

## Research Article

# The Prognostic Value of Preoperative Fibrinogen in Patients with Radical Cholecystectomy

Yuchen Wu <sup>1</sup>, Shengjie Zhang <sup>1</sup>, Xu Qian <sup>1</sup>, Jing Zhu <sup>1</sup>, Shuang Zheng <sup>2</sup>,  
Jian Wu <sup>3</sup> and Xiaodan Huang <sup>4</sup>

<sup>1</sup>Department of Laboratory Medicine, The Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital), Institute of Basic Medicine and Cancer (IBMC), Chinese Academy of Sciences, Hangzhou, Zhejiang 310022, China

<sup>2</sup>Department of Laboratory Medicine, The First Affiliated Hospital, Zhejiang University School of Medicine, 79 Qingchun Rd., Hangzhou City 310003, China

<sup>3</sup>Department of Clinical Laboratory, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, 242 Guangji Road, Suzhou 215008, Jiangsu, China

<sup>4</sup>Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

Correspondence should be addressed to Jian Wu; wujianglinxing@163.com and Xiaodan Huang; xdhuang@uchicago.edu

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**Background and Aims.** There is currently a lack of suitable hematological markers as a complement to pathological factors in predicting the prognosis of patients with gallbladder cancer. The study aimed to investigate the clinical value of preoperative fibrinogen levels in assessing the prognosis of patients with gallbladder cancer after radical surgery. **Methods.** The study retrospectively analyzed 260 gallbladder cancer patients who underwent radical resection. Time-dependent receiver operating characteristic (ROC) curves were used to calculate the optimal cut-off values of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and fibrinogen. Besides, univariate and multivariate analyses of disease-free survival (DFS) and overall survival (OS) were performed to determine independent markers in peripheral blood. Then, subgroup analyses of fibrinogen in different degrees of differentiation, age, gender, and BMI were performed by log-rank test. **Result.** The cut-off values of fibrinogen, CEA, and CA19-9 were set at 2.97 g/L, 2.17 ng/mL, and 41.1 U/mL, respectively. The results showed that the preoperative fibrinogen level was associated with tumor size, degree of differentiation, TNM stage, and CA19-9 levels. Multivariate analyses indicated that advanced TNM stage, excessive fibrinogen, CEA, and CA19-9 levels were independent risk factors for postoperative DFS. And gallbladder neck tumors, poor differentiation, cancer nodules, advanced TNM stage, excessive fibrinogen, and CEA levels were independent adverse factors for postoperative OS. Notably, the preoperative excessive fibrinogen was an independent adverse factor for both DFS ( $p = 0.044$ , HR = 1.629, 95% CI = 1.014 – 2.618) and OS ( $p = 0.006$ , HR = 2.328, 95% CI = 1.272 – 4.261) in patients with gallbladder cancer. The subgroup analyses further indicated that patients with high-level of fibrinogen had both poorer DFS ( $p = 0.002$ ) and OS ( $p = 0.005$ ) than patients with poorly-differentiated gallbladder cancer. And for well-differentiated gallbladder cancer, patients with high fibrinogen levels had poorer OS ( $p = 0.004$ ), but no significant difference in DFS ( $p = 0.062$ ). Besides, fibrinogen was more significant in GBC patients with higher BMI (>22.62) or older age (>60 yrs) and was not affected by gender. **Conclusion.** Elevated preoperative fibrinogen level was independently associated with poor postoperative DFS and OS in patients with gallbladder cancer, especially for poor differentiation.

## 1. Introduction

Gallbladder cancer (GBC) is the most common malignant tumor (about 80% to 95%) in the biliary tract and causes approximately 40,700 deaths worldwide annually in China [1, 2]. The prognosis of GBC is extremely poor although it is a rare incident (about 1.2% of all cancers), and the median survival time of all patients is only around 6 months [3–5]. Surgery is the main stream intervention for GBC, especially for the radical cholecystectomy with R0 resection has displayed certain benefits in prognosis [6, 7]. In principle, simple cholecystectomy could effect a radical cure in patients at T1a assessed preoperatively. For patients at T1b and above, radical cholecystectomy should be performed. Radical cholecystectomy requires additional partial liver resection and regional lymph node dissection, and if necessary, widening the resection to reach R0 margins [8]. However, even in patients with GBC undergoing radical surgery, there is still a high possibility of recurrence (about 25%–65%) after surgery [9, 10]. Currently, the absolute survival benefits after preoperative neoadjuvant chemotherapy and postoperative adjuvant chemotherapy with operable GBC are not clear [11, 12]. Meanwhile, insufficient evidence has supported the benefit of chemotherapy or radiotherapy in unresectable advanced GBC [13–15]. It is difficult to improve the prognosis even with multidisciplinary treatments including reoperation or chemoradiotherapy once GBC recurs. Therefore, a reasonable and effective assessment of recurrence and survival is crucial for postoperative management.

The postoperative survival of patients with GBC is mainly evaluated based on postoperative pathological factors. Studies have shown that postoperative pathological factors such as margin status, TNM stage, histological type, and degree of differentiation of gallbladder cancer were associated with the survival of patients [16]. However, the definitions of TNM stages of GBC varied in four editions of the American Joint Committee on Cancer (AJCC) Staging Manual published from 1997 to 2017, and the definition of regional lymph nodes of GBC in the Japanese TNM staging system was different from AJCC staging system [17, 18]. Therefore, pathological factors could not fully reflect recurrence and mortality in patients undergoing radical cholecystectomy.

Hematological markers can assess early disease burden and optimize disease management conveniently, even in preoperation. Representative tumor markers like carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are commonly used to evaluate the prognosis in malignant tumors [19]. Fibrinogen is a homodimeric glycoprotein composed of 2A $\alpha$ , 2B $\beta$ , and 2 $\gamma$  peptide chains. In healthy individuals, fibrinogen is mainly synthesized by the liver and circulates at concentrations of 2-5 mg/mL. [20] As the most abundant coagulation factor in plasma, fibrinogen plays an important role in the process of coagulation. During thrombosis, fibrinogen is converted into insoluble fibrin catalyzed by coagulation factors (e.g. thrombin), and the latter forms a fibrin meshwork structure to complete hemostasis [21]. In addition to coagulation and hemostasis, fibrinogen is also a response protein in the acute phase,

which is closely related to the progression of inflammation and cancer [22]. It was reported that the elevated fibrinogen before treatment was an adverse prognostic factor in solid tumors, such as breast cancer, lung cancer, and gastrointestinal cancer [23–26]. It is uncertain about the value of preoperative fibrinogen level to postoperative recurrence and prognostic in patients with GBC. Therefore, developing potential indicators (e.g. hematological markers) will be a good supplement to pathological factors in predicting the prognosis of patients with GBC.

This study retrospectively analyzed 260 GBC patients who underwent radical resection and explored the clinical value of preoperative fibrinogen level in assessing postoperative recurrence and mortality.

## 2. Materials and Methods

**2.1. General Information.** The data of 260 patients with GBC were collected with treatment between January 2015 and December 2020. Among them, 205 cases were from The First Affiliated Hospital, Zhejiang University School of Medicine, and 55 cases were from Zhejiang Cancer Hospital. The inclusion criteria were as follows: underwent treatment of radical cholecystectomy, without any anticancer treatment before surgery, had no other malignancies and/or blood disorders, diagnosed as gallbladder cancer by histopathology, postoperative pathology showed R0 resection margin, and complete clinical data. The ethical approvals were obtained by the Ethics Committees of The First Affiliated Hospital, Zhejiang University School of Medicine (IRB-2014-272) and Zhejiang Cancer Hospital (IRB-2021-276).

**2.2. Data Collection.** The metadata including gender, age, height, weight, smoking history, drinking history, and body mass index (BMI) were collected by using the hospitals' information system. Meanwhile, preoperative blood indexes and postoperative pathological information were collected. Follow-up data including specific dates of recurrence and death were collected by the hospital system or by telephone.

Serum samples were used for the detection of CEA and CA19-9, and sodium citrate anticoagulated plasma was used for the detection of fibrinogen. All the blood samples were collected from patients in a fasting state within one week before surgery and tested in strict accordance with the instructions of equipment and reagents. And the preoperative hematological indicators contained CEA, CA19-9, and fibrinogen. CEA and CA19-9 were determined by Abbott ALinity i automatic chemiluminescence immunoassay analyzer (USA) and original supporting reagents, and the detection method was chemiluminescence microparticle immunoassay; and Fibrinogen was determined by Sysmex CS-5100 automatic coagulation analyzer (Japan) and original supporting reagents, and the detection method was coagulation method (Clauss method).

**2.3. Surgical Strategy.** All patients underwent radical surgery for GBC after strict imaging evaluation and the surgery complied with the expert consensus on diagnosis and treatment of gallbladder carcinoma in China [27]. All

TABLE 1: Correlation between fibrinogen level and clinical characteristics.

Clinical characteristics	Number of patients (%)	Preoperative fibrinogen levels (g/L)	<i>p</i> value
Gender			
Male	86 (33.08)	3.46 (2.71,4.47)	0.225
Female	174 (66.92)	3.25 (2.67,3.99)	
Age (yrs)			
≤60	78 (30.00)	3.19 (2.53,3.74)	0.017
>60	182 (70.00)	3.41 (2.72,4.42)	
Smoking history			
Yes	55 (21.15)	3.39 (2.72,4.30)	0.714
No	205 (78.85)	3.32 (2.67,4.20)	
Drinking history			
Yes	47 (18.08)	3.46 (2.84,4.59)	0.115
No	213 (81.92)	3.25 (2.67,4.01)	
BMI (kg/m <sup>2</sup> )			
>22.62	104 (50.98)	3.25 (2.66,4.06)	0.503
≤22.62	100 (49.02)	3.32 (2.72,4.20)	
Tumor location			
Neck	50 (24.75)	3.46 (2.80,4.44)	0.162
Body+fundus	152 (75.25)	3.17 (2.56,4.00)	
Tumor size (cm)			
>3.5	114 (45.60)	3.16 (2.55,3.84)	0.001
≤3.5	136 (54.40)	3.50 (2.97,4.51)	
Differentiation			
Poorly	133 (51.15)	3.46 (1.80,6.50)	0.013
Well	127 (48.85)	3.22 (2.56,3.87)	
Pathological type			
Adenocarcinoma	239 (91.92)	3.28 (2.65,4.16)	0.114
Others	21 (8.08)	3.52 (2.24,12.80)	
Vascular invasion			
Positive	54 (20.77)	3.43 (2.78,4.34)	0.604
Negative	206 (79.23)	3.32 (2.64,4.17)	
Nerve invasion			
Positive	64 (24.62)	3.46 (3.03,4.45)	0.157
Negative	196 (75.38)	3.26 (2.64,4.14)	
Cancerous node			
Positive	13 (5.00)	3.32 (2.87,3.80)	0.888
Negative	247 (95.00)	3.33 (2.65,4.33)	
TNM stage			
I+II	98(37.69)	2.84 (2.40,3.55)	<0.001
III+IV	162 (62.31)	3.51 (3.00,4.48)	
CEA (ng/mL)			
>2.17	164 (63.08)	3.37 (2.69,4.30)	0.4543
≤2.17	96 (36.92)	3.25 (2.58,4.20)	
CA19-9 (U/mL)			
>41.1	114 (43.85)	3.55 (3.07,4.48)	<0.001
≤41.1	146 (56.15)	3.16 (2.51,3.99)	

Note: *p* values were calculated by the Mann–Whitney U method; BMI: body mass index; CEA: carcinoembryonic antigen, CA19-9: carbohydrate antigen 19-9.

patients met the R0 resection criteria confirmed by pathological examination.

**2.4. Histopathological Examination.** After surgical resection, the tissues/parts of the organ were sent for histopathological examination to find out various histopathological conditions including maximum tumor diameter, tumor location, pathological type, tumor differentiation, the extent of primary tumor invasion, and lymph node metastasis. Pathological examinations were performed by professional pathologists. All the cases underwent TNM staging based on pathological data according to the AJCC Cancer Staging Manual 8<sup>th</sup> [28].

**2.5. Follow-Up Data.** Disease-free survival (DFS) was defined as the time between patients undergoing surgery and cancer recurrence. Overall survival (OS) was defined as the time from patients undergoing surgery to death. Follow-up ended on January 22, 2022, the maximum follow-up period was 80 months and the median follow-up period was 27 (14, 43) months.

**2.6. Statistical Analysis.** Normally distributed data was expressed as mean  $\pm$  standard deviation (SD) and non-normally distributed data was expressed as median (25<sup>th</sup> percentile, 75<sup>th</sup> percentile) after being tested by the Shapiro-Wilk method. The “survival ROC” package of R Programming Language 4.1.2 was used to calculate the optimal cut-off value of each hematological index by drawing time-dependent receiver operating characteristic (ROC) curves, then the hematological indexes were divided into high-level and low-level groups. Mann-Whitney *U* test was used to analyze the relationship between postoperative clinical characteristics and fibrinogen. Univariate and multivariate analyses of DFS and OS were performed by using the COX proportional hazards model, which aimed to find independent factors of DFS and OS and calculate their *p* values, hazard ratios (HRs), and 95% confidence intervals (CI) for HR. And subgroup analyses of fibrinogen in different degrees of differentiation were performed by log-rank test. The above statistics were completed by SPSS 25.0. Statistical results with a two-sided *p* < 0.05 were considered statistically significant.

### 3. Results

**3.1. Correlation between Fibrinogen Level and Clinical Characteristics.** A total of 260 patients with GBC were enrolled in the study, including 86/260 (33.07%) males and 174/260 (66.93%) females. The median age was 64.5 years old (range 40-92) and the median BMI was 22.62 (20.82, 24.71). There were 50 (24.75%), 42 (20.79%), and 110 (54.46%) tumors located in the gallbladder neck, gallbladder body, and gallbladder floor, respectively. The median of CEA, CA19-9, and fibrinogen were, respectively, 2.80 (1.80, 5.50) ng/mL, 28.40 (7.60, 191.30) U/mL, and 3.33 (2.67, 4.23) g/L. Postoperative pathology showed that there were 239 (91.92%) cases of adenocarcinoma, 10 (3.85%) cases of squamous cell carcinoma, and 11 (4.23%) cases of neuroendocrine carcinoma among the enrolled patients. For the degrees of differentiation, 133 (51.15%) cases were poorly differentiated, and 127 (48.85%) cases were well differenti-

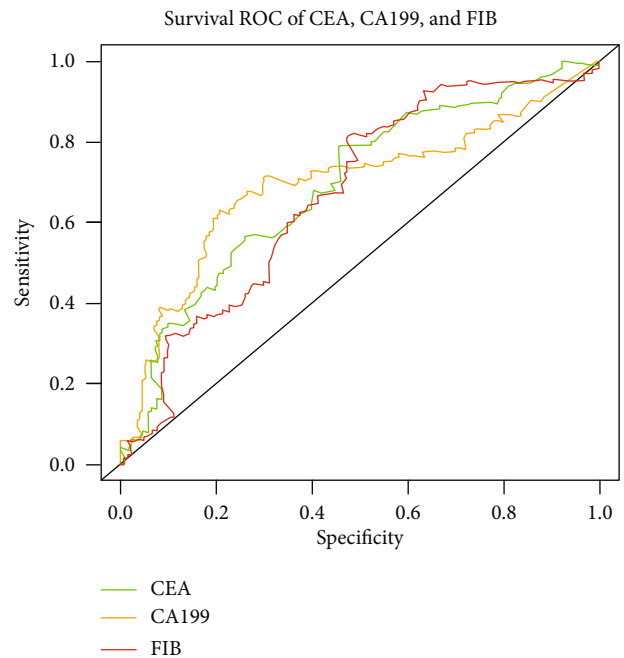


FIGURE 1: Time-dependent ROC curves of CEA, CA19-9, and fibrinogen—with the expected time—were 60 months, and the survival model was Kaplan-Meier.

ated. Tumor size was expressed as the largest tumor diameter, with a median of 3.5 (2.3, 5.0) cm. The positive numbers of vascular invasion, nerve invasion, and cancer nodules were, respectively, 54 (20.77%), 64 (24.62%), and 13 (5.00%). TNM stages were identified by pathological characteristics, and the number of stages I, II, III, and IV was 29 (10.12%), 69 (26.85%), 110 (42.80%), and 52 (20.23%) patients, respectively (Table 1).

Table 1 also showed that preoperative fibrinogen level was associated with age, tumor size, degree of differentiation, TNM stage, and CA19-9 level. Briefly, patients with age > 60 (*p* = 0.017), tumors size > 3.5 cm (*p* = 0.001), poor differentiation (*p* = 0.013), advanced TNM stage (*p* < 0.001), and high concentration of CA19-9 (*p* < 0.001) were higher in fibrinogen levels. The results suggested that a high level of FIB was related to the degree of malignancy and progression of GBC cancer.

**3.2. Optimal Cut-Off Values of CEA, CA19-9, and Fibrinogen.** As shown in Figure 1, according to the principle of the largest Youden index, the best cut-off values of CEA, CA19-9, and fibrinogen were 2.17 ng/mL, AUC = 0.692; 41.1 U/mL, AUC = 0.694; and 2.97 g/L, AUC = 0.671, respectively. The time-dependent ROC curve, whose expected time was 60 months, decreased the bias caused by different follow-up times. Then CEA, CA19-9, and fibrinogen were grouped based on the cut-off values listed above (Table 1).

**3.3. Univariate and Multivariate Analyses of DFS and OS in Patients with Gallbladder Cancer.** The 1-year, 3-year, and 5-year recurrence rates of 260 patients with GBC were 36.6%, 53.9%, and 59.3%, having survival rates of 80.8%,

TABLE 2: Univariate and multivariate analyses of DFS in patients with GBC.

Factors	Univariate analysis			Multivariate analysis		
	<i>p</i> value	HR	95% CI	<i>p</i> value	HR	95% CI
Gender (male vs. female)	0.873	1.030	0.716-1.481			
Age ( $\leq 60$ vs. $>60$ )	0.172	0.7780	0.543-1.115			
BMI ( $\leq 22.62$ vs. $>22.62$ )	0.550	1.018	0.960-1.079			
Smoking history (no vs. yes)	0.823	1.049	0.688-1.602			
Drinking history (no vs. yes)	0.599	1.128	0.720-1.770			
Tumor location (body + fundus vs. neck)	0.023	1.619	1.067-2.456	0.353	1.241	0.787-1.957
Tumor size ( $\leq 3.5$ cm vs. $>3.5$ cm)	0.127	1.068	0.918-1.163			
Pathological type (adenocarcinoma vs. others)	0.007	0.489	0.289-0.826	0.402	0.774	0.425-1.410
Differentiation (poor vs. well)	$<0.001$	0.435	0.305-0.620	0.078	0.690	0.457-1.043
Vascular invasion (negative vs. positive)	$<0.001$	2.219	1.528-3.225	0.227	1.329	0.837-2.110
Nerve invasion (negative vs. positive)	$<0.001$	2.538	1.753-3.676	0.290	1.278	0.812-2.010
Cancerous node (negative vs. positive)	$<0.001$	3.459	1.804-6.630	0.071	2.115	0.938-4.769
TNM stage (I+II vs. III+IV)	$<0.001$	5.517	3.292-8.077	$<0.001$	3.508	2.007-6.131
CEA ( $\leq 2.17$ ng/mL vs. $>2.17$ ng/mL)	$<0.001$	2.050	1.400-3.003	0.009	1.856	1.167-2.951
CA19-9 ( $\leq 41.10$ U/mL vs. $>41.10$ U/mL)	$<0.001$	2.686	1.892-3.805	0.031	1.639	1.046-2.567
Fibrinogen ( $\leq 2.97$ g/L vs. $>2.97$ g/L)	$<0.001$	2.160	1.461-3.194	0.044	1.629	1.014-2.618

Note: HR: hazard ratio; CI: confidence interval; BMI: body mass index; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.

59.8%, and 48.8%, respectively. No significant difference in postoperative DFS ( $p = 0.279$ , HR = 0.769, 95% CI = 0.478 – 1.237) and OS ( $p = 0.635$ , HR = 1.120, 95% CI = 0.702 – 1.785) were found in the two medical centers. Univariate analysis showed that tumor location, pathological type, tumor differentiation, nerve invasion, vascular invasion, cancer nodules, TNM stage, and the levels of CEA, CA19-9, and fibrinogen were associated with shorter postoperative DFS and OS in patients with GBC (Tables 2 and 3). Notably, multivariate analysis indicated that high-level preoperative fibrinogen was an independent adverse factor for DFS ( $p = 0.044$ , HR = 1.629, 95% CI = 1.014 – 2.618) and OS ( $p = 0.006$ , HR = 2.328, 95% CI = 1.272 – 4.261) in patients with GBC. In addition to fibrinogen, advanced TNM stage, CEA  $>2.17$  U/mL, and CA19-9  $>41.10$  U/mL were independent risk factors for postoperative DFS in patients with GBC (Table 2). While gallbladder neck tumors, poor differentiation, cancer nodules, advanced TNM stage, and CEA  $>2.17$  U/mL were independent adverse factors for postoperative OS in patients with GBC (Table 3).

**3.4. Subgroup Analyses of Fibrinogen by Different Degrees of Differentiation.** For patients with low fibrinogen levels ( $\leq 2.97$  g/L), the 1-year, 3-year, and 5-year recurrence rates were 23.2%, 39.9%, and 39.9% and having survival rates of 92.6%, 77.6%, and 75.6%. And for patients with high fibrinogen level ( $>2.97$  g/L), the 1-year, 3-year, and 5-year recurrence rates were 44.6%, 56.4%, and 70.2% and having survival rates of 74.4%, 51.2%, and 35.8%. The DFS and OS of the high-level fibrinogen group were significantly lower than those of the low-level group (Figure 2).

All patients were divided into two groups (poor differentiation and well differentiation) and the Kaplan-Meier (log-rank test) was used for subgroup analyses. The results

showed that patients with high-level fibrinogen were both poorer in DFS ( $p = 0.002$ , Figure 3(a)) and OS ( $p = 0.005$ , Figure 3(b)) for poorly-differentiated gallbladder cancer. For well-differentiated gallbladder cancer, patients with high fibrinogen levels had poorer OS ( $p = 0.004$ , Figure 3(d)) but no significant difference in DFS ( $p = 0.062$ , Figure 3(c)). The results suggested that it is best to execute personalized evaluation by combining preoperative fibrinogen with differentiation when assessing DFS in patients with GBC, while OS is not affected by the degree of differentiation.

**3.5. Subgroup Analyses of Fibrinogen by Different Age, Gender, and BMI.** Among GBC patients aged  $>60$  yrs, those with higher fibrinogen levels had worse DFS ( $p < 0.001$ , Figure S1C) and OS ( $p < 0.001$ , Figure S1D). For GBC patients aged  $\leq 60$  yrs, those with high fibrinogen levels had poorer DFS ( $p = 0.034$ , Figure S1A) but no significant difference in OS ( $p = 0.11$ , Figure S1B); and both male and female, patients with higher fibrinogen levels had worse DFS and OS (Figure S2). There was no significant difference in DFS ( $p = 0.082$ , Figure S3A) and OS ( $p = 0.81$ , Figure S3B) among different fibrinogen levels in patients with BMI  $\leq 22.62$ . But in patients with BMI  $>22.62$ , those with higher fibrinogen levels had worse DFS ( $p < 0.001$ , Figure S3C) and OS ( $p < 0.001$ , Figure S3D).

## 4. Discussions

Radical cholecystectomy is still the only way to cure gallbladder cancer although new therapies (e.g. immunotherapy and chemoradiotherapy) have been popularized in clinical practice [29, 30]. The gallbladder has a special anatomical structure, which lacks peritoneal coverage on the side facing the liver, so that it makes early GBC also prone to

TABLE 3: Univariate and multivariate analyses of OS in patients with GBC.

Factors	Univariate analysis			Multivariate analysis		
	<i>p</i> value	HR	95% CI	<i>p</i> value	HR	95% CI
Gender (male vs. female)	0.435	1.178	0.781-1.778			
Age ( $\leq 60$ vs. $>60$ )	0.566	1.1400	0.729-1.782			
BMI ( $\leq 22.62$ vs. $>22.62$ )	0.778	1.073	0.657-1.752			
Smoking history (no vs. yes)	0.349	1.270	0.770-2.095			
Drinking history (no vs. yes)	0.493	1.167	0.750-1.815			
Tumor location (body+fundus vs. neck)	0.002	2.069	1.320-3.241	0.042	1.701	1.019-2.841
Tumor size ( $\leq 3.5$ cm vs. $>3.5$ cm)	0.401	1.046	0.942-1.161			
Pathological type (adenocarcinoma vs. others)	0.001	0.382	0.220-0.663	0.211	0.665	0.352-1.259
Differentiation (poor vs. well)	$<0.001$	0.343	0.224-0.524	0.017	0.556	0.343-0.902
Vascular invasion (negative vs. positive)	$<0.001$	2.174	1.143-3.345	0.197	1.397	0.840-2.322
Nerve invasion (negative vs. positive)	$<0.001$	2.410	1.599-3.632	0.871	0.958	0.575-1.599
Cancerous node (negative vs. positive)	$<0.001$	4.277	2.143-8.537	0.009	3.529	1.378-9.038
TNM stage (I+II vs. III+IV)	$<0.001$	5.265	3.074-9.016	0.001	2.985	1.551-5.746
CEA ( $\leq 2.17$ ng/mL vs. $>2.17$ ng/mL)	0.001	2.318	1.452-3.700	0.025	1.880	1.081-3.271
CA19-9 ( $\leq 41.10$ U/mL vs. $>41.10$ U/mL)	$<0.001$	2.851	1.902-4.274	0.104	1.516	0.918-2.503
Fibrinogen ( $\leq 2.97$ g/L vs. $>2.97$ g/L)	$<0.001$	3.021	1.812-5.038	0.006	2.328	1.272-4.261

Note: HR: hazard ratio; CI: confidence interval; BMI: body mass index; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.

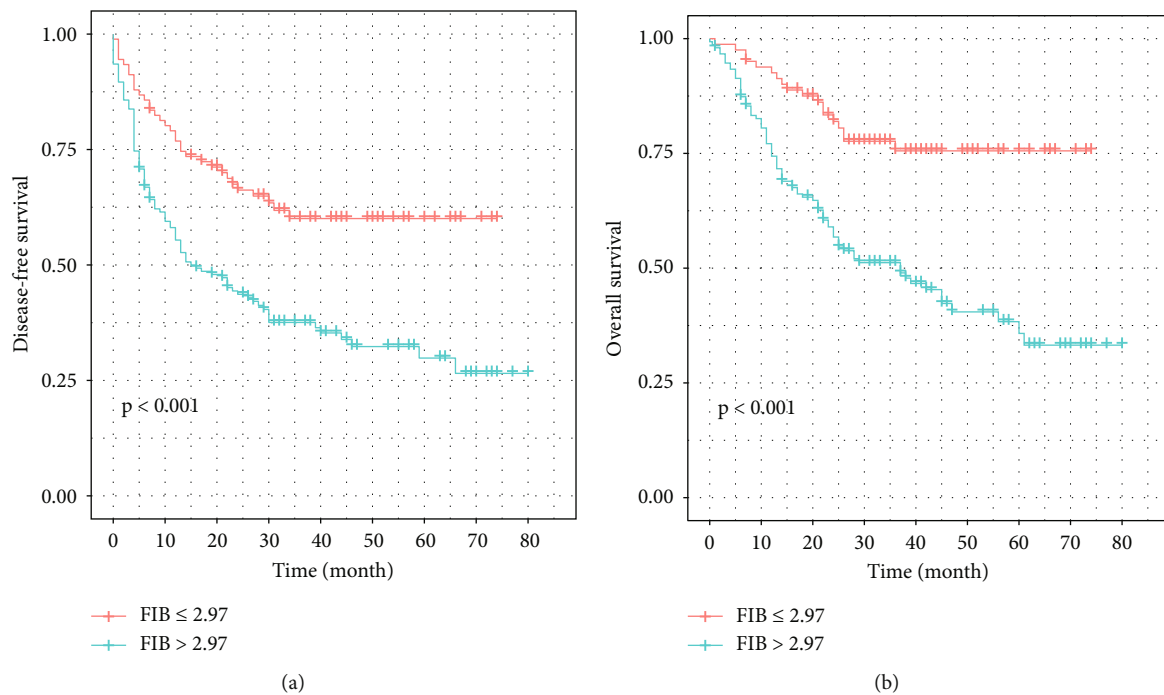


FIGURE 2: Kaplan-Meier curve analysis of DFS and OS in patients with gallbladder cancer. (a) DFS in patients with gallbladder cancer. (b) OS in patients with gallbladder cancer.

unavoidable micrometastases or metastases [31], greatly increasing the recurrence and mortality of GBC patients after radical surgery. Searching for promising biomarkers that affect the recurrence and prognosis of gallbladder cancer from convenient blood parameters is beneficial for the efficient assessment, subsequent therapies, and the postoperative management of GBC patients.

In this study, we demonstrated the preoperative fibrinogen level in patients with gallbladder cancer were correlated to tumor size, differentiation, TNM stage, and CA19-9 level, which indicates the probable promotion of fibrinogen on tumor growth and metastasis. Emerging evidence showed that fibrinogen promotes the metastasis and invasion of cancer cells through multiple pathways. Fibrinogen accelerates

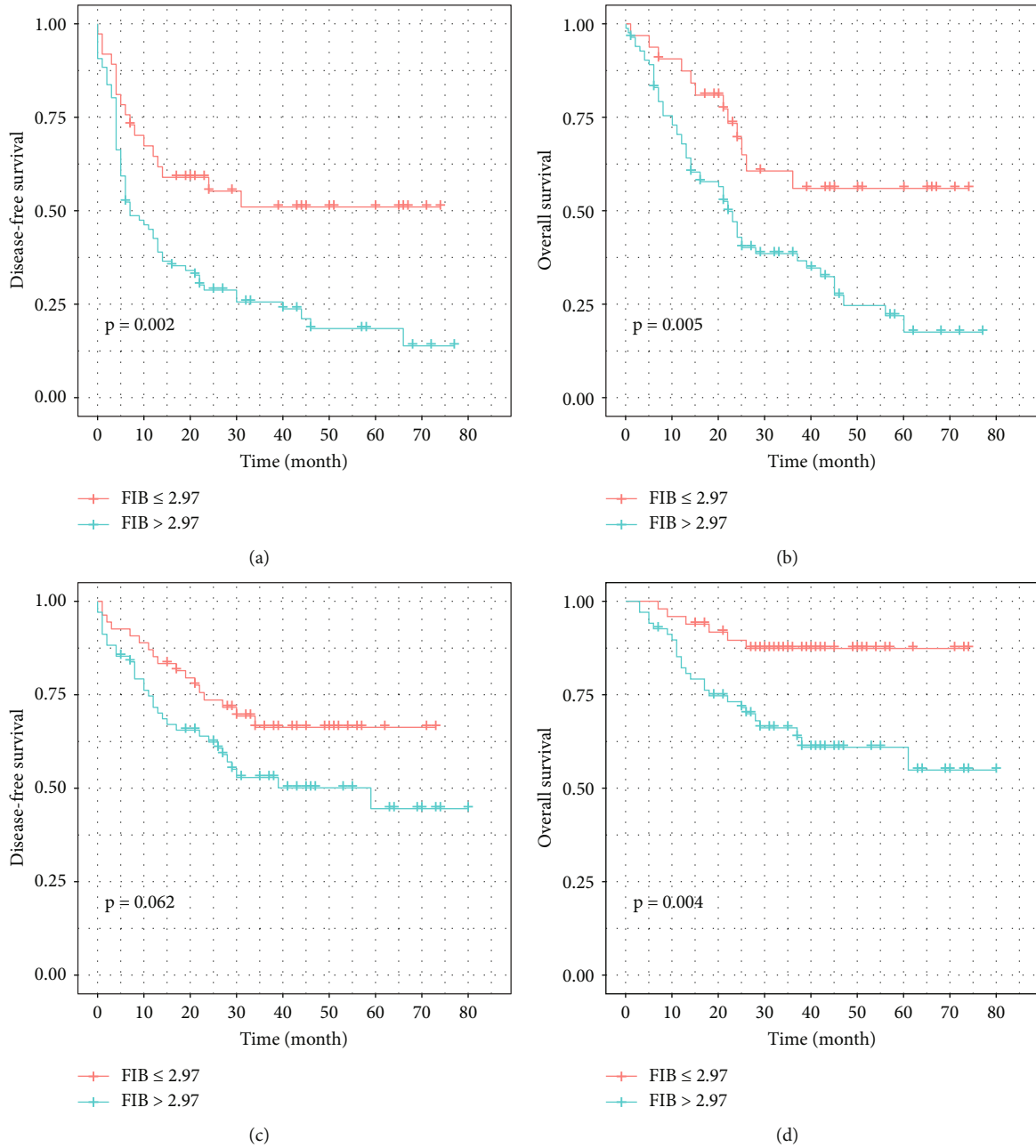


FIGURE 3: Kaplan-Meier curve analysis of DFS and OS of GBC patients stratified by degree of differentiation and preoperative fibrinogen level. (a) DFS in patients with poorly differentiated gallbladder cancer. (b) OS in patients with poorly differentiated gallbladder cancer. (c) DFS in patients with well-differentiated gallbladder cancer. (d) OS in patients with well-differentiated gallbladder cancer.

tumor angiogenesis by regulating fibroblast growth factor (FGF-2), thus providing nutrients and gas exchange for cancer cells [32], and ultimately leading to tumor enlargement. In addition, fibrinogen interacts with platelets in plasma to form microthrombi, which act as physical barriers to protect tumor cells from eliminating by natural killer cells, thereby increasing metastatic potential. [33] Studies have shown that fibrinogen enhances cell adhesion through integrin and non-integrin receptors to bridge vascular endothelium and specific receptors on tumor cells, and promotes tumor cells metastasis to adjacent target organs [34, 35]. In poorly differentiated and aggressive tumors, fibrinogen was mediated

by tumor development-related proinflammatory cytokines or synthesized and secreted by tumor cells directly [36]. As a consequence, the advanced TNM stage and poor tumor differentiation of GBC patients were causally related to a high level of preoperative fibrinogen.

Several publications have confirmed the prognostic value of CEA and CA19-9 in patients with GBC [37, 38], while few discoveries were found on the relevance of fibrinogen with gallbladder cancer. This study reveals that high levels of CEA were an independent adverse factor for OS and DFS in GBC patients, and CA19-9 was an independent risk factor for DFS but not OS. Unexpectedly, high-level preoperative

fibrinogen was an independent adverse factor both for DFS and OS in patients with GBC, and the hazard ratio of fibrinogen to OS (OS: HR = 2.328 CI: 1.272-4.261) was even higher than CEA (OS: HR = 1.880, CI:1.081-3.271). The results showed that fibrinogen was a better blood index other than tumor markers, which can early assess the recurrence and prognosis of gallbladder cancer. A combination of fibrinogen with pathological factors after surgery can further evaluate the DFS and OS of patients with GBC comprehensively. It was reported CEA  $\geq 3.02$  ng/mL was an independent prognostic marker for gallbladder cancer but not CA19-9 [39], which is consistent with our results. The value of CEA in the prediction of the prognosis in gallbladder cancer was confirmed despite the varied cut-off value of CEA. In this study, the time-dependent ROC curves were used to set the cut-off values of fibrinogen, CEA, and CA19-9 at 2.97 g/L, 2.17 ng/mL, and 41.1 U/mL, respectively, which could reduce the bias caused by different follow-up times. It is worthy to verify the cut-off values of the above markers by expanding the sample size.

Figure 2 further confirms the value of preoperative fibrinogen to assess postoperative DFS and OS in patients with gallbladder cancer. GBC patients with low preoperative FIB levels ( $\leq 2.97$  g/L) had no recurrence 3 years after surgery (1-year, 3-year, and 5-year recurrence rates were 23.2%, 39.9%, and 39.9%, respectively). The 3- to 5-year overall survival rate was reduced by only 2% (1-year, 3-year, and 5-year survival rates were 92.6%, 77.6%, and 75.6%, respectively); while the recurrence rate and mortality rate of GBC patients with high preoperative FIB levels ( $>2.97$  g/L) were increasing year by year. Recurrence and death even occur in those patients who have received surgeries more than five years ago. Currently, there is no uniform postoperative monitoring strategy for GBC patients undergoing surgical treatment [40, 41]. This study proposes that individualized postoperative management strategies can be formulated according to the preoperative FIB levels of GBC patients. GBC patients with high preoperative FIB levels should be monitored more closely and the follow-up monitoring period should be appropriately extended, while GBC patients with low preoperative FIB level should be appropriately relaxed with the extension of time to save medical resources.

However, subgroup analyses of fibrinogen by differentiation revealed that preoperative fibrinogen was more valuable for recurrence and survival in patients with poorly differentiated gallbladder cancer (Figure 3). There is reason to believe that preoperative fibrinogen can be a good marker for assessing OS of GBC patients regardless of tumor differentiation. When evaluating the DFS of GBC patients, it is best to make a comprehensive judgment based on tumor differentiation. In addition, we observed the suitability of preoperative fibrinogen in different subgroups of age, gender, and BMI. The results found that fibrinogen was more significant in GBC patients with higher BMI ( $>22.62$ ) or older age ( $>60$  yrs), and was not affected by gender (Figure S1–S3). If necessary, further comprehensive evaluation can be performed according to the patient's age and BMI. Thence, it is recommended to conduct individualized evaluations according to the clinical characteristics of patients, and

formulate more reasonable postoperative management plans. Furthermore, evidence was supporting that inhibiting the pathways of tumor-associated coagulation cascade activation could improve the outcomes of patients [42, 43]. As one of the most critical factors in the coagulation pathway, the preoperative fibrinogen level in patients with gallbladder cancer deserves further study.

Studies of gallbladder cancer tend to small sample size, limited by the low incidence. The subjects of this study came from two professional medical institutions, which held relatively large-scale tissue banks and clinical abilities of diagnosis and treatment for cancer patients. However, the study was also with certain limitations. Although the period of the cases in the retrospective analysis is large enough, the selection bias and potential confounding factors in the study cannot be eliminated, and the date of the patient's outcome events cannot be completely accurate. This may be the reason why the area under the ROC curves of CEA, CA19-9, and fibrinogen on the prognosis of gallbladder cancer is not very high. It is necessary to open further prospective studies with a large multicenter sample cohort to solve the issues.

## 5. Conclusions

A retrospective analysis of 260 GBC patients who underwent radical cholecystectomy uncovered that patients with high preoperative fibrinogen levels had poor postoperative DFS survival and OS, especially for poor differentiation. Therefore, fibrinogen is probably a potential prognostic biomarker for gallbladder cancer.

## Data Availability

Data relevant to the study are available and included in the manuscript.

## Ethical Approval

This study was performed according to the Helsinki Declaration. The Ethics Committees of the First Affiliated Hospital, Zhejiang University School of Medicine, and Zhejiang Cancer Hospital approved this study, and the approval number is NO: IRB-2014-272, IRB-2021-276.

## Consent

The authors have obtained the informed consents from all participants and their families.

## Conflicts of Interest

The authors have no competing interest to declare.

## Authors' Contributions

Y.W., J.W., and X.H. were responsible for the study concept and design, data acquisition, and manuscript drafting. S.Z., X.Q., and J.Z. were responsible for data interpretation and statistical analysis. S.Z. and J.Z. were responsible for the



collection of samples. J.W. and X.H. were responsible for study concept and design, supervision, and manuscript critical revision. The manuscript has been checked and approved by all the authors.

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## Supplementary Materials

Figure S1 Kaplan-Meier curve analysis of DFS and OS of GBC patients stratified age and preoperative fibrinogen level. (A) DFS in patients aged $\leq$ 60. (B) OS in patients aged $\leq$ 60. (C) DFS in patients aged $>$ 60. (D) OS in patients aged $>$ 60. Figure S2 Kaplan-Meier curve analysis of DFS and OS of GBC patients stratified gender and preoperative fibrinogen level. (A) DFS in male patients. (B) OS in male patients. (C) DFS in female patients. (D) OS in female patients. Figure S3 Kaplan-Meier curve analysis of DFS and OS of GBC patients stratified BMI and preoperative fibrinogen level. (A) DFS in patients with BMI $\leq$ 22.62. (B) OS in patients with BMI $\leq$ 22.62. (C) DFS in patients with BMI $>$ 22.62. (D) OS in patients with BMI $>$ 22.62. (*Supplementary Materials*)

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