Original Article

Preoperative elevated plasma fibrinogen level predicts tumor recurrence and poor prognosis in patients with hepatocellular carcinoma

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Background: Elevated plasma fibrinogen has been reported to be associated with poor prognosis in several cancers. The aim of present study was to investigate the prognostic value of preoperative plasma fibrinogen in hepatocellular carcinoma (HCC) patients.

Methods: Data were collected retrospectively from 302 HCC patients who received hepatectomy. The association between fibrinogen and clinicopathological characteristics was evaluated. Both univariate and multivariate analyses were performed to identify prognostic factors for disease-free survival (DFS) and overall survival (OS). And accordingly, the nomograms were constructed.

Results: Elevated plasma fibrinogen (>4 g/L) was correlated with larger tumor diameter, the presence of vascular invasion, lower MELD score, higher NLR, advanced Barcelona Clinic Liver Cancer stage and poor-moderate pathological differentiation. On multivariate analysis, the elevated plasma fibrinogen was found independently associated with poor DFS (HR =1.575, P=0.024) and OS (HR =2.051, P=0.025). And the nomograms including fibrinogen were constructed to predict DFS and OS for HCC patients. Both DFS and OS in patients with plasma fibrinogen >4 g/L were significantly lower than those with fibrinogen ≤4 g/L (1-, 3-, 5-year DFS: 34.2%, 19.5% and 0.0% *vs.* 60.4%, 34.2% and 30.2%; 1-, 3-, 5-year OS: 83.4%, 62.7% and 48.8% *vs.* 95.4%, 84.3% and 75.8%, both P<0.001). Besides, subgroup analyses also showed the prognostic values of fibrinogen in HCC patients with/without cirrhosis or high AFP levels, and in those with single tumor and BCLC 0-A stage.

Conclusions: Preoperative elevated plasma fibrinogen was an independent prognostic factor associated with poor prognosis in HCC patients receiving liver resection.

Keywords: Fibrinogen; hepatocellular carcinoma (HCC); recurrence; prognosis

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide, and China alone accounts for about half of total cases (1). The high prevalence of chronic hepatitis B virus (HBV) infection and HBV-related cirrhosis are the major risk factors for HCC development in China (2). Due to the asymptomatic feature of early HCC, many patients are found with advanced stages when diagnosed and lose their opportunities to get radical treatments, including liver transplantation, surgical resection and ablation (3). With the development of diagnostic techniques and extensive surveillance programs for populations with high risk, the proportion of patients diagnosed with early stage and receiving curative therapies are increasing, but the long-term prognosis of these patients are still poor, especially with high rate of recurrence (4). Therefore, it's necessary to look for some novel biomarkers to recognize and stratify patients with high risk of recurrence and allocate individual follow-up algorithms and preventive measures to improve the clinical outcomes.

A specific relationship between blood coagulation system and cancer has been revealed for decades. Tumor cells may possess procoagulant activities and could facilitate local activation of coagulation system, which may promote the tumor progression, angiogenesis and hematogenous metastasis (5-8). Fibrinogen is a kind of plasma glycoprotein synthesized by hepatocytes, which acts as an important coagulation factor and can be converted into fibrin by thrombin (9). And fibrinogen also involves in inflammatory reaction in response to injury, infection or inflammation (10). Previous clinical studies have revealed that the levels of pretreatment plasma fibrinogen are elevated and are associated with tumor progression and poor prognosis in several malignant tumors (11-17). As for HCC, Zhu *et al.* reported that the mRNA expression levels of fibrinogen gamma were up-regulated both in HCC cell lines and tissues, and elevated plasma fibrinogen levels were correlated with the presence of tumor thrombosis (18). Then, Kinoshita *et al.* found that elevated plasma fibrinogen levels were independently associated with poor prognosis in HCC patients (19). And several following studies have focused on the fibrinogen as a prognostic predictor for overall survival (OS) and tumor recurrence in HCC patients (20-22). But the evidences are limited, and the patients enrolled in these studies usually with an early Barcelona Clinic Liver Cancer (BCLC) stage HCC. Besides, our center first investigated the prognostic value of fibrinogen in HCC patients receiving liver transplantation and demonstrated it as a new predictor of HCC recurrence (23). The present study aimed to investigate the association between plasma fibrinogen and clinicopathological characteristics and clarify the prognostic value of plasma fibrinogen in tumor recurrence and OS in HCC patients with a wider range of BCLC stages after surgical resection.

Methods

Patients

A total of 466 patients with primary liver cancer from March 2005 to May 2013 were retrospectively collected. The eligibility criteria are as follows: (I) histologically diagnosed with HCC; (II) no extrahepatic or distant metastases; (III) received liver resection without pre-/intra-operative treatment; (IV) no other concomitant malignances or hematological diseases; (V) age equal to or greater than 18 years old; (VI) complete clinical and laboratory data were available; (VII) followed up adequately. According to the eligibility rules, 302 HCC patients were included and analyzed in our study, while 164 cases were excluded: 57 cases received preoperative treatment, 73 cases undergone ablation treatment due to multiple tumor nodules, 13 cases were histologically proven to be not HCC, 20 cases were found presence with tumor rupture, and one case at seventeen years old was also excluded. Before surgery, all patients were examined by an enhanced computed tomography (CT) or magnetic resonance imaging (MRI) and clinically diagnosed with HCC. When these images were reviewed, tumor-related parameters, such as tumor diameter, the number of tumor lesions and vascular invasion, were evaluated and collected respectively.

Preoperative plasma fibrinogen and other variables

Blood samples were obtained within 7 days before surgery and tested in the clinical laboratory of our hospital. Plasma fibrinogen levels were measured by the Clauss method with fibrinogen reagent kit (Diagnostica Stago, Asnières sur Seine, France) and the normal range was defined at the levels between 2.0 and 4.0 g/L. Besides, other indexes including neutrophil, lymphocyte and platelet (PLT) count, alanine aminotransferase (ALT), albumin (ALB), glutamyl transpeptidase (GGT), α-fetoprotein (AFP) and HBV-DNA were also collected. Neutrophil-lymphocyte ratio (NLR)

was calculated by neutrophil and lymphocyte count. The Model for End-stage Liver Disease (MELD) score was calculated and rounded to an integer value by the equation with 3.8 × ln[bilirubin (mg/dL)] + 11.2 × ln(INR) + 9.6 × ln[creatinine (mg/dL)] + 6.4. The presence of liver cirrhosis was identified histologically with specimen obtained in operation. The Child-Pugh grade and BCLC stage were also evaluated respectively.

Follow-up

All patients were followed up regularly at the outpatient office after liver resection. The serum AFP levels were measured every month in the first year and then every three months for the next two years. Abdominal ultrasound and dynamic enhanced CT or MRI were performed every three months in the first two years and followed by once every six months in the third year. The follow-up programme began at the date of operation and ended with death or time of the last follow-up encompassed by this study (December 2016). At the end of follow-up period, patients didn't occur tumor recurrence or HCC-related death were considered as failure events for DFS and OS respectively, while patients lost to follow up or died due to other non-HCC related deaths during follow-up period were considered as censored events.

Statistical analysis

Continuous variables were presented as median and ranges. Categorical variables were presented as numbers and percentages. The cut-off values of NLR and MELD score were determined by the receiver operating characteristic (ROC) curve with Youden's index, while the cut-off value of fibrinogen was determined by the upper reference limit which recommended by the fibrinogen reagent kit, which was mainly in consideration of clinical easy application (14). The Pearson chi-square (χ^2) test or Fisher's exact test was used to analyze the association between plasma fibrinogen levels and other clinicopathological factors.

The disease-free survival (DFS) and OS rates were calculated by Kaplan-Meier method and compared by the log-rank test. Univariate Cox analysis was performed to identify potential related factors for HCC recurrence or OS. In consideration of other confounding factors and the impact of a suppressor effect, variables with a significant value of P<0.1 were subjected to a multivariate Cox proportional hazard analysis and further screened by forward selection method to evaluate their independent effect. All the variables selected for multivariate COX analyses were satisfied with the proportional hazard assumptions (data not shown). Hazard ratio (HR) and 95% confidence interval (CI) were also calculated for each other. Besides, nomograms based on the results of multivariate analysis were constructed to estimate the 3-, 5-year DFS and OS of HCC patients. The prediction performance was evaluated by a concordance index (C-index) and showed with a calibration plot.

All P values resulted from two-sided statistical testing. And a P value <0.05 was considered statistically significant. All data analyses were performed using the SPSS software package version 19.0 (SPSS, Chicago, IL, USA) and R version 3.2.3 with the package of "rms".

Results

Patient characteristics

The baseline characteristics of enrolled 302 HCC patients are listed in *Table 1*. In this study, 266 (88.1%) patients were males and 36 (11.9%) were females. The median age of these patients was 51 years (range, 42.0–59.0 years). All patients were found with positive hepatitis B surface antigen, and six (2.0%) patients were present with antibodies to hepatitis C virus concurrently, while only one (0.3%) patient was diagnosed with coinfection of HIV. Two hundred and four (66.7%) patients were confirmed with liver cirrhosis by pathology. Besides, 58 (19.2%) and 96 (31.8%) patients had the history of alcohol consumption and smoking, respectively. All the patients, with well-preserved liver function at Child-Pugh grade A (92.7%) and B (7.3%), received surgical resection as their first treatment. And the median MELD score for overall HCC patients was 5 (range, 3.0–7.0). According to the Barcelona Clinic Liver Cancer (BCLC) system, 154 (51%) patients were classified with very early (BCLC 0) and early-stage (BCLC A) HCC, while 122 (40.4%) and 26 (8.6%) patients belonged to intermediate (BCLC B) and advanced-stage (BCLC C) HCC respectively. During the follow-up period (median =24.2 months), 193 (63.9%) patients developed tumor recurrence and 51 (16.9%) patients died.

Comparison of preoperative plasma fibrinogen levels and clinicopathological characteristics

The median value of preoperative plasma fibrinogen

Table 1 The baseline characteristics of 302 hepatocellular carcinoma patients

Variables	n=302
Gender (male/female)	266 (88.1%)/36 (11.9%)
Age (years)	51 (42.0–59.0)
Smoking (no/yes)	206 (68.2%)/96 (31.8%)
Drinking (no/yes)	244 (80.8%)/58 (19.2%)
Diabetes (no/yes)	274 (90.7%)/28 (9.3%)
Cirrhosis (no/yes)	98 (32.5%)/204 (67.5%)
Tumor diameter (cm)	4.3 (3.0–7.5)
Tumor number (solitary/multiple)	213 (70.5%)/89 (29.5%)
Vascular invasion (no/yes)	187 (61.9%)/115 (38.1%)
HBV-DNA (≤1,000/>1,000, copies/mL)	152 (50.3%)/150 (49.7%)
Fibrinogen (g/L)	2.95 (2.45–3.62)
PLT (10^9/L)	168 (118.0–219.0)
AFP (ng/mL)	73.71 (7.70–932.75)
NLR	1.92 (1.46–2.72)
ALT (U/L)	37 (26.0–51.0)
ALB (U/L)	39.9 (37.4–42.5)
GGT (U/L)	60.5 (34.8–104.5)
Child-Pugh grade (A/B)	280 (92.7%)/22 (7.3%)
MELD score	5 (3.0–7.0)
BCLC stage (0/A/B/C)	24 (8.0%)/130 (43.0%)/122 (40.4%)/26 (8.6%)
Pathological differentiation (well/poor-moderate)	55 (18.2%)/247 (81.8%)
Blood transfusion (no/yes)	170 (56.3%)/132 (43.7%)

HBV, hepatitis B virus; PLT, platelet; AFP, α-fetoprotein; NLR, neutrophil-lymphocyte ratio; ALT, alanine aminotransferase; ALB, albumin; GGT, glutamyl transpeptidase; MELD, Model for End-stage Liver Disease; BCLC, Barcelona Clinic Liver Cancer.

level was 2.95 g/L. Patients were divided into two groups (≤4 g/L, n=258; >4 g/L, n=44) based on the upper limit of the normal range of plasma fibrinogen level. The associations of preoperative plasma fibrinogen levels with clinicopathological characteristics are presented in *Table 2*. The findings showed that the elevated plasma fibrinogen levels were associated with larger tumor size (P<0.001), higher NLR level (P=0.005), lower MELD score (P=0.001), the presence of vascular invasion (P<0.001), advanced BCLC stage (P<0.001) and poor-moderate pathological differentiation (P=0.020). No other clinicopathological parameters were found associated with plasma fibrinogen levels, such as gender, age, smoking, drinking, diabetes, cirrhosis, tumor number, HBV-DNA, AFP or Child-Pugh grade (all P>0.05).

Prognostic factors of DFS in HCC patients

The 1-, 3-, 5-year DFS and OS rates were 56.6%, 32.0%, 25.9%. To identify the prognostic factors of DFS, both univariate and multivariate analyses were used to evaluate the effect of preoperative plasma fibrinogen and other clinicopathological variables. The results suggested that preoperative plasma fibrinogen (P<0.001), tumor diameter (P<0.001), tumor number (P<0.001), vascular invasion (P<0.001), AFP (P=0.027), NLR (P=0.008), GGT (P=0.001), smoking (P=0.037), BCLC stage (P<0.001), tumor differentiation (P=0.001) and blood transfusion (P=0.001)

Table 2 Comparison of preoperative plasma fibrinogen with clinicopathological characteristics in 302 hepatocellular carcinoma patients

Variables	Cases	Fibrinogen, n (%)		χ^2	P
		≤4 g/L	>4 g/L		
Gender				0.000	1.000
Male	266	227 (85.3)	39 (14.7)		
Female	36	31 (86.1)	5 (13.9)		
Age (years)				0.665	0.415
≤50	151	126 (83.4)	25 (16.6)		
>50	151	132 (87.4)	19 (12.6)		
Smoking				3.729	0.053
No	206	182 (88.3)	24 (11.7)		
Yes	96	76 (79.2)	20 (20.8)		
Drinking				0.412	0.521
No	244	210 (86.1)	34 (13.9)		
Yes	58	48 (82.8)	10 (17.2)		
Diabetes				0.638	0.424
No	274	236 (86.1)	38 (13.9)		
Yes	28	22 (78.6)	6 (21.4)		
Cirrhosis				0.006	0.938
No	98	83 (84.7)	15 (15.3)		
Yes	204	175 (85.8)	29 (14.2)		
Tumor diameter (cm)				32.776	<0.001*
≤5	177	169 (95.5)	8 (4.5)		
>5	125	89 (71.2)	36 (28.8)		
Tumor number				2.630	0.105
Single	213	187 (87.8)	26 (12.2)		
Multiple	89	71 (79.8)	18 (20.2)		
Vascular invasion				27.970	<0.001*
No	187	176 (94.1)	11 (5.9)		
Yes	115	82 (71.3)	33 (28.7)		
HBV-DNA (copies/mL)				1.414	0.234
≤1,000	152	134 (88.2)	18 (11.8)		
>1,000	150	124 (82.7)	26 (17.3)		
AFP (ng/mL)				0.281	0.596
≤400	206	178 (86.4)	28 (13.6)		
>400	96	80 (83.3)	16 (16.7)		

Table 2 (*continued*)

Table 2 (*continued*)

Variables	Cases	Fibrinogen, n (%)		χ^2	P
		≤4 g/L	>4 g/L		
NLR				7.930	0.005*
≤1.86	145	133 (91.7)	12 (8.3)		
>1.86	157	125 (79.6)	32 (20.4)		
PLT (10^9/L)				7.376	0.004*
<100	49	48 (98.0)	1 (2.0)		
≥100	253	210 (83.0)	43 (17.0)		
ALT (U/L)				0.426	0.514
≤45	205	177 (86.3)	28 (13.7)		
>45	97	81 (83.5)	16 (16.5)		
ALB (g/L)				0.460	0.498
≤35	29	26 (89.7)	3 (10.3)		
>35	273	232 (85.0)	41 (15.0)		
GGT (U/L)				12.876	<0.001*
≤60	151	140 (92.7)	11 (7.3)		
>60	151	118 (78.1)	33 (21.9)		
HBV-DNA (copies/mL)				1.829	0.176
≤1,000	152	134 (88.2)	18 (11.8)		
>1,000	150	124 (82.7)	26 (17.3)		
Child-Pugh grade				1.145	0.285
A	280	237 (84.6)	43 (15.4)		
B	22	21 (95.5)	1 (4.5)		
MELD score				10.641	0.001*
≤5	151	119 (78.8)	32 (21.2)		
>5	151	139 (92.1)	12 (7.9)		
BCLC stage				25.935	<0.001*
0	24	23 (95.8)	1 (4.2)		
A	130	122 (93.8)	8 (6.2)		
B	122	98 (80.3)	24 (19.7)		
C	26	15 (57.7)	11 (42.3)		
Pathological differentiation				5.429	0.020*
Well	55	53 (96.4)	2 (3.6)		
Poor-moderate	247	205 (83.0)	42 (17.0)		
Blood transfusion				0.556	0.456
No	170	148 (87.1)	22 (12.9)		
Yes	132	110 (83.3)	22 (16.7)		

*, P<0.05. HBV, hepatitis B virus; PLT, platelet; AFP, α-fetoprotein; NLR, neutrophil-lymphocyte ratio; ALT, alanine aminotransferase; ALB, albumin; GGT, glutamyl transpeptidase; MELD, Model for End-stage Liver Disease; BCLC, Barcelona Clinic Liver Cancer.

Table 3 Univariate and multivariate analyses of prognostic factors for disease-free survival in 302 hepatocellular carcinoma patients

Variables	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Gender (male/female)	0.780	0.485–1.253	0.304			
Age, years (≤50/>50)	0.851	0.641–1.129	0.264			
Smoking (no/yes)	1.371	1.108–1.846	0.037*	–	–	–
Drinking (no/yes)	1.148	0.805–1.638	0.446			
Diabetes (no/yes)	1.416	0.898–2.230	0.134			
Cirrhosis (no/yes)	1.125	0.826–1.533	0.455			
Tumor diameter, cm (≤5/>5)	2.092	1.572–2.782	<0.001*	1.658	1.222–2.249	0.001*
Tumor number (single/multiple)	2.184	1.629–2.928	<0.001*	1.936	1.438–2.605	<0.001*
Vascular invasion (no/yes)	1.701	1.278–2.264	<0.001*	–	–	–
HBV-DNA, copies/mL (≤1,000/>1,000)	1.313	0.989–1.743	0.059	–	–	–
AFP, ng/mL (≤400/>400)	1.399	1.039–1.884	0.027*	–	–	–
Fibrinogen, g/L (≤4/>4)	2.202	1.523–3.184	<0.001*	1.660	1.124–2.451	0.011*
NLR (≤1.86/>1.86)	1.476	1.108–1.964	0.008*	–	–	–
PLT, 10^9/L (<100/≥100)	0.940	0.646–1.367	0.745			
ALB, g/L (<35/≥35)	0.984	0.612–1.582	0.948			
ALT, U/L (≤45/>45)	1.237	0.921–1.661	0.157			
GGT, U/L (≤60/>60)	1.615	1.215–2.146	0.001*	–	–	–
MELD score (≤5/>5)	1.047	0.789–1.389	0.752			
Child-Pugh grade (A/B)	1.138	0.700–1.851	0.603			
BCLC stage (0-A/B-C)	1.983	1.488–2.643	<0.001*	–	–	–
Pathological differentiation (well/poor-moderate)	1.992	1.317–3.014	0.001*	–	–	–
Blood transfusion (no/yes)	1.656	1.243–2.207	0.001*	1.544	1.156–2.061	0.003*

*, P<0.05. HBV, hepatitis B virus; PLT, platelet; AFP, α-fetoprotein; NLR, neutrophil-lymphocyte ratio; ALT, alanine aminotransferase; ALB, albumin; GGT, glutamyl transpeptidase; MELD, Model for End-stage Liver Disease; BCLC, Barcelona Clinic Liver Cancer.

were related to DFS. The multivariate analysis showed that preoperative plasma fibrinogen (P=0.011), tumor diameter (P=0.001), tumor number (P<0.001), and blood transfusion (P=0.003) were independent factors to DFS (shown in *Table 3*).

Furthermore, we constructed a nomogram for DFS prediction with four variables significant in multivariate analysis (*Figure 1*). The model displays a C-index of 0.656 (95% CI: 0.588–0.724).

Prognostic factors of OS in HCC patients

The 1-, 3-, 5-year OS rates were 93.8%, 81.5%, 71.8%. The results of univariate analysis showed that the preoperative plasma fibrinogen (P<0.001), tumor diameter (P<0.001), vascular invasion (P=0.001), AFP (P=0.002), GGT (P=0.009), HBV-DNA (P=0.002), age (P=0.022) and BCLC stage (P=0.002) were associated with OS. Furthermore, in multivariate analysis, preoperative plasma fibrinogen (P=0.025), tumor diameter (P=0.017), AFP (P=0.006) and HBV-DNA (P=0.005) were found to be independent prognostic factors of OS (shown in *Table 4*).

The nomogram was developed to predict the OS with above four variables significant in multivariate analysis (*Figure 2*). And the C-index of this model is 0.720 (95% CI: 0.637–0.803).

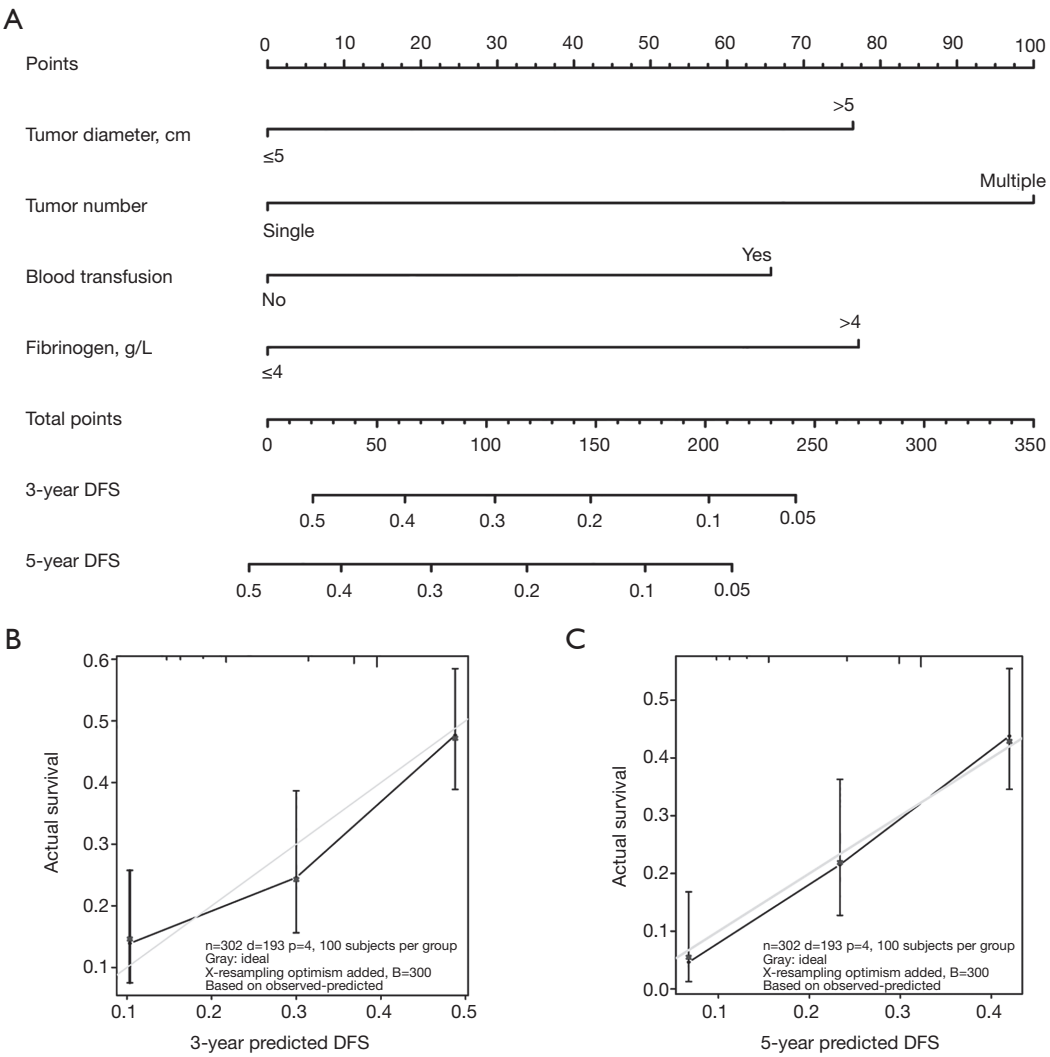

Figure 1 Nomography to predict DFS of HCC patients received liver resection. (A) Prognostic nomogram for DFS; (B,C) the calibration curves for predicting 3-, 5-year DFS. DFS, disease-free survival; HCC, hepatocellular carcinoma.

Prognostic values of preoperative plasma fibrinogen in HCC patients

The 1-, 3-, 5-year DFS rates in patients with elevated plasma fibrinogen levels >4 g/L were 34.2%, 19.5% and 0.0%, respectively. These survival rates were significantly lower than the rates in patients with normal plasma fibrinogen level ≤4 g/L, which were 60.4%, 34.2% and 30.2%, respectively (P<0.001; *Figure 3A*). Similarly, the 1-, 3-, 5-year OS rates were also significantly lower in patients with elevated plasma fibrinogen levels >4 g/L than in patients with plasma fibrinogen levels ≤4 g/L (83.4%, 62.7% and 48.8% *vs.* 95.4%, 84.3% and 75.8%, respectively, P<0.001; *Figure 3B*).

Furthermore, relevant subgroup analyses were also performed to analyze the prognostic values of preoperative plasma fibrinogen levels in specific HCC patients. We found that preoperative plasma fibrinogen was a prognostic factor of DFS (P=0.049, *Figure 4A*) in patients without cirrhosis. And as expected, similar results were also found in patients with cirrhosis (P<0.001, *Figure 4B*). Besides, in patients with different levels of AFP (≤400, >400 ng/mL), elevated plasma fibrinogen still showed as a prognostic factor with lower DFS rates (P=0.001, and P=0.006; *Figure 4C,D*). Both in patients with single tumor and BCLC 0-A stage, the elevated plasma fibrinogen levels

Table 4 Univariate and multivariate analyses of prognostic factors for overall survival in 302 hepatocellular carcinoma patients

Variables	Univariate			Multivariate		
	HR	95% CI	P value	HR	95% CI	P value
Gender (male/female)	0.760	0.301–1.918	0.562			
Age, years (≤50/>50)	0.507	0.283–0.907	0.022*	–	–	–
Smoking (no/yes)	1.514	0.863–2.657	0.148			
Drinking (no/yes)	0.904	0.439–1.858	0.783			
Diabetes (no/yes)	0.708	0.254–1.972	0.509			
Cirrhosis (no/yes)	0.923	0.516–1.653	0.798			
Tumor diameter, cm (≤5/>5)	3.081	1.743–5.447	<0.001*	2.097	1.143–3.847	0.017*
Tumor number (single/multiple)	1.416	0.789–2.539	0.243			
Vascular invasion (no/yes)	2.545	1.461–4.435	0.001*	–	–	–
HBV-DNA, copies/mL (≤1,000/>1,000)	2.478	1.379–4.453	0.002*	2.341	1.288–4.255	0.005*
AFP, ng/mL (≤400/>400)	2.387	1.374–4.145	0.002*	2.217	1.256–3.915	0.006*
Fibrinogen, g/L (≤4/>4)	2.954	1.612–5.411	<0.001*	2.051	1.093–3.851	0.025*
NLR (≤1.86/>1.86)	1.473	0.843–2.576	0.174			
PLT, 10^9/L (<100/≥100)	0.863	0.405–1.839	0.703			
ALB, g/L (<35/≥35)	0.195	0.027–1.415	0.106			
ALT, U/L (≤45/>45)	1.026	0.572–1.839	0.932			
GGT, U/L (≤60/>60)	2.159	1.215–3.839	0.009*	–	–	–
MELD score (≤5/>5)	0.695	0.398–1.215	0.202			
Child-Pugh grade (A/B)	0.484	0.117–1.991	0.314			
BCLC stage (0-A/B-C)	2.446	1.372–4.361	0.002*	–	–	–
Pathological differentiation (well/poor-moderate)	1.852	0.826–4.149	0.135			
Blood transfusion (no/yes)	1.040	0.588–1.838	0.894			

*, P<0.05. HBV, hepatitis B virus; PLT, platelet; AFP, α-fetoprotein; NLR, neutrophil-lymphocyte ratio; ALT, alanine aminotransferase; ALB, albumin; GGT, glutamyl transpeptidase; MELD, Model for End-stage Liver Disease; BCLC, Barcelona Clinic Liver Cancer; HR, hazard ratio; CI, confidence interval.

also presented significantly prognostic values with lower DFS rates (P=0.004, *Figure 4E*; BCLC 0-A stage: P=0.010, *Figure 4F*).

Discussion

In the present study, we examined the relationships between preoperative plasma fibrinogen and clinicopathological characteristics and its prognostic significance in HCC patients. We found that an elevated plasma fibrinogen level was positively associated with larger tumor size, the presence of vascular invasion, advanced BCLC stage, lower MELD score, poorer pathological differentiation, and higher NLR. Moreover, our results demonstrated that patients with elevated preoperative plasma fibrinogen levels possessed worse OS and DFS. And in subgroup analyses, we found that elevated fibrinogen levels also related to poorer DFS in patients without cirrhosis, single tumor nodule, low AFP level and early stages, respectively. Our findings indicate that preoperative plasma fibrinogen level might reflect the tumor burden and progression, and act as a significantly independent prognostic marker in HCC.

Hyperfibrinogenemia has been recognized in many solid malignancies as a prognostic indicator with tumor

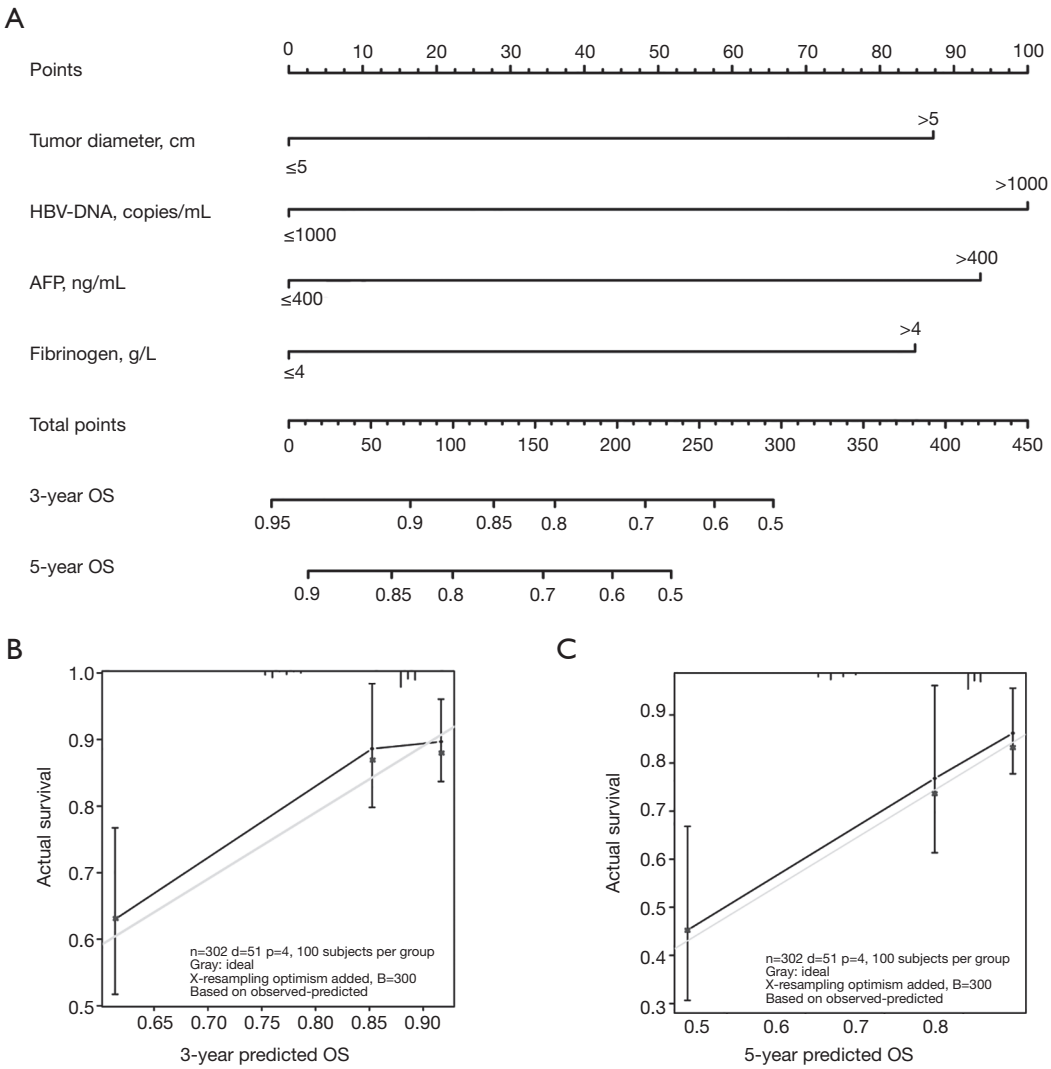

Figure 2 Nomography to predict OS of HCC patients received liver resection. (A) Prognostic nomogram for OS; (B,C) the calibration curves for predicting 3-, 5-year OS. OS, overall survival; HCC, hepatocellular carcinoma.

progression and poor survival outcomes (11-17). And results from a meta-analysis also supported that pretreatment plasma fibrinogen represents as a biomarker of worse survival in tumor patients (24). Zhu *et al.* first reported elevated plasma fibrinogen levels correlating with advanced tumor stages and the presence of tumor thrombosis in HCC patients (18), which is consistent with our study. Moreover, recent clinical studies have also investigated the prognostic role of plasma fibrinogen in HCC patients (19-23). And in the current study, we further evaluated the prognostic value of preoperative plasma fibrinogen in several specific subgroups of HCC patients, such as patients without cirrhosis, with low AFP level, single tumor nodule, and early BCLC stages, and the results showed similar significant prognostic value as in the whole cohort, which indicated the universally applicable prognostic feature of fibrinogen.

Fibrinogen is a soluble 340-kDa glycoprotein synthesized by hepatocytes. A growing body of evidence has shown that fibrinogen participates in tumor development at different stages (25). Fibrinogen could bind directly to some growth factors, such as transforming growth factor-B (TGF-B), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth

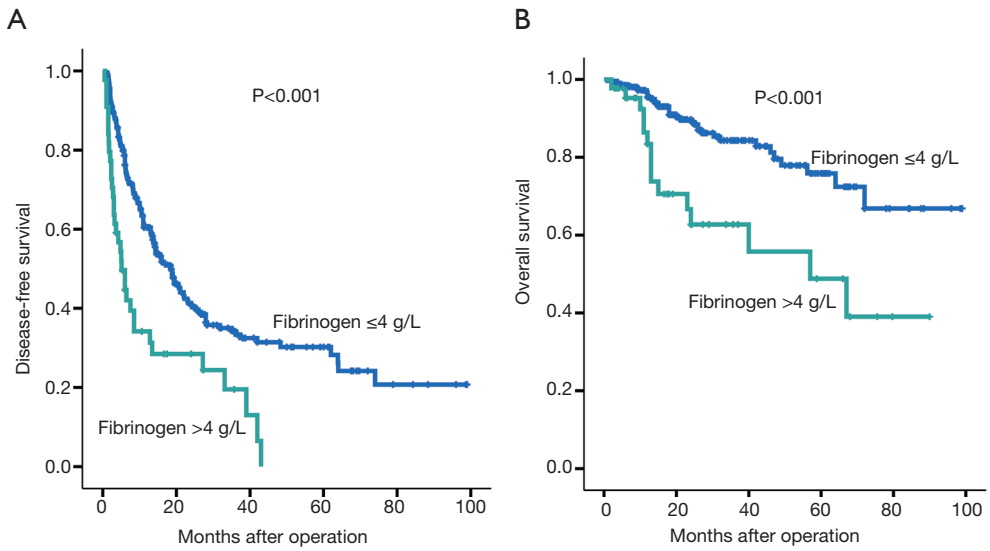

Figure 3 Kaplan-Meier curves of DFS and OS in HCC patients. (A) DFS of HCC patients with plasma fibrinogen >4 g/L was significantly lower than those with fibrinogen ≤4 g/L (P<0.001); (B) OS of HCC patients with plasma fibrinogen >4 g/L was significantly lower than those with fibrinogen ≤4 g/L (P<0.001). DFS, disease-free survival; OS, overall survival; HCC, hepatocellular carcinoma.

factor (PDGF) families, and contact with tumor cells to regulate tumor cell proliferation, inhibition of apoptosis, angiogenesis, and metastasis (26,27). Besides, as a prominent component in the coagulation pathway, fibrinogen could be converted to fibrin by the activated thrombin and facilitate platelet aggression by conjunction with platelets. It has been recognized that high levels of fibrinogen receptors expressed in some tumor cells, including intercellular adhesion molecule-1 (ICAM-1) and the $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins (28,29). Thereby fibrinogen promotes the contact between platelets and circulating tumor cells (CTCs) as a bridge, helping platelets adhesion to tumor cells (30). With the formation of such matrix barrier, the CTCs escape from the natural killer-cell induced elimination (31). Fibrinogen could also mediate the endothelial adhesion of CTCs by ICAM-1 on endothelial cells (32). Thus, fibrinogen may take an important part in the adhesive interaction among tumor cells, platelets, or endothelial cells, leading to hematogenous metastasis (33,34). On the other hand, Palumbo *et al.* demonstrated that spontaneous hematogenous and lymphatic metastasis was diminished in fibrinogen-deficient mice and indicated a therapeutic strategy focusing on hemostatic factors in controlling solid tumor metastasis (33). A recent study has shown that some anticoagulants possess the antitumor and antimetastatic properties both *in vivo* and *in vitro* (35). Furthermore, several studies have found that fibrinogen could be synthesized by tumor cells endogenously and induce the epithelial-to-mesenchymal transition (EMT), which involves in tumor cell migration, invasion, and metastasis (11,36).

In addition, fibrinogen also acts as an acute phase protein in response to infection or systemic inflammation (10). Previous studies have demonstrated that fibrinogen could regulate the inflammatory response by producing several pro-inflammatory cytokines (IL-1b, IL-6, and TNF-a) or inducing the interaction between leukocyte and endothelial cells (34,37). And the fibrinogen-modulated inflammatory response has been identified with cancer progression in tumor microenvironment (38). In our study, we also found that elevated plasma fibrinogen level is correlated with higher NLR, a noteworthy marker of inflammatory response, which is in accordance with previous studies (22,39). And Fu *et al.* have shown an enhanced prognostic value by combining preoperative fibrinogen and NLR in patients with HCC after liver transportation (22).

With various evidence from experimental and clinical studies, fibrinogen shows the potential to become a promising predictor for long-term prognosis of patients with malignant tumors. And some researchers have indicated the possibility of fibrinogen to be applied in cancer staging and patient stratification, aiming to improve

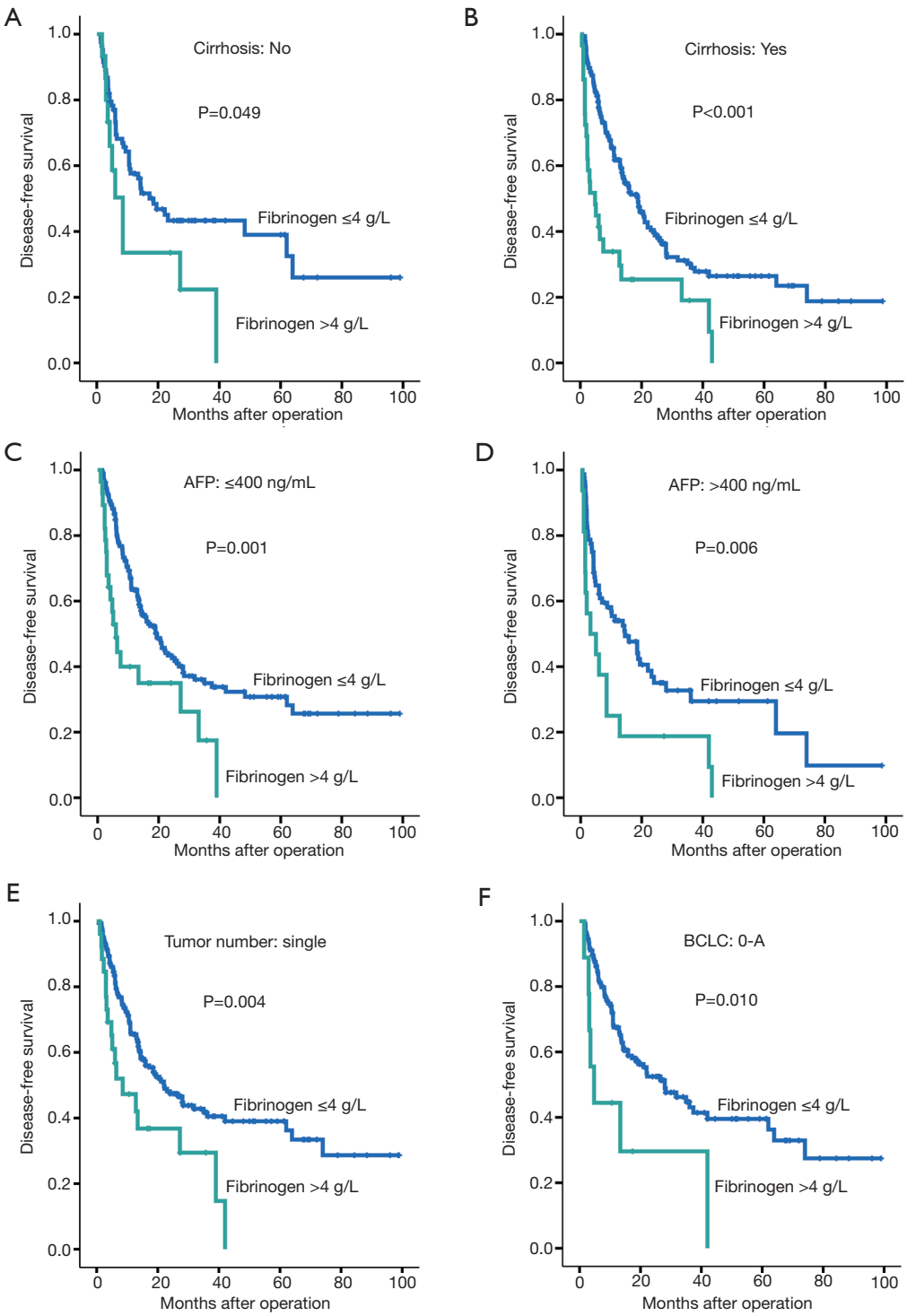

Figure 4 Kaplan-Meier curves of DFS in specific subgroups of HCC patients. DFS of patients with plasma fibrinogen >4 g/L was significantly lower than those with fibrinogen ≤4 g/L both in HCC patients without cirrhosis (A, P=0.049) and with cirrhosis (B, P<0.001); DFS of patients with fibrinogen >4 g/L was significantly lower than those with fibrinogen ≤4 g/L both in AFP ≤400 ng/mL (C, P=0.001) and >400 ng/mL (D, P=0.006); DFS of patients with plasma fibrinogen >4 g/L was significantly lower than those with fibrinogen ≤4 g/L in HCC patients with single tumor (E, P=0.004) and BCLC 0-A stage (F, P=0.010). DFS, disease-free survival; HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer.

personalized treatment selection, recurrence prevention and survival extension. Besides, plasma fibrinogen level is routinely tested before operation in clinical practice, which makes it available to be widely used.

There are several potential limitations in our study. First, as a retrospective study, all patients are enrolled from our single center and data missing is inevitable, and a limited sample size may cause an inappropriate conclusion. Second, except for fibrinogen, other inflammatory indicators such as C-reactive protein, were not included and analyzed, which may result in some statistical biases. Third, the plasma fibrinogen level after surgery and the expression level in tumor weren't tested or collected. However, such work may be our following plan, and large-scale multi-center prospective validation study is further required.

Conclusions

In conclusion, our study has demonstrated that preoperative elevated plasma fibrinogen levels were associated with larger tumor size, the presence of vascular invasion, and advanced BCLC stages in patients with HCC. And it could serve as a significant useful prognostic predictor in HCC patients with poor long-term OS and DFS.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University and written informed consent was obtained from all patients.

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