Research Paper

Systemic inflammation score predicts survival in patients with intrahepatic cholangiocarcinoma undergoing curative resection

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Abstract

Inflammation has a critical role in the development and progression of cancers. We developed a novel systemic inflammation score (SIS) based on lymphocyte, monocyte, and CA19-9 and explored its prognostic value in intrahepatic cholangiocarcinoma (ICC). From January 2005 to December 2011, 322 consecutive ICC patients who underwent curative resection in our center were included in this study, and validated in a retrospective study of 126 patients enrolled from 2012 to 2014. Clinicopathological variables including preoperative serum CA19-9 and LMR were analyzed. The cutoff values of CA19-9 and LMR were determined based on receiver operating characteristics curve analysis in the primary cohort. Kaplan-Meier curves and multivariate Cox-regression analyses were calculated for time to recurrence (TTR) and overall survival (OS). In univariate analysis of all patients, all three inflammatory and tumor marker including NLR ≥ 2.49 (P<0.001), LMR ≤ 4.45 (P=0.002), and CA19-9≥89 (P<0.001) were associated with poor prognoses. When omitting SIS in multivariate analysis, preoperative LMR (P =0.006) and serum CA19-9 (P<0.001) were independent predictors of OS. In addition, elevated CA19-9 (P=0.001), multiple tumors (P<0.001), and lymph node metastasis (P<0.001) were significant predictors of worse recurrence free survival. Moreover, high SIS was significantly associated with aggressive tumor behaviours including large tumor size (P<0.001), multiple tumors (P=0.033), lymphonodus node metastasis (P=0.001), and high TNM stage (P<0.0001). Finally, univariate and multivariate analyses revealed the SIS was an independent predictor for TTR (HR=2.077, 95% CI, 1.365-3.162, P=0.001) and OS (HR=3.133 95% CI, 2.058-4.769, P<0.001). These results were further confirmed in the validation cohort. In conclusions, our findings demonstrate that the SIS as a potentially powerful prognostic biomarker in ICC that predicts poor clinical outcomes and is a promising tool for ICC treatment strategy decisions.

Key words: lymphocyte-monocyte ratio, CA19-9, systemic inflammation, intrahepatic cholangiocarcinoma, liver resection, prognosis

Introduction

Intrahepatic cholangiocarcinoma (ICC), originating from the intrahepatic biliary tree, is the second most common primary hepatic malignancy tumor. It accounts for 5% to 30% of all primary liver cancers [1, 2]. The incidence and mortality of ICC has been rapidly increasing in the United States [3, 4]. ICC

is one of the most fatal cancers, surgical resection remains the mainstay of curative intent treatment for ICC [5, 6]. Unfortunately, prognosis after partial hepatic resection is unsatisfactory, with 5-year survival rates of 30% to 40% after hepatectomy and ICC patients with unresectable disease have a median survival of only 12-15 months [7-9]. Owing to treatment-refractory disease with dismal outcome, the early detection of ICC and identification prognostic and protective factors are beneficial for improving ICC patients' outcome.

In the last decade, cancer-related inflammation has been verified to be involved in cancer development and progression, and in particular facilitates tumor cell proliferation, survival, angiogenesis, and metastasis [10, 11]. Most ICC patients have a background of hepatitis B virus and/or hepatitis C virus infection, choledocholithiasis, and primary sclerosing cholangitis [12-14], suggesting a potential link between chronic inflammation and ICC development. Therefore, studies on the mechanisms of cancer-related inflammation and tumor progression in ICC remain to be extensively investigated.

Cancer-related inflammation has emerged as the seventh hallmark of cancers, and the key determinant effects of tumor-associated innate immune cells have been suggested in a numerous cancers [15, 16]. There is recent evidence that systemic inflammatory response, such as neutrophil–lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR), modified Glasgow prognostic score (mGPS), prognostic nutritional index (PNI), and C-reactive protein (CRP) had been proposed and investigated as prognostic biomarkers in patients with several solid tumors [17-24]. In this study, we created a novel prognostic score named systemic inflammation score (SIS) basing on preoperative serum CA19-9 and LMR, which were both verified to be independently associated with ICC patients' outcomes. Furthermore, the prognostic utility of the SIS in ICC patients who underwent curative resection was evaluated in two independent cohorts. We found that the SIS was a promising independent predictive factor for prognosis of patients with ICC after curative surgical resection and that the increased recurrence rate in patients with high SIS score.

Materials and Methods

Patients and follow up

This retrospective study included 322 consecutive patients with ICC (the primary cohort, *n*=322), who underwent curative intent hepatic resection at our institution (Liver Surgery Department of Zhongshan Hospital, Fudan University, Shanghai, China) between January 2005 and December 2011. From 2012 to 2014, a validation cohort of patients with ICC (the validation cohort, *n*=126) undergoing resection was recruited. The eligibility criteria were as follows: complete resection of macroscopic liver tumors and histopathological diagnosis of ICC, no history of previous anti-cancer therapies and other malignancies, availability of data on all clinical and laboratory features and treatments given, as well as outcome, and follow-up. Patients who underwent preoperative therapies (e.g., transcatheter arterial chemoembolization, percutaneous ethanol injection, or, radiofrequency ablation) were excluded from this study. Part of the physical examinations, serum tumor markers included CA19-9, and α-fetoprotein (AFP), routine blood and liver function tests were performed within one week prior to surgery. The Research Ethics Committee of Zhongshan Hospital approved this study, and written informed consent was obtained from all patients. The study was censored on August 1, 2018.

Follow-up Strategy and Recurrence Pattern

The patients follow-up and postoperative treatment were administrated as described previously according to our established guidelines [25, 26]. Briefly, after discharge, all patients were followed up once every two months for the first two years after hepatectomy and thereafter every three to six months interval or until death. At each of the follow-up visits, peripheral blood was routinely taken for routine blood, liver function tests and serum tumor markers including CA19-9, and AFP, and an abdominal ultrasound was performed. Contrast-enhanced CT or MRI was performed once every six months or earlier if tumor recurrence or metastasis was suspected. Further investigation was carried out when clinically indicated, such as positron emission tomography CT (PET-CT). While ICC recurrence was being confirmed, a second hepatectomy, radiofrequency ablation, percutaneous ethanol injection, transcatheter arterial chemoembolization, or external radiotherapy were administered according to the number, size, and site of the recurrent tumors [27]. Overall survival (OS) and time to recurrence (TTR) were used as primary end points. OS was calculated from the dates of operation to the dates of death or the dates of last follow-up. TTR was calculated from the interval between the dates of operation and the first recurrence or from the dates of operation to the dates of last follow-up (for the patients without recurrence).

Statistical Analysis

Statistical analyses were assessed using SPSS 19.0 software (SPSS, Chicago, IL, USA). Data were presented as the means ± standard deviation. The

optimal cutoff values of pretreatment LMR and CA19-9 were evaluated using receiver operating curve (ROC) analysis. Categorical data were analyzed by the $\chi2$ test or Fisher's exact test. Kaplan-Meier survival curves were used for calculating the OS and TTR. Univariate and multivariate analyses were performed using the Cox proportional hazards regression model. Differences were considered statistically significant at *P*<0.05.

Results

Patient Characteristics

The clinicopathologic features of ICC patients were presented in Table 1. In the training cohort, the mean age of the patients was 57.9 years (range, 27.0-81.0 years); the hepatitis etiology was the hepatitis B virus in 38.2% of the patients and the hepatitis C virus in 0.62% the patients; and 28.6% of the patients exhibited either macrovascular invasion (14.3%) or lymphonodus node metastasis (17.4%). The median follow-up time was 44.0 months (range, 2.7-100.5 months), 187 (58.1%) patients had died and 191 (59.3%) patients were confirmed with tumor recurrence at last follow-up. The postoperative 1-, 3-, and 5-year survival rates were 75.0 %, 47.8%, and 35.2%, respectively. The postoperative 1-, 3-, and 5-year recurrence free survival rates were 56.6 %, 39.0 %, and 32.3 %, respectively (Fig. 1).

In the validation cohort (*n*=126), the median follow-up time was 38.3 months (range, 1.0 to 80 months). The median TTR was 18 months (range, 2 to 76 months), and the postoperative 1- and 3-year recurrence rates were 43.1 % and 63.1%, respectively. The median OS time was 29.3 months (range, 3.5 to 80 months), and the 1-and 3-year OS rates were 72.8% and 45.3%, respectively. The clinicopathologic characteristics were similar between the two cohorts, with the exception of HBsAg and AFP. The primary cohort included more HBsAg and AFP than those in the validation cohort (Supplementary Table 1).

The Optimal Cut-Offs of LMR and CA19-9

Next, a LMR of 4.45 was the optimal cutoff point for postoperative prognosis, using time-dependent receiver operating characteristic curve. Therefore, all the patients were stratified into two groups: a low (<4.45) LMR group (*n*=210) and a high (≥4.45) LMR group (*n*=112). Similarly, the optimal cutpoint of 89 U/ml was identified for the CA19-9. High and low groups were created as for the LMR using the same method.

Table 1. Correlation between the factors and clinicopathologic characteristics in ICC (Cohort 1, *n*=322)

Clinicopathological Indexes		LMR			CA19-9			SIS			
		low	high	*P*	low	high	*P*	I	II	III	*P*
Age(year)	≤50	23	62	**0.000**	57	28	0.782	23	40	22	0.953
	>50	187	50		155	82		61	116	60	
Sex	female	76	52	0.074	74	54	0.138	39	58	31	0.346
	male	134	60		128	66		45	98	51	
HBsAg	negative	135	64	0.21	125	74	0.146	49	88	62	**0.011**
	positive	75	48		87	36		35	68	20	
HCV	negative	209	111	1*	211	109	1*	84	155	81	0.746*
	positive	1	1		1	1		0	1	1	
AFP (ng/ml)	≤20	186	100	0.846	188	98	0.911	84	128	74	**0.000***
	>20	24	12		24	12		0	28	8	
Child-Pugh	A	202	109	0.753*	208	103	**0.05***	83	151	77	0.183*
	B or C	8	3		4	7		1	5	5	
Liver cirrhosis	no	156	80	0.581	149	87	0.090	58	113	65	0.317
	yes	54	32		63	23		26	43	17	
Tumor size (cm)	≤5	90	55	0.283	115	30	**0.000**	46	77	22	**0.000**
	>5	120	57		97	80		38	79	60	
Tumor number	single	163	81	0.291	164	80	0.358	72	110	62	**0.033**
	multiple	47	31		48	30		12	46	20	
Lymphonodus node metastasis	yes	41	15	0.167	25	31	**0.000**	5	29	22	**0.001***
	no	169	97		187	79		79	127	60	
Microvascular invasion	yes	33	13	0.316	29	17	0.666	9	24	13	0.562
	no	177	99		183	93		75	132	69	
Tumor differentiation[a]	poor	46	22	0.903	49	19	0.207	17	37	14	0.249
	moderated	130	71		133	68		52	100	49	
	well	34	19		30	23		15	19	19	
TNM stage[b]	I+II	155	93	0.061	178	70	**0.000**	75	121	52	**0.000**
	III+IVA	55	19		34	40		9	35	30	

Boldface type indicates significant values.

Abbreviations: AFP, alpha-fetoprotein; CA19-9, carbohydrate antigen 19-9; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; LMR, lymphocyte-to-monocyte ratio; TNM, tumor-node-metastasis; SIS, combination of preoperative serum CA19-9 and LMR. I, CA19-9[Low]/LMR[High]; II, CA19-9 [High]/LMR[High] and CA19-9 [Low]/LMR [Low]; III, CA19-9 [High]/LMR [Low].

[a] Tumor differentiation was determined according to the "British Society of Gastroenterology guidelines on the management of cholangiocarcinoma".

[b] TNM stage: American Joint Committee on Cancer 7th edition staging for intrahepatic cholangiocarcinoma. *Fisher's exact tests; chi-square tests for all other analyses.
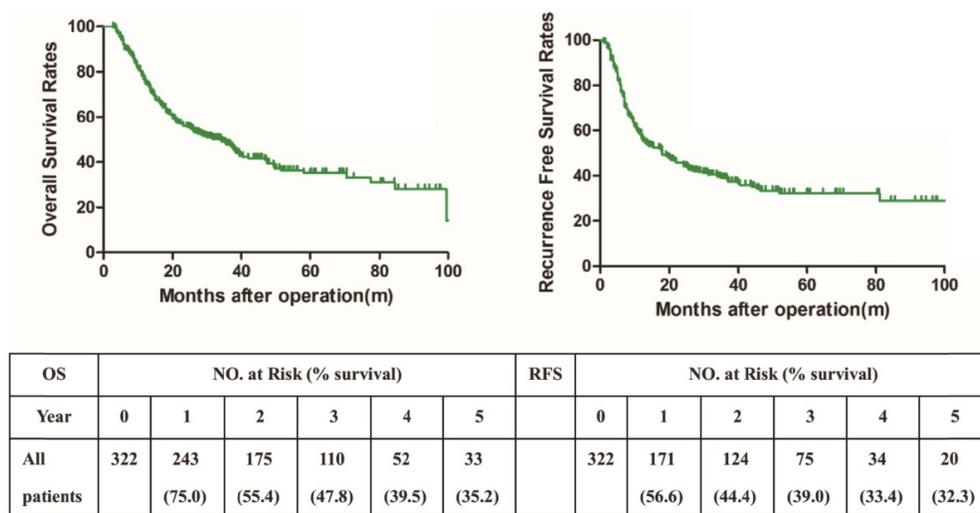
OS	NO. at Risk (% survival)						RFS	NO. at Risk (% survival)					
Year	0	1	2	3	4	5		0	1	2	3	4	5
All patients	322	243 (75.0)	175 (55.4)	110 (47.8)	52 (39.5)	33 (35.2)		322	171 (56.6)	124 (44.4)	75 (39.0)	34 (33.4)	20 (32.3)

Figure 1. Kaplan-Meier curve showing overall survival and recurrence-free survival of patient undergoing curative resection for ICC (Cohort 1, n=322).

For the CA19-9, 212 (65.8%) patients were in the low group, whereas 110 (34.2%) were in the high group. In addition, the discrimination ability of inflammatory and tumor markers were compared by the area under the ROC curve (AUC) for OS. The AUCs for LMR and CA19-9 were 0.57 (95% CI, 0.51-0.64, P=0.034), and 0.63 (95% CI, 0.57-0.69, P=0.0001), respectively (Supplementary Fig. 1).

Associations of LMR, CA19-9, and SIS with clinicopathological parameters

The relationships between the LMR, CA19-9, and SIS and clinicopathologic parameters are summarized in Table 1. We found that a low LMR was associated with older age (P<0.001, Table 1). We noted that patients with a low LMR were likely to be older than 50 years (89.0%) compared to those with high LMR (44.6%). Scatter diagram was used to investigate the correlation between perioperative LMR and NLR. Scatter-plot analyses showed a significant negative linear correlation between perioperative LMR and NLR (n=322, r=-0.3870, P<0.0001; Supplementary Fig. 2). Specifically, high NLR was more likely in the low LMR group (72.4%) than in the high LMR group (22.3%). In addition, CA19-9≥89 U/ml was associated with high Child-Pugh scores (P=0.05), large tumor size (P<0.001), lymphonodus node metastasis (P<0.001), and high TNM stage (P<0.001, Table 1). An elevated SIS score was associated with HBsAg positive (P=0.011), high AFP level (P <0.001), large tumor size (P<0.001), and multiple tumors (P=0.033), lymphonodus node metastasis (P=0.001), and high TNM stage (P<0.0001, Table 1).

The prognostic significance of SIS in ICC patients in the training cohort

The Kaplan-Meier curves indicated that the high LMR was significantly associated with prolonged OS (P=0.0017, Fig. 2A) and RFS (P=0.0453, Fig. 2D). In addition, our results indicated that the 1-, 3-, and 5-OS rates in the LMR[High] ICC patients were significantly increased than the survival rates in the LMR[Low] patients (82.0% versus 71.3%, 62.2 % versus 41.1%, and 43.7% versus 31.0%, respectively; P=0.0017, Fig. 2A). Similarly, LMR[Low] ICC patients had the worse prognosis at 1-, 3-, and 5-years, with worse RFS rates than LMR[High] patients (53.1 versus 62.8%, 35.8% versus 44.7%, and 27.8% versus 40.3%, respectively; P=0.0453, Fig. 2D).

The Kaplan-Meier curves revealed that the high CA19-9, and SIS score were both significantly associated with shorter OS (P<0.0001, and P<0.0001, respectively; Fig.2B and C) and RFS (P<0.0001, and P<0.0001, respectively; Fig. 2E and F). Our results revealed that for the low CA19-9 group, the 1-, 3-, and 5-year OS rates were 85.8%, 58.1%, and 44.2%, respectively. These results were significantly better compared with the high CA19-9 group who had rates of 54.3%, 27.8%, and 18.1% for 1-, 3-, and 5-year OS, respectively (P<0.0001, Fig.2B). The results for RFS rates for ICC patients in the low and high CA19-9 groups are shown in Fig. 4E. The 1-, 3-, and 5-year RFS rates were significantly lower in the high CA19-9 group (36.1%, 23.9%, and 20.5%, respectively) compared with the low CA19-9 group (66.6%, 46.2%, and 37.8%, respectively; P<0.0001, Fig. 2E). Thus, these data indicated that ICC patients after hepatic resection with high CA19-9 ≥89 U/ml should be closely monitored for ICC recurrence.

When evaluating the combined effect of preoperative serum CA19-9 and LMR on ICC prognosis, our results demonstrated that the 1-, 3-, and 5-year OS rates for CA19-9 [High]/LMR [Low] patients were 48.8%, 23.0%, and 15.6%, respectively, and were significantly lower than the OS rates in the

CA19-9^{Low}/LMR^{High} patients (85.7%, 65.7%, and 48.9%, respectively; Fig. 2C). The 1-, 3-, and 5-year RFS rates in the CA19-9^{Low}/LMR^{High} patients were 67.6%, 50.9%, and 45.3 %, respectively, which were significantly higher than those in the CA19-9 ^{High}/LMR ^{Low} patients (30.7%, 24.8%, and 19.9 %, respectively; Fig. 2F).

Results from our univariate analysis indicated that tumor size, tumor number, lymphonodus node metastasis, TNM stage, NLR , LMR, CA19-9, and SIS were prognostic factors of OS and TTR, multivariate analyses revealed that SIS were independent prognostic factors for both OS (HR=3.133 95% CI, 2.058-4.769, *P*<0.001; Table 2) and TTR (HR=2.077, 95% CI, 1.365-3.162, *P*=0.001; Table 2), together with multiple tumors and tumor-node metastasis (Table 2). Whereas age, gender, hepatitis history, AFP, Child-Pugh score, liver cirrhosis, and tumor differentiation had no prognostic significance for OS and TTR (Table 2). When omitting SIS in multivariate analysis, preoperative serum CA19-9 and LMR were both verified to be independently associated with ICC patients' prognosis (Table 3). Elevated LMR was associated with better OS (HR= 0.636, 95% CI: 0.461-0.878, *P*=0.006, Table 3). While, multiple tumors, lymphonodus node metastasis, high TNM stage, elevated NLR and CA19-9 were independent predictors of worse OS (Table 3). Meanwhile, patients with elevated CA19-9, multiple tumors, and lymphonodus node metastasis were significant predictors of worse

recurrence free survival (Table 3). In addition, the discrimination ability of the SIS, as assessed by AUC, was 0.652 (0.593-0.712, *P*< 0.001) for OS (Fig. 3), which was higher than other clinical indexes (CA19-9, LMR, NLR, and PLR) (Fig. 3). Previous study shown that PNI had been identified as independent prognostic factors in ICC advance patients [17]. However, we found that the PNI was not a promising independent predictive factor for prognosis of patients with ICC after surgery (Supplementary Fig. 3).

Validation of the SIS in an independent cohort

In the present study, the prognostic utilities of the preoperative LMR, serum CA19-9, and SIS were further confirmed in an independent validation cohort of patients with ICC (the validation cohort, *n*=126). The results were similar to those obtained from the training cohort (Fig. 2). The high LMR also significantly correlated with prolonged OS (*P*=0.0064; Fig. 4A) and RFS (*P*=0.0122; Fig. 4D). The high serum CA19-9 and SIS remained associated with shorter OS (*P*<0.0001 and *P*<0.01, respectively; Fig. 4B and 4C) and RFS (*P*<0.0001 and *P*<0.01, respectively; Fig. 4E and 4F). Univariate and multivariate analyses demonstrated that the high SIS was significantly correlated with shorter TTR (HR, 2.083; 95% CI, 1.091-3.976; *P*=0.026; HR, 3.946; 95% CI, 1.913-8.138, respectively; *P*< 0.0001) and OS (HR, 3.213; 95% CI, 1.365-7.565; *P*=0.008; HR, 9.666; 95% CI, 3.411-27.394; *P*< 0.0001, respectively; Supplementary Table 2).
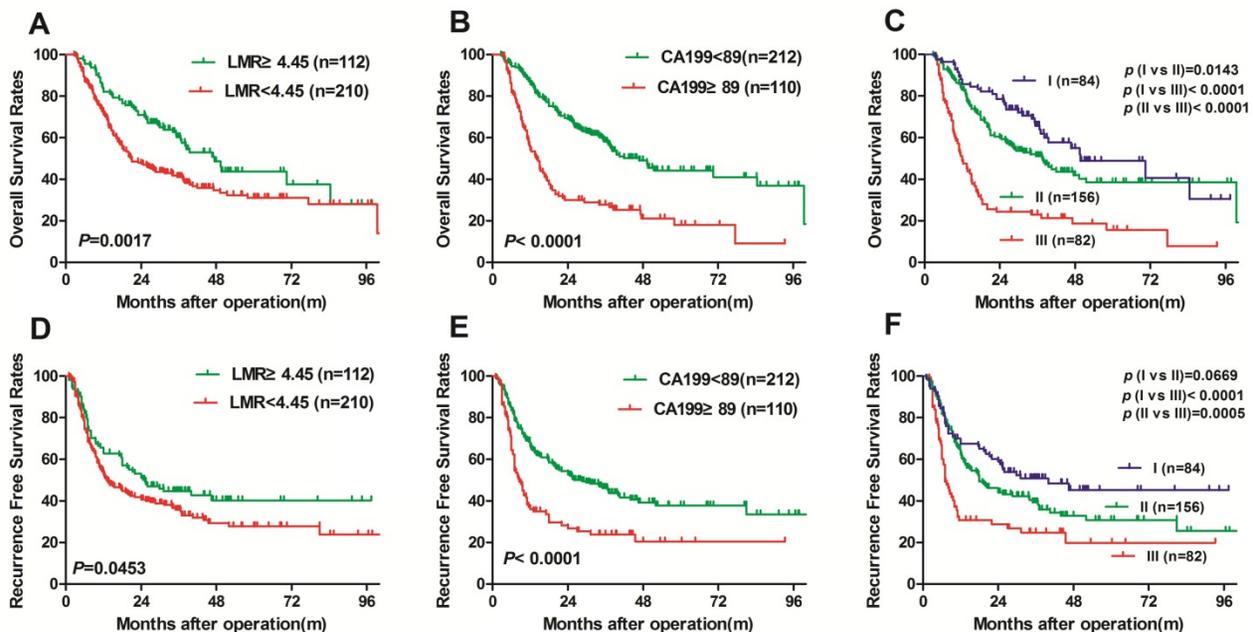
Figure 2. Prognostic values of preoperative LMR and serum CA19-9 using Kaplan-Meier analysis in the training cohort (Cohort 1, n=322). Kaplan-Meier analysis for overall survival according to (A) preoperative LMR, (B) preoperative serum CA19-9, (C) combination of preoperative LMR and serum CA19-9. Kaplan-Meier analysis for recurrence-free survival according to (D) preoperative LMR, (E) preoperative serum CA19-9, (F) combination of preoperative LMR and serum CA19-9. SIS, combination of preoperative LMR and serum CA19-9. I, CA19-9^{Low}/LMR^{High}; II, CA19-9 ^{High}/LMR^{High} and CA19-9 ^{Low}/LMR ^{Low}; III, CA19-9 ^{High}/LMR ^{Low}.

Table 2. Univariate and Multivariate analyses of prognostic factors in ICC (Cohort 1, *n*=322)

Variable	TTR		OS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Univariate analysis				
Age, year (≤50 vs. >50)	1.160 (0.841- 1.598)	0.366	1.079 (0.780- 1.493)	0.647
Sex (female vs. male)	1.063 (0.794- 1.422)	0.683	1.143 (0.850- 1.538)	0.376
HBsAg (negative vs. positive)	0.660 (0.271- 1.171)	0.360	1.090 (0.505- 2.352)	0.826
HCV (negative vs. positive)	0.645 (0.206- 2.018)	0.451	1.333 (0.330- 5.380)	0.686
AFP, ng/ml (≤20 vs. >20)	0.897 (0.558- 1.441)	0.652	0.897 (0.558- 1.442)	0.654
Child-Pugh (A vs. B or C)	0.660 (0.271- 1.605)	0.360	1.090 (0.505- 2.352)	0.826
Liver cirrhosis (no vs. yes)	1.254 (0.915- 1.719)	0.159	1.187 (0.863- 1.633)	0.292
Tumor size, cm (≤5 vs. >5)	1.349 (1.012- 1.798)	**0.042**	1.486 (1.107- 1.994)	**0.008**
Tumor number (single vs. multiple)	1.839 (1.345- 2.515)	**0.000**	1.636 (1.191- 2.248)	**0.002**
Lymphonodus node metastasis (no vs. yes)	2.532 (1.792- 3.577)	**0.000**	2.895 (2.066- 4.058)	**0.000**
Microvascular invasion (no vs. yes)	1.545 (1.066- 2.240)	**0.022**	1.272 (0.856- 1.892)	0.234
Tumor differentiation[a] (P vs. M,W)	1.304 (0.976- 1.740)	0.072	1.126 (0.839- 1.511)	0.428
TNM stage[b] (I+II vs. III+IVA)	2.042 (1.483- 2.813)	**0.000**	2.459 (1.798- 3.364)	**0.000**
NLR (low vs. high)	1.426 (1.069- 1.902)	**0.016**	1.782 (1.322- 2.402)	**0.000**
LMR (low vs. high)	0.735 (0.542- 0.997)	**0.048**	0.604 (0.439- 0.831)	**0.002**
CA19-9, U/ml (≤89 vs. >89)	1.960 (1.460-2.632)	**0.000**	2.536 (1.894- 3.395)	**0.000**
SIS				
I	1 (Referent)		1 (Referent)	
II	1.369 (0.950-1.973)	0.092	1.442 (0.975- 2.133)	0.067
III	2.429 (1.621-3.640)	**0.000**	3.577 (2.378- 5.382)	**0.000**
Multivariate analysis[1]				
Tumor size, cm (≤5 vs. >5)	1.030 (0.743- 1.426)	0.860	1.014 (0.733- 1.402)	0.934
Tumor number (single vs. multiple)	1.742 (1.269- 2.393)	**0.001**	1.610 (1.168- 2.219)	**0.004**
Lymphonodus node metastasis (no vs. yes)	2.030 (1.415- 2.912)	**0.000**	2.335 (1.646- 3.312)	**0.000**
Microvascular invasion (no vs. yes)	1.292 (0.885- 1.886)	0.185	1.521 (0.919- 2.515)	0.103
TNM[b] (I+II vs. III+IVA)	1.219 (0.632- 2.350)	0.554	1.483 (0.808- 2.723)	0.203
NLR (low vs. high)	1.121 (0.800- 1.571)	0.506	1.286 (0.919- 1.800)	0.142
LMR (low vs. high)	1.205 (0.720- 2.017)	0.479	0.890 (0.570- 1.389)	0.609
CA19-9, U/ml (≤89 vs. >89)	1.650 (1.216- 2.238)	**0.001**	1.302 (0.747- 2.270)	0.352
SIS				
I	1 (Referent)		1 (Referent)	
II	1.262 (0.871- 1.829)	0.219	1.307 (0.878- 1.947)	0.187
III	2.077 (1.365- 3.162)	**0.001**	3.133 (2.058- 4.769)	**0.000**

Boldface type indicates significant values.

[1] Analysis including LMR, CA19-9, and SIS.

Analyses were conducted using univariate analysis or multivariate Cox proportional hazards regression.

Abbreviations: AFP, alpha-fetoprotein; CA19-9, carbohydrate antigen 19-9; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; TTR, time to recurrence; OS, overall survival; TNM, tumor-node-metastasis; CI, confidential interval; HR, hazard ratio; P, poor differentiation; M, moderated differentiation; W, well differentiation; SIS, combination of preoperative serum CA19-9 and LMR. I, CA19-9[Low]/LMR[High]; II, CA19-9 [High]/LMR[High] and CA19-9 [Low]/LMR [Low]; III, CA19-9 [High]/LMR[Low].

[a] Tumor differentiation was determined according to the "British Society of Gastroenterology guidelines on the management of cholangiocarcinoma".

[b] TNM stage: American Joint Committee on Cancer 7th edition staging for intrahepatic cholangiocarcinoma.

Table 3. Multivariate analyses of prognostic factors in ICC (Cohort 1, *n*=322)

| Variable	TTR		OS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Multivariate analysis[2]				
Tumor size, cm (≤5 vs. >5)	1.034 (0.751- 1.422)	0.838	1.019 (0.736- 1.409)	0.912
Tumor number (single vs. multiple)	1.761 (1.281- 2.421)	**0.000**	1.701 (1.235- 2.345)	**0.001**
Lymphonodus node metastasis (no vs. yes)	1.972 (1.375- 2.829)	**0.000**	2.177 (1.533- 3.091)	**0.000**
Microvascular invasion (no vs. yes)	1.582 (0.971- 2.577)	0.066	1.627 (0.985- 2.689)	0.058
TNM[b] (I+II vs. III+IVA)	1.289 (0.671- 2.478)	0.446	2.065 (1.498- 2.848)	**0.000**
NLR (low vs. high)	1.113 (0.793- 1.562)	0.536	1.504 (1.108- 2.043)	**0.009**
LMR (low vs. high)	0.758 (0.557- 1.032)	0.079	0.636 (0.461- 0.878)	**0.006**
CA19-9, U/ml (≤89 vs.>89)	1.657 (1.220- 2.250)	**0.001**	2.138 (1.584- 2.886)	**0.000**

Boldface type indicates significant values.

[2] Analysis including LMR and CA19-9 (omitting SIS)

Analyses were conducted using univariate analysis or multivariate Cox proportional hazards regression.

Abbreviations: CA19-9, carbohydrate antigen 19-9; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; TTR, time to recurrence; OS, overall survival; TNM, tumor-node-metastasis; CI, confidential interval; HR, hazard ratio.

[a] Tumor differentiation was determined according to the "British Society of Gastroenterology guidelines on the management of cholangiocarcinoma".

[b] TNM stage: American Joint Committee on Cancer 7th edition staging for intrahepatic cholangiocarcinoma.
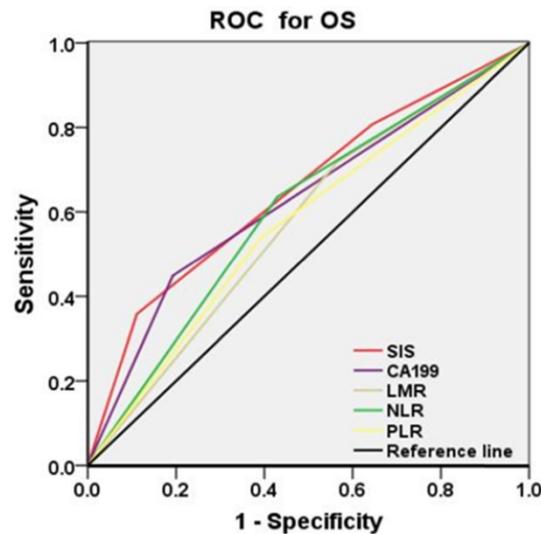
Figure 3. Predictive ability of the SIS was compared with other clinical parameters by ROC curves in the training cohort (Cohort 1, *n*=322). The AUCs for SIS, CA19-9, LMR, NLR, and PLR were 0.652 (0.593-0.712, *P*< 0.001), 0.628 (0.567-0.689, *P*< 0.001), 0.577 (0.513-0.640, *P*=0.019), 0.603 (0.541-0.666, *P*=0.002), and 0.574 (0.511-0.637, *P*=0.024), respectively. Abbreviations: CA19-9, carbohydrate antigen 19-9; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio, PLR, platelet-lymphocyte ratio, SIS, systemic inflammation score. AUC, indicates area under the ROC curve.
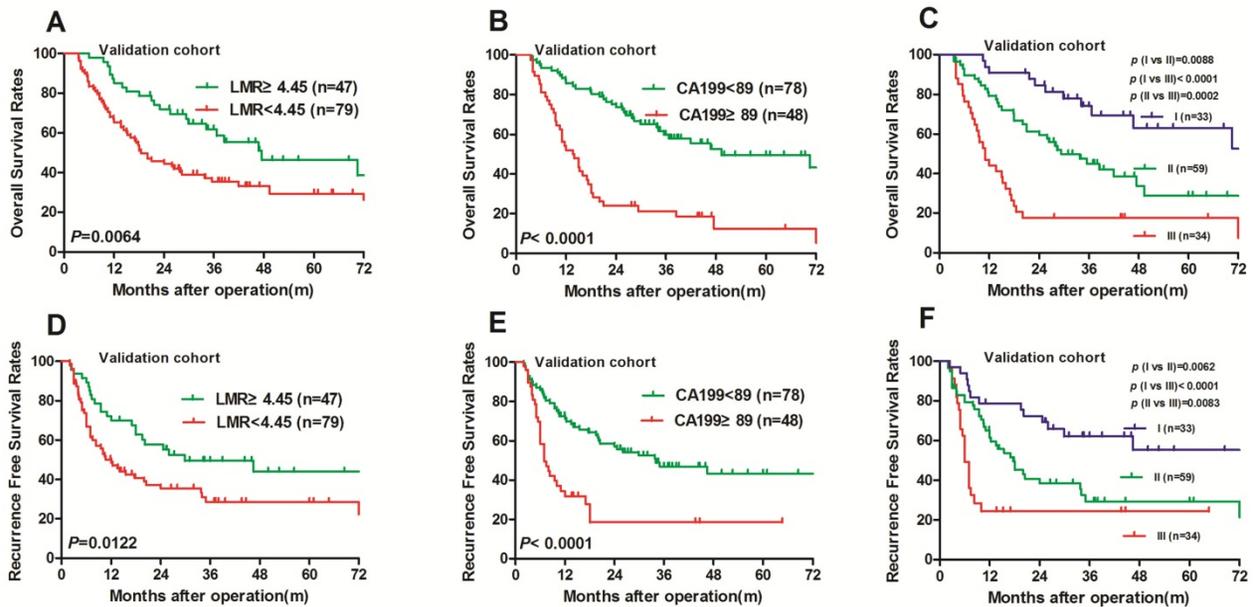
Figure 4. Prognostic values of preoperative LMR and serum CA19-9 using Kaplan-Meier analysis in the validation cohort (Cohort 2, *n*=126). Kaplan-Meier analysis for overall survival according to (A) preoperative LMR, (B) preoperative serum CA19-9, (C) combination of preoperative LMR and serum CA19-9. Kaplan-Meier analysis for recurrence-free survival according to (D) preoperative LMR, (E) preoperative serum CA19-9, (F) combination of preoperative LMR and serum CA19-9. SIS, combination of preoperative LMR and serum CA19-9. I, CA19-9Low/LMRHigh; II, CA19-9 High/LMRHigh and CA19-9 Low/LMR Low; III, CA19-9 High/LMR Low.

Discussion

Growing evidence indicates a crucial role of cancer-related inflammation in cancer metastasis and progression. The hallmark of cancer-associated inflammation involves in the infiltration of inflammatory cells and the production of inflammatory mediators in tumor tissues, which participates in promotion of angiogenesis, extracellular matrix restructuring, and pre-metastatic niche formation [16]. Moreover, cancer-related inflammation generates alteration and polarization the tumor microenvironment that can increase

propensity for tumor recurrence and metastasis [28, 29]. Serological inflammatory markers including CRP, NLR, LMR, and platelet-lymphocyte ratio (PLR) as well as tumor marker serum CA19-9, which reflect the host systemic inflammatory response have certain prognostic utilities in various cancers [24, 26, 30-32]. Therefore, the inflammatory mediators and inflammatory cells of tumor microenvironment may be reflected in the peripheral circulation.

Currently, there is no serum or bile tumor markers are specific for ICC but may be of diagnostic values. In the present study, our result identified

serum CA19-9 as an independent predictor for RFS and OS. We chose the optimal cutoff value for serum CA19-9 was more than 89 U/ml, which demonstrated the best discriminating power for distinguishing ICC patient clinical outcome in our large cohort. Furthermore, high CA19-9 was significantly correlated with high Child-Pugh score, large tumor size, lymphonodus node metastasis, and high TNM stage. This finding parallels previous studies have identified serum CA19-9 greater than 100 U/ml was significantly correlated with inferior RFS after hepatic resection [33]. Therefore, elevated serum CA19-9 may facilitate the selection of the ICC patients with an aggressive tumor biology. This group of patients should be monitor and further postoperative adjuvant treatment be carried out.

The development of tumor metastasis comprises a complex cascade steps that involves the multiple intercellular interaction between the tumor cells and the host-derived stromal cells that includes factors that facilitate angiogenesis and pre-metastatic niche formation. Several studies have demonstrated the prognostic utilities of NLR, PLR, and CA19-9 in ICC patients after surgery [24, 26, 34]. Although previous study examining the LMR in ICC, had been limited both in size and scope to select populations within locally advanced or metastatic ICC [17]. In the present study, ROC and AUC analysis identified serum CA19-9 and LMR as a predictor for OS. Our results revealed that elevated serum CA19-9 and low LMR were independent and worse predictors of OS and TTR in two independent cohorts. Furthermore, multivariate analysis identified preoperative serum CA19-9 and LMR were also independent predictor for OS and TTR, after excluding SIS. Consistent with the recent reports, evaluating the convenient clinical application of LMR as an easily measurable inflammatory biomarker in a variety of solid tumors, such as HCC, lung, and colon cancer [31, 35-37].

In the present study, we constructed a novel prognostic score named SIS basing on serum CA19-9 and LMR, which were both confirmed to be correlated with ICC patients' outcome. Furthermore, we also assessed the prognostic utility of combined serum CA19-9 and LMR in patients with ICC by direct comparisons of prognosis among three subgroups (I, CA19-9^{Low}/LMR^{High}; II, CA19-9 ^{High}/LMR^{High} and CA19-9 ^{Low}/LMR ^{Low}; III, CA19-9 ^{High}/LMR^{Low}) in 322 ICC patients. Our results demonstrated that ICC patients who had both elevated serum CA19-9 and low LMR were more prone to early recurrence and suffered poor survival rates after hepatectomy. Conversely, the ICC patients who had both low serum CA19-9 and high LMR had the best prognosis. Multivariate analysis indicated that high SIS score

was a significant independent inferior predictor for OS and TTR, together with tumor number and tumor-node metastasis stage. Consistent with our previous findings demonstrated that high systemic immune-inflammation index was a powerful prognostic indicator of poor outcome in HCC patients [38]. Moreover, elevated SIS score was significantly associated with aggressive tumor biological phenotypes such as high TNM stage, large tumor size, multiple tumors, lymphonodus node metastasis, and high AFP level, as well as HBsAg positive. Therefore, the predictive significance of the SIS score in those subgroups may facilitate clinicians identifying patients with high risk of recurrence and enable targeted rational adjuvant therapy after hepatic resection.

As an integrated indicator basing on serum tumor marker CA19-9 and LMR, the potential mechanisms behind the prognostic utility of SIS might be elucidated by the function of the serum CA19-9, lymphocytes and monocytes. Here, we confirmed the elevated CA19-9 was powerful prognostic indicator of poor outcome in patients with ICC, and was also in keeping with those previous findings [22, 32]. Actually, serum CA19-9 is not specific for ICC but may be of diagnostic utility. Serum tumor markers CA19-9 and CEA are significant overlapped with other benign diseases. In addition, acute cholangitis or bile duct obstruction may affect serum CA19-9 level. Previous literature had shown that lymphocytes can strengthen cancer immune-surveillance to suppress tumor cell proliferation, invasion and metastasis [39]. Lymphopenia has been associated with worse outcomes in cancer patients [40, 41]. Recent studies demonstrates that serum monocytes can be recruited in tumor microenvironment and differentiate into tumor-associated macrophages (TAMs), which participate in cancer angiogenesis, immunosuppression, and metastasis [42, 43]. In line with the literatures, our earlier results confirmed that myeloid-derived suppressor cells are a subset of circulating leucocytes known to have immunosuppressive activity in HCC [31, 44]. Therefore, a low circulating lymphocyte amounts indicate impaired immunity, which may facilitate tumorigenesis and subsequent tumor growth. An increased circulating serum levels of monocytes may indicate an elevated levels of TAMs as a marker of high tumor burden.

This is the first study, to our knowledge, to describe the use of systemic inflammation score (SIS) based on preoperative serum CA19-9 and LMR, which are routine laboratory parameters that reflect the systemic inflammatory response as a biomarker for ICC patients undergoing curative intent resection.

Meanwhile, the measure of SIS is based on standard laboratory measurements of total monocyte, and lymphocyte counts as well as serum tumor marker CA19-9, which are routinely performed in clinical practice. Thus, our results indicated that the SIS may be a powerful prognostic biomarker for ICC recurrence and treatment response surveillance, which may allow early therapeutic intervention according to characteristics of individual tumors.

Previously, we have observed that decreased PNI, another inflammatory marker, predicted poor postoperative prognosis of advance ICC patients, especially in those with metastatic ICC [17]. However, our current results indicated that PNI was not a promising independent prognostic factor. Our results were consistent with the previous studies [45]. The exclusion criteria of ICC patients enrolled in our cohorts included intrahepatic metastasis of extrahepatic cholangiocarcinoma, which may explain why preoperative PNI was not an independent prognostic factor in current study. In addition, we did not evaluate mGPS in our analyses, because serum CRP was not routinely performed at our institution. The prognostic utility of mGPS in combination with SIS should be evaluated in the further studies.

The current study has several limitations. First, it was a retrospective analysis. There may have been a selection bias regarding the diagnosis and treatment of patients with ICC in the study. Second, most ICC patients (*n*=123 [38.2%] in primary cohort) in China were seropositive for hepatitis B surface antigen, whereas one patient (*n*=1 [0.31%] in primary cohort) had anti HCV positivity, which differs greatly from the patient population in previous studies in United States, Europe, and Japan. Because the key detrimental factors in carcinogenesis of ICC are primary sclerosing cholangitis and HCV infection, especially in western countries. Therefore, the prognostic significance of the SIS needs to be validated in patients with ICC from other populations in the future. Nonetheless, more prospective studies should be conducted to further validate the predicting probability of SIS.

Conclusions

In conclusion, we created an easily obtained systemic inflammatory biomarker named SIS basing on preoperative serum CA19-9 and LMR. Our results have demonstrated the SIS qualifies as a novel, independent prognostic predictor of patients with ICC after hepatectomy in two independent cohorts. Our results confirmed that the elevated SIS was associated with early recurrence and poor prognosis in patients with ICC after curative resection. The preoperative serum CA19-9 and the blood tests be routinely performed in the clinical practice, which make the SIS a promising tool for assessing ICC prognosis and personalizing management in future clinical practice.

Abbreviations

AFP, alpha-fetoprotein; ICC, Intrahepatic Cholangiocarcinoma; HR, hazard ratio; OS, overall survival; Systemic inflammation score, SIS.

Supplementary Material

Supplementary figures and tables.
http://www.jcancer.org/v10p0494s1.pdf

Acknowledgements

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Competing Interests

The authors have declared that no competing interest exists.

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