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Original Article

Hyperfibrinogen is associated with the systemic inflammatory response and predicts poor prognosis in intrahepatic cholangiocarcinoma

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Abstract: Objective: This study aimed to investigate the prognostic prediction significance of plasma fibrinogen levels in patients with intrahepatic cholangiocarcinoma (ICC). Methods: A total of 173 patients with ICC were retrospectively recruited. We analyzed the association between initial plasma fibrinogen and overall survival (OS). The correlation between plasma fibrinogen level and the systemic inflammatory response (SIR) markers: the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and lymphocyte-monocyte ratio (LMR) were also assessed. Results: Elevated plasma fibrinogen levels were significantly correlated with shorter OS and poor prognosis in ICC patients (6.7 months for patients with high fibrinogen levels compared with 12.4 months for patients with low fibrinogen levels; log rank, 9.274; *P*=0.0023). In addition, plasma fibrinogen level was positively correlated with NLR and PLR and negatively correlated with LMR. Conclusions: Hyperfibrinogen is associated with the SIR and can serve as an independent factor for predicting poor prognosis in patients with ICC.

Keywords: Fibrinogen, intrahepatic cholangiocarcinoma, prognosis, systemic inflammation response

Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common liver tumor following hepatocellular carcinoma and is the most frequent malignancy of the biliary tract [1, 2]. ICC is very aggressive and is associated with a dismal prognosis. Studies have demonstrated that the incidence and mortality of ICC are remarkably increasing [3-6]. In contrast to other tumors of the liver and gastrointestinal tract, the pathogenesis of ICC remains obscure [7]. Moreover, patients with ICC have an unsatisfactory median survival of no more than 1year, even after different treatments such as surgery, systemic chemotherapy or radiotherapy [8]. However, clinical data have shown that survival rates vary significantly among patients with ICC. The commonly acknowledged factors that may serve as predictors for survival include intrahepatic satellite lesions, lymph node invasion and distant metastasis [9-11].

Growing evidence has shown that tumor growth, stroma formation and tumor invasion can be facilitated by activation of the tumor cells' hemostatic and coagulation systems. An activated hemostatic system can be measured by thrombocytosis, elevated D-dimer levels and hyperfibrinogenemia, as some studies have suggested [12].

Fibrinogen, an indispensable hemostatic factor, is converted to fibrin by activated thrombin. It is produced by hepatocytes in the liver and secreted into the circulation. In response to inflammatory mediators, its level will be augmented and it can be secreted from non-hepatocytes such as epithelial cells, carcinoma cells and trophoblasts [13-15]. Recent studies have demonstrated that elevated plasma fibrinogen levels are closely associated with tumor progression and poor outcomes in several types of cancer [16-18]. However, the prognostic value of plas-

Table 1. Clinical characteristics of intrahepatic cholangiocarcinoma patients (N=173)

Variables		N=173
Age (years)	Mean ± SD	58.83±11.31
Gender	Male	107 (38.2)
	Female	66 (61.8)
Stage	Locally advanced	36 (20.8)
	Metastatic	137 (79.2)
CA19-9	<1000 IU/ml	109 (63.0)
	≥1000 IU/ml	64 (37.0)
Plasma fibrinogen level	Median (Range)	3.53 (1.62-8.07)

ma fibrinogen levels in ICC patients remains to be investigated.

Therefore, in this study, we investigated the correlation between plasma fibrinogen and overall survival (OS) in patients with ICC. In addition, the association between plasma fibrinogen with systemic inflammatory response (SIR) markers was also analyzed.

Materials and methods

Patients' characteristics and clinical features

This study was approved by the Ethics Committee of Fudan University Shanghai Cancer Center, Shanghai, China, and written informed consent was obtained from each participant according to institutional guidelines. A total of 173 patients who were pathologically diagnosed with ICC were included in this retrospective study. All patients were treated between October 2011 and October 2015 at the Department of Integrative Oncology, Fudan University, Shanghai Cancer Center, Shanghai, China. Standard radiological studies included contrast-enhanced abdominal CT scans, magnetic resonance imaging (MRI), and MR-cholangiopancreatography (MRCP). Among the 173 patients enrolled in the study, 107 (61.8%) were male. According to the American Joint Committee on Cancer staging, 36 (20.8%) patients had a diagnosis of locally advanced cancer, while the remaining patients were at the metastatic stage. A total of 64 (37.0%) patients had CA199 of 1000 IU/ml or greater. A total of 99 patients received gemcitabine-based chemotherapy, while 74 patients were subjected to 5-fluorouracil-based chemotherapy. Detailed clinicopathological characteristics are depicted in **Table 1**.

Plasma fibrinogen measurements

Plasma fibrinogen levels were measured as a routine examination to exclude coagulation disorders or acute infections before cancer diagnostic interventions or treatments. Preintervention and pretreatment fibrinogen levels were used in the statistical analyses. Patients with acute infectious diseases were excluded from all analyses if fibrinogen increased in the acute phase response. Fibrinogen was measured by peripheral venous punctures at the first visit to our hospital. Fibrinogen levels were measured according to the Claus method described previously [19].

Statistical analyses

The results of fibrinogen analysis are reported as mean ± standard deviation. Kaplan-Meier analysis was used to compare OS between patients in different groups and the log-rank test was used to estimate the difference in survival. Univariate and multivariate analyses were performed using the Cox proportional hazards regression model. Hazard ratios estimated from the Cox analysis were reported as relative risks with corresponding 95% confidence intervals. $P<0.05$ was considered significant. All these tests were performed using SPSS 17.0 software.

Results

Plasma fibrinogen levels were associated with OS in patients with ICC

We first examined the correlation between plasma fibrinogen levels and OS in the ICC patients. Clinicopathological characteristics of the ICC patients are shown in **Table 1**. The median fibrinogen level of these ICC patients was 3.53 g/l and was selected as the cutoff value. Kaplan-Meier analyses showed that high plasma fibrinogen levels were significantly correlated with shorter OS in ICC patients (6.7 months for patients with high fibrinogen levels vs 12.4 months for patients with low fibrinogen levels; log rank, 9.274; $P=0.0023$; **Figure 1**). In addition, the association between fibrinogen and ICC were further investigated in the different stages of ICC. Kaplan-Meier curves for OS stratified for fibrinogen plasma concentration

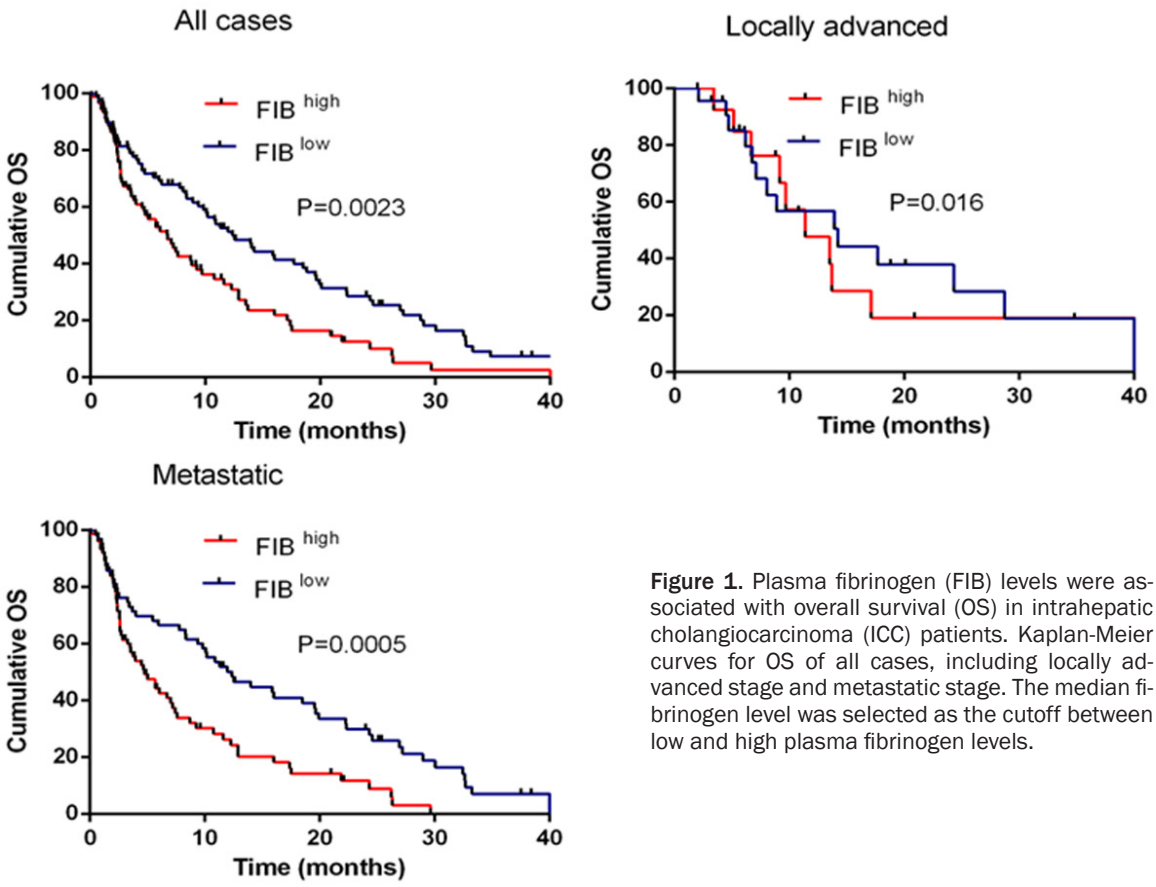

Figure 1. Plasma fibrinogen (FIB) levels were associated with overall survival (OS) in intrahepatic cholangiocarcinoma (ICC) patients. Kaplan-Meier curves for OS of all cases, including locally advanced stage and metastatic stage. The median fibrinogen level was selected as the cutoff between low and high plasma fibrinogen levels.

Table 2. Correlation of fibrinogen with OS in patients with intrahepatic cholangiocarcinoma patients (N=173)

Variables	Case number	Hazard Ratio (95% CI)	*P*-value*
Univariate analysis			
Age (<60 vs ≥60 years)	79/94	0.851 (0.607-1.193)	0.349
Gender (male vs female)	107/66	1.141 (0.809-1.625)	0.441
Cancer stage (locally advanced vs metastatic)	36/137	1.668 (1.063-2.617)	0.026
CA199 (<1000 vs ≥1000 IU/ml)	109/64	2.273 (1.584-3.264)	0.0001
Plasma fibrinogen level (low vs high)	86/87	1.707 (1.205-2.419)	0.003
Multivariate analysis			
Cancer stage (locally advanced vs metastatic)	36/137	1.636 (1.039-2.576)	0.033
CA199 (<1000 vs ≥1000 IU/ml)	109/64	2.276 (1.583-3.274)	0.0001
Plasma fibrinogen level (low vs high)	86/87	1.775 (1.247-2.527)	0.001

Abbreviations: OS, overall survival; CI, confidence interval.

differ significantly in the locally advanced group and in the metastatic cohort (**Figure 1**).

Plasma fibrinogen level is a prognostic predictor for OS

Univariate analyses were performed to assess the prognostic value of plasma fibrinogen levels for OS. Tumor stage (locally advanced vs metastatic), CA19-9 (<1000 vs ≥ 1000 IU/ml), and high plasma fibrinogen levels were all prognostic predictors for poor OS. Considering the impact of tumor stage and CA19-9 on prognosis, as reflected from the univariate analyses, multivariate analysis was also conducted using Cox proportional hazards regression. The results demonstrated that the plasma fibrinogen level was an independent prognostic predictor

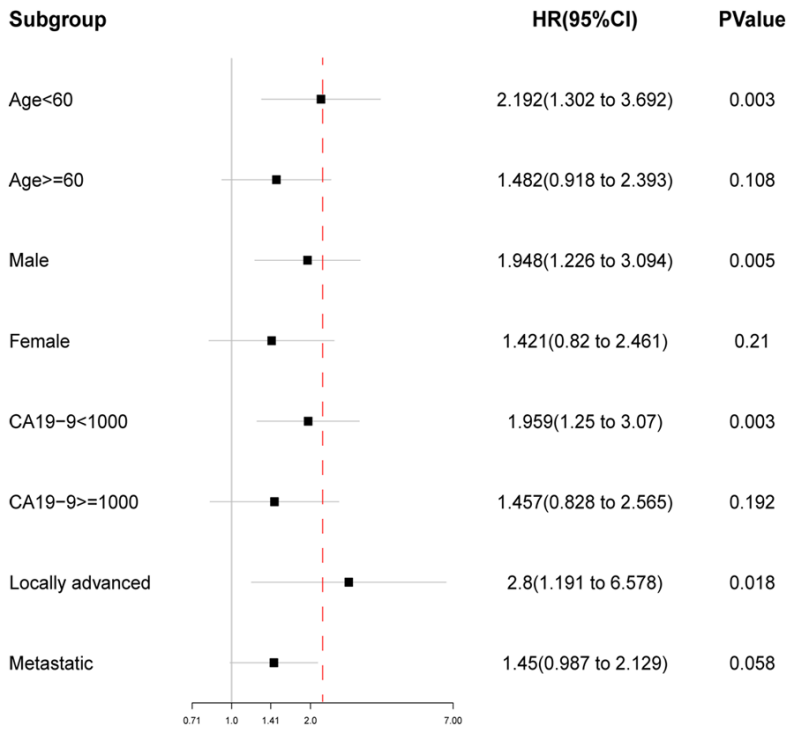

Figure 2. Predictive value of plasma fibrinogen (FIB) level for overall survival (OS). A, Hazard ratios (HRs) of plasma fibrinogen levels were calculated for OS in different patient subgroups. HRs of fibrinogen for OS were calculated by comparing patients with low plasma fibrinogen levels to those with high plasma fibrinogen levels. Hazard ratio >1.0 indicates a worse outcome. The median plasma fibrinogen level was selected as the cutoff.

for OS (hazard ratio, 1.775; 95% confidence interval, 1.247-2.527; *P*=0.001; **Table 2**).

Predictive value of plasma fibrinogen level for OS

Additionally, the prognostic value of plasma fibrinogen level in the subgroups of the ICC patients was analyzed. The ICC patients were grouped according to age, gender, CA19-9 level and tumor stage. Further analyses demonstrated that plasma fibrinogen level was associated with OS in each subgroup (**Figure 2**).

Plasma fibrinogen level correlated with systemic inflammatory response markers

An increasing body of evidence has confirmed the vital role of fibrinogen in regulating the inflammatory process. Considering that the proinflammatory role of fibrinogen has been reported in many studies, we evaluated the correlation between fibrinogen and the systemic inflammatory response markers: neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and lymphocyte/monocyte ratio (LMR). Results showed that plasma fibrinogen levels were positively correlated with NLR (r=0.332, *P*=0.0001) and PLR (r=0.360, *P*=0.0001) but negatively correlated with LMR (r=-0.246, *P*=0.001) (**Figure 3**). These results indicated that elevated plasma fibrinogen levels were associated with the systemic inflammatory response in ICC (Supplementary Material).

Discussion

ICC is an aggressive malignancy and one of the most devastating diseases of the biliary tract. The incidence and mortality rates of ICC are on the rise worldwide [1, 2]. More concerning is that the prognosis of ICC patients is very poor, with OS of less than 1 year [20]. In clinical practices, variables, such as CA19-9, and tumor stage have been employed to evaluate the outcomes of patients with ICC. However, such examinations are expensive and laborious. Therefore, there is a need for a more convenient and effective means to assess the prognosis of ICC patients.

It has been recognized for many years that hemostatic factors play a critical role in the progression of carcinoma [21]. Consequently, factors involved in the coagulation cascade, such as tissue factor, thrombin and fibrinogen have been under extensive investigations in recent years. Such studies have found that upregulated expression levels of fibrinogen-related products in most solid tumors in humans and experimental animals. For example, an increasing amount of evidences have revealed an elevated production of fibrinogen in some types of cancers including esophageal cancer, non-small lung cancer, gastric cancer [16-18]. Moreover, in vitro experiments have also con-

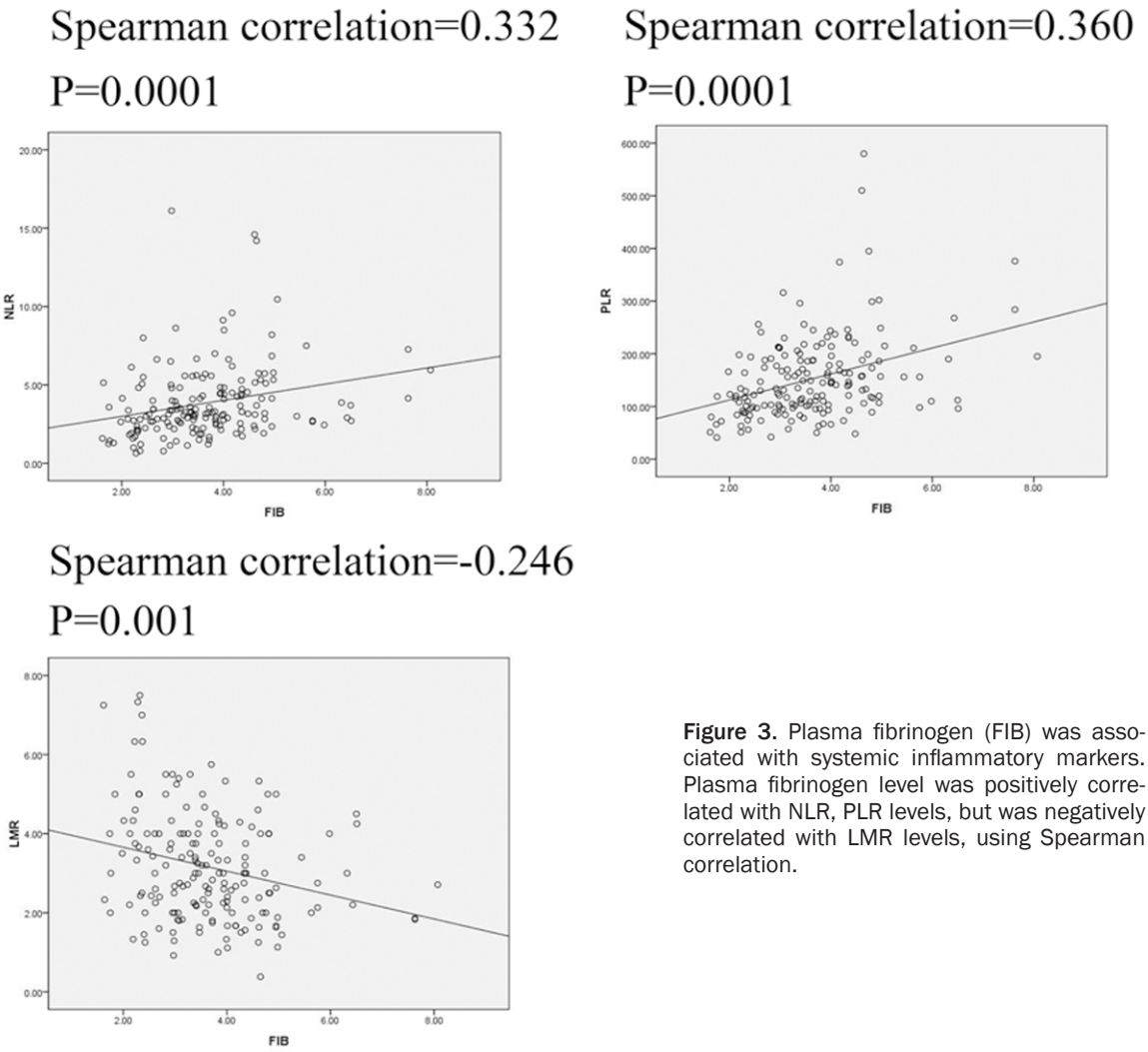

Figure 3. Plasma fibrinogen (FIB) was associated with systemic inflammatory markers. Plasma fibrinogen level was positively correlated with NLR, PLR levels, but was negatively correlated with LMR levels, using Spearman correlation.

firmed fibrin matrices can promote the proliferation and migration of various types of cancers [22, 23]. However, to the best of our knowledge, there are fewer reports concerning the role of fibrinogen in ICC.

In this study, we examined plasma fibrinogen levels in patients with ICC and their association with the prognosis. We found that high plasma fibrinogen levels were closely related to shortened OS and that plasma fibrinogen level was an independent prognostic predictor for OS in patients with ICC.

The median value of the fibrinogen levels (3.53 g/l) as chosen as the cutoff and the patients enrolled were categorized into the low- or high-fibrinogen group. Compared with the high fibrinogen group, the mean survival time of the low fibrinogen group was longer (12.4 months vs 6.7 months, *P*=0.0023). ICC patients with elevated plasma fibrinogen (>3.53 g/l) levels had 1.707 times the risk of death compared to patients with lower fibrinogen. In our study, we also tested the correlation between fibrinogen and OS in different cohorts of ICC patients. Patients were divided into the locally advanced group and the metastatic group according to their tumor stages. Results have shown that boosted fibrinogen levels were indicative of decreased OS, both in the locally advanced group and the metastatic group.

The reason for the association between elevated fibrinogen levels and decreased OS remains to be explored. The effect of fibrinogen in the context of cancers stems from its role as a pro-inflammatory marker. Fibrinogen has long been viewed as one of the major acute phase proteins [24]. Surmounting evidence has indicated

a potential role for fibrinogen in tumor growth and progression [23, 25]. Indeed, tumor progression is a consequence of complex interaction between tumor cells and the microenvironment. The possible mechanisms can be concluded as follows: 1) Fibrinogen provides a stable framework to the tumor extracellular matrix, thereby promoting metastasis [26]. 2) The interaction between the tumor cells and host cells can be enhanced by fibrinogen. To date, numerous studies have focused on the association between fibrinogen and inflammation, leading fibrinogen to be considered as a mediator of inflammatory disease. For example, it can act on different cell types through cell-specific integrin and non-integrin receptors to induce specific inflammatory functions in a variety of diseases with an inflammatory component [27, 28]. Furthermore, fibrinogen can bind directly to inflammatory and tumor cells, resulting in production of proinflammatory cytokines. It may boost the binding of many growth factors to tumor cells, which might be of biologic relevance to tumor progression [29, 30]. For instance, research has confirmed the proinflammatory of fibrinogen using fibrinogen-deficient mice in models of fibrotic diseases and acute endotoxemia. Their studies implied a possible role for fibrinogen in the early acute inflammation stage and the engagement of the innate immune system [31]. Therefore, the role of fibrinogen in cancer progression can be largely attributed to its involvement in inflammation.

The role of inflammation in cancer has also been widely studied. It has been reported that inflammation can promote tumor growth, metastasis and angiogenesis [6, 32]. The inflammatory status can be reflected in the infiltration of white blood cells, neutrophils, lymphocytes and platelets. Various SIR markers have been employed to assess the inflammatory status and to evaluate the prognosis of cancer patients. The commonly used and widely accepted SIR markers include: NLR, PLR and LMR. Studies have found that an elevated preoperative NLR predicts poor prognosis in ICC patients undergoing hepatectomy [33]. Additionally, the negative impact of preoperative PLR on outcome after hepatic resection for ICC was also observed [34]. Given the possible role of fibrinogen in inflammation and cancer, we also evaluated the correlation between fibrinogen and these SIR markers. Spearman rank correlation analysis revealed that fibrinogen was positively correlated with NLR and PLR, but was negatively correlated with LMR. These results support our proposal that fibrinogen can serve as a prognostic marker for patients with ICC, which is partly attributed to its role as a mediator of inflammation.

For ICC patients, the measurement of fibrinogen has many advantages. First, it is convenient and inexpensive because it can be obtained from routine blood tests and can be measured repeatedly. Second, the prediction of prognosis can be enhanced with the combination of the fibrinogen with some traditional markers (CA19-9 and tumor stage).

This study inevitably has limitations, which can be listed as follows: First, because cytokines and other mediators can be induced by large tumor loads, the elevated fibrinogen could be a result of large tumor size. However, we failed to analyze the association between tumor size and fibrinogen levels due to lack of information on patients' tumor size. Second, information on the inflammatory marker C-reactive protein (CRP) was not available in our study because the CRP was not routinely examined in our patient cohort. Third, the patients enrolled in this study were in advanced stages of the diseases, thus large cohort and multicenter trials are needed to further evaluate the role of fibrinogen in ICC patients at early stages. Furthermore, considering that fibrinogen is a critical participant in coagulation, elevated fibrinogen levels might impair patients' survival by modulating thromboembolism events. Therefore, decreased survival can be attributable to the occurrence of thromboembolism events. In our study, thromboembolic events were not observed among the ICC patients, therefore such possibilities can be excluded.

Taken together, our study confirmed the prognostic significance of fibrinogen in ICC, and found its association with SIR markers. Additional studies are warranted to validate whether fibrinogen could serve as a therapeutic target for ICC treatment, which might provide more strategies for clinicians to perform individualized therapies in the future.

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Disclosure of conflict of interest

None.

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