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Clinical Significance of Alpha-Fetoprotein in Alpha-Fetoprotein Negative Hepatocellular Carcinoma Underwent Curative Resection

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Abstract

Background The clinical value of alpha-fetoprotein (AFP) in patients with AFP-negative (< 20 ng/ml) hepatocellular carcinoma (HCC) who underwent curative resection remained controversial.

Aims To investigate clinical relevance and prognostic effect of preoperative serum AFP level in this subgroup.

Methods A total of 1879 patients with AFP-negative HCC who underwent curative resection were included in the study. Overall survival (OS) and disease-free survival (DFS) rate were displayed by Kaplan–Meier method and compared by log-rank test. Multivariate cox proportional hazard regression analysis was used to identify the independent prognostic factors. The prognostic predictive performance was analyzed by time-dependent areas under receiver operating characteristic curve (AUC).

Results Even in AFP-negative HCC, patients with high preoperative serum AFP level tended to have multiple tumor ($P < 0.001$), poorer differentiation of tumor cell ($P < 0.001$), presence of satellite nodules ($P < 0.001$), and MVI ($P = 0.002$). Kaplan–Meier analysis showed the adverse impact of AFP level on prognosis, especially for DFS. Multivariate analysis identified AFP as the independent unfavorable factor for OS and DFS ($P < 0.001$ for both). Time-dependent AUC analysis showed that the combination with AFP could improve the prognostic predictive performance of 8th AJCC and BCLC staging system.

Conclusions AFP was still the surrogate of aggressive behavior of HCC and independent prognostic factor for patients with AFP-negative HCC underwent curative resection. Even combining with such a low level of AFP could significantly improve the predictive performance of conventional staging system.

Keywords Alpha-fetoprotein · Hepatocellular carcinoma · Resection · Prognosis

Abbreviations

AFP	Alpha fetoprotein
HBsAg	Hepatitis B virus surface antigen
HCVAb	Anti-hepatitis c virus antibody
HBV-HCC	HBV associated hepatocellular carcinoma
HCV-HCC	HCV associated hepatocellular carcinoma
HB/CV-HCC	HBV, HCV associated hepatocellular carcinoma
NBNC-HCC	Non-B, non-C hepatocellular carcinoma
WBCs	White blood cells
RBCs	Red blood cells
Hb	Hemoglobin
ALT	Alanine transaminase
GGT	Gamma-glutamyl transpeptidase
ALBI	Albumin–bilirubin
BCLC	Barcelona Clinic Liver Cancer staging system
AJCC	American Joint Committee on Cancer
MVI	Microvascular invasion

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Introduction

Hepatocellular carcinoma (HCC), which accounts for 90% liver cancer, ranks as the fifth most common cancer, and also the third leading cause of cancer-related death worldwide [1]. Despite the improvement of diagnosis and treatment over the years, the long-term prognosis remains unsatisfactory due to high incidence of postoperative recurrence

and metastasis [2]. To address the issue, several prognostic markers with abilities to classify and prognosticate HCC were identified to provide guidance for treatment strategies and postoperative follow-up managements.

Although numerous biomarkers have been identified, alpha-fetoprotein (AFP) is still the most widely accepted serum biomarker for HCC in a daily clinical practice [3]. The clinical application of AFP was mainly focused on the following four aspects: screening and diagnosing, predicting prognosis, and monitoring response to treatment [4]. For healthy adults, elevated AFP in serum was the indication of HCC; furthermore, for the patients diagnosed as HCC, higher AFP was associated with more aggressive tumor characteristics, poorer outcomes, and poorer therapy responses [5–7].

The subgroup without elevated AFP is account for around 38.1–39.4% in the group of HCC patients [8, 9]. Some studies have classified the subgroup as the AFP-negative HCC and have also researched the clinical characteristic and risk factors of the subgroup. However, the clinical value of AFP in the subgroup remained controversial [10, 11]. Zhang [10], Gan [11], and Wang et al. [12] investigated the prognostic factors of AFP-negative HCC, but none of their studies included AFP as the prognostic factor. Besides, Blank [13] and Lu et al. [14] reported the controversial prognostic effect of AFP. Blank et al. reported that AFP was an independent prognostic factor for HBV-HCC patients underwent hepatectomy, and slight changes of AFP within the range of normality may affect prognosis of HBV-HCC; however, Lu et al. failed to find the prognostic effect of AFP in their study.

It was worth noting that AFP-negative was classified by the cutoff point of AFP for the purpose of HCC screening rather than HCC prognostic. Therefore, this study, which included a large cohort of patients with AFP-negative HCC underwent curative resection, aims to investigate the clinical relevance and prognostic effect of preoperative AFP level in serum in this subgroup.

Methods

Patients

The data of patients who met following criteria underwent hepatectomy between June, 20, 2008, and December, 30, 2014, were extracted from Primary Liver Cancer Big Data (PLCBD). The inclusion criteria of patients in the study were as follows: (1) accepted hepatectomy as the primary treatment for HCC; (2) with a preoperative serum AFP level lower than 20 ng/ml; (3) with tolerable preoperative liver function (Child–Pugh A or B7); (4) without distant metastasis; and (5) receipt of R0 resection, which means the complete removal and histological tumor-free surgical

margins of all detectable tumor nodes. The exclusion criteria used in the study were as follows: (1) receipt of palliative tumor resection or preoperative anti-HCC treatment; (2) with medical history of any other malignant diseases; and (3) incomplete clinicopathologic data. All data in this study were verified by three independent researchers (Kongying Lin, Jianxing Zeng, and Qizhen Huang), and the study was conducted to the ethical guideline of the 1975 Declaration of Helsinki and was approved by the institutional ethics committee of Mengchao Hepatobiliary Hospital of Fujian Medial University. Informed consent was obtained from each patient for their data to be used for research purposes.

Preoperative Assessment, Hepatectomy, and Follow-Up

Preoperative assessments of patients contained routine examination of liver, renal, cardiopulmonary function, AFP, and hepatitis B/C immunology. Imaging examinations included abdominal ultrasonography, contrast-enhanced magnetic resonance (MRI) or computed tomography (CT) of abdomen, and X-ray check or CT scan of chest. The diagnosis of HCC complied with practice guidelines which recommended by American Association for the study of Liver Diseases [15]. The adoption of anatomical or partial hepatectomy depends on the tumor variables, such as diameter, number and location, and patient's liver function status. Intraoperative ultrasound test was routinely performed to ensure all detectable tumor nodes were removed.

The followed-up procedure was described as follows. In simple terms, patients routinely accepted serum AFP test, abdominal ultrasonography every 2–3 months in the first 2 years after surgery and later every 3–6 months. The contrast-enhanced CT or MRI was routinely performed every 6 months, or earlier if patients with the result suggestive of recurrence. The diagnosis of recurrence of tumor was similar to the initial diagnostic criteria. The end-points of study were overall survival (OS) and disease-free survival (DFS). Overall survival (OS) was defined as the interval between the date of resection and the date of either death or the last follow-up taken. Disease-free survival (DFS) was defined as the interval between date of resection and the date of recurrence, metastasis, or last follow-up.

Clinicopathologic Variables

The preoperative serologic AFP level and other serologic variables of patients selected in the study were the most recent test within 15 days prior to surgery. The formula of albumin–bilirubin score (ALBI) was reported in previous studies, and we further divided patients into three grades according to the previous cutoff point (grade $1 \leq -2.60$, grade $2 > -2.60 \sim -1.39$; grade $3 > -1.39$) [6, 16]. The

pathological examination of surgical specimens was performed by three independent pathologists. Tumor diameter means the largest diameter of the largest tumor nodule. The differentiation of tumor cells was based on the Edmondson–Steiner classification, and if there were multiple tumor nodes, the differentiation of tumor cells was depended on which was worst. Vascular invasion was divided into macrovascular invasion and microvascular invasion (MVI).

Statistical Analysis

Continuous variables were expressed as the median (25th to 75th percentiles), and the comparison of continuous variables was tested by the Student's t test or the Mann–Whitney U test. Categorical variables were expressed by number of patients (percentage) and compared by the χ^2 test or Fisher exact test. The comparisons of OS and DFS rate were displayed by Kaplan–Meier method and tested by log-rank test. The independent risk factors for OS and DFS were acquired using the univariate and multivariate cox proportional hazard regression analysis. The predictive performance was analyzed by time-dependent areas under the receiver operating characteristic cure (AUC). Time-dependent AUC of AFP, 8th AJCC, and BCLC staging system for OS and DFS at each time point was calculated by the function of "timeROC" using "timeROC" R package, and the comparison between two time-dependent AUCs for each time point was tested by the function of "compare" using "timeROC" R package [17]. All statistical tests were two-sided tests, and the P value < 0.05 was regarded statistically significant. The SPSS software 20.0 and R software 3.0 ("rms," "survival," and "timeROC") were used in this study.

Results

Patients' Characteristics

According to the inclusion and exclusion criteria accepted in the study, 1879 patients with AFP-negative HCC were included for further analysis. The basic clinicopathologic characteristics of patients are shown in Table 1. The median AFP level of the patients was 4.6 (25th to 75th percentiles: 2.8, 8.2) ng/ml. Most patients were male (91.0%) and HBsAg positive (82.2%). Liver cirrhosis was present in 65.7% of patients. In ALBI grading, the majority of patients showed well liver function, 77.2% of patients were ALBI grade1 and 22.8% of patients were ALBI grade 2/3. (We grouped together ALBI grades 2 and 3 for further statistical analysis, because only a few patients (2 patients) were classified as ALBI 3 grade.) Regarding to tumor characteristics, most of patients were BCLC A stage (78.6%) or AJCC I stage (66.5%). The median diameter of tumor was 4.6 (25th to

Table 1 Baseline clinical characteristics of patients with AFP-negative HCC

Variable	Value
Age (years)	54.0 (46.0, 61.0)
Gender	
Female	170 (9.0%)
Male	1709 (91.0%)
HBsAg	
Negative	335 (17.8%)
Positive	1544 (82.2%)
HCVAb	
Negative	1840 (97.9%)
Positive	39 (2.1%)
Etiology	
HBV-HCC	1531 (81.5%)
HCV-HCC	26 (1.4%)
HB/CV-HCC	13 (0.7%)
NBNC-HCC	309 (16.4%)
Cirrhosis	
No	645 (34.3%)
Yes	1234 (65.7%)
Diabetes	
No	1715 (91.3%)
Yes	164 (8.7%)
Hypertension	
No	1588 (84.5%)
Yes	291 (15.5%)
WBCs (10^9/L)	5.20 (4.29, 6.39)
RBCs (10^9/L)	4.61 (4.31, 4.93)
Hb (g/L)	142 (133, 151)
Platelets (10^9/L)	157 (116, 198)
Albumin (g/L)	41.9 (39.4, 44.4)
ALT (U/L)	34.0 (23.8, 51.0)
Total bilirubin (μmol/L)	13.2 (10.3, 17.0)
GGT (U/L)	59.0 (34.0, 108)
ALBI	
1 grade	1451 (77.2%)
2/3 grade	428 (22.8%)
AFP (ng/ml)	4.60 (2.80, 8.20)
BCLC staging system	
0	95 (5.1%)
A	1476 (78.6%)
B	201 (10.7%)
C	107 (5.7%)
AJCC staging system 8th	
I	1250 (66.5%)
II	393 (20.9%)
III	236 (12.6%)
Intraoperative blood loss (ml)	
< 800	1786 (95.1%)
≥ 800	93 (4.9%)
Intraoperative blood transfusion	

Table 1 (continued)

Variable	Value
No	1695 (90.2%)
Yes	184 (9.8%)
Tumor diameter (cm)	4.60 (3.20, 7.10)
Tumor number	
Solitary	1611 (85.7%)
Multiple	268 (14.3%)
Satellite nodules	
Absent	1313 (69.9%)
Present	566 (30.1%)
Tumor differentiation	
I/II	505 (26.9%)
III/IV	1374 (73.1%)
Tumor capsule	
Complete	600 (31.9%)
Incomplete	983 (52.3%)
None	296 (15.8%)
MVI	
Absent	1419 (75.5%)
Present	460 (24.5%)
Macrovascular invasion	
Absent	1772 (94.3%)
Present	107 (5.7%)

HCC hepatocellular carcinoma, *AFP* alpha-fetoprotein, *HBsAg* hepatitis B virus surface antigen, *HCVAb* Anti-hepatitis c virus antibody, *HBV-HCC* HBV associated hepatocellular carcinoma, *HCV-HCC* HCV associated hepatocellular carcinoma, *HB/CV-HCC* HBV, HCV associated hepatocellular carcinoma, *NBNC-HCC* non-B, non-C hepatocellular carcinoma, *WBCs* white blood cells, *RBCs* red blood cells, *Hb* hemoglobin, *ALT* Alanine transaminase, *GGT* gamma-glutamyl transpeptidase, *ALBI* albumin–bilirubin, *BCLC* Barcelona Clinic Liver Cancer staging system, *AJCC* American Joint Committee on Cancer, *MVI* microvascular invasion

75th percentiles: 3.2, 7.1) cm, 85.7% of patients presented solitary tumor, 24.5% of patients presented MVI, and 5.7% of patients presented macrovascular invasion.

Association Between AFP and Clinicopathologic Characteristics

As shown in Figure (S1-2), AFP level was significantly different within different contexts of liver or tumor characteristics. The patients with higher AFP level were associated with undesirable liver characteristic, such as positive HBsAg ($P < 0.001$), liver cirrhosis ($P < 0.001$), decreased albumin ($P = 0.015$), decreased platelets ($P < 0.001$), high ALT level ($P < 0.001$), and undesirable ALBI grade ($P < 0.001$) (Figure S1). In addition, higher AFP was also associated with aggressive pathologic characteristics of HCC even within such low level. Higher AFP level was significantly associated with multiple tumor nodules ($P < 0.001$), poorer

differentiation of tumor cell ($P < 0.001$), presence of satellite nodules ($P < 0.001$), and MVI ($P = 0.002$) (Figure S2).

Prognostic Effect of AFP

The median of follow-up was 49.1 months (range 1.1–119.9 months). We classified patients into two cohorts based on the median AFP level (4.6 ng/ml) of the whole cohort. Kaplan–Meier analysis showed that the OS and DFS were significantly poorer for the higher AFP level cohort than the lower AFP level cohort ($P < 0.001$ for both) (Fig. 1a, b). The 1-, 3-, and 5-year OS rates of higher AFP level cohort were 91.6%, 75.0%, 53.7%, respectively, and the corresponding DFS rates were 66.3%, 44.9%, 30.5%, respectively, whereas the postoperative 1-, 3, 5-year OS rates of lower AFP level cohort were 93.4%, 81.4%, 64.0%, respectively, and the corresponding DFS rates were 76.5%, 56.5%, 42.3%, respectively. Although the OS and DFS rates between two cohorts were both significantly different, the difference was relatively small in OS rates, while the difference in the DFS rates was more distinctive. The DFS curve between two cohorts was distinctly different within 1 year after resection, and then almost parallel thereafter.

After stratification according to cirrhosis, higher AFP level was associated with poorer outcome regardless of cirrhosis (Fig. 1c–f). When the prognostic analysis was conducted in the subgroup stratified by etiology, the OS and DFS rates in higher AFP level cohort were both poorer than in lower AFP level cohort, excepting the OS in HCV-HCC (Fig. 2a–f). The distinctive DFS curve trend mentioned above was both found in HBV-HCC and NBNC-HCC cohorts, especially clear in NBNC-HCC cohort. In further prognostic analysis that performed in the subgroup stratified by the 8th AJCC staging system, the higher AFP level cohort had a significantly poorer OS and DFS than lower AFP level cohort in the stage I and II patients (Fig. 3a–f).

Prognostic Factors for OS and DFS

Univariate and multivariate cox proportional hazard regression analysis was used to acquire independent risk factor of OS and DFS (Tables 2, 3). Multivariate analysis for OS showed that higher AFP level remained as independent risk factor of OS (HR with 95% CI, 1.036 (1.022–1.051), $P < 0.001$), and other variables were albumin [0.961 (0.943–0.979), $P < 0.001$], tumor diameter [1.067 (1.047–1.087), $P < 0.001$], multiple tumor [1.472 (1.228–1.764), $P < 0.001$], poorer tumor differentiation [1.299 (1.080–1.563), $P = 0.005$], tumor capsule [1.248 (1.051–1.482), $P = 0.011$, 1.618(1.297–2.018), $P < 0.001$], MVI [1.545 (1.300–1.836), $P < 0.001$], and macrovascular invasion [2.057 (1.576–2.685), $P < 0.001$] (Table 3).
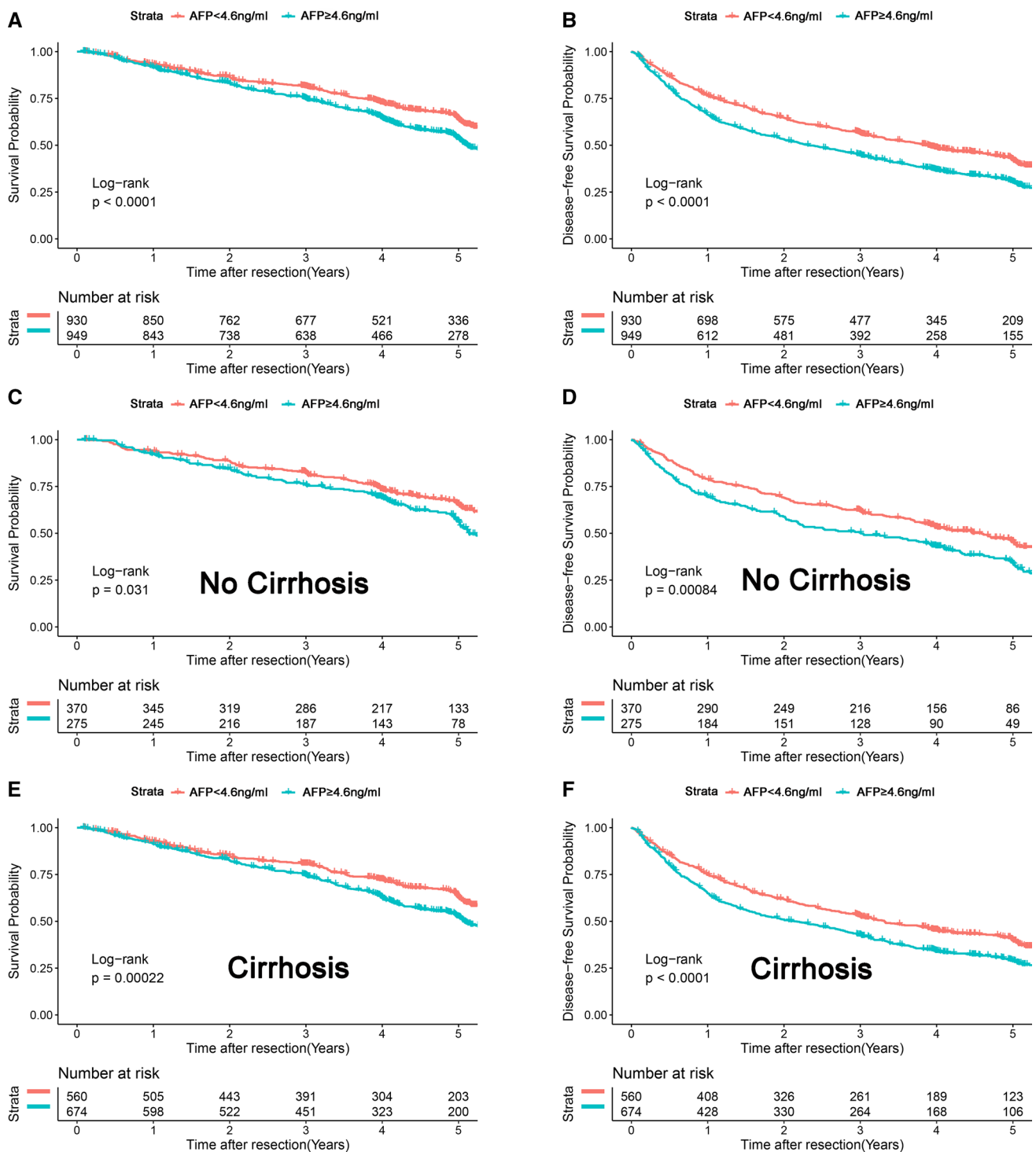
Fig. 1 Comparisons of OS and DFS rate of patients subdivided according to the median of preoperative serologic AFP level. **a** OS, total cohort; **b** DFS, total cohort; **c** OS, no cirrhosis cohort; **d** DFS, no cirrhosis cohort; **e** OS, cirrhosis cohort; **f** DFS, cirrhosis cohort

Multivariate analysis for DFS also identified AFP level as the independent risk factor for DFS [1.031 (1.019–1.043), $P < 0.001$], in addition to albumin [0.977 (0.961–0.993), $P = 0.005$], cirrhosis [1.335 (1.175–1.517), $P < 0.001$], tumor diameter [1.075 (1.059–1.092),

$P < 0.001$], multiple tumor [1.600 (1.376–1.860), $P < 0.001$], tumor capsule [1.289 (1.126–1.476), $P < 0.001$, 1.511 (1.259–1.814), $P < 0.001$], MVI [1.290
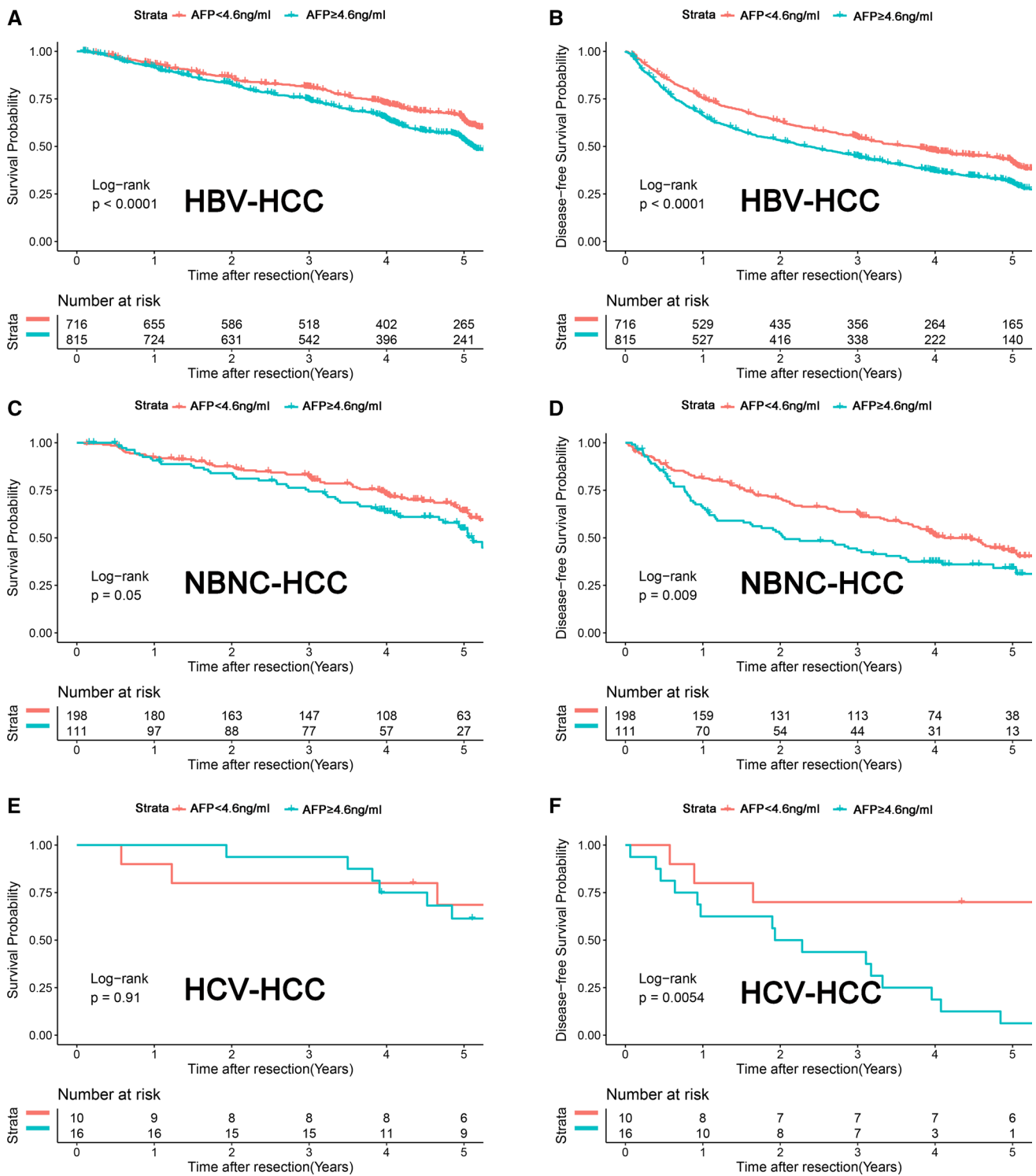
Fig. 2 Comparisons of OS and DFS rate of patients subdivided according to preoperative serologic AFP level among HBV-HCC cohort (**a**, **b**), NBNC-HCC cohort (**c**, **d**), and HCV-HCC (**e**, **f**)
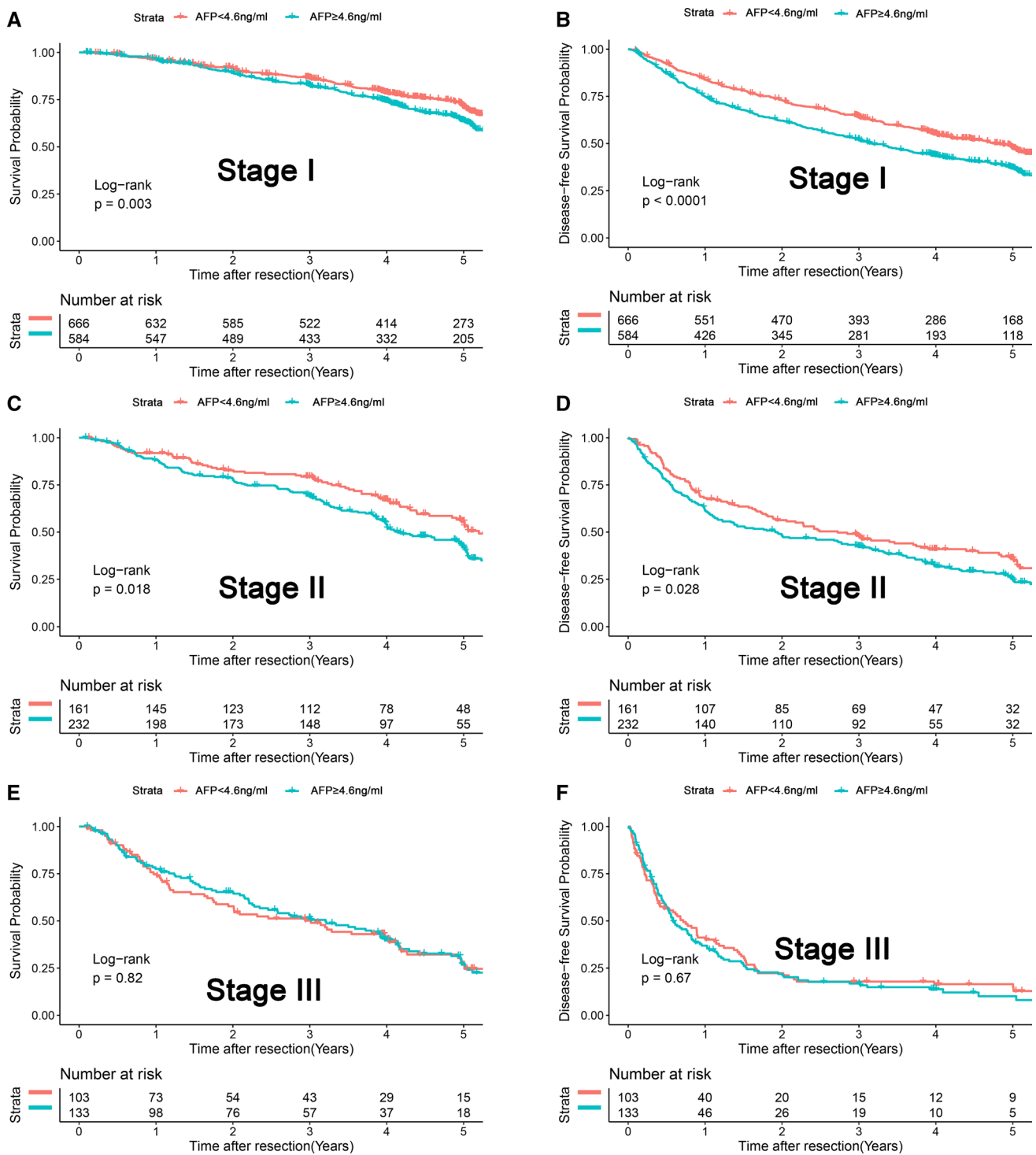
Fig. 3 Comparisons of OS and DFS rate of patients subdivided according to preoperative serologic AFP level among a different 8th AJCC staging system. **a**, **b** Stage I; **c**, **d** Stage II; **e**, **f** Stage III

(1.118–1.489), $P < 0.001$], and macrovascular invasion [2.433 (1.913–3.093), $P < 0.001$] (Table 3).

Predictive Effect of AFP for Prognosis

The time-dependent AUC analysis was performed to assess the predictive effect of the AFP on OS and DFS (Fig. 4a, b). The median time-dependent AUC of AFP was 0.572

Table 2 Univariate cox regression analysis of factors for overall and disease-free survival

Variable	OS		DFS	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (years)	1.000 (0.993–1.006)	0.922	0.998 (0.993–1.004)	0.520
Gender (male vs. female)	1.148 (0.889–1.482)	0.291	1.201 (0.978–1.476)	0.080
HBsAg (positive vs. negative)	1.051 (0.872–1.266)	0.603	1.137 (0.976–1.325)	0.099
HCVAb (positive vs. negative)	0.985 (0.624–1.553)	0.947	1.129 (0.776–1.642)	0.527
Cirrhosis (yes vs. no)	1.149 (0.988–1.335)	0.071	1.229 (1.088–1.388)	0.001
Diabetes (present vs. absent)	1.196 (0.951–1.503)	0.126	1.070 (0.881–1.299)	0.494
Hypertension (present vs. absent)	0.969 (0.797–1.177)	0.749	0.944 (0.808–1.104)	0.474
WBCs (10^9/L)	0.985 (0.947–1.024)	0.435	0.996 (0.965–1.027)	0.779
RBCs (10^9/L)	0.744 (0.646–0.856)	<0.001	0.825 (0.737–0.925)	0.001
Hb (g/L)	0.989 (0.984–0.993)	<0.001	0.992 (0.989–0.996)	<0.001
Platelets (109/L)	1.000 (0.999–1.001)	0.977	0.999 (0.999–1.000)	0.225
Albumin (g/L)	0.936 (0.919–0.953)	<0.001	0.953 (0.938–0.968)	<0.001
ALT (U/L)	1.002 (1.001–1.003)	0.002	1.002 (1.001–1.003)	<0.001
Total bilirubin (μmol/L)	1.005 (1.000–1.011)	0.049	1.003 (0.997–1.008)	0.328
GGT (U/L)	1.001 (1.001–1.001)	<0.001	1.001 (1.001–1.001)	<0.001
ALBI (2/3 grade vs. 1 grade)	1.576 (1.347–1.844)	<0.001	1.464 (1.285–1.668)	<0.001
AFP (ng/ml)	1.047 (1.033–1.062)	<0.001	1.042 (1.030–1.054)	<0.001
Intraoperative blood loss (≥800 vs. 800)	1.999 (1.526–2.619)	<0.001	1.904 (1.509–2.403)	<0.001
Intraoperative blood transfusion (yes vs. no)	1.747 (1.416–2.156)	<0.001	1.738 (1.457–2.073)	<0.001
Tumor diameter (cm)	1.091 (1.073–1.110)	<0.001	1.077 (1.061–1.092)	<0.001
Tumor number (multiple vs. solitary)	1.728 (1.444–2.068)	<0.001	1.868 (1.609–2.169)	<0.001
Satellite nodules (present vs. absent)	1.786 (1.545–2.064)	<0.001	1.638 (1.454–1.846)	<0.001
Tumor differentiation (I/II vs. III/IV)	1.856 (1.561–2.208)	<0.001	1.522 (1.333–1.737)	<0.001
Tumor capsule		<0.001		<0.001
Complete	Ref	Ref	Ref	Ref
Incomplete	1.485 (1.257–1.755)	<0.001	1.369 (1.199–1.562)	<0.001
None	2.070 (1.683–2.545)	<0.001	1.739 (1.466–2.062)	<0.001
MVI (present vs. absent)	2.364 (2.037–2.744)	<0.001	1.824 (1.609–2.069)	<0.001
Macrovascular invasion (present vs. absent)	4.392 (3.476–5.549)	<0.001	4.175 (3.371–5.170)	<0.001

OS overall survival, *DFS* disease-free survival, *HBsAg* hepatitis B virus surface antigen, *HCVAb* anti-hepatitis c virus antibody, *WBCs* white blood cells, *RBCs* red blood cells, *Hb* hemoglobin, *ALT* alanine transaminase, *GGT* gamma-glutamyl transpeptidase, *ALBI* albumin–bilirubin, *AFP* alpha-fetoprotein, *MVI* microvascular invasion

(range 0.558–0.576) for OS and 0.587 (range 0.575–0.594) for DFS, respectively. In addition to conventional BCLC and 8th AJCC staging systems, we further added the factor of AFP to analyze the time-dependent AUCs and compared it with the conventional ones. As shown in Fig. 4c, d, the combination with AFP could significantly improve the predictive capability of 8th AJCC and BCLC staging systems. As shown in Table S1, by comparing the original BCLC and 8th AJCC staging systems and the staging systems combined with APF factor, the time-dependent AUCs of DFS showed significantly different for each time points (*P* < 0.001 for both). Concerning the time-dependent AUCs of OS, the comparison showed significantly different at 3-, 4-, and 5-year time point (Table 1).

Discussion

The present study included a large cohort of patients with AFP-negative HCC who underwent curative resection into the investigation, in order to find out the clinicopathologic and prognostic relevance of AFP in the subgroup. Our results showed that preoperative AFP level was still associated with the aggressive behavior of tumor cells and poor prognostic outcome in AFP-negative HCC.

High serologic AFP level was not only clinically indication of HCC, but also the surrogate for tumor aggressive biology [5, 18]. Some studies have also reported the association among AFP level and different molecular subclasses of HCC [19, 20]. In our study, we showed that preoperative serologic AFP level was also significantly associated

Table 3 Multivariate cox regression analysis of factors for overall and disease-free survival

Variable	HR (95% CI)	P value
OS		
AFP (ng/ml)	1.036 (1.022–1.051)	<0.001
Albumin (g/L)	0.961 (0.943–0.979)	<0.001
Tumor diameter (cm)	1.067 (1.047–1.087)	<0.001
Tumor number (multiple vs. solitary)	1.472 (1.228–1.764)	<0.001
Tumor differentiation* (I/II vs. III/IV)	1.299 (1.080–1.563)	0.005
Tumor capsule		<0.001
Complete	Ref	Ref
Incomplete	1.248 (1.051–1.482)	0.011
None	1.618 (1.297–2.018)	<0.001
MVI (present vs. absent)	1.545 (1.300–1.836)	<0.001
Macrovascular invasion (present vs. absent)	2.057 (1.576–2.685)	<0.001
DFS		
AFP (ng/ml)	1.031 (1.019–1.043)	<0.001
Albumin (g/L)	0.977 (0.961–0.993)	0.005
Cirrhosis (yes vs. no)	1.335 (1.175–1.517)	<0.001
Tumor diameter (cm.)	1.075 (1.059–1.092)	<0.001
Tumor number (multiple vs. solitary)	1.600 (1.376–1.860)	<0.001
Tumor capsule		<0.001
Complete	Ref	Ref
Incomplete	1.289 (1.126–1.476)	<0.001
None	1.511 (1.259–1.814)	<0.001
MVI (present vs. absent)	1.290 (1.118–1.489)	<0.001
Macrovascular invasion (present vs. absent)	2.433 (1.913–3.093)	<0.001

OS overall survival, *DFS* disease-free survival, *AFP* alpha-fetoprotein, *MVI* microvascular invasion

with more frequency of multiple tumor numbers, satellite nodules, poorly differentiation, and MVI. The findings may demonstrate that AFP is still the surrogate of aggressive characteristic of tumor cells in AFP-negative HCC.

The Kaplan–Meier analysis showed the adverse impact of AFP level on prognosis, especially for DFS. The DFS curves between higher and lower AFP level cohorts were distinctly different within 1 year after resection and then almost parallel thereafter. There were two different recurrence types of HCC: One is the "early recurrence" which was mainly caused by the initial tumor, and the other is the "late recurrence" which was mainly due to clonal origin [21]. The distinctive trend of DFS curve between different AFP levels suggested that the frequency of early recurrence was higher in higher AFP cohort. Some studies have reported that early recurrence of HCC is mainly associated with aggressive tumor behavior, such as multiple tumors nodules, poorly differentiation, and MVI [6, 22]. The findings may suggest that the adverse impact of AFP on prognosis of AFP-negative HCC was caused by its association of tumor aggressive behavior.

Interestingly, the prognostic analysis according to etiology showed that the changed trend of DFS curve was more distinctive in NBNC-HCC rather than the one in HBV-HCC.

It may be caused by the difference pathogenic mechanisms of hepatocarcinogenesis between HBV-HCC and NBNC-HCC. Chronic hepatitis B virus infection was unique pathogenic mechanisms of hepatocarcinogenesis in HBV-HCC. The role of HBV in elevation of AFP has been previously reported in some studies [23, 24]. Our results also showed higher AFP level in HBsAg-positive patients. Zhang et al. [24] reported that HBX could directly upregulate the expression of AFP via binding to and activate the promoter of AFP gene. In view of this, the prognostic effect of serum AFP level in HBV-HCC may be affected by HBV factors, such as HBV DNA load. However, more detailed mechanism of such difference between HBV-HCC and NBNC-HCC remains unknown and needs further study.

The study also analyzed the prognostic effect of AFP in the subgroup stratified by 8th AJCC staging system. The AFP level showed a significantly adverse effect on prognosis in the stage I and II. The AJCC staging system was worldwide accepted and conventionally used in a daily clinical practice [25]. However, given the heterogeneity of tumor cells, the patients, even within same stage, may manifest different prognoses [26]. The result showed that AFP may be an additional biomarker, which could be joint with conventional AJCC staging system for clinical usage, in order
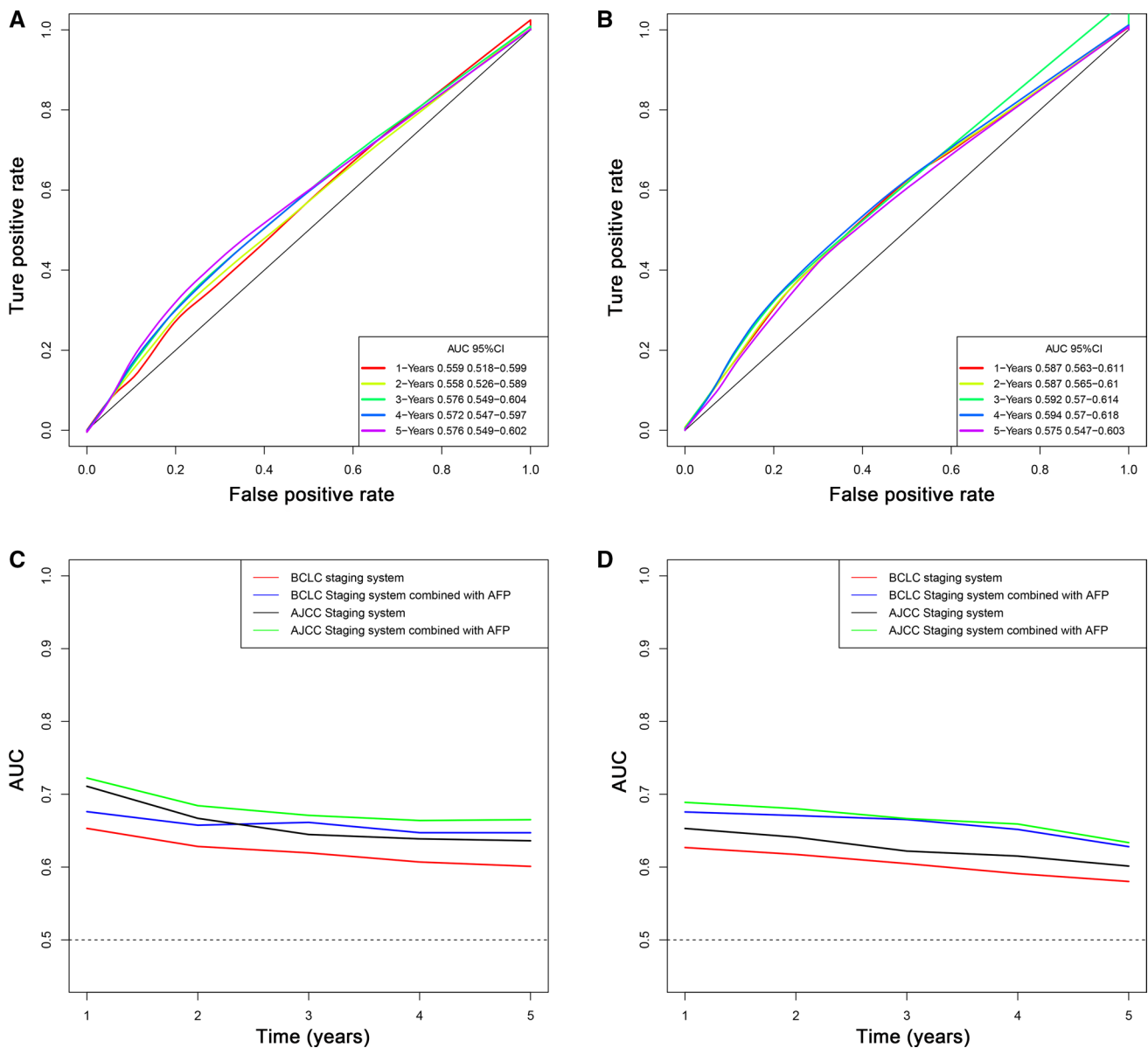
Fig. 4 Time-dependent AUCs of AFP for OS (**a**) and DFS (**b**); time-dependent AUCs of conventionally used staging systems combined with or without AFP for OS (**c**) and DFS (**d**)

to identify the high-risk patients. This may be valuable to guide the postoperative adjuvant therapy and monitoring.

Furthermore, the present study analyzed the prognostic predictive effect of AFP. The prognostic effect of AFP has been acknowledged by serval scoring systems, such as BALAD score, [27] Cancer of the Liver Italian Program (CLIP) score [28], Chinese University Prognostic Index (CUPI) [29], and The Taipei Integrated score (TIS) [30]. However, the cutoff point of AFP accepted in these scoring systems was in the range from 400 to 500 ng/ml, which may be unable to differentiate between patients with low AFP level. In the study, we also showed that the time-dependent AUC of AFP in AFP-negative HCC was 0.572

(range 0.558–0.576) for OS and 0.587 (range 0.575–0.594) for DFS, respectively, which were approximated to those reported in previous studies that included the HCC with various ranges of AFP level [31, 32]. In consideration that HCC is a complicated disease with diverse pathogenic mechanisms caused by various risk factors, a single biomarker may be difficult to stably predict prognosis. Hence, the study further analyzed the predictive performance of conventionally used BCLC and 8th AJCC staging system combined with AFP factor. The result showed that even combining with such a low level of AFP could significantly improve the predictive performance of conventional 8th AJCC and BCLC staging systems.

The study has several limitations. The first one is single-center retrospective study only, and thus, the selection bias was unavoidable. The second one is that the patients included in the study were from a hepatitis B virus endemic area, the majority of patients were suffering from HBV infection, and only 26 patients were HCV-associated HCC, and thus, the clinicopathologic relevance and prognostic effect of AFP in HCV-associated HCC need further study. The third one is that although present study demonstrated that AFP was still the surrogate of aggressive behavior of tumor cell and independent risk prognostic factor for patients with AFP-negative HCC, the time-dependent AUC suggested that AFP alone was not a powerful prognostic predictor. Combination with other serologic biomarkers, such as AFP-L3 and PIVKA-II, may improve predictive performance of AFP. In addition, the study included only the patients who underwent resection and the results need to be further verified in patients who received other treatments.

Conclusion

AFP was still the surrogate of aggressive behavior of HCC and independent risk factor of prognosis in patients with AFP-negative HCC who underwent curative resection. Even combining with such a low level of AFP could significantly improve the predictive performance of conventional staging system.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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