Small Hepatocellular Carcinomas and Thrombocytopenia

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Key Words

Hepatocellular carcinoma · Thrombocytopenia · Portal vein thrombosis · Alpha-fetoprotein

Abstract

Background: Clinical phenotypes of small and large hepatocellular carcinomas (HCCs) are not well characterized. **Aim:** To evaluate the characteristics of small HCCs diagnosed by screening. **Method:** A cohort of 430 small HCCs that were diagnosed through screening, were dichotomized according to a size of ≤ 3 cm or > 3 cm maximum tumor diameter and compared for radiological and blood-test parameters. **Results:** There were 330 males and 100 females. A higher percent of females had smaller tumors. The majority of patients had single tumors, but 15% of those with larger tumors had portal vein thrombosis (PVT) compared to 5% of those with smaller tumors. Significant differences between the tumor-size groups included alpha-fetoprotein (AFP) values and platelet counts, with thrombocytopenia and elevated bilirubin levels being associated with smaller tumors. In comparing PVT-positive and PVT-negative patients, AFP levels and platelet counts were also significantly different between the 2 groups. A mean multinomial multiple logistic regression model was developed for maximum tumor diam-

eter plus PVT. **Conclusions:** The finding of larger tumors being associated with normal platelets and bilirubin levels in comparison to smaller tumors having thrombocytopenia reveals 2 different patterns of HCC presentation.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide (with an increasing incidence in several developed countries) and the third most frequent cause of death from cancer. HCC that is not diagnosed early, usually by screening, is often advanced at presentation, with incidence and survival rates being nearly equal [1, 2]. It is often not amenable to surgery with curative intent. Major progress in HCC survival is therefore likely to come from prevention strategies, such as hepatitis B (HBV) vaccination, or from early diagnosis and the surgical removal for those patients who have tumors at an early stage of evolution. Given the importance for of the need to diagnose HCC at the early stages when possible, in order to be able to conduct definitive treatment, we evaluated a large group of patients with relatively small HCCs, who were diagnosed via a

Table 1. Descriptive statistics of demographic and serum variables (mean and SD or absolute frequency and percentage) according to MTD

	MTD		p value ^a
	≤3 cm (n = 216)	>3 cm (n = 214)	
Sex, female	57 (26.4)	43 (20.1)	0.122
Age, years	69.9 ± 10.6	73.0 ± 10.2	0.003
PVT	11 (5.1)	33 (15.4)	<0.001
Total mass, RU	3.8 ± 3.3	13.0 ± 17.3	<0.0001
Tumor number			0.015
1–3 (n = 387)	202 (93.5)	185 (86.4)	
>3 (n = 43)	14 (6.5)	29 (13.5)	
Alcohol use	64 (29.6)	65 (30.4)	0.866
HBV	55 (25.5)	63 (29.4)	0.356
HCV	75 (34.7)	73 (34.1)	0.894
Child-Pugh score			0.944
A	150 (69.4)	150 (70.1)	
B	49 (22.7)	49 (22.9)	
C	17 (7.9)	15 (7.0)	
AFP, ng/ml	274.0 ± 1,805.2	2,081.5 ± 8,807.5	0.0008
PT INR	1.3 ± 0.2	1.2 ± 0.2	0.014
Albumin, g/l	3.5 ± 0.6	3.4 ± 0.6	0.335
Total bilirubin, mg/100 ml	1.9 ± 2.1	1.9 ± 2.6	0.388
ALT, IU/l	88.9 ± 79.6	77.8 ± 68.3	0.163
AST, IU/l	91.4 ± 75.8	85.1 ± 71.5	0.409
GGTP, IU/l	124.9 ± 116.3	169.4 ± 230.8	0.023
Hemoglobin, g/dl	13.5 ± 1.8	13.4 ± 1.8	0.618
Platelet, 10 ⁹ /l	103.3 ± 55.0	141.5 ± 90.8	<0.0001

Results are presented as means ± SD or numbers with percentages in parentheses. ALT = Alanine aminotransferase; AST = aspartate aminotransferase; PT INR = prothrombin time international normalized ratio; RU = relative units.

^a χ^2 test for categorical variables and Mann-Whitney test for continuous variables.

screening program, and we subjected their baseline clinical parameters to statistical evaluation in relation to the tumor mass, to characterize this important outcome-associated phenotype. We compared patients with tumors of ≤3 cm maximum diameter to those with tumors of >3 cm. This bivariate dichotomization was chosen because 3 cm is generally accepted as a size that can be treated curatively with radio-frequency ablation or resection, whereas a greater size tends to be associated with more recurrences [3–7]. A mean multinomial multiple logistic regression model for our data showed statistically significant differences in the 2 dichotomized tumor-size groups with respect to baseline serum alpha-fetoprotein (AFP),

the presence or absence of portal vein thrombosis (PVT) and blood platelet levels. The lower platelet levels were found in patients with smaller tumors.

Methods

A cohort of 430 Italian HCC patients, who were diagnosed by surveillance screening, as they had a history of HBV, hepatitis C (HCV), alcoholic or other liver disease, were the subjects of this comparative study. Tumor sizes were derived using CT-scan-based electronic measurements of the maximum diameters of the largest tumor mass and were recorded to the nearest 0.1 cm. Tumor numbers and the presence or absence of PVT were also recorded. Patients were then rank-ordered according to maximum tumor diameter (MTD) the data processed. Baseline standard liver function test values, complete blood count and serum AFP values were recorded. All the clinical descriptors were those obtained at the time of HCC diagnosis. Nodules were considered as HCC according to the criteria of the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases [8, 9]. This study was approved by the institutional review board for the retrospective examination of de-identified HCC data.

Statistical Analysis

Mean and standard deviation (SD) for continuous variables and relative frequency for categorical variables, were used as indices of centrality and dispersion of the distribution for clinical parameters. The χ^2 test for categorical variables and Mann-Whitney test for continuous variables were used to test bivariate associations of tumor size or PVT with clinical parameters. A multiple logistic regression model was used to evaluate the associations between MTD and tumor number or PVT, respectively, and single variables. The model was corrected for sex, age and history of alcohol use. Multinomial multiple logistic regression was subsequently used to evaluate the associations between MTD and PVT combined with serum AFP levels and peripheral blood platelet count values in the model, corrected for sex, age and alcohol use. When testing the null hypothesis of no association, 2-tailed tests on 0.05 significance level were used. All the statistical computations were made using STATA 10.0 statistical software (StataCorp, College Station, Tex., USA).

Tercile Analysis

The total dataset of MTD, serum AFP and platelet count values was log-transformed, in order to linearize relationships between parameters. Histograms of the log-transformed tumor sizes were composed for the ‘terciles’ that were then identified in the log (tumor-size) axis. Thus, each tercile contained one third (430/3) or 143 patients in the sequence, rank-ordered by MTD. The platelets and the total bilirubin for the two ‘wings’ or outside groupings, were then examined in relation to the terciles.

Results

Table 1 shows descriptive statistics. The data of the 430 patients were dichotomized for analysis into those with tumors with an MTD of ≤3 cm or >3 cm. There were 330

Fig. 1. Distribution of platelets in the individual categories of Tumor Size (tertiles) and comparisons of the differences between the Tertiles for platelet numbers. * Kruskal-Wallis rank test and ** Mann-Whitney rank test.

males and 100 females. Fifty-seven females (26% of the patients), i.e. 57% of the females in this dataset had smaller tumors, whereas 48% of the males had larger tumors. These differences were not significant ($p < 0.12$). The population with larger tumors was significantly older on average 73 years old – versus 69.9 years old for those with smaller tumors. We used tumor mass (maximum tumor size $\times$ maximum tumor number) as an estimate of total liver tumor burden [10, 11] and found significant differences between the 2 groups ($p < 0.0001$), unsurprisingly, as diameter was used for dichotomization. However, when the tumor number subsets were examined, the majority of patients had a small number of tumor nodules, i.e. 1–3 in 93.5% of those with small tumors and 86.4% with larger tumors. Although the sample size was small, there was double the number of patients with >3 tumor

nodules in the larger-tumor group than in the small-tumor group, i.e. 13.5 versus 6.5%. PVT was significantly more frequent in the patients with larger tumors, than in those with smaller tumors ($p < 0.001$). The 2 groups were similar with respect to etiology. However, within the alcohol subset, males predominated, with 34% of the larger-tumor group being alcoholic males versus 13.9% being female ($p < 0.09$). There was little difference between the 2 tumor-size groups and the liver function tests, but AFP mean values were much higher for the larger-tumor group ($p < 0.0008$). Platelets were also significantly different between the 2 groups ($p < 0.0001$) with only the small-tumor patients having thrombocytopenia, which has a much-cited association with HCC [12–14]. However, the means for the platelet counts in the larger-tumor group in our dataset were within the normal range.

Fig. 2. Distribution of total bilirubin in the individual categories of Tumor Size (tertiles) and comparisons of the differences between the Tertiles for total bilirubin values. * Kruskal-Wallis rank test and ** Mann-Whitney rank test.

The patient data were then divided into tertiles according to MTD and these were examined for both platelet values and total serum bilirubin levels (fig. 1, 2). The tumor-size groups identified in this way had different platelets mean values, with the larger-tumor group having platelet counts within the normal range, but with thrombocytopenia being manifest in the small-tumor group ($p = 0.0001$; fig. 1). To determine whether the thrombocytopenia reflected portal hypertension as expected, we then examined the tumor-size groups in relation to total serum bilirubin levels (fig. 2). We found significant differences in mean serum bilirubin levels ($p = 0.03$) between the smallest- and largest-tumor tertiles, with the smaller-tumor patients having the higher bilirubin values.

The dataset was next dichotomized into the presence or absence of PVT and analyzed according to the same clinical parameters as for MTD. Significance was found for both tumor size and platelet counts, respectively (table 2). Thrombocytopenia was found predominantly in the group without PVT ($p < 0.006$) comprised predominantly of patients with tumors of a small size.

A multiple logistic regression analysis was performed to relate MTD, tumor number and the presence of PVT to baseline clinical parameters (table 3). We found significance within the larger-tumor group for PVT, AFP and platelet counts. In the tumor number analysis, there was significance for the presence of PVT and tumor mass only. Within the PVT analysis, there was significance for MTD and platelets. We then devised a mean multinomial multiple logistic regression model for MTD and

Table 2. Descriptive statistics of demographic and serum variables (mean and SD absolute frequency and percentage) according to the presence or absence of PVT

	PVT		p value ^a
	no (n = 386)	yes (n = 44)	
Sex, female	90 (23.3)	10 (22.7)	0.930
Age, years	71.6 ± 10.5	69.7 ± 10.2	0.245
Alcohol use	117 (30.3)	12 (27.3)	0.677
Maximum diameter	3.86 ± 2.73	6.69 ± 4.11	<0.0001
MTD, >3 cm	181 (46.9)	33 (75.0)	<0.001
Total mass	7.2 ± 9.9	18.7 ± 27.3	<0.0001
HBV	99 (25.6)	19 (43.2)	0.014
HCV	140 (36.3)	8 (18.2)	0.017
Child-Pugh score			0.441
A	271 (70.2)	29 (65.9)	
B	85 (22.0)	13 (29.5)	
C	30 (7.8)	2 (4.5)	
AFP	1,054.6 ± 6,400.3	2,216.7 ± 6,380.1	0.002
PT INR	1.3 ± 0.2	1.2 ± 0.1	0.682
Albumin	3.5 ± 0.6	3.4 ± 0.5	0.227
Total bilirubin	1.9 ± 2.5	1.7 ± 1.2	0.710
ALT	83.4 ± 74.1	83.1 ± 77.7	0.817
AST	87.8 ± 74.9	92.6 ± 62.5	0.322
GGTP	143.0 ± 181.5	183.1 ± 199.8	0.093
Hemoglobin	13.4 ± 1.8	13.3 ± 1.9	0.569
Platelet	118.7 ± 75.2	153.9 ± 88.8	0.006

For abbreviations and laboratory values, see table 1. Results are presented as means ± SD or numbers with percentages in parentheses.

^a x² test for categorical variables and Mann-Whitney test for continuous variables.

PVT combined (table 4), these being amongst the most important features for clinical (surgical) decision-making. We found that tumors of >3 cm maximum diameter with PVT were significantly correlated with AFP and platelet values, whereas in the cases of an MTD of >3 cm without PVT, only the AFP values were significant.

Discussion

We previously examined a large cohort of patients with advanced and unresectable HCC and found several factors to be of prognostic significance, including age, gender, tumor size, serum AFP and gamma glutamyl transpeptidase (GGTP) levels [11, 19, 20]. The current work focusses on smaller HCCs that were diagnosed by surveillance screening in Italy.

Meaningful analysis of clinical-practice data is in general a complicated task, given the heterogeneity of both tumor subsets and the influence of the tumor microenvironment (underlying liver for HCC). Most HCC arises on the basis of chronic hepatitis or cirrhosis or both. The prognosis is considered to be significantly associated with tumor factors such as serum AFP levels, tumor size and PVT, and liver factors such as serum bilirubin, GGTP and transaminase levels. It is likely that there is even an interaction between these processes [21], since cirrhosis is a premalignant condition, leading to either liver failure, HCC or both. This dual set of influences was initially reflected in the staging system of Okuda [15] and subsequently by many other systems in which greater phenotypic complexity was taken into account, in an effort to identify prognostic subsets for survival [16, 17]. However, in addition to the nontumor liver being important in the sum of the prognostic factors, it has recently become apparent that the nontumor liver may actually influence the tumor behavior and has a characteristic 'molecular signature' [21, 22]. AFP is included amongst many of these HCC scoring and classification systems, as elevated serum levels have been shown (in many published series) to represent a worse prognosis [18].

We dichotomized our patient dataset, using a ≤3 cm or >3 cm cut-off, since smaller tumors are generally considered curable as single lesions in the presence of normal liver function. Our descriptive statistics showed that the 2 tumor-size groups were similar in etiology, that the larger tumors typically occurred in older patients and that the percentage of females with smaller tumors was greater than that of males (table 1). Gender differences in terms of survival have been noted by many authors, with females having a lower incidence rate than males and a better prognosis. Patients with larger tumors also had more tumor nodules (>3) than patients with smaller tumors. The major significant differences in the descriptive statistics of the 2 tumor-size groups were in the levels of serum AFP, the presence of PVT and blood platelet levels. An increase in the incidence of PVT with increased HCC size has been noted previously [23] and was a trend in our patients, as was elevated AFP levels and tumor size. We found that thrombocytopenia, which has previously been noted in association with cirrhosis and HCC [12–14, 29, 30], was significantly different between the 2 tumor-size groups and was unexpectedly more pronounced in patients with smaller tumors than in those with the larger ones.

We had previously observed the heterogeneity of clinical HCC phenotypes and identified several phenotypic

Table 3. Multiple logistic regression model of MTD (≤ 3 cm vs. >3 cm), tumor number (≤ 3 vs. >3) or PVT (no vs. yes) on single variables corrected for sex, age and use of alcohol (abbreviations and laboratory values as in table 1)

	MTD (>3 cm)			Tumor number (>3)			PVT		
	OR (SE)	p value	95% CI	OR (SE)	p value	95% CI	OR (SE)	p value	95% CI
MTD, >3 cm	–	–	–	2.21 (0.77)	0.023	1.12–4.36	3.78 (1.40)	<0.001	1.83–7.82
PVT	3.78 (1.40)	<0.001	1.83–7.80	3.36 (1.38)	0.003	1.50–7.51	–	–	–
Maximum diameter	–	–	–	1.14 (0.05)	0.002	1.05–1.25	1.25 (0.05)	<0.001	1.15–1.35
Total mass	1.34 (0.05)	<0.001	1.24–1.46	1.31 (0.05)	<0.001	1.21–1.42	1.04 (0.01)	<0.001	1.02–1.06
HBV	1.42 (0.33)	0.13	0.90–2.23	1.69 (0.62)	0.15	0.82–3.47	2.10 (0.72)	0.03	1.07–4.11
HCV	0.89 (0.19)	0.60	0.58–1.37	0.44 (0.18)	0.05	0.19–1.0005	0.37 (0.15)	0.02	0.16–0.84
Child-Pugh score	1.08 (0.18)	0.65	0.78–1.48	1.45 (0.36)	0.13	0.90–2.35	0.99 (0.26)	0.97	0.59–1.66
AFP	1.0002 (0.0001)	0.014	1.00003–1.0003	1.00 (0.00002)	0.09	1.00–1.0001	1.00002 (0.00002)	0.25	0.99999–1.00005
PT INR	0.66 (0.29)	0.35	0.28–1.56	0.93 (0.67)	0.92	0.22–3.85	0.34 (0.28)	0.19	0.07–1.69
Albumin	0.79 (0.13)	0.15	0.57–1.09	0.65 (0.18)	0.12	0.38–1.12	0.73 (0.19)	0.22	0.44–1.21
Total bilirubin	1.01 (0.04)	0.75	0.93–1.10	1.06 (0.05)	0.22	0.96–1.17	0.93 (0.09)	0.49	0.77–1.13
ALT	1.00 (0.001)	0.13	0.995–1.001	0.998 (0.003)	0.43	0.993–1.003	0.9997 (0.002)	0.89	0.995–1.004
AST	1.00 (0.001)	0.44	1.00–1.002	1.002 (0.002)	0.35	0.998–1.005	1.001 (0.002)	0.75	0.996–1.005
GGTP	1.002 (0.001)	0.02	1.0002–1.003	1.0004 (0.001)	0.62	0.999–1.002	1.001 (0.0006)	0.18	0.9996–1.0022
Hemoglobin	0.94 (0.05)	0.31	0.85–1.05	0.90 (0.08)	0.24	0.76–1.07	0.95 (0.08)	0.57	0.80–1.13
Platelet	1.007 (0.002)	<0.001	1.004–1.011	0.998 (0.002)	0.46	0.993–1.003	1.005 (0.002)	0.004	1.001–1.008

Table 4. Mean multinomial multiple-logistic regression model, corrected for sex, age and use of alcohol (backward method)

	OR	SE(OR)	p value	95% CI
MTD >3 cm without PVT				
AFP (>30)	2.01	0.45	0.002	1.30–3.13
Platelets (>125)	1.50	0.33	0.072	0.96–2.32
MTD ≤ 3 cm with PVT				
AFP (>30)	1.84	1.21	0.349	0.51–6.67
Platelets (>125)	0.57	0.47	0.494	0.12–2.82
MTD >3 cm with PVT				
AFP (>30)	4.49	1.89	<0.001	1.96–10.27
Platelets (>125)	7.16	3.19	<0.001	2.99–17.14

patient subsets, based on clinical data alone [21], which will likely become better defined with the use of molecular signature profiling [24]. In order to better understand the relationship of tumor size to thrombocytopenia, we log-transformed our MTD and clinical parameter data as done previously [21], and used MTD to divide our dataset into terciles, to see more clearly the 2 extremes or wings of smallest and largest tumors (fig. 1, 2). Significantly lower platelet levels were found in the patients with smaller tumors than in those with larger tumors ($p < 0.001$; fig. 1), noted in the dichotomization. We supposed that the thrombocytopenia was likely due to the cirrho-

sis-associated splenomegaly, and so we repeated the tercile analysis by plotting tumor size against total serum bilirubin levels. Unlike the dichotomization seen in table 1, however, an increase in bilirubin levels in the smaller tumors compared to the larger ones ($p = 0.02$; fig. 2) was revealed. The difference in the results of dichotomization and tercile analysis was likely due to the heterogeneity of the patient population; differences were obscured when only data dichotomization was used. Thus, patients with smaller tumors had lower platelets and higher average bilirubin levels, consistent with portal hypertension.

The major new findings in this analysis are a lower platelet count and higher bilirubin in patients with tumors of a smaller diameter compared to those of a larger diameter. We had thought that there is a progression from cirrhosis to small tumors and then to larger tumors. However, if this were true, it would be expected that as tumors become larger and occupy more space in the underlying liver, there would be a worse thrombocytopenia and higher bilirubin levels, with the growing tumors destroying more of the liver. The data showed the opposite. In order to explain this, we hypothesize that the larger tumors might develop from a subset of the smaller tumors, which develop in patients with better liver function. Possibly, the better liver function permits increased growth of tumors that are detected later or are larger in their development. Tumors growing in more advanced cirrhosis, as reflected in concomitant thrombocytopenia,

would otherwise cause liver failure from the liver parenchyma that is destroyed in order to accommodate the enlarging tumor mass. If these theories are correct, then they suggest that the pathways for hepatocarcinogenesis that result in smaller tumors in cirrhotic patients could be different from the pathways that lead to larger tumors (which also have more satellites, as noted in the tumor numbers in table 1). There are many reports of long survival after resection for large HCCs [24], although increasing tumor size tends to worsen prognosis [25, 26]. We considered, but rejected, the possibility that these results could be explained by growing HCCs contributing to hepatic synthetic function, since sulfur-colloid scans typically show HCCs as 'cold' spots. The results from technetium [27] and positron-emission tomography scans [28] that relate to tumor differentiation, make this an unlikely explanation, although current scans do not assess HCC liver function. The finding of lower bilirubin levels in some patient groups with larger tumors was also reported in another patient population [11].

A multiple logistic regression analysis model was created, relating the 3 parameters of maximum tumor size,

tumor number and PVT to other clinical parameter descriptors. For maximum tumor mass, AFP levels and platelets were significant. For tumor number, neither AFP levels nor platelet counts were significant and for PVT, platelet counts were significant, but not AFP levels (table 3). Given the importance of small tumor size and absence of PVT for definitive therapy, we also constructed a mean multinomial multiple regression model of MTD and PVT combined. In this model, AFP was significant for identifying a tumor size of >3 cm and platelets were significant for recognizing a tumor size of >3 cm with PVT, but not in the group without PVT (table 2).

Conclusions

This prospectively collected and retrospectively analyzed dataset shows the significance of AFP levels and platelet counts. Patients with HCC ≤ 3 cm have increased thrombocytopenia and increased serum bilirubin levels.

References

- 1 Bosch FX, Ribes J, Diaz M, et al: Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004;127:S5–S16.
- 2 Venook AP, Papandreou C, Furuse J, de Guevara LL: The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist* 2010;15(suppl 4):5–13.
- 3 Aii S, Okamoto E, Imamura M: Registries in Japan: current status of hepatocellular carcinoma in Japan. *Liver Cancer Study Group of Japan. Semin Surg Oncol* 1996;12:204–211.
- 4 Nathan H, Schulick RD, Choti MA, Pawlik TM: Predictors of survival after resection of early hepatocellular carcinoma. *Ann Surg* 2009;249:799–805.
- 5 Zhou Y, Zhao Y, Li B, Xu D, et al: Meta-analysis of radiofrequency ablation versus hepatic resection for small hepatocellular carcinoma. *BMC Gastroenterol* 2010;9:78–84.
- 6 Rhim H, Lim HK, Choi D: Current status of radiofrequency ablation of hepatocellular carcinoma. *World J Gastrointest Surg* 2010;2:128–136.
- 7 Tiong L, Maddern GJ: Systematic review and meta-analysis of survival and disease recurrence after radiofrequency ablation for hepatocellular carcinoma. *Br J Surg* 2011;98:1210–1224.
- 8 Bruix J, Sherman M: Practice Guidelines Committee, American Association for the Study of Liver Diseases: Management of hepatocellular carcinoma. *Hepatology* 2005;42:1208–1236.
- 9 Lencioni R, Llovet JM: Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010;30:52–60.
- 10 Carr BI, Pancoska P, Branch RA: HCC in young adults. *Hepatogastroenterology* 2010;57:436–440.
- 11 Carr BI, Lu SN, Pancoska P: Small hepatocellular carcinoma in Chinese patients. *Hepatogastroenterology* 2011;58:1334–1342.
- 12 Lu SN, Wang JH, Liu SL, et al: Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. *Cancer* 2006;107:2212–2222.
- 13 Wan DW, Tzimas D, Smith JA, et al: Risk factors for early-onset and late-onset hepatocellular carcinoma in Asian immigrants with hepatitis B in the United States. *Am J Gastroenterol* 2011;106:1994–2000.
- 14 Azzaroli F, Colecchia A, Lodato F, et al: A statistical model predicting high hepatocyte proliferation index and the risk of developing hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. *Aliment Pharmacol Ther* 2006;24:129–136.
- 15 Okuda K, Ohtsuki T, Obata H, et al: Natural history of hepatocellular carcinoma and prognosis in relation to treatment. *Cancer* 1985;56:918–928.
- 16 Grieco A, Pompili M, Caminiti G, et al: Prognostic factors for survival in patients with early-intermediate hepatocellular carcinoma undergoing non-surgical therapy: comparison of Okuda, CLIP, and BCLC staging systems in a single Italian centre. *Gut* 2005;54:411–418.
- 17 Guglielmi A, Ruzzenente A, Pachera S, et al: Comparison of seven staging systems in cirrhotic patients with hepatocellular carcinoma in a cohort of patients who underwent radiofrequency ablation with complete response. *Am J Gastroenterol* 2008;103:597–604.
- 18 Nomura F, Ohnishi K, Tanabe Y: Clinical features and prognosis of hepatocellular carcinoma with reference to serum alpha-fetoprotein levels. Analysis of 606 patients. *Cancer* 1989 Oct 15;64:1700–1707.
- 19 Carr BI, Pancoska P, Branch RA: Low alpha-fetoprotein hepatocellular carcinoma. *J Gastroenterol Hepatol* 2010;25:1543–1549.
- 20 Carr BI, Buch SC, Kondragunta V, Pancoska P, Branch RA: Tumor and liver determinants of prognosis in unresectable hepatocellular carcinoma: a case cohort study. *J Gastroenterol Hepatol* 2008;23:1259–1266.

- 21 Pancoska P, Carr BI, Branch RA: Network-based analysis of survival for unresectable hepatocellular carcinoma. *Semin Oncol* 2010;37:170–181.
- 22 Hoshida Y, Villanueva A, Kobayashi M, et al: Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008;359:1995–2004.
- 23 Pawlik TM, Delman KA, Vauthey JN, et al: Tumor size predicts vascular invasion and histologic grade: implications for selection of surgical treatment for hepatocellular carcinoma. *Liver Transpl* 2005;11:1086–1092.
- 24 Woo HG, Park ES, Thorgeirsson SS, Kim YJ: Exploring genomic profiles of hepatocellular carcinoma. *Mol Carcinog* 2011;50:235–243.
- 25 Ramacciato G, Mercantini P, Petrucciani N, et al: Does surgical resection have a role in the treatment of large or multinodular hepatocellular carcinoma? *Am Surg* 2010;76:1189–1197.
- 26 Hao K, Luk JM, Lee NP, et al: Predicting prognosis in hepatocellular carcinoma after curative surgery with common clinicopathologic parameters. *BMC Cancer* 2009;9:389.
- 27 Calvet X, Pons F, Bruix J, et al: Technetium-99m DISIDA hepatobiliary agent in diagnosis of hepatocellular carcinoma: relationship between detectability and tumor differentiation. *J Nucl Med* 1988;29:1916–1920.
- 28 Seo S, Hatano E, Higashi T, Hara T, Tada M, Tamaki N, et al: Fluorine-18 fluorodeoxyglucose positron emission tomography predicts tumor differentiation, P-glycoprotein expression, and outcome after resection in hepatocellular carcinoma. *Clin Cancer Res* 2007;13:427–433.
- 29 Kawamura Y, Arase Y, Ikeda K, et al: Large-scale long-term follow-up study of Japanese patients with non-alcoholic fatty liver disease for the onset of hepatocellular carcinoma. *Am J Gastroenterol* 2012;107:253–261.
- 30 Lu SN, Wang JH, Chen PF, et al: Community-based mass ultrasonographic screening of hepatocellular carcinoma among thrombocytopenic adults. *Cancer Epidemiol Biomarkers Prev* 2008;17:1813–1821.