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Preoperative serum ferritin is an independent prognostic factor for liver cancer after hepatectomy

Si-Jia Wu[a,1], Zun-Zhen Zhang[b], Nan-Sheng Cheng[c], Xian-Ze Xiong[c], Luo Yang[a,*]

[a] Department of Surgery, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, 610041, Sichuan Province, China
[b] Department of Environmental Health and Occupational Medicine, West China School of Public Health, Sichuan University, Chengdu, 610041, Sichuan Province, China
[c] Department of Bile Duct Surgery, West China Hospital, Sichuan University, Chengdu, 610041, Sichuan Province, China

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ABSTRACT

Background and aims: Serum ferritin (SF) may have a close relationship with the tumor. But no study has investigated the prognostic value of SF in hepatocellular carcinoma (HCC) patients receiving curative resection yet. Aim of this study is to explore the role of preoperative SF in survival outcomes of such patients.
Methods: We retrospectively analyzed 427 HCC patients who received curative hepatic resection in our medical center. Significant clinical and pathological data along with the association between SF and clinicopathological parameters were compared and analyzed. The prognostic significance of SF was determined by Kaplan-Meier analysis and the Cox proportional hazards regression model.
Results: The optimal cut-off value of SF for overall survival (OS) was 267 ng/ml. Preoperative SF level could predict OS ($P = 0.001$, HR = 1.651, 95%CI: 1.213–2.247) and recurrence-free survival (RFS) ($P < 0.001$, HR = 1.570, 95%CI: 1.221–2.018) independent of other prognostic factors. Patients with a low SF were more likely to have both favorable OS and RFS (both $P < 0.001$), and vice versa. The 1-, 3-, and 5-year OS and RFS rates were 91.4%, 80.1%, 71.7%, and 78.0%, 53.0%, 47.3% in low SF group, and 91.6%, 60.2%, 45.2%, and 61.3%, 36.4%, 29.0% in high SF group, respectively.
Conclusions: Preoperative SF was a simple, inexpensive, convenient and reliable prognostic factor that could predict survival outcomes in HCC patients who received radical hepatic resection.

1. Introduction

Representing the fifth newly-confirmed cancer and the second cause of tumor-associated mortality, hepatocellular carcinoma (HCC) is a highly prevalent liver malignant tumor in Asia, and whose morbidity and mortality are increasing rapidly these years, even in Western countries [1–5]. As HCC is insensitive to radiotherapy and chemotherapy, radical hepatic resection is still the prior curative therapeutic option for HCC patients, especially in China [6–9]. The long-term survival outcomes of HCC remain dismal due to the high rate of recurrence and what's striking is that some studies have reported that the recurrence rate of HCC patients after hepatic resection can be higher than 50% [10–15]. Therefore, predicting postoperative recurrence and survival outcomes in such patients must be enforced.

As is known to all, tumor pathological factors, such as pathologic type and differentiation degree, tumor size, number of tumors and vascular invasion, can affect and predict the prognosis of HCC patients [10,12,13,16–19]. Besides, these key pathological parameters constitute the most widely used tumor staging systems, such as the tumor node metastasis (TNM) system, the Barcelona Clinic Liver Cancer (BCLC) staging system, etc [20–23]. However, these pathological factors can be only obtained after surgery. Oppositely, several inflammation-based prognostic scores and models that can be obtained before surgery have attracted a lot of attention and been widely explored up to now, for instance, neutrophil-to-lymphocyte ratio (NLR), inflammation-based score, platelet-to-lymphocyte ratio, etc. Yet their predictability in surgically-treated HCC patients' prognosis has not reached a consensus, and application of these prognostic scores in clinical practice still has lots more to work on [11,24–28]. Consequently, there is an urgent need to search for reliable and convenient prognostic variables that can be available before surgery.

Serum ferritin (SF), a primary iron-binding protein, plays an important role in tumor proliferation, angiogenesis and immunoregulation [29–34]. An elevated SF may forebode either body iron overload

* Corresponding author.
E-mail addresses: 43311458@qq.com (S.-J. Wu), 3425183157@qq.com (L. Yang).
[1] First Author.

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conditions or some severe diseases (e.g. inflammation, infection, liver diseases, tumor, liver dysfunction, even deterioration and progress of the disease) [29–34]. Simultaneously, raised SF level also infers intrahepatic iron accumulation, lack of hepcidin and endotoxemia, which may bring on liver injury and hepatocarcinogenesis [33,34]. Previous studies have demonstrated that SF has a close relationship with tumor, and the prognostic value of SF has also been proved in a variety of malignancies, such as lung cancer, pancreatic cancer, colorectal cancer, HCC and so forth [29–37]. In addition, as a routinely tested and inexpensive biochemical parameter, SF can be easily obtained before surgery. From the above, we hypothesized that preoperative SF might have fine prognostic value in HCC patients treated with radical hepatic resection. However, for all we know, no study in respect of the prognostic value of SF in HCC patients receiving curative resection has been published yet. As a consequence, we performed this study for the first time to investigate the role of preoperative SF in survival outcomes of HCC patients who underwent radical hepatic resection and whether preoperative SF could be an independent prognostic biomarker in such patients.

2. Methods

2.1. Patients and study design

Between January 2009 and December 2014, 626 HCC patients' hospital serial numbers were randomly generated from the medical records database of West China Hospital of Sichuan University. Then, they were randomly matched up on a scale of 1 to 1 and divided into either training cohort or validation cohort. The clinical data of these 626 patients (313 in training cohort and 313 in validation cohort) was collected and retrospectively reviewed. Written informed consent was obtained from all patients. Curative hepatic resection was defined as macroscopic complete removal of all detected tumors. Our inclusion criteria were as follows: resectable primary HCC confirmed by pathological diagnosis, received no previous anticancer therapy and underwent curative hepatic resection as the initial therapy, Childs-Pugh-Turcotte score A or B, appropriate Model for end-stage liver disease scores (less than 13) and with good general status. Inversely, HCC combined with other tumors, recurrent HCC, unresectable primary HCC, loss of follow-up and patients with incomplete data were exclusion criteria. We performed this study in accordance with the ethical standards of the responsible committee on human experimentation (Institutional or regional) and the Helsinki Declaration, and this study was approved by the Ethics Committee of West China Hospital, and West China School of Public Health and West China Fourth Hospital, Sichuan University.

2.2. Perioperative procedure and data collection

Patients' demographics data, including age, gender, weight, height, concomitant diseases, detailed present history, previous medical history, family history and detailed physical examination results were recorded at the time of the hospitalization. Laboratory measurement including complete blood count, biochemistry parameters, SF, liver and renal function, coagulation tests, hepatitis B and C immunology and tumor markers were performed and recorded. If a patient was identified as Hepatitis B virus (HBV) or Hepatitis C virus (HCV) infection, further HBV DNA load or HCV-RNA test was carried out to estimate whether the patient should take antiviral drugs. Preoperative chest radiography, electrocardiogram and ultrasonography were routinely executed. An enhanced computed tomography (CT) or magnetic resonance imaging (MRI) was also performed to help diagnose and determine surgical procedures. The diagnosis of HCC was based on clinical imaging findings, alpha fetoprotein (AFP) levels and previous history, and finally was confirmed by postoperative pathological examination. Either micro-vascular invasion or macro-vascular invasion was deemed as pathological vascular invasion which was finally confirmed by postoperative pathological examination. All preoperative routine laboratory and imaging examination were done 2-4 days before the surgery. Pulmonary function test, echocardiography and other additional examinations were executed to exclude surgical contraindications when necessary. Intraoperative details and postoperative clinicopathologic parameters were also collected. The TNM (7th edition) and BCLC system were used to categorize HCC stage. Postoperative conditions of patients were closely monitored and recorded, and effective treatments were also offered to each patient according to the patient's specific situations until hospital discharge.

2.3. Follow-up and recurrence treatment

All patients received regular surveillance at the1st month after the surgery and then every 3 months in the first 3 postoperative years and every 6 months in the subsequent postoperative years. During the follow-up period, basic physical examination, complete blood count, liver and renal function, serum AFP level and abdominal ultrasonography were routinely carried out at each visit. If the recurrence HCC was suspected, abdominal enhanced CT, MRI and hepatic artery angiography examination were selectively performed, depending on the case. Overall survival (OS) and recurrence-free survival (RFS) were the primary endpoints of our study. OS was defined as the interval between the surgery and death or the last follow-up, and RFS was defined as the interval from the date of surgery to the date of confirmed HCC recurrence or the last follow-up. Our study was censored on 30 June 2018.

Patients with confirmed HCC recurrence received further individual treatment, including repeated liver resection, transarterial chemoembolization, radiofrequency ablation (RFA), liver transplantation, percutaneous ethanol injection or sorafenib therapy, depending on tumor size and number, location of recurrence, liver function, residual liver volume, general conditions and patient's decision.

2.4. Statistical analysis

Categorical variables were described as count and percentage and were compared by the Pearson's chi-square analysis or Fisher exact test. Continuous data were presented as median and range and were compared by the Mann-Whitney *U* test. The best cut-off values of SF were determined by the receiver operating characteristic (ROC) curve and the point on ROC curve with both maximum sensitivity and specificity was selected for the best cut-off value. Survival curves were developed using the Kaplan–Meier method and comparisons carried out using the log-rank test. Univariate and multivariate analysis of independent prognostic factors for OS and RFS were performed by the Cox proportional hazard model. Statistical significance for all the analyses was established at P-value < 0.05 derived from two-tailed test and statistical analysis was performed using SPSS 17.0 software (version 17.0; SPSS Inc., Chicago, IL, United States).

3. Results

3.1. Patients' characteristics

After screening, 199 patients (108 in training cohort and 91 in validation cohort) were excluded for the following reasons: incomplete data (n = 67), loss to follow-up (n = 71), recurrent HCC receiving repeated liver resection (n = 19), received other treatments before surgery (n = 26), combined multiple organs resection (n = 9) and other reasons (n = 7). Finally, 427 patients were involved (training cohort (n = 205), validation cohort (n = 222), and total cohort (n = 427)). Our total cohort involved 362 men (84.78%) and 65 women (15.22%), with a mean age of 47.5 years (range 18–82 years). Postoperative pathological examination demonstrated that 295 patients (69.09%) had cirrhosis, 73 patients (17.10%) showed a poor pathological

Table 1
Baseline demographic and clinicopathological characteristics of patients.

Variables	Training cohort (n = 205)	Validation cohort (n = 222)	P-value
Age (years, median (range))	48 (19–82)	47 (18–79)	0.762
Gender (Male/Female, n (%))	179 (87.3%)/26 (12.7%)	183 (82.4%)/39 (17.6%)	0.179
Etiology (HBV/HCV/Other, n (%))	156 (76.1%)/27 (13.2%)/22 (10.7%)	176 (79.2%)/29 (13.1%)/17 (7.7%)	0.526
Cirrhosis (absent/present, n (%))	60 (29.3%)/145 (70.7%)	72 (32.4%)/150 (67.6%)	0.531
ALT (IU/L, ≤45/ > 45, n (%))	104 (50.7%)/101 (49.3%)	119 (53.6%)/103 (46.4%)	0.562
AST (IU/L, ≤45/ > 45, n (%))	96 (46.8%)/109 (53.2%)	116 (52.3%)/106 (47.7%)	0.287
Albumin (g/L, > 40/≤40, n (%))	118 (57.6%)/87 (42.4%)	121 (54.5%)/101 (45.5%)	0.559
Total bilirubin (umol/L, ≤28/ > 28, n (%))	195 (95.1%)/10 (4.9%)	208 (93.7%)/14 (6.3%)	0.538
Platelet count (10^9/L, median (range))	121 (64–272)	123.5 (62–276)	0.967
NLR (median (range))	2.25 (0.91–25.71)	2.30 (0.53–8.42)	0.996
Monocyte count (10^9/L, median (range))	0.34 (0.07–1.55)	0.34 (0.09–1.47)	0.593
AFP (ng/ml, < 200/≥200, n (%))	135 (65.9%)/70 (34.1%)	137 (61.7%)/85 (38.3%)	0.421
SF (ng/mL, median (range))	233.4 (18.6–1908)	260.2 (6.8–1272)	0.477
Prothrombin time (sec, median (range))	11.9 (9.6–16.9)	11.9 (9.6–15.2)	0.556
Differentiation (well and moderate/poor, n (%))	170 (82.9%)/35 (17.1%)	184 (82.9%)/38 (17.1%)	1.000
Tumor necrosis (absent/present, n (%))	128 (62.4%)/77 (37.6%)	128 (57.7%)/94 (42.3%)	0.325
Tumor number (single/multiple, n (%))	167 (81.5%)/38 (18.5%)	186 (83.8%)/36 (16.2%)	0.609
Vascular invasion (absent/present, n (%))	162 (79.0%)/43 (21.0%)	177 (79.7%)/45 (20.3%)	0.907
Tumor size (cm, ≤5/ > 5, n (%))	127 (62.0%)/78 (38.0%)	139 (62.6%)/83 (37.4%)	0.921
TNM stage (I and II/III and IV, n (%))	184 (89.8%)/21 (10.2%)	198 (89.2%)/24 (10.8%)	0.876
BCLC stage (0 and A/B/C, n (%))	140 (68.3%)/22 (10.7%)/43 (21.0%)	148 (66.7%)/29 (13.0%)/45 (20.3%)	0.627

HBV: hepatitis B virus; HCV: hepatitis C virus; ALT: alanine aminotransferase; AST: aspartate aminotransferase; NLR: neutrophil to lymphocyte ratio; AFP: alfa-fetoprotein; TNM: tumor node metastasis; BCLC: Barcelona Clinic Liver Cancer; SF: serum ferritin.

differentiation, 74 patients (17.33%) had multiple nodules and 88 patients (20.61%) had pathological vascular invasion. Of the total, 382 patients (89.46%) were classified as TNM stage I and II, and 288 patients (67.45%) were classified as BCLC stage 0 and A. As shown in Table 1, there was no significant difference in clinicopathologic variables between the training and validation cohorts.

3.2. Prognostic cut-off value for SF

The best cut-off value of preoperative SF for OS in each cohort was determined depending on the ROC analysis, respectively. In training cohort, the optimal cut-off value of SF for OS was estimated to be as 267.2 ng/ml, with the area under the curve (AUC) as 0.578 and 95% confidence interval (CI) as 0.524 to 0.633 (P = 0.006) (Fig. 1A). Homoplastically, the optimal cut-off value of SF for OS in validation cohort was also 267.2 ng/ml, and the AUC was 0.592 (95%CI: 0.535–0.649, P = 0.001) (Fig. 1B). Finally, in total cohort, an SF of 267.3 ng/ml with a sensitivity of 61.5% and a specificity of 68.3% was chosen as the best cut-off point for OS (AUC: 0.630, 95%CI: 0.554–0.707, P = 0.001) (Fig. 1C). Therefore, we determined the best cut-off value of SF for OS as 267 ng/ml and divided patients in each cohort into either low SF group or high SF group.

3.3. Correlation between SF and clinicopathological variables

Grounded on the best cut-off value of SF, altogether 427 patients were divided into low SF group (SF ≤ 267 ng/ml, n = 247) and high SF group (SF > 267 ng/ml, n = 180). We next investigate the relationship between preoperative SF and clinicopathological parameters. As shown in Table 2, TNM and BCLC stage were closely related to preoperative SF rather than other clinicopathological variables. Lower preoperative SF level was closely correlated with better TNM stage (P = 0.016) and BCLC stage (P = 0.010). Details of the relationship between clinicopathological variables and preoperative SF levels were summarized in Table 2.

3.4. Determination of prognostic factors for OS and RFS

Due to the fact that SF could be affected by age and sex, we included age and sex in the multivariate Cox model regardless of their results of the univariate analysis and the following results were adjusted for age and gender (Table 3, Table 4 and Table 5).

In training cohort, albumin concentration (P < 0.001), NLR (P = 0.044), SF level (P = 0.038), tumor differentiation (P = 0.035) and vascular invasion (P < 0.001), rather than tumor number (P = 0.091) and tumor size (P = 0.057), were identified as significant independent prognostic factors for OS in the multivariate Cox proportional hazards analysis (Table 3). While albumin concentration (P = 0.015), SF level (P = 0.004), tumor number (P = 0.031), vascular invasion (P < 0.001) and tumor size (P < 0.001) were regarded as significant independent predictors associated with RFS (Table 3). The detailed results of Cox univariate/multivariate analyses of prognostic factors for OS and RFS in training cohort were presented in Table 3.

Gender (P = 0.022), albumin concentration (P = 0.046), NLR

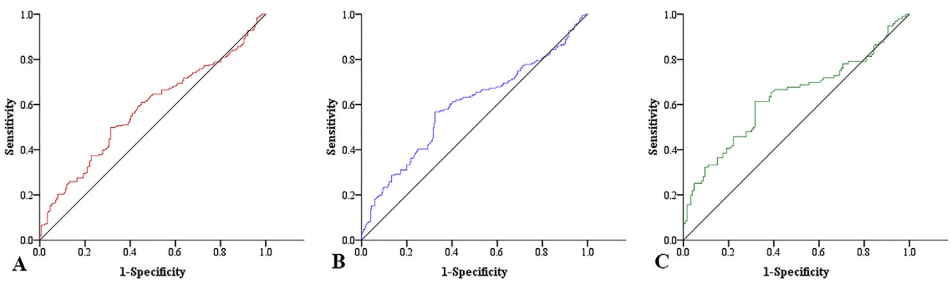

Fig. 1. Receiver operating characteristics (ROC) curves to evaluate the optimal cut-off value of serum ferritin (SF) for overall survival (OS). A: ROC curve of SF in training cohort. B: ROC curve of SF in validation cohort. C: ROC curve of SF in total cohort.

Table 2
Association of preoperative serum ferritin level with clinicopathological parameters (Total cohort, N = 427).

Variables	Preoperative Serum Ferritin (SF)		P-value
	Low SF Group (≤267 ng/ml) (n = 247)	High SF Group (> 267 ng/ml) (n = 180)	
Age (years, median (range))	48 (22–81)	47 (18–79)	0.110
Gender (Male/Female, n (%))	209 (84.6%)/38 (15.4%)	153 (85.0%)/27 (15.0%)	1.000
Etiology (HBV/HCV/Other, n (%))	191 (77.3%)/37 (15.0%)/19 (7.7%)	141 (78.3%)/19 (10.6%)/20 (11.1%)	0.426
Cirrhosis (absent/present, n (%))	78 (31.6%)/169 (68.4%)	54 (30.0%)/126 (70.0%)	0.751
ALT (IU/L, ≤45/ > 45, n (%))	132 (53.4%)/115 (46.6%)	91 (50.6%)/89 (49.4%)	0.558
AST (IU/L, ≤45/ > 45, n (%))	127 (51.4%)/120 (48.6%)	85 (47.2%)/95 (52.8%)	0.433
Albumin (g/L, > 40/≤40, n (%))	140 (56.7%)/107 (43.3%)	99 (55.0%)/81 (45.0%)	0.767
Total bilirubin (umol/L, ≤28/ > 28, n (%))	234 (94.7%)/13 (5.3%)	169 (93.9%)/11 (6.1%)	0.832
Platelet count (10^9/L, median (range))	127 (65–273)	121.5 (62–264)	0.838
NLR (median (range))	2.19 (0.66–25.71)	2.36 (0.53–9.00)	0.145
Monocyte count (10^9/L, median (range))	0.33 (0.07–1.47)	0.35 (0.09–1.55)	0.313
AFP (ng/ml, < 200/≥200, n (%))	163 (66.0%)/84 (34.0%)	109 (60.6%)/71 (39.4%)	0.263
Prothrombin time (sec, median (range))	11.9 (9.6–16.9)	11.85 (9.6–15)	0.585
Differentiation (well and moderate/poor, n (%))	204 (82.6%)/43 (17.4%)	150 (83.3%)/30 (16.7%)	0.897
Tumor necrosis (absent/present, n (%))	105 (42.5%)/142(57.5%)	66 (36.7%)/114 (63.3%)	0.232
Tumor number (single/multiple, n (%))	205 (83.0%)/42 (17.0%)	148 (82.2%)/32 (17.8%)	0.897
Vascular invasion (absent/present, n (%))	204 (82.6%)/43 (17.4%)	135 (75.0%)/45 (25.0%)	0.069
Tumor size (cm, ≤5/ > 5, n (%))	162 (65.6%)/85 (34.4%)	104 (57.8%)/76 (42.2%)	0.107
TNM stage (I and II/III and IV, n (%))	229 (92.7%)/18 (7.3%)	153 (85.0%)/27 (15.0%)	**0.016**
BCLC stage (0 and A/B/C, n (%))	181 (73.3%)/23 (9.3%)/43 (17.4%)	107 (59.4%)/28(15.6%)/45 (25.0%)	**0.010**

SF: serum ferritin; HBV: hepatitis B virus; HCV: hepatitis C virus; ALT: alanine aminotransferase; AST: aspartate aminotransferase; NLR: neutrophil to lymphocyte ratio; AFP: alfa-fetoprotein; TNM: tumor node metastasis; BCLC: Barcelona Clinic Liver Cancer.

(P = 0.002), SF level (P = 0.025), tumor number (P = 0.025), vascular invasion (P < 0.001) and tumor size (P < 0.001) were found to be significant independent prognostic predictors associated with OS in validation cohort (Table 4). Analogously, albumin concentration (P = 0.041), NLR (P = 0.001), SF level (P = 0.022), tumor number (P = 0.042), vascular invasion (P < 0.001) and tumor size (P < 0.001) resulted as significant independent prognostic factors for RFS (Table 4). The details of Cox univariate/multivariate analyses of prognostic factors for OS and RFS in validation cohort were shown in Table 4.

The multivariate Cox proportional hazards analysis in total cohort indicated that SF level could predict OS (P = 0.002, HR = 1.646, 95%CI: 1.209–2.242) independent of albumin concentration (P = 0.002, HR = 1.703, 95%CI: 1.207–2.403), NLR (P = 0.002, HR = 1.107, 95%CI: 1.037–1.182), tumor number (P = 0.001, HR = 1.865, 95%CI: 1.285–2.708), vascular invasion (P < 0.001, HR = 2.970, 95%CI: 2.092–4.216) and tumor size (P < 0.001, HR = 2.242, 95%CI: 1.598–3.146), and predict RFS (P < 0.001, HR = 1.570, 95%CI: 1.221–2.020) independent of albumin concentration (P = 0.025, HR = 1.375, 95%CI: 1.040–1.817), NLR (P = 0.008, HR = 1.075, 95%CI: 1.019–1.133), tumor number (P < 0.001, HR = 1.800, 95%CI: 1.320–2.453), vascular invasion (P < 0.001, HR = 3.221, 95%CI: 2.380–4.360) and tumor size (P < 0.001, HR = 2.371, 95%CI: 1.804–3.115) (Table 5). The details of Cox univariate/multivariate analyses of prognostic factors for OS and RFS in total cohort were summarized in Table 5.

Table 3
Cox univariate/multivariate analyses for overall and recurrence-free survival in training cohort (n = 205).

Variables	Overall Survival				Recurrence-Free Survival			
	Univariate	Multivariate analyses			Univariate	Multivariate analyses		
	P-value	HR	95%CI	P-value	P-value	HR	95%CI	P-value
Age (years)	0.851	1.004	0.984–1.025	0.675	0.900	1.003	0.987–1.019	0.688
Gender (Female/Male)	0.403	0.599	0.313–1.148	0.123	0.951	0.878	0.502–1.534	0.647
Cirrhosis (present/absent)	0.212				0.503			
ALT (IU/L, > 45/≤45)	0.409				0.441			
AST (IU/L, > 45/≤45)	0.156				0.190			
Albumin (g/L, ≤40/ > 40)	**< 0.001**	**2.590**	**1.543–4.350**	**< 0.001**	**< 0.001**	**1.657**	**1.102–2.492**	**0.015**
Total bilirubin (umol/L, > 28/≤28)	0.718				0.650			
Platelet count (10^9/L)	0.394				0.670			
NLR (≥2.31/ < 2.31)	**0.031**	**1.093**	**1.002–1.193**	**0.044**	0.194			
Monocyte count (10^9/L)	0.164				0.557			
AFP (ng/ml, ≥200/ < 200)	0.425				0.072			
SF (ng/mL, > 267/≤267)	**0.011**	**1.637**	**1.028–2.605**	**0.038**	**0.004**	**1.738**	**1.190–2.538**	**0.004**
Prothrombin time (sec)	0.111				0.085			
Differentiation (poor/well and moderate)	**0.006**	**1.834**	**1.044–3.224**	**0.035**	**0.003**	1.463	0.938–2.282	0.093
Tumor necrosis (present/absent)	0.664				0.678			
Tumor number (multiple/single)	**0.002**	1.622	0.926–2.839	0.091	**< 0.001**	**1.657**	**1.047–2.621**	**0.031**
Vascular invasion (present/absent)	**< 0.001**	**3.304**	**1.865–5.856**	**< 0.001**	**< 0.001**	**3.087**	**1.909–4.990**	**< 0.001**
Tumor size (cm, > 5/≤5)	**0.002**	1.617	0.986–2.650	0.057	**< 0.001**	**2.160**	**1.473–3.166**	**< 0.001**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; AFP: alfa-fetoprotein; NLR: neutrophil to lymphocyte ratio; HR: hazard ratio; CI: confidence interval; SF: serum ferritin.
For binary variables, previous data in parentheses is the basis of comparison.

Table 4
Cox univariate/multivariate analyses for overall and recurrence-free survival in validation cohort (n = 222).

Variables	Overall Survival				Recurrence-Free Survival			
	Univariate	Multivariate analyses			Univariate	Multivariate analyses		
	P-value	HR	95%CI	P-value	P-value	HR	95%CI	P-value
Age (years)	0.053	0.999	0.982–1.015	0.865	0.136	0.999	0.984–1.015	0.940
Gender (Female/Male)	**0.017**	**0.456**	**0.233–0.895**	**0.022**	0.067	0.651	0.397–1.067	0.088
Cirrhosis (present/absent)	0.118				0.498			
ALT (IU/L, > 45/≤45)	0.717				0.647			
AST (IU/L, > 45/≤45)	0.183				**0.044**	1.043	0.786–1.626	0.667
Albumin (g/L, ≤40/ > 40)	**0.001/ < 0.001**	**1.422**	**1.067–2.117**	**0.046**	**0.001/ < 0.001**	**1.401**	**1.058–2.001**	**0.041**
Total bilirubin (umol/L, > 28/≤28)	0.423				0.064			
Platelet count (10^9/L)	0.208				0.397			
NLR (≥2.31/ < 2.31)	**0.006**	**1.238**	**1.082–1.416**	**0.002**	**0.002**	**1.220**	**1.085–1.371**	**0.001**
Monocyte count (10^9/L)	**0.036**	1.615	0.571–4.567	0.366	**0.039**	1.711	0.669–4.186	0.239
AFP (ng/ml, ≥200/ < 200)	**0.017**	1.083	0.708–1.659	0.713	0.891			
SF (ng/mL, > 267/≤267)	**< 0.001**	**1.636**	**1.064–2.516**	**0.025**	**0.005**	**1.571**	**1.086–2.437**	**0.022**
Prothrombin time (sec)	0.180				0.286			
Differentiation (poor/well and moderate)	0.943				0.881			
Tumor necrosis (present/absent)	**< 0.001**	0.903	0.538–1.518	0.701	**< 0.001**	1.207	0.783–1.860	0.394
Tumor number (multiple/single)	**0.038**	**1.832**	**1.079–3.109**	**0.025**	**0.041**	**1.601**	**1.017–2.529**	**0.042**
Vascular invasion (present/absent)	**< 0.001**	**3.254**	**2.026–5.228**	**< 0.001**	**< 0.001**	**3.754**	**2.480–5.683**	**< 0.001**
Tumor size (cm, > 5/≤5)	**< 0.001**	**2.988**	**1.779–5.020**	**< 0.001**	**< 0.001**	**2.399**	**1.580–3.643**	**< 0.001**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; AFP: alfa-fetoprotein; NLR: neutrophil to lymphocyte ratio; HR: hazard ratio; CI: confidence interval; SF: serum ferritin.
For binary variables, previous data in parentheses is the basis of comparison.

However, in multivariate Cox proportional hazards analysis, TNM and BCLC stage were not included since they were composed of tumor number, tumor size and vascular invasion. Otherwise, the disturbance of collinearity factors and clinical bias may increase.

To sum up, the above findings proved that preoperative SF was a significant independent prognostic predictor for both OS and RFS.

3.5. Survival analysis

By the final follow up date, 251 patients (58.78%) encountered with confirmed HCC recurrence and 171 patients (40.05%) died. The median follow-up time was 45 months (range: 2–101 months). In total cohort, the OS rate was 91.5%, 71.7%, 60.2% at 1, 3, and 5 years, respectively, and the RFS rate was 71.0%, 46.0%, 39.4% at 1, 3, and 5 years, respectively.

In training cohort, patients in low SF group had better OS and RFS rates than those in high SF group (OS: P = 0.009, RFS: P = 0.003) (Fig. 2A and B). As shown in Fig. 2C and D, similar situation was confirmed in validation cohort: primary survival outcomes (OS and RFS) were advantageous in the patients with a lower SF level (OS: P < 0.001, RFS: P = 0.004) (Fig. 2C and D). In total cohort, the OS and RFS rates in low SF group were significantly better than those in high SF group (both P < 0.001). The 1-, 3-, and 5-year OS and RFS rates were 91.4%, 80.1%, 71.7%, and 78.0%, 53.0%, 47.3% in low SF group, and

Table 5
Cox univariate/multivariate analyses for overall and recurrence-free survival in total cohort (N = 427).

Variables	Overall Survival				Recurrence-Free Survival			
	Univariate	Multivariate analyses			Univariate	Multivariate analyses		
	P-value	HR	95%CI	P-value	P-value	HR	95%CI	P-value
Age (years)	0.148	1.001	0.988–1.014	0.842	0.292	1.000	0.989–1.011	0.961
Gender (Female/Male)	0.184	0.853	0.542–1.342	0.491	0.170	0.850	0.589–1.226	0.384
Cirrhosis (present/absent)	0.052				0.355			
ALT (IU/L, > 45/≤45)	0.816				0.396			
AST (IU/L, > 45/≤45)	0.062				**0.022**	1.133	0.878–1.464	0.337
Albumin (g/L, ≤40/ > 40)	**< 0.001**	**1.703**	**1.207–2.403**	**0.002**	**< 0.001**	**1.375**	**1.040–1.817**	**0.025**
Total bilirubin (umol/L, > 28/≤28)	0.403				0.091			
Platelet count (10^9/L)	0.139				0.360			
NLR (≥2.31/ < 2.31)	**0.002**	**1.107**	**1.037–1.182**	**0.002**	**0.007**	**1.075**	**1.019–1.133**	**0.008**
Monocyte count (10^9/L)	**0.014**	1.529	0.768–3.044	0.227	0.059			
AFP (ng/ml, ≥200/ < 200)	**0.020**	0.986	0.718–1.353	0.930	0.176			
SF (ng/mL, > 267/≤267)	**< 0.001**	**1.646**	**1.209–2.242**	**0.002**	**< 0.001**	**1.570**	**1.221–2.020**	**< 0.001**
Prothrombin time (sec)	**0.034**	1.107	0.949–1.293	0.196	**0.047**	1.116	0.988–1.261	0.079
Differentiation (poor/well and moderate)	0.060				**0.035**	1.082	0.785–1.491	0.630
Tumor necrosis (present/absent)	**0.003**	1.011	0.719–1.422	0.950	**< 0.001**	1.026	0.777–1.355	0.857
Tumor number (multiple/single)	**< 0.001**	**1.865**	**1.285–2.708**	**0.001**	**< 0.001**	**1.800**	**1.320–2.453**	**< 0.001**
Vascular invasion (present/absent)	**< 0.001**	**2.970**	**2.092–4.216**	**< 0.001**	**< 0.001**	**3.221**	**2.380–4.360**	**< 0.001**
Tumor size (cm, > 5/≤5)	**< 0.001**	**2.242**	**1.598–3.146**	**< 0.001**	**< 0.001**	**2.371**	**1.804–3.115**	**< 0.001**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; AFP: alfa-fetoprotein; NLR: neutrophil to lymphocyte; HR: hazard ratio; CI: confidence interval; SF: serum ferritin.
For binary variables, previous data in parentheses is the basis of comparison.

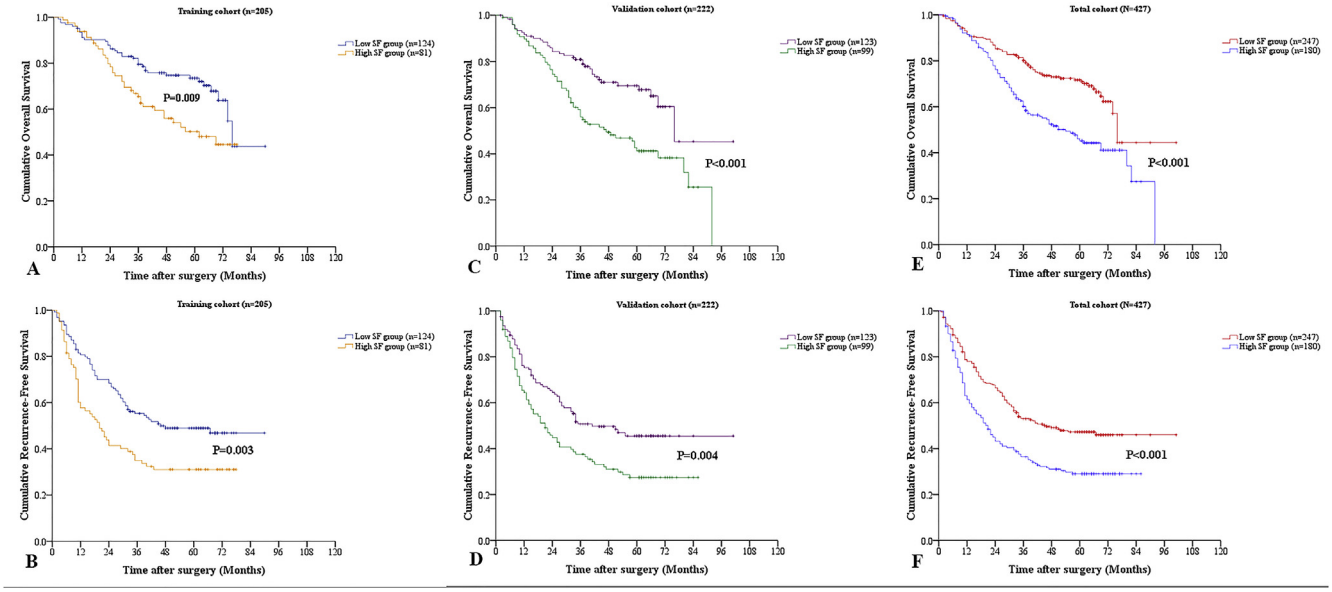

Fig. 2. Comparison of overall survival (OS) and recurrence-free survival (RFS) rates between low serum ferritin (SF) group and high SF group by Kaplan-Meier survival curves. A: OS curve of training cohort. B: RFS curve of training cohort. C: OS curve of validation cohort. D: RFS curve of validation cohort. E: OS curve of total cohort. F: RFS curve of total cohort. High preoperative SF level predicted poor survival outcomes in hepatocellular carcinoma patients who received curative hepatectomy.

91.6%, 60.2%, 45.2%, and 61.3%, 36.4%, 29.0% in high SF group, respectively (Fig. 2E and F). In brief, elevated SF level was associated with worse OS and RFS in all study cohorts (Fig. 2).

3.6. Subgroup analysis

In the previous section we found that lower preoperative SF level was closely correlated with better TNM stage (P = 0.016) and BCLC stage (P = 0.010) (Table 2). Therefore, we performed subgroup analysis to assess the prognostic value of SF in TNM stage I and II subgroup and BCLC stage 0 and A subgroup, respectively. As shown in Fig. 3A and B, in BCLC stage 0 and A subgroup, patients with low SF level had a prolonged OS (P = 0.002) and RFS (P = 0.015) rather than those patients with high SF level (Fig. 3A and B). Similar results that low SF level was linked with better OS (P < 0.001) and RFS (P < 0.001) were also confirmed in TNM stage I and II subgroup (Fig. 3C and D). The data of subgroup analysis indicated that preoperative SF acted as a useful independent prognostic factor even in better TNM stage and BCLC stage subgroups, which also increases the strength of our research.

4. Discussion

SF is an iron storage protein that plays a key role in keeping iron balance [29–38]. Not only is an elevated SF symbolic of body iron overload, infectious diseases, organ dysfunction, inflammation, many liver diseases (virus hepatitis, steatohepatitis, severe hepatopathy and cirrhosis), it is also relevant to tumor [29–39]. In 1977, Hazard and Drysdale first found that ferritin had a close relationship with cancer [37,38]. From then on, the relationship between SF and cancer has drawn great attention and been studied. Kalousová M et al. tested the significance of SF in the prognosis of patients with pancreas cancer and found that SF was an independent mortality predictor and high level of SF at the time of diagnosis of pancreas cancer indicated bad prognosis [36]. In the same year, Jézéquel P et al. studied 268 node-negative breast cancer patients, and then they reported that tumor-associated macrophage ferritin light chain had a predictive value in such patients [40]. In 2014, SF as an adverse prognostic marker for tumor patients was confirmed by two studies [33,35]. In 103 HCC patients treated with percutaneous RFA, SF could predict prognosis and was a reversed risk factor for survival and recurrence [33]. Shi et al. also found that SF levels in healthy subjects were significantly lower than patients with advanced non-small cell lung cancer and SF level had a very close relationship with distant metastasis and regional lymph node metastasis [35]. Moreover, the overall response rate to platinum-based chemotherapy was significantly higher in normal SF group when compared with high SF group, which confirmed the prognostic value of SF in predicting the tumor progression for advanced non-small cell lung cancer patients [35]. In the last two years, the fact that an elevated SF level in patients with malignant tumors is associated with poor survival outcomes was also proved in colorectal cancer and peripheral T-cell lymphoma [30,37,39]. In a word, the above evidence fully embodied the prognostic value of SF in tumor patients.

However, as far as we know, no study has studied the prognostic role of SF in HCC patients who underwent curative resection. Therefore, for the first time, we decided to explore this subject. The patients' baseline and clinicopathologic data between two cohorts were compared and no significant difference was found. As a novel prognostic indicator, there is no ready-made optimal cut-off value of SF. Thus the first step in our analysis was to determine the best cut-off value by ROC analysis. We performed ROC analysis in training cohort, validation cohort and total cohort, respectively, and finally defined 267 ng/ml as the best cut-off point of SF in our study. Different from us, Facciorusso et al. used 244 ng/ml as the best cut-off point of SF [33]. The reason for this difference can be attributed to different study populations and treatment measures. Be that as it may, our cut-off point of SF was very close to Facciorusso's result, which may mean that the optimal cut-off value of SF that suitable for HCC patients verges on our results. Then we investigated the correlation between SF level and clinicopathological variables, and found that TNM and BCLC stage were closely related to preoperative SF rather than other clinicopathological variables. While Facciorusso et al. reported that SF did not correlate with BCLC stage in HCC patients treated with RFA [33]. In their study, only patients who were BCLC stage B, not suitable for hepatectomy or liver transplantation and treated with RFA were included [33]. Thus, we inferred that different inclusion criterias and treatment measures may explain this discordant result. But the same thing as our research is that Facciorusso et al. also found other laboratory indices, such as NLR and AFP, were

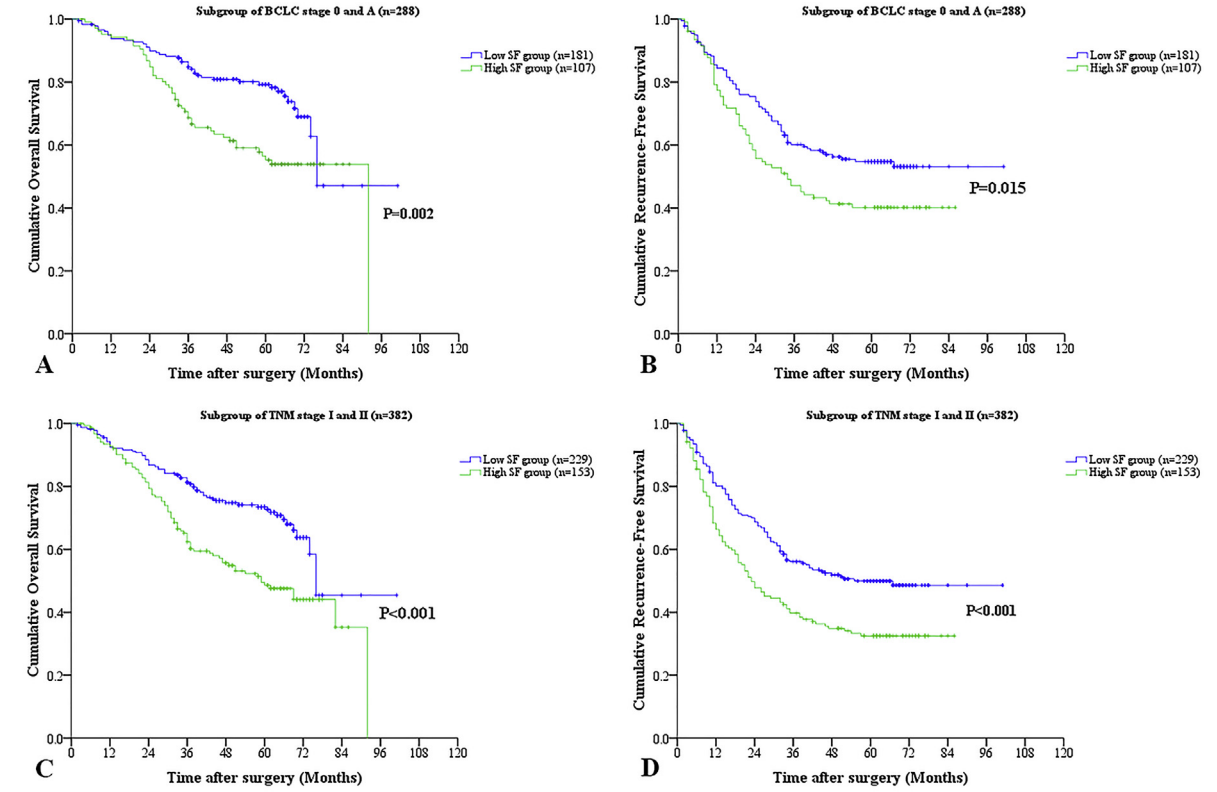

Fig. 3. Comparison of overall survival (OS) and recurrence-free survival (RFS) rates between low serum ferritin (SF) group and high SF group by Kaplan-Meier survival curves in different subgroups. A: OS curve of BCLC stage 0 and A subgroup. B: RFS curve of BCLC stage 0 and A subgroup. C: OS curve of TNM stage I and II subgroup. D: RFS curve of TNM stage I and II subgroup. High preoperative SF level also predicted poor survival outcomes in different subgroups.

not related to SF [33]. Similarly, Shi et al. also reported that there was a statistically significant difference between distant and regional lymph node metastasis and SF levels, and no correlation was found between SF levels and other clinical characteristics [35]. Our findings were not in contradiction with the above results, which suggests that SF may be a specific marker that can reflect the progress of tumor disease independent of other clinicopathological indicators and has a close correlation with the formation and development of tumor. After that, the multivariate Cox proportional hazards analysis was carried out to determine independent prognostic factors for OS and RFS in each cohort, and our results revealed that preoperative SF level resulted as a significant independent prognostic factor that could predict both OS and RFS independent of other prognostic predictors in all cohorts (training cohort, validation cohort and total cohort). Finally, our survival analysis in any cohort all indicated that patients with a low SF were more likely to have both favorable OS and RFS, even in our subgroup analysis, and vice versa. In addition, our results were also consistent with above evidences [30,33,35–37,39,40], and we concluded that our findings were credible. All in all, the above evidence along with our findings proved the fine prognostic value of SF in tumor patients from the clinical point of view.

Although the underlying biological mechanisms of the prognostic significance of SF remain unclear, we can still get some evidence of basic medical research and inspiration from existing studies. Iron is very important to cell proliferation and DNA synthesis, and tumor cells require more iron than normal cells due to the differences of cell reproductive capacity [39]. Some studies reported that iron levels had something to do with the formation and progress of tumor, and iron overload may result in cancer including HCC [33,41–43]. The metabolism and storage of iron is mainly carried out by the liver, thus iron homeostasis cannot be maintained when various liver diseases occur [41]. Except for storing iron, keeping iron homeostasis and reflecting body iron level, SF is also involved in immunoregulation, angiogenesis and tumor proliferation [29–34,37]. Furthermore, an elevated SF caused by tumor can be attributed to two aspects. On the one hand, macrophages and hepatocytes can lead to SF synthesis, and ferritin can be over-expressed in tumor-associated macrophages [29–31,39]. For instance, in breast cancer, tumor-associated macrophages can secrete ferritin, which can result in a rise in SF by affecting the expression of related molecules through a series of complex mechanisms and causing stromal reactions [30]. On the other hand, some tumor tissue can secrete SF directly [30,44]. For example, some scholars had confirmed that human ferritin could be detected in nude mice bearing human neuroblastoma xenografts, and SF level returned to normal when the tumor was removed [30,44]. Another example is that patients with metastatic tumors often had higher SF than patients without metastasis and SF usually dropped after surgery [30,44]. In addition, apart from iron overload, an elevated SF usually is seen in some severe liver diseases [29–34]. There are many reasons for this phenomenon, but the most important one is the lack of hepcidin. Hepcidin, an iron-regulating hormone secreted by the liver, has the function of regulating the concentration of ferritin and iron [29–34]. Hepcidin is deficiency when liver dysfunction occurs, which spontaneously lead to a rise in SF [29–34]. An increased SF as also able to cause liver damage and increase the risk of HCC, thus patients who trapped in this vicious cycle often have poor prognosis [29–39,41–43]. Consequently, the underlying mechanisms of SF could predict survival outcomes in tumor patients may be reasonably explained.

Several limitations regarding our present study must be recognized. First, although our study suggested a cut-off value of SF, all data was collected from a single medical center and relevant biases were inevitable. Second, our research was a retrospective study, and selective bias, withdraw bias and other clinical bias were also inevitable. Third, basic medical research was not performed to investigate the underlying

biological mechanisms of the prognostic significance of SF. However, our study for the first time proved the prognostic value of SF in surgically treated HCC patients and provided evidence and inspiration for future research. In the future, multicenter randomized controlled trials are needed to prove our findings and basic medical studies are also urgently needed to clarify the specific mechanisms on this topic.

As a whole, inexpensive, convenient and reliable marker, preoperative SF was a significant independent prognostic factor for both OS and RFS in HCC patients who received curative hepatectomy. Elevated preoperative SF signified poor survival outcomes in such patients. Patients who may have poor prognosis can be identified for appropriate individual treatments before surgery by this prognostic predictor.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.suronc.2019.05.013.

Author contributions

Si-Jia Wu, Zun-Zhen Zhang and Luo Yang designed this study; Si-Jia Wu wrote the manuscript; Si-Jia Wu, Nan-Sheng Cheng and Xian-Ze Xiong collected and analyzed data; Si-Jia Wu, Nan-Sheng Cheng and Xian-Ze Xiong critically revised the content; Si-Jia Wu, Zun-Zhen Zhang and Luo Yang approved the final version of the manuscript.

Conflicts of interest

Si-Jia Wu, Zun-Zhen Zhang, Nan-Sheng Cheng, Xian-Ze Xiong and Luo Yang have no potential conflicts of interest.

Disclosure

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