Serum levels of preoperative α-fetoprotein and CA19-9 predict survival of hepatic carcinoma patients after liver transplantation

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Objective **The aim of this study was to assess serum levels of presurgical α-fetoprotein (AFP) and carbohydrate antigen 19-9 (CA19-9) as prognostic markers in patients with hepatic carcinoma after liver transplantation (LT).**

Methods **A total of 226 patients were recruited for the analysis of serum AFP and CA19-9 levels, on the basis of which the tumor marker type (TMT) was defined and evaluated for prognostic prediction. Overall survival (OS) and relapse-free survival (RFS) were analyzed using Kaplan–Meier curves, and univariate and multivariate Cox models.**

Results **One-year and 5-year OS were 79.0 and 58.0%, respectively, whereas RFS were 70.3 and 62.2%, respectively, in this cohort of patients. There were six variables predicting both OS and RFS, including TMT, tumor size, number of tumor lesions, extrahepatic or vascular invasion, and histopathological grade. Among these, TMT, tumor size, and extrahepatic invasion were all independent predictors of OS and RFS among these patients. Further, on the basis of TMT, novel LT selection criteria for patients with hepatic carcinoma, which supplemented the Milan criteria, were adopted, because the patients within the Milan criteria ($n=107$) and those exceeding Milan but fulfilling the proposed criteria ($n=30$) had similar 5-year OS (77.8 vs. 79.3%, $P=0.862$) and RFS (85.5 vs. 75.1%, $P=0.210$) rates.**

Conclusion **The data from this study showed that serum levels of preoperative AFP and CA19-9 were able to predict survival of patients with hepatic carcinoma after LT. This study included novel criteria, adding serum AFP and CA19-9 levels to the selection criteria for LT eligibility of patients, in addition to the Milan criteria.** ***Eur J Gastroenterol Hepatol*** **26:553–561 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: biomarker, CA19-9, α-fetoprotein, hepatocellular carcinoma, liver cancer, liver transplantation, survival

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Introduction

Liver cancer is a significant worldwide health problem and is the sixth most frequently diagnosed cancer in the world. Infection with hepatitis B or C virus is the major risk factor for liver cancer, which accounts for more than 85% of cases in developing countries. The incidence rates of liver cancer are increasing in many parts of the world including the USA and Central Europe, possibly because of the obesity epidemic and the rise in hepatitis C virus infection [1]. A significant proportion of cases of liver cancer are accompanied by serious cirrhosis or liver dysfunction. Liver transplantation (LT) is considered to be the optimal therapy for small-sized hepatic carcinomas in patients with decompensated liver cirrhosis. To date, the Milan criteria have been adopted by the United Network of Organ Sharing (UNOS) as the standard LT selection criteria for patients with hepatocellular carcinoma (HCC) [2,3]. Recently, it has been heavily investigated whether we can expand the Milan criteria to enable more patients to qualify as transplant candidates. Indeed, previous studies [4–9] have shown that moderate expansion of the Milan criteria could yield favorable outcomes.

α-Fetoprotein (AFP) has been widely accepted in the screening of HCC and in the identification of high-risk populations [10], and carbohydrate antigen 19-9 (CA19-9), also called sialylated Lewis (a) antigen, is a tumor marker for screening of different human cancers in the digestive system [11]. Moreover, our own experience with long-term follow-up of hepatic carcinoma patients also confirmed that elevated preoperative levels of AFP or CA19-9 predicted a poor prognosis in such patients after LT. Thus, in the current study, we assessed presurgical serum levels of AFP and CA19-9 as prognostic markers in the prediction of overall survival (OS) and relapse-free survival (RFS) for patients with hepatic carcinoma after LT. Thereafter we

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tried to add more LT selection criteria for such patients, in addition to the Milan criteria.

Patients and methods

Study population and data collections

From January 2007 to June 2010, a total of 237 consecutive patients with histologically proven primary hepatic carcinoma underwent LT at the Department of Liver Surgery, Ren Ji Hospital (Shanghai, China). Eleven patients were excluded from the current study because of the following reasons: (i) seven patients had possible metastatic disease before LT; (ii) two patients had coexistence of HCC and gallbladder carcinoma, confirmed pathologically after LT; (iii) one patient had undergone additional left nephrectomy for concurrent renal carcinoma; and (iv) one patient had undergone combined liver–kidney transplantation. Ultimately, 226 patients met the eligibility criteria and were enrolled in this study.

The clinicopathological data from our prospective LT database were retrospectively reviewed. Salvage LT was performed in patients who developed recurrent hepatic carcinoma after the primary liver resection. Preoperative downstaging treatment for tumor size reduction included transcatheter arterial chemoembolization, radiofrequency ablation, percutaneous ethanol injection, and stereotactic body radiation therapy (gamma knife). Tumor size was measured as the maximal diameter of the largest tumor in the resected specimens. Histopathologic differentiation of the tumors was carried out according to the Edmondson–Steiner criteria [12] (grade I, well-differentiated; grade II, moderately differentiated; and grade III, poorly differentiated). The latest measurement results of AFP and CA19-9 before LT were recorded in the database, and in most patients both results were obtained within 7 days before surgery. A serum CA19-9 level greater than 500 U/ml was rare, thus higher values were truncated at this threshold.

Liver transplantation surgery

All the surgical procedures were performed by specialists with experience in the LT technique at the Department of Liver Surgery, Ren Ji Hospital. All the surgeons participating in this study were from the same surgical team. Surgery was performed using standard techniques. Classic orthotopic LT was the only surgical technique for deceased donor LT. All patients undergoing living donor LT were operated upon using right liver grafts without the middle hepatic vein. Organ donation or transplantation in the study was strictly implemented under the regulation of Shanghai Organ Transplant Committee and the Declaration of Helsinki. Ethical approval was obtained from the Committee of Ethics at Ren Ji Hospital. All of the living organs were donated with informed consent, and cadaveric donors involved in the study were brain-dead donors or those with no heart beat.

Immunosuppressive treatment

After LT, a triple-drug regimen of tacrolimus or cyclosporine (CsA) combined with methylprednisolone and/or mycophenolate mofetil (MMF) was used. Immunosuppression was started during surgery with 500 mg methylprednisolone; this dose was tapered from 240 mg on postoperative day 1 to 40 mg on postoperative day 6. Maintenance prednisone at an initial dose of 20 mg daily was gradually reduced every week and was withdrawn 3 months after transplantation. The initial dose of tacrolimus was 0.06–0.15 mg/kg/day with a target trough level of 8–10 ng/ml during the first 30 days. MMF was administered orally after LT at 0.5–0.75 g twice daily. If tacrolimus did not reach the target level, it would be replaced by CsA at 6–10 mg/kg/day. The target C0 and C2 levels for CsA were 150–200 and 800–1200 ng/ml, respectively.

Patients' follow-up and study endpoint

All patients were followed up at our outpatient clinic or through a telephone interview. The surviving patients were regularly followed up at the clinic: monthly during the first 6 months after LT, every 3 months from the seventh to the 18th month, and every 6 months thereafter. Serum levels of AFP and CA19-9, chest radiographs, and abdominal ultrasound scans were routinely assessed at each follow-up visit, and abdominal contrast-enhanced computed tomography was performed every 6 months during the first 2 years and annually thereafter. An increased AFP or CA19-9 level alone was not identified as being indicative of tumor recurrence, but once tumor recurrence had been confirmed, the date at which the AFP or CA19-9 level began to increase was taken as the date of recurrence. The endpoint of this study was estimation of OS and RFS. OS was calculated from the time of LT until death or the last follow-up contact; the cut-off date of follow-up was 1 September 2013. RFS was defined as the duration from LT to the date of a suspected tumor recurrence in patients with eventually confirmed tumor recurrence or to the last follow-up contact in patients without tumor recurrence. The follow-up period ranged from 1 to 78 months, with a median of 36 months.

Statistical analysis

Statistical analysis was carried out using SPSS for Windows version 13.0 (SPSS Inc., Chicago, Illinois, USA). Categorical data were analyzed using the χ^2-test. Normality of all related variables was checked using the Shapiro–Wilk method, and mean ± SD was used to describe the central tendency and dispersion of the measurement data with a normal distribution, whereas median (range) was applied to the data without a normal distribution. Kaplan–Meier curves were used to estimate the cumulative OS and RFS rates. The equality of survival distributions among different patient groups was tested using the log-rank method. Univariate analysis was used to analyze each factor that might have

influenced the prognosis of patients with hepatic carcinoma after LT, and any variable identified as statistically significant ($P < 0.05$) in univariate analysis was subjected to multivariate Cox analysis, which assessed the independent predictors for OS and RFS. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Patient characteristics

The clinicopathological characteristics of the 226 patients are summarized in Table 1. Specifically, there were 192 male (85.0%) and 34 female (15.0%) patients, with a mean age of 50.2 (±8.8) years. Two hundred and twenty-three (98.7%) patients were confirmed to have had liver cirrhosis during LT. Salvage LT was performed in 28 (12.4%) patients. Eighty-five (37.6%) patients had a preoperative serum AFP level higher than 400 ng/ml and 32 (14.2%) patients had a preoperative CA19-9 level greater than 100 U/ml. Further, according to the Child–Pugh classification, 86 (38.1%) patients were of Child's class A, 100 (44.2%) patients were of Child's class B, and 40 (17.7%) patients were of Child's class C. The majority of the patients (54.9%) had a model for end-stage liver disease score of 10–19. Eighty-six (38.1%) patients underwent downstaging treatment before LT. The most common etiology of cirrhosis was hepatitis B virus infection, which accounted for 217 of 226 cases (96.0%). There were two (0.9%) patients with hepatitis C infection, one (0.4%) with hepatitis B and C coinfection, two (0.9%) with alcoholic liver disease, one (0.4%) with autoimmune hepatitis, and three (1.3%) with idiopathic liver cirrhosis.

Table 2 shows the details of the entire cohort's histopathologic features. There were 119 (52.7%) patients who did not fulfill the Milan criteria. Fifty-four (23.9%) patients and 25 (11.1%) patients had a maximum tumor size of 5–10 cm and greater than 10 cm, respectively, whereas 40 (17.7%) patients were identified as having a vascular invasion and 21 (9.3%) patients as having an extrahepatic invasion. One-year and 5-year OS rates among these 226 patients were 79.0 and 58.0%, respectively, whereas RFS rates were 70.3 and 62.2%, respectively. A total of 78 (34.5%) patients showed tumor recurrence after LT.

Table 1 Baseline characteristics of patients ($n = 226$)

Variable	Number of patients (%)
Age (mean years±SD)	50.2±8.8
Sex	
Male	192 (85.0)
Female	34 (15.0)
Liver cirrhosis	223 (98.7)
Salvage LT	28 (12.4)
Surgical technique	
LDLT	37 (16.4)
DDLT	189 (83.6)
Preoperative AFP (ng/ml)	
≤ 400	141 (62.4)
>400	85 (37.6)
Preoperative CA19-9 (U/ml)	
≤ 100	194 (85.8)
>100	32 (14.2)
Child–Pugh class	
A	86 (38.1)
B	100 (44.2)
C	40 (17.7)
MELD score	
<10	78 (34.5)
10–19	124 (54.9)
≥ 20	24 (10.6)
Preoperative downstaging treatment	86 (38.1)
Etiology of liver disease	
Hepatitis B	217 (96.0)
Hepatitis C	2 (0.9)
Hepatitis B + C	1 (0.4)
Alcoholic	2 (0.9)
Autoimmune	1 (0.4)
Idiopathic	3 (1.3)

AFP, α-fetoprotein; CA19-9, carbohydrate antigen 19-9; DDLT, deceased donor liver transplantation; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, model for end-stage liver disease.

Table 2 Histopathologic features of patients ($n = 226$)

Variable	Number of patients (%)
Within Milan criteria	107 (47.3)
Outside Milan criteria	119 (52.7)
Tumor size (cm)	
≤ 5	147 (65.0)
5–10	54 (23.9)
>10	25 (11.1)
Tumors number	
Single	148 (65.5)
Multiple	78 (34.5)
Vascular invasion	40 (17.7)
Extrahepatic invasion	21 (9.3)
Tumor pathological type	
HCC	219 (96.1)
ICC or cHCC-CC	7 (3.1)
Histopathologic grading	
I–II	158 (69.9)
III	68 (30.1)

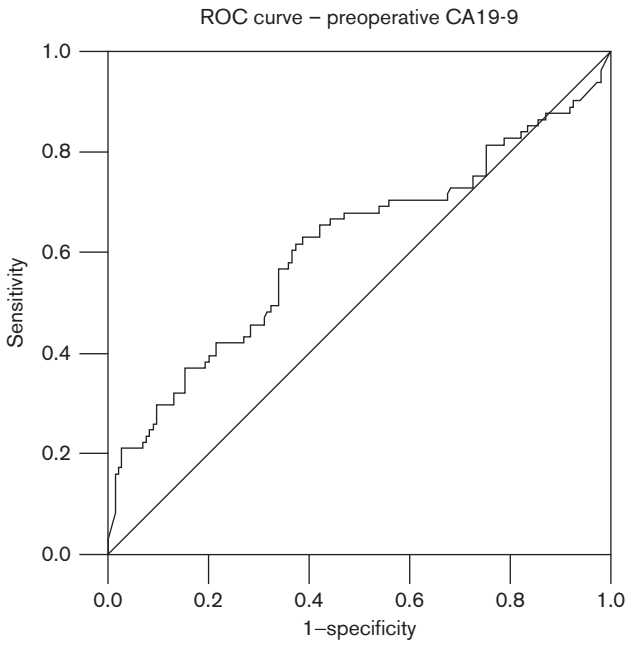
cHCC-CC, combined hepatocellular carcinoma–cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

Definition of the tumor marker type (TMT)

To define TMT, we assessed OS and RFS of these patients with different preoperative serum levels of AFP or CA19-9 by interaction between preoperative AFP and CA19-9 levels and OS and RFS rates. We first generated a receiver operating characteristic (ROC) curve for CA19-9 levels. As shown in Fig. 1, the area under the ROC curve (area = 0.613, $P = 0.005$) showed that an elevated preoperative serum level of CA19-9 was a useful predictor for high mortality of hepatic carcinoma patients within 3 years after LT. To prevent false-positive results in such a high-risk population of tumor recurrence, we used 100 U/ml as the cut-off value for the preoperative serum level of CA19-9 in this study. The data showed that those patients with a preoperative CA19-9 level greater than 100 U/ml had a significantly worse prognosis than those with a CA19-9 level of 100 U/ml or lower (5-year OS: 32.4 vs. 62.2%, $P < 0.001$; 5-year RFS: 35.1 vs. 66.1%,

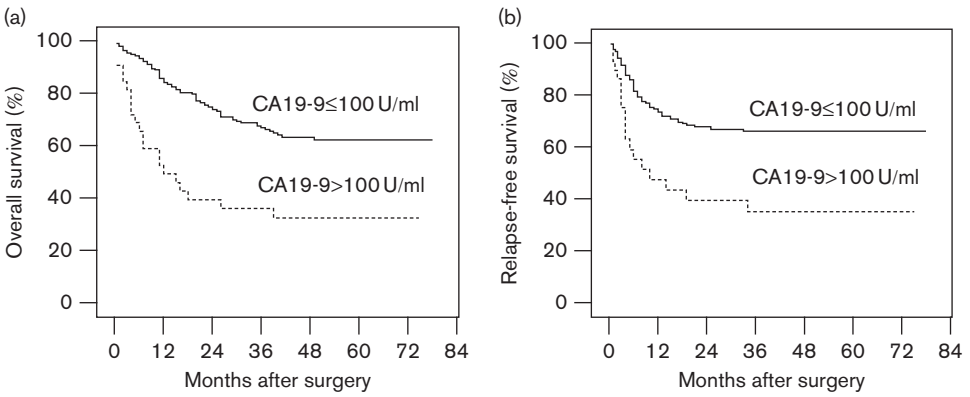
$P < 0.001$; Fig. 2). However, 26 of 32 patients (81.3%) with an elevated CA19-9 level fell outside the Milan criteria. Further, there were seven non-HCC patients enrolled in this study, including five patients with intrahepatic cholangiocarcinoma (ICC) and two patients with combined HCC-cholangiocarcinoma (cHCC-CC). The mean levels of preoperative CA19-9 in HCC and non-HCC ($n = 219$ vs. 7) patients were 65.9 and 259.8 U/ml, respectively ($P = 0.052$). In addition, we adopted a cut-off value of 400 ng/ml for the AFP level. Serum levels of preoperative AFP of 400 ng/ml or lower versus greater than 400 ng/ml showed a significant survival benefit on both 5-year OS (69.5 vs. 38.7%, $P < 0.001$) and RFS (76.1 vs. 40.3%, $P < 0.001$; Fig. 3). Thereafter, we analyzed OS and RFS of these patients according to AFP and CA19-9 levels, which led to their segregation into four groups: group 1 patients ($n = 13$) had elevated levels of both preoperative AFP (> 400 ng/ml) and CA19-9 (> 100 U/ml); group 2 ($n = 72$) had a preoperative AFP level of greater than 400 ng/ml but a CA19-9 level of 100 U/ml or lower; group 3 ($n = 19$) had a preoperative AFP level of 400 ng/ml or lower, but a high level (> 100 U/ml) of preoperative CA19-9; and group 4 ($n = 122$) had low levels of both AFP and CA19-9 ($\leq$ 400 ng/ml and $\leq$ 100 U/ml, respectively). The data on OS and RFS of these four groups of patients are summarized in Table 3 and Fig. 4. Patients in group 4 reached 1-year and 5-year OS rates of 89.9 and 74.6%, respectively, whereas the RFS rates were 84.9 and 78.5%, respectively. The data showed that these patients had significant survival advantages compared with those in each of the other three groups (OS: $P_{1-4} < 0.001$, $P_{2-4} < 0.001$, $P_{3-4} < 0.001$; RFS: $P_{1-4} < 0.001$, $P_{2-4} < 0.001$, $P_{3-4} = 0.021$; Table 3). Moreover, both 5-year OS (41.2 vs. 36.8%, $P = 0.118$) and RFS (45.3 vs. 56.1%, $P = 0.649$) rates were equivalent between groups 2 and 3. However, patients in group 1 (5-year OS and RFS rates = 25.4 and 15.4%) showed the worst prognosis among these four groups of patients. Thus, on the basis of these data, TMT was defined, and hepatic carcinoma patients could be classified on the basis of TMT as follows: type negative (N), 122 patients

Fig. 1



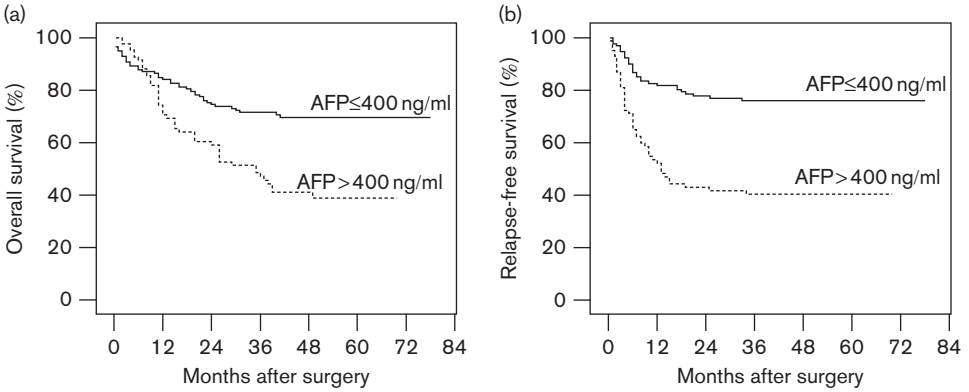
The ROC curve of the serum level of preoperative CA19-9 and patient survival within 3 years after LT. The area under the ROC curve (area = 0.613, $P = 0.005$) showed that an elevated preoperative CA19-9 level was a significant predictor for high mortality of patients within 3 years after LT. CA19-9, carbohydrate antigen 19-9; LT, liver transplantation; ROC, receiver operating characteristic.

Fig. 2



Comparison of OS and RFS between patients with a preoperative CA19-9 level $\leq$ 100 U/ml and those with a preoperative CA19-9 level >100 U/ml. (a) The OS rates (5-year: 62.2 vs. 32.4%, $P < 0.001$); (b) The RFS rates (5-year: 66.1 vs. 35.1%, $P < 0.001$). CA19-9, carbohydrate antigen 19-9; OS, overall survival; RFS, relapse-free survival.

Fig. 3



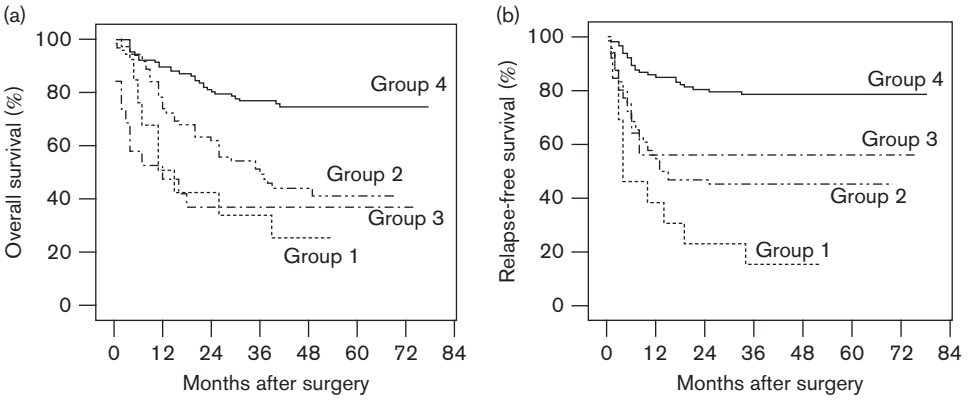
Comparison of OS and RFS between patients with a preoperative AFP level $\leq$ 400 ng/ml and those with a preoperative AFP level >400 ng/ml. (a) The OS rates (5-year: 69.5 vs. 38.7%, $P<0.001$); (b) the RFS rates (5-year: 76.1 vs. 40.3%, $P<0.001$). AFP, α-fetoprotein; OS, overall survival; RFS, relapse-free survival.

Table 3 OS and RFS rates of group 1 to 4 of patients

Group	1-year OS (%)	5-year OS (%)	*P*-value[a]	1-year RFS (%)	5-year RFS (%)	*P*-value[a]
Group 1 ($n=13$)	50.8	25.4	$P_{1-2}=0.120$	38.5	15.4	$P_{1-2}=0.037$
Group 2 ($n=72$)	73.9	41.2	$P_{2-3}=0.118$	54.6	45.3	$P_{2-3}=0.649$
Group 3 ($n=19$)	47.4	36.8	$P_{1-3}=0.922$	56.1	56.1	$P_{1-3}=0.071$
Group 4 ($n=122$)	89.9	74.6	$P_{3-4}<0.001$	84.9	78.5	$P_{3-4}=0.021$
			$P_{1-4}<0.001$			$P_{1-4}<0.001$
			$P_{2-4}<0.001$			$P_{2-4}<0.001$
All ($n=226$)			$P<0.001$			$P<0.001$

OS, overall survival; RFS, relapse-free survival.
[a]P_{a-b} expresses the significance between groups a and b.

Fig. 4



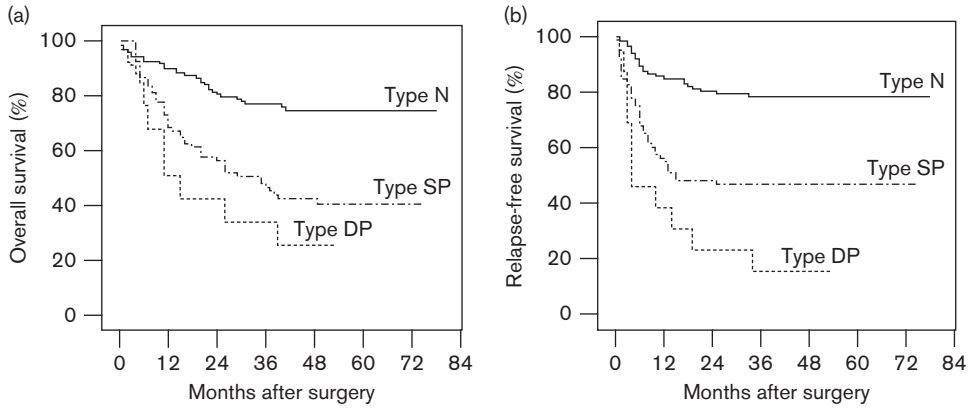
Comparison of OS and RFS among patients of groups 1 to 4. (a) The OS rates ($P_{1-2}=0.120$, $P_{2-3}=0.118$, $P_{1-3}=0.922$, $P_{3-4}<0.001$, $P_{1-4}<0.001$, $P_{2-4}<0.001$); (b) the RFS rates ($P_{1-2}=0.037$, $P_{2-3}=0.649$, $P_{1-3}=0.071$, $P_{3-4}=0.021$, $P_{1-4}<0.001$, $P_{2-4}<0.001$). Group 1 ($n=13$), AFP of >400 ng/ml + CA19-9 of >100 U/ml; group 2 ($n=72$), AFP of >400 ng/ml + CA19-9 of $\leq$ 100 U/ml; group 3 ($n=19$), AFP of $\leq$ 400 ng/ml + CA19-9 of >100 U/ml; group 4 ($n=122$), AFP of $\leq$ 400 ng/ml + CA19-9 of $\leq$ 100 U/ml. AFP, α-fetoprotein; CA19-9, carbohydrate antigen 19-9; OS, overall survival; RFS, relapse-free survival.

(group 4); type single positive (SP), 91 patients (groups 2 and 3); and type double positive (DP), 13 patients (group 1). The OS and RFS rates of patients in the N, SP, and DP groups are shown in Fig. 5.

Predictors for survival

We entered these TMT and clinicopathological data into multiple Cox regression models of OS and RFS as covariates. A total of 14 variables that might affect the

Fig. 5



Comparison of OS and RFS among patients in the N, SP, and DP groups. (a) The OS rates (5-year, 74.6 vs. 40.5 vs. 25.4%; $P_{N-SP}<0.001$, $P_{N-DP}<0.001$, $P_{SP-DP}=0.233$); (b) the RFS rates (5-year, 78.5 vs. 47.1 vs. 15.4%; $P_{N-SP}<0.001$, $P_{N-DP}<0.001$, $P_{SP-DP}=0.026$). DP, double positive; N, negative; OS, overall survival; RFS, relapse-free survival; SP, single positive.

OS or RFS of patients with hepatic carcinoma were subjected to univariate analysis, including age (≤ 50 or >50 years), sex (male or female), Child–Pugh class (A, B, or C), model for end-stage liver disease score (<10, 10–19, or ≥ 20), primary or salvage LT, preoperative downstaging treatment (yes or no), surgical technique (living donor LT or deceased donor LT), TMT (type N, SP, or DP), tumor pathological type (HCC or non-HCC), tumor size (≤ 5, 5–10, or >10 cm), tumor number (single or multiple), extrahepatic invasion (presence or absence), vascular invasion (presence or absence), and histopathologic grading (I–II or III). We found that six variables were significant predictors for both OS and RFS, including TMT (type N, SP, or DP), tumor size (≤ 5, 5–10, or >10 cm), tumor number (single or multiple), extrahepatic invasion (presence or absence), vascular invasion (presence or absence), and histopathologic grading (I–II or III). In addition, tumor pathological type (HCC or non-HCC) was also a significant predictor for OS (Table 4). The multivariate Cox analysis showed that TMT, tumor size, and extrahepatic invasion were all independent predictors for OS and RFS of these patients, whereas vascular invasion was an independent predictor for RFS (Table 5).

Table 4 Univariate analysis of variables that significantly affected OS or RFS

	OS		RFS	
Variable	5-year (%)	*P*-value	5-year (%)	*P*-value
TMT		<0.001		<0.001
Negative	74.6		78.5	
Single positive	40.5		47.1	
Double positive	25.4		15.4	
Tumor pathological type		<0.001		
HCC	59.1			
Non-HCC	19.0			
Tumor size (cm)		<0.001		<0.001
≤5	71.5		77.5	
5–10	40.2		45.1	
>10	9.5		6.0	
Tumor number		0.043		0.015
Single tumor	63.4		67.9	
Multiple tumor	46.2		50.2	
Extrahepatic invasion		<0.001		<0.001
Absence	62.2		67.2	
Presence	14.1		15.2	
Vascular invasion		<0.001		<0.001
Absence	64.2		70.7	
Presence	20.0		20.6	
Histopathologic grading		0.004		<0.001
Grade I–II	65.1		69.3	
Grade III	40.8		44.1	

HCC, hepatocellular carcinoma; OS, overall survival; RFS, relapse-free survival; TMT, tumor marker type.

The proposed criteria for liver transplantation candidates

To propose LT eligibility criteria for patients with hepatic carcinoma, in addition to the Milan criteria, using our current data, we further exploited TMT as a prognostic predictor for survival in these patients and found that the N-group patients were a special subgroup with a favorable prognosis. Therefore, we proposed additional criteria for LT eligibility for hepatic carcinoma patients who did not meet the Milan criteria, which could consist of those within the N group, who were free from vascular invasion and extrahepatic metastasis, regardless of tumor size and number. The OS rates of patients meeting the Milan criteria ($n=107$) and those exceeding the Milan criteria but fulfilling the proposed criteria ($n=30$) were 87.7 versus 96.6% for 1 year and 77.8 versus 79.3% for 5 years, respectively ($P=0.862$), whereas the RFS rates were 90.7 versus 86.0% for 1 year and 85.5 versus 75.1% for 5 years, respectively ($P=0.210$). In contrast, patients who did not fulfill both criteria ($n=89$) showed poor prognostic outcomes (1-year OS and RFS rates = 61.7 and 39.8%, and 5-year OS and RFS rates = 23.6 and

Table 5 Independent variables in the multivariate analysis for OS and RFS

	OS		RFS	
Variable	RR (95% CI)	*P*-value	RR (95% CI)	*P*-value
TMT (vs. negative)				
Single positive	2.669 (1.646–4.329)	<0.001	2.959 (1.754–4.990)	<0.001
Double positive	2.775 (1.264–6.092)	0.011	3.497 (1.643–7.447)	0.001
Tumor size (vs. ≤ 5 cm)				
5–10 cm	1.879 (1.125–3.139)	0.016	2.632 (1.525–4.541)	0.001
>10 cm	6.079 (3.228–11.450)	<0.001	6.643 (3.332–13.246)	<0.001
Extrahepatic invasion	2.302 (1.263–4.197)	0.007	2.529 (1.398–4.572)	0.002
Vascular invasion			2.152 (1.283–3.609)	0.004

CI, confidence interval; OS, overall survival; RFS, relapse-free survival; RR, relative risk; TMT, tumor marker type.

28.8%). The median tumor size in patients exceeding the Milan criteria but fulfilling the proposed criteria was 5.8 cm (range from 1.0 to 14.0 cm), and multiple tumor lesions occurred in 18 (60%) patients. Only two patients with a tumor greater than 10 cm were found among these 30 newly proposed eligible patients for LT, with one patient achieving long-term survival and the other dying of tumor recurrence 19 months after LT.

Discussion

In 1996, Mazzaferro *et al.* [2] introduced the Milan criteria (i.e. single nodule ≤ 5 cm, or no more than three nodules, with each measuring 3 cm or less) on the basis of a retrospective study of 48 patients who underwent LT for HCC, and the Milan criteria have been used thereafter as a guideline for candidate selection for LT in many transplant centers worldwide. Thus, patients with liver cancer who meet the Milan criteria are expected to have a low rate of tumor recurrence. It is true that more stringent selection criteria for LT could achieve a lower tumor recurrence rate, but at the expense of excluding more patients from receiving LT. A previous multicenter study conducted at seven US transplant centers showed that only ~65% of HCC patients who underwent LT met the Milan criteria in the USA [13]. Moreover, Japanese studies had expanded the criteria to include HCC patients with more than three lesions [6,7], and more than half of the HCC patients who underwent LT exceeded the Milan criteria in Japan [14]. In the current study, 119 (52.7%) patients exceeded the Milan criteria.

Further, two additional criteria were proposed by Fan *et al.* [8] (Shanghai criteria) and Zheng *et al.* [9] (Hangzhou criteria) on the basis of LT for hepatitis B-related HCC patients in mainland China. Both Shanghai and Hangzhou criteria lack validation studies on other cohorts of patients. In addition, the Hangzhou criteria could not avoid the 'dilute effect' – that is, a majority of HCC patients who fulfilled the Milan criteria were included in the Hangzhou group, and a separate comparison between the newly proposed eligible patients for LT and the Milan group of patients was absent in the study. Indeed, our previous study [15] verified both the expanded criteria in patients with hepatitis B-related HCC using a prospectively collected database, and the data suggested that the 1-, 3-, and 5-year recurrence rates of the newly eligible patients selected using the Shanghai or Hangzhou criteria were significantly higher than those among patients fulfilling the Milan criteria. Thus, it is important to efficiently and accurately identify the patients with favorable prognosis from those outside the Milan criteria.

Toward this, the serum level of AFP is the most commonly used biomarker to assist in HCC diagnosis and is used as a screening tool for HCC in patients with chronic liver disease [16,17]. A previous study showed that a persistently elevated AFP level is a risk factor for HCC development and helps identify high-risk populations [10]. However, AFP lacks specificity in HCC diagnosis because its levels may also be high in patients with liver cirrhosis [18]. A higher cut-off value of AFP may increase its specificity, whereas the sensitivity drops remarkably [16]. Thus, use of the serum AFP level alone is not recommended for the diagnosis of HCC, whereas the AFP level has been demonstrated to have a predictive value for prognosis in patients with liver cancer. In the prognostic scoring system proposed by the Cancer of the Liver Italian Program group (CLIP) on the basis of retrospective evaluation of 435 Italian HCC patients, AFP was used as an important prognostic factor for HCC patients and was included in the CLIP scoring system [19]. It was advised by the recent EASL–EORTC clinical practice guidelines to test the level of AFP for poor prognosis of HCC patients using greater than 400 ng/ml as a predictor [20]. Thus, in the current study, we used the cut-off value of 400 ng/ml and our data further confirm the prognostic value of AFP.

Further, CA19-9 has important diagnostic value in the detection of cholangiocarcinoma in primary sclerosing cholangitis [21–23], and similar data have also been obtained for patients without primary sclerosing cholangitis [24]. Moreover, persistently elevated CA19-9 levels had a strong predictive value for a poor prognosis of hepatobiliary malignancy [25–28]. Our current data also supported the predictive value of CA19-9 in hepatic carcinoma patients. Nevertheless, the sensibility and specificity in tumor diagnosis increased considerably by combination of two or more serum tumor markers [21,29].

In our study, the area under the ROC curve showed that the preoperative CA19-9 level significantly affected the post-LT survival rate of patients. The 5-year OS and RFS rates of patients with isolated increase in CA19-9 (group 3) were significantly lower than in those with neither biomarker elevated (group 4). Conceivably, a single measurement of the serum AFP cannot be used as an accurate predictive marker for the prognosis of liver cancer patients, whereas the combination of AFP and CA19-9 will greatly improve the prognostic prediction. We therefore proposed selection of patients with hepatic carcinoma who exceeded the Milan criteria using preoperative serum AFP and CA19-9 levels. It should be noted that an elevated CA19-9 level occurred more commonly in ICC or cHCC-CC patients. However, when we removed the seven non-HCC patients from the study, the new results based on the 219 HCC patients were almost the same as the initial findings of the study. In addition, the conclusion was also found to be applicable to patients with ICC or cHCC-CC. Therefore, we did not exclude this group of patients in the current study. However, being a retrospective study, we could not obtain AFP or CA19-9 levels at different time points before and after surgery to perform a time-dependent analysis. Moreover, the difference in OS rates between DP and SP groups of patients failed to reach statistical significance probably because of a limited patient number of the DP group, although TMT correlated extremely well with the RFS rates after LT. Further large-scale prospective trials are in progress to address these issues.

In addition, in the current study, tumor size also remained an independent factor for the survival of patients on the basis of the multivariate Cox analysis. Using newly established LT eligibility criteria, two patients with a tumor larger than 10 cm were found among the 30 newly eligible patients for LT, one of whom with a maximum tumor size of 14 cm achieved long-term survival. Most high-risk patients for tumor recurrence with large tumors had been filtered out by the proposed criteria, and thus, we did not set a limitation of tumor size and number in the criteria. As expected, the 5-year OS and RFS rates were similar between patients within the Milan criteria and the newly eligible patients fulfilling the proposed criteria.

The prognostic relevance of other serum markers (such as des-γ-carboxyprothrombin, AFP-L3 fraction, vascular endothelial growth factor, and angiopoietin 2) was also investigated [30–32], but none of these markers were recommended to survey patients for risk for developing HCC at present. Further studies may evaluate their prognostic values in liver cancer patients.

Conclusion

In conclusion, data from this study show that the combination of AFP and CA19-9 is able to predict OS and RFS of hepatic carcinoma patients after LT and that the TMT based on preoperative serum levels of AFP and CA19-9 could be a useful tool to select hepatic carcinoma patients for LT, especially those exceeding the Milan criteria. However, further prospective studies conducted on a large scale are needed to verify the new criteria and to use these two markers to predict survival of patients with hepatic carcinoma.

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Conflicts of interest

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