The Prognostic Role of a Combined Fibrinogen and Neutrophil-to-Lymphocyte Ratio Score in Patients with Resectable Hepatocellular Carcinoma: A Retrospective Study

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Background: Inflammation and activation of the coagulation cascades have a role in the pathogenesis of malignancy, including hepatocellular carcinoma (HCC). This retrospective study aimed to investigate the prognostic role of the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) in patients with resectable HCC.

Material/Methods: This retrospective study included 292 patients with HCC who underwent surgical resection. The receiver operating characteristic (ROC) curve was used to determine the cut-off value of preoperative fibrinogen (Fib) levels and the neutrophil-to-lymphocyte ratio (NLR). The. Hyperfibrinogenemia was >3.35 g/L, and an increased NLR was ≥2.47. The F-NLR was calculated for all patients. Kaplan-Meier survival curves, univariate analysis, multivariate analysis, and subgroup analysis were used to identify independent prognostic factors for overall survival (OS) and disease-free survival (DFS). The receiver operating characteristic (ROC) curve analysis of the F-NLR score and OS, according to the Barcelona Clinic Liver Cancer (BCLC) stage, was performed.

Results: Increased F-NLR scores were significantly associated with the presence of tumor thrombus (P=0.001), larger tumor diameter (P<0.001), vascular invasion (P<0.001), and increased BCLC stage (P<0.001). Multivariate analysis showed that the F-NLR score was an independent predictor of OS (P<0.001) and DFS (P=0.002). The prognostic role of F-NLR was significant for BCLC stage 0–I (P=0.004; P<0.001) and BCLC stage II–III (P=0.026; P=0.005) for OS and DFS, respectively.

Conclusions: In patients with resectable HCC, the combined F-NLR score, a new indicator of systemic inflammation, was an independent prognostic indicator.

MeSH Keywords: Carcinoma, Hepatocellular • Fibrinogen • Neutrophils • Prognosis

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Background

Worldwide, hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death and is the sixth most common cause of malignancy [1]. Due to the high rate of hepatitis B virus (HBV) infection, the number of cases of HCC diagnosed in China represent approximately 50% of total Europe and Northern America cases [2]. Surgical resection of the primary tumor is considered to provide good clinical outcome for patients with HCC in the early stages. However, despite significant advances in surgical techniques, the recurrence rates and mortality rates in patients with HCC after liver resection remain high. Currently, alpha-fetoprotein (AFP) is used as a serum tumor biomarker for patients with HCC and is used following surgery to monitor recurrence, but its diagnostic efficacy is unreliable [3]. Therefore, it is necessary to find new biomarkers to evaluate prognosis in patients with HCC following hepatic resection.

In recent decades, studies have shown that systemic inflammatory response may have a role in tumor progression [4,5]. The prognostic role of indicators of a systemic including the monocyte-to-lymphocyte ratio (MLR), the platelet-to-lymphocyte ratio (PLR), and the neutrophil-to-lymphocyte ratio (NLR) have been demonstrated in many tumors [6–13]. Also, previous studies have shown that fibrinogen plays an important role in systemic inflammatory response and tumorigenesis [14–17]. Sun et al. showed that an increased NLR, PLR, and reduced LMR were significantly associated with cancer-specific survival (CSS), overall survival (OS) and with malignant phenotypes in patients with esophageal squamous cell carcinoma [6]. Ji et al. showed that increased level of plasma fibrinogen was significantly associated with poor prognosis in tumors of the gastrointestinal tract [17].

Recent studies have shown that the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) score was a prognostic marker for outcome in patients with several tumors, including gastric cancer, non-small cell lung cancer (NSCLC), ovarian cancer, and esophageal cancer [18–22]. However, the prognostic role of the F-NLR score for patients with HCC remains unknown. Therefore, this retrospective study aimed to investigate the prognostic role of the combined F-NLR score in patients with resectable HCC at a single center.

Material and Methods

Patients

This retrospective study included 292 patients with primary hepatocellular carcinoma (HCC) who underwent liver resection at the Third Affiliated Hospital of Sun Yat-sen University between 2009 and 2015. The study was conducted according to the standards of the Helsinki Declaration and was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University.

Study inclusion and exclusion criteria

All patients had a histologically confirmed diagnosis of primary HCC and were staged according to the Barcelona Clinic Liver Cancer (BCLC) staging system [3]. The Child-Pugh class was determined for the severity of liver damage, with class A (no liver damage), class B (moderate liver damage), and class C (severe liver damage). The study inclusion criteria were patients diagnosed with primary HCC, which was confirmed by two pathologists, with no other concurrent malignant tumors, and who had negative tumor margins on surgical resection. Patients included in the study also had no history of infection or inflammatory disease one month before surgery, and all patients had complete laboratory data. Study exclusion criteria included preoperative anti-inflammatory, immunosuppressive, or anti-tumor therapy, or a history of other primary malignancies.

Clinical data evaluated for patients with HCC who underwent liver resection

Perioperative blood transfusion was defined as the infusion of red blood cell concentrate before, during, or after surgery. Patients who were infused other blood products, such as albumin, platelets, and fresh frozen plasma were not considered to have had a transfusion. After surgical resection, patients were followed every three months for the first three years, and then for every six months. Routine laboratory tests were recorded, including liver function tests and measurement of serum alpha-fetoprotein (AFP). Imaging studies were performed for all patients and included abdominal ultrasound, and magnetic resonance imaging (MRI), or thoracoabdominal computed tomography (CT). A bone scan was performed if the recurrence of HCC was suspected.

Evaluation of the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR)

The results of laboratory investigations performed one week before surgery were recorded from the patient medical records. Data were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), fibrinogen (Fb), prealbumin (PA), albumin, gamma-glutamyl transpeptidase (GGT), the neutrophil count, the platelet count, the lymphocyte count, and the monocyte count. The neutrophil count divided by the lymphocyte count was defined as the neutrophil-to-lymphocyte ratio (NLR). The platelet count, divided by the lymphocyte count, was defined as the platelet-to-lymphocyte ratio (PLR). The monocyte count divided by

Table 1. Clinical and demographic characteristics of the patients with hepatocellular carcinoma (HCC) included in this study.

Characteristic	Patients (%)	Characteristic	Patients (%)
Age (years)		AFP	
≤50	151 (51.7)	≤400	200 (68.5)
>50	141 (48.3)	>400	92 (31.5)
Gender		Vascular invasion	
Male	258 (88.4)	No	174 (59.6)
Female	34 (11.6)	Yes	118 (40.4)
HBsAg		Differentiation	
Negative	34 (11.6)	1	52 (17.8)
Positive	258 (88.4)	2	219 (75.0)
Cirrhosis		3	21 (7.2)
No	89 (30.5)	BCLC	
Yes	203 (69.5)	0	21 (7.2)
Transfusion		A	96 (32.9)
No	160 (54.8)	B	57 (19.5)
Yes	132 (45.2)	C	118 (40.4)
Child-Pugh grade		ALT (U/L)	38.0 (26.0–51.8)
A	270 (92.5)	AST (U/L)	37.0 (28.0–51.8)
B	22 (7.5)	ALP (U/L)	79.0 (67.0–101.0)
Tumor thrombus		Fib (g/L)	3.02 (2.45–3.67)
No	264 (90.4)	PA (mg/L)	179.5 (139.3–223.0)
Yes	28 (9.6)	ALB (g/L)	39.8±4.1
Tumor diameter (cm)		GGT (U/L)	57.5 (36.0–112.0)
≤5	162 (55.5)	NLR	1.94 (1.48–2.72)
>5	130 (44.5)	PLR	10.54 (72.63–135.27)
Tumor number		MLR	0.26 (0.19–0.36)
Single	207 (70.9)		
Multiple	85 (29.1)		

AFP – alpha fetoprotein; BCLC – Barcelona-Clinic Liver Cancer staging system; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; Fib – fibrinogen; PA, prealbumin; ALB – albumin; GGT – gamma-glutamyl transpeptidase; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MLR – monocyte-to-lymphocyte ratio.

the lymphocyte count was defined as the monocyte-to-lymphocyte ratio (MLR).

Optimal cut-off values for fibrinogen and the NLR,

The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was used to identify the optimal cut-off value as the continuous variable with the largest Youden index. Based on the ROC curve analysis, the optimal cut-off value for fibrinogen was 3.35 g/L, the optimal cut-off value for the NLR was 2.47. The AUC for fibrinogen was 0.640, and the AUC for the NLR was 0.604.
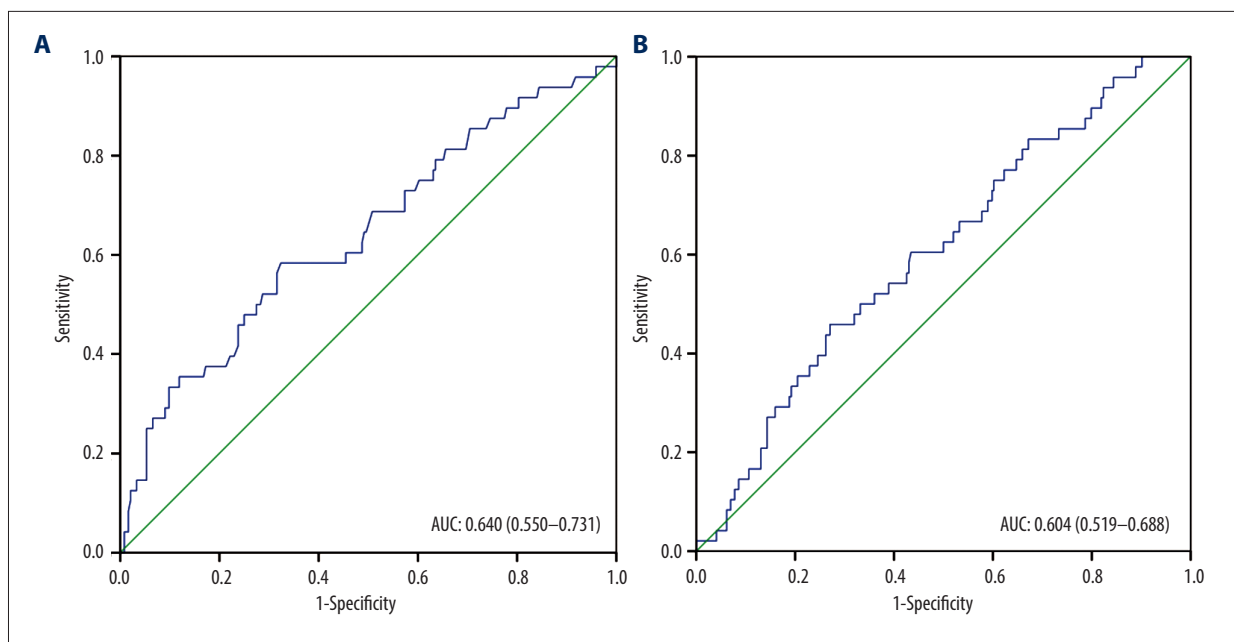
Figure 1. Receiver operating characteristic (ROC) curve analysis of a combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) score and overall survival (OS) in patients with resectable hepatocellular carcinoma (HCC). (**A**) ROC curve analysis for OS and fibrinogen. (**B**) ROC curve analysis for OS and NLR. ROC – receiver operating characteristic; NLR – neutrophil-to-lymphocyte ratio; OS – overall survival.

The F-NLR score

The F-NLR scores were 2, 1, and 0. Patients with a fibrinogen level >3.35 g/L and an increased NLR >2.47 had an F-NLR score of 2. Patients with only one of the two criteria had an F-NLR score of 1. Patients with none of these criteria had an F-NLR score of 0.

Follow-up, disease-free survival (DFS), and overall survival (OS)

Disease-free survival (DFS) and overall survival (OS) were the primary study outcomes. DFS was defined as the interval between the time of surgery and first tumor progression or death or date of the last follow-up. OS was defined as the interval between the time of surgery and the time of death or last follow-up.

Statistical analysis

Data were analyzed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). The categorical variables were described by frequency (%). If the continuous variables satisfied a normal distribution, they were expressed as the mean±standard deviation (SD) using the 95% confidence interval (CI). If the data did not conform to a normal distribution, the median and the 25–75% quartiles were used. Differences between the F-NLR score and the clinicopathological features or laboratory parameters were evaluated using the chi-squared (χ^2) test and the Kruskal-Wallis test, respectively. The Kaplan-Meier method and the log-rank test were used to compare the survival data between groups. Cox proportional hazard regression analysis was used to screen for independent prognostic factors for OS and DFS. Significant parameters in the univariate regression analysis were validated using multivariate analysis using a forward step-wise method. A p-value <0.05 was considered as statistically significant.

Results

Baseline characteristics of patients with resectable HCC

There were 292 patients with hepatocellular carcinoma (HCC) who underwent liver resection. The study group included 141 patients (48.3%) who were >50 years of age, and 34 female patients (11.6%). There were 258 patients (88.4%) who were positive for hepatitis B surface antigen (HBsAg), 132 patients (45.2%) had a blood transfusion, 22 patients (7.5%) had Child-Pugh class B liver function, and 85 patients (29.1%) had multiple lesions. The median age was 50 years (range, 41.3–58.8 years) at the time of diagnosis. Using the Barcelona Clinic Liver Cancer (BCLC) class, there were 21 (7.2%), 96 (32.9%), 57 (19.5%), and 118 (40.4%) patients with BCLC class 0, class A, class B, and class C, respectively. Other baseline data, including laboratory and hematological parameters, are shown in Table 1.

Table 2. Associations between the clinicopathological characteristics of the patients with hepatocellular carcinoma (HCC) included in this study and the fibrinogen and neutrophil-to-lymphocyte ratio (NLR).

Variables	Fibrinogen			NLR		
	Low Fib group, n=185	High Fib group, n=107	P-value	Low NLR group, n=204	High NLR group, n=88	P-value
Age (years)			0.168			0.525
≤50	90	61		103	48	
>50	95	46		101	40	
Gender			0.862			0.372
Male	163	95		178	80	
Female	22	12		26	8	
HBsAg			0.336			0.274
Negative	19	15		21	13	
Positive	166	92		183	75	
Cirrhosis			0.092			0.182
No	50	39		67	22	
Yes	135	68		137	66	
Transfusion			0.928			**0.009**
No	101	59		122	38	
Yes	84	48		82	50	
Child-Pugh grade			0.062			**0.035**
A	167	103		193	77	
B	18	4		11	11	
Tumor thrombus			**0.001**			**0.048**
No	175	89		189	75	
Yes	10	18		15	13	
Tumor diameter (cm):			**<0.001**			0.080
≤5	125	37		120	42	
>5	60	70		84	46	
Tumor number			0.446			0.914
Single	134	73		145	62	
Multiple	51	34		59	26	
AFP			0.852			0.940
<400	126	74		140	60	
≥400	59	33		64	28	
Vascular invasion			**<0.001**			0.094
No	130	44		128	46	
Yes	55	63		76	42	
Differentiation			**0.043**			0.187
Well/moderate	176	95		192	79	
Poor	9	12		12	9	
BCLC stage			**<0.001**			0.087
0	18	3		18	3	
A	74	22		73	23	
B	38	19		37	20	
C	55	63		76	42	

AFP – alpha fetoprotein; BCLC – Barcelona-Clinic Liver Cancer staging system.

Table 3. Hematological parameters of the patients with hepatocellular carcinoma (HCC) included in this study and the fibrinogen and neutrophil-to-lymphocyte ratio (NLR).

Variables	Fibrinogen		P-value	NLR		P-value
	Low Fib group, n=185	High Fib group, n=107		Low NLR group, n=204	High NLR group, n=88	
ALT (U/L)	37.0 (26.0–52.0)	39.0 (29.0–53.0)	0.315	37.0 (26.0–50.8)	38.0 (30.0–59.0)	0.312
AST (U/L)	35.0 (28.0–48.0)	40.0 (30.0–56.0)	0.096	36.0 (28.0–49.0)	39.0 (29.0–58.0)	0.122
ALP (U/L)	73.0 (61.0–98.0)	84.0 (75.0–120.0)	**<0.001**	77.0 (64.0–98.0)	81.0 (71.3–118.0)	**0.005**
Fib (g/L)	2.61 (2.20–2.95)	3.89 (3.63–4.63)	**<0.001**	2.92 (2.38–3.43)	3.51 (2.64–4.14)	**<0.001**
PA (mg/L)	181.0 (142.0–214.5)	177.0 (137.0–229.0)	0.948	183.5 (143.3–222.3)	169.5 (134.0–224.5)	0.383
ALB (g/L)	39.9±4.3	39.6±3.8	0.606	40.0±4.1	39.4±4.2	0.273
GGT (U/L)	51.0 (33.0–88.5)	77.0 (47.0–138.0)	**<0.001**	53.5 (34.0–101.8)	72.5 (39.3–127.0)	**0.010**
NLR	1.79 (1.35–2.41)	2.32 (1.74–3.55)	**<0.001**	1.67 (1.25–2.02)	3.55 (2.92–4.71)	**<0.001**
PLR	89.4 (67.8–113.4)	129.7 (95.8–165.1)	**<0.001**	90.2 (68.1–113.5)	133.5 (104.9–195.0)	**<0.001**
MLR	0.24 (0.18–0.32)	0.31 (0.22–0.44)	**<0.001**	0.23 (0.17–0.30)	0.38 (0.29–0.52)	**<0.001**

AFP – alpha fetoprotein; BCLC – Barcelona-Clinic Liver Cancer staging system; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; Fib – fibrinogen; PA – prealbumin; ALB – albumin; GGT – gamma-glutamyl transpeptidase; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MLR – monocyte-to-lymphocyte ratio.

The receiver operating characteristic (ROC) curves for fibrinogen and the neutrophil-to-lymphocyte ratio (NLR) for overall survival (OS) are shown in Figure 1. The area under the ROC curve for fibrinogen and NLR were 0.640 and 0.604, respectively.

The associations between the clinicopathologic features and laboratory parameters and fibrinogen or NLR in patients with resectable HCC

The associations between fibrinogen or NLR and clinicopathological features in patients with HCC are shown in Table 2. Fibrinogen levels were significantly correlated with the presence of tumor thrombus in HCC (P=0.001), tumor diameter (P<0.001), vascular invasion (P<0.001), differentiation (P=0.043), and the Barcelona Clinic Liver Cancer (BCLC) class (P<0.001). Also, the value for the NLR was significantly associated with a history of blood transfusion (P=0.009), the Child-Pugh grade (P=0.035), and tumor thrombus (P=0.048). The association between fibrinogen and NLR and the clinical and laboratory parameters in patients with HCC are shown in Table 3. Fibrinogen levels were significantly correlated with levels of alkaline phosphatase (ALP) (P<0.001), fibrinogen (Fib) (P<0.001), gamma-glutamyl

transpeptidase (GGT), (P<0.001), NLR (P<0.001), platelet-to-lymphocyte ratio (PLR) (P<0.001), and monocyte-to-lymphocyte ratio (MLR) (P<0.001). Also, the value of NLR was significantly associated with ALP (P=0.005), Fib (P<0.001), GGT (P=0.010), NLR (P<0.001), PLR (P<0.001), and MLR (P<0.001).

The associations between the patient clinicopathologic features, laboratory parameters, and the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) groups (scores 2, 1, and 0) in patients with resectable HCC

The results of the associations between the three groups of F-NLR (scores 2,1, and 0) and the laboratory parameters in patients with resectable HCC are presented in Tables 4 and 5. In the three here were significant associations between the F-NLR and tumor thrombus (P=0.001), tumor diameter (P<0.001), vascular invasion (P<0.001), BCLC stage (P<0.001), ALP (P<0.001), Fib (P<0.001), ALB (P=0.027), GGT (P<0.001), NLR (P<0.001), PLR (P<0.001), and MLR (P<0.001) were statistically different between the three groups of F-NLR (scores 2, 1, and 0).

Table 4. Association between the clinicopathologic features and the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR).

Variables	F-NLR			P-value
	0	1	2	
Age (years)				0.315
≤50	68	57	26	
>50	76	44	21	
Gender				0.119
Male	128	85	45	
Female	16	16	2	
HBsAg				0.190
Negative	12	16	6	
Positive	132	85	41	
Cirrhosis				0.241
No	40	37	12	
Yes	104	64	35	
Transfusion				0.181
No	83	57	20	
Yes	61	44	27	
Child-Pugh grade				0.942
A	133	94	43	
B	11	7	4	
Tumor thrombus				**0.001**
No	136	92	36	
Yes	8	9	11	

Variables	F-NLR			P-value
	0	1	2	
Tumor diameter (cm)				**<0.001**
≤5	98	49	15	
>5	46	52	32	
Tumor number				0.202
Single	107	65	35	
Multiple	37	36	12	
AFP				0.936
<400	99	68	33	
≥400	45	33	14	
Vascular invasion				**<0.001**
No	101	56	17	
Yes	43	45	30	
Differentiation				0.069
Well/moderate	137	94	40	
Poor	7	7	7	
BCLC stage				**<0.001**
0	15	6	0	
A	60	27	9	
B	26	23	8	
C	43	45	30	

AFP – alpha fetoprotein; BCLC – Barcelona Clinic Liver Cancer.

Table 5. Relationship between hematological parameters and the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR).

Variables	F-NLR						P-value
	0		1		2		
ALT (U/L)	35.0	(25.3–49.0)	41.0	(27.0–60.5)	38.0	(31.0–46.0)	0.435
AST (U/L)	35.0	(28.0–47.8)	37.0	(27.5–55.5)	44.0	(31.0–59.0)	0.107
ALP (U/L)	70.0	(60.0–96.8)	80.0	(69.5–99.5)	97.0	(76.0–133.0)	**<0.001**
Fib (g/L)	2.62	(2.19–2.95)	3.43	(2.78–3.89)	4.13	(3.68–5.23)	**<0.001**
PA (mg/L)	186.0	(145.0–216.0)	172.0	(133.0–2186.0)	173.0	(138.0–232.0)	0.375
ALB (g/L)	39.7±4.2		40.5±4.2		38.5±3.6		**0.027**
GGT (U/L)	50.5	(32.3–91.8)	59.0	(37.0–103.0)	98.0	(56.0–175.0)	**<0.001**
NLR	1.60	(1.23–1.96)	2.18	(1.74–3.14)	3.69	(3.02–4.89)	**<0.001**
PLR	83.3	(62.4–105.3)	114.9	(91.4–146.5)	152.9	(120.2–230.9)	**<0.001**
MLR	0.22	(0.16–0.28)	0.29	(0.22–0.36)	0.44	(0.31–0.55)	**<0.001**

AFP – alpha fetoprotein; BCLC – Barcelona-Clinic Liver Cancer staging system; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; Fib – fibrinogen; PA – prealbumin; ALB – albumin; GGT – gamma-glutamyl transpeptidase; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MLR – monocyte-to-lymphocyte ratio.
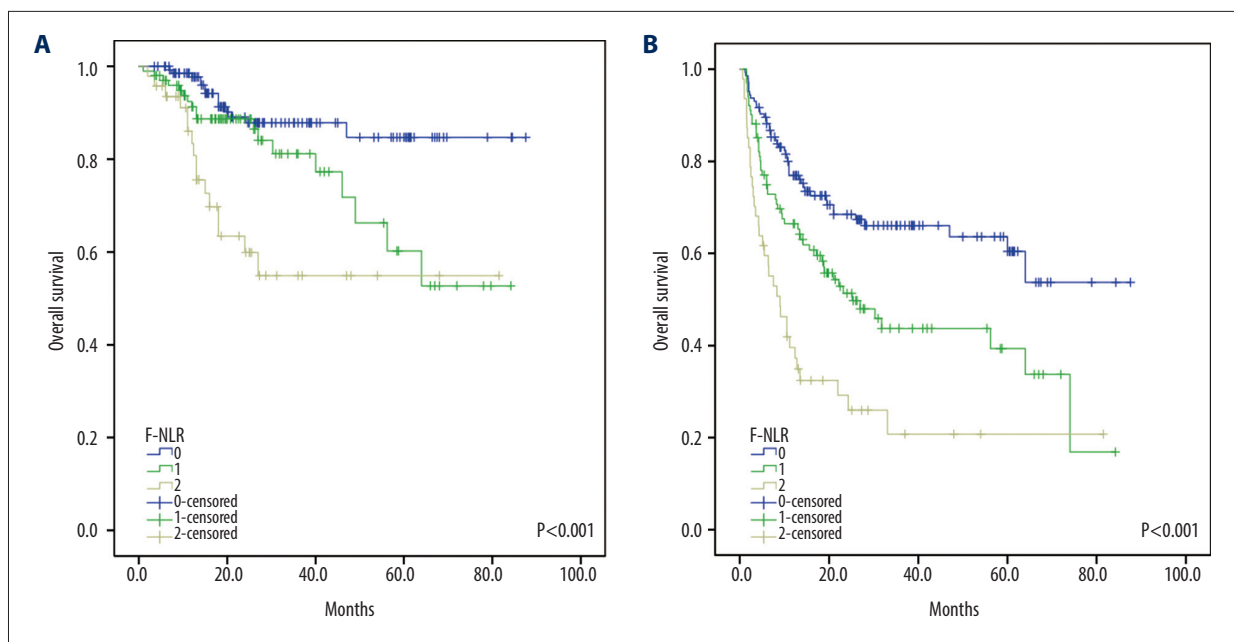
Figure 2. Kaplan-Meier survival curves for overall survival (OS) and disease-free survival (DFS) using the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR), in patients with resectable hepatocellular carcinoma (HCC). (**A**) Kaplan-Meier survival curve for overall survival (OS) and the F-NLR. (**B**) Kaplan-Meier survival curve for disease-free survival (DFS) and the F-NLR. OS – overall survival; DFS – disease-free survival; F-NLR – combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR).

Survival curve analysis of F-NLR for OS and DFS

The Kaplan-Meier method and log-rank test evaluated the survival differences among the three groups of F-NLR (scores 2, 1, and 0). Survival curves showed that the different scores of F-NLR distinguished between the survival curves of patients with HCC with three separate groups for OS (P<0.001) and DFS (P<0.001) (Figure 2). Subgroup analysis of the prognostic role of the F-NLR in patients with HCC was evaluated for different tumor stages, which showed that F-NLR had a prognostic role in OS and DFS in patients with HCC, regardless of subgroups of BCLC stage (Figure 3).

Univariate and multivariate analyses of OS

Univariate regression analysis showed that patient age (P=0.006), tumor thrombus (P=0.046), tumor diameter (P=0.002), AFP (P=0.013), vascular invasion (P=0.003), BCLC stage (P=0.002), ALT (P=0.045), AST (P=0.039), ALP (P=0.003), Fib (P=0.001), GGT (P=0.003), NLR (P=0.001), PLR (P=0.002), MLR (P=0.001), and F-NLR (P<0.001) were significantly associated with OS. These variables were included in multivariate regression analysis using a forward step-wise method. The results showed that patient age (P=0.013), AFP (P=0.021), GGT (P=0.014), and F-NLR (P<0.001) were independent factors for OS in patients with resectable HCC in this study (Table 6).

Univariate and multivariate analyses for DFS

Univariate regression analysis showed that patient age (P=0.011), a history of blood transfusion (P=0.002), tumor thrombus (P<0.001), tumor diameter (P<0.001), tumor number (P<0.001), AFP (P=0.023), vascular invasion (P<0.001), BCLC stage (P<0.001), ALT (P=0.038), AST (P=0.005), ALP (P<0.001), Fib (P<0.001), GGT (P=0.003), NLR (P<0.001), PLR (P<0.001), MLR (P<0.001), and F-NLR (P<0.001) were significantly associated with DFS. These variables were included in multivariate regression analysis using a forward step-wise method. The results showed that patient age (P=0.015), a history of blood transfusion (P=0.016), tumor thrombus (P=0.006), tumor number (P=0.007), ALP (P=0.010), MLR (P=0.019), and F-NLR (P=0.002) were independent factors for DFS in patients with resectable HCC in this study (Table 7).

Discussion

Although there have been recent developments in the diagnosis and treatment of hepatocellular carcinoma (HCC), the 5-year overall survival (OS) rate of patients remains unsatisfactory [2]. The progression of tumors are not only determined by the biological characteristics of the tumor, but may be influenced by preoperative biochemical and hematological parameters [4,5]. The use of appropriate prognostic indicators can provide risk stratification for patients with HCC and guidance for the choice
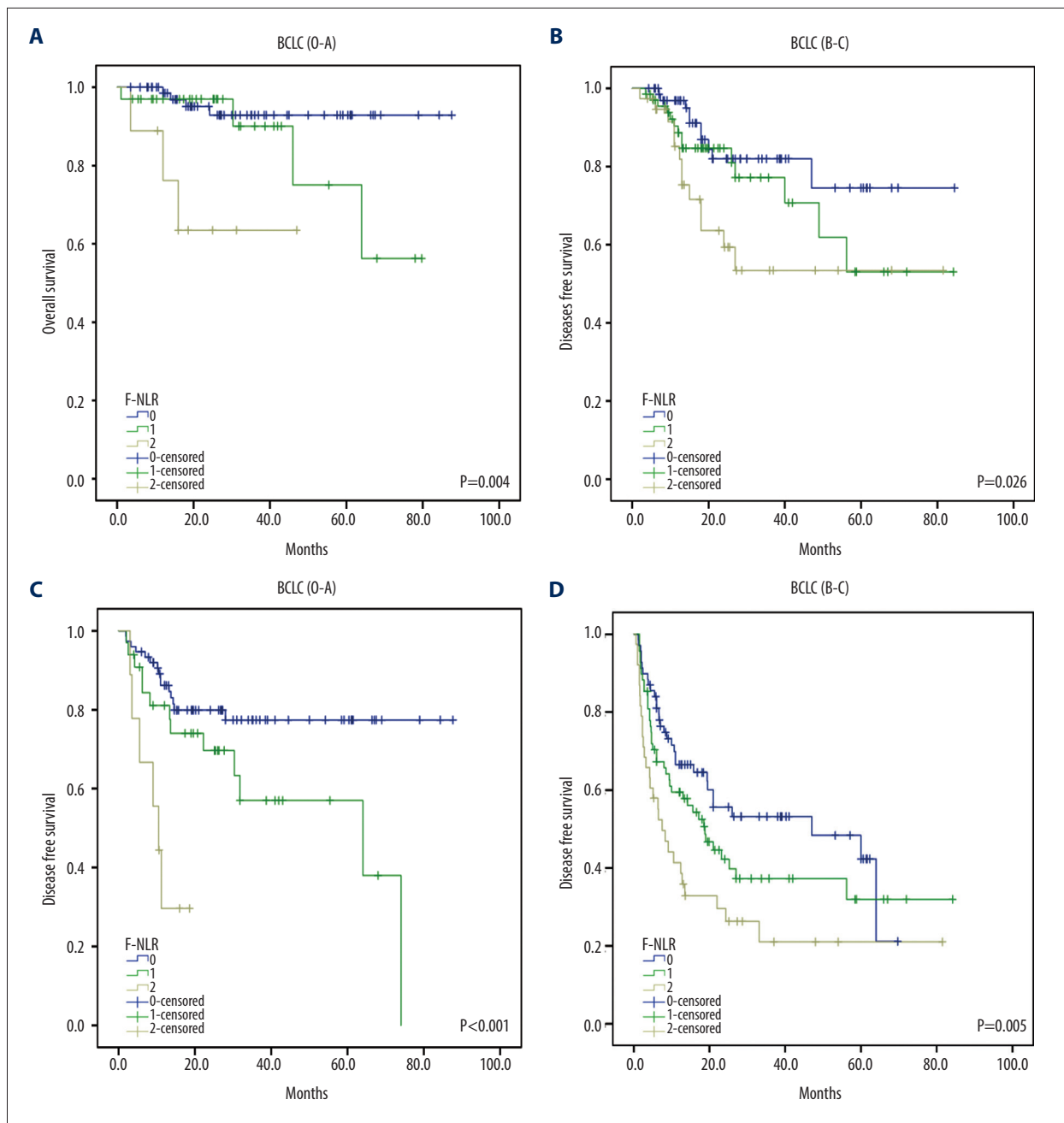
Figure 3. Kaplan-Meier survival curves for overall survival (OS) and disease-free survival (DFS) using the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) according to the Barcelona Clinic Liver Cancer (BCLC) stage in patients with resectable hepatocellular carcinoma (HCC). (**A, B**) Kaplan-Meier survival curve for overall survival (OS) using the F-NLR according to the Barcelona Clinic Liver Cancer (BCLC) stage. (**C, D**) Kaplan-Meier survival curve for disease-free survival (DFS) using the F-NLR according to the BCLC stage. OS – overall survival; DFS – disease-free survival; BCLC – Barcelona Clinic Liver Cancer stage.

of treatment. The aim of this retrospective study was to investigate the prognostic role of the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) in patients with resectable HCC at a single center. Demographic, clinicopathological, and laboratory parameters of 292 patients with HCC were analyzed correlated with the three groups of F-NLR (scores 2, 1, and 0).

Inflammation is recognized to be associated with tumor development and progression. The inflammatory response promotes the production of inflammatory mediators in tumor cells and the tumor microenvironment that facilitate tumor cell growth, invasion, and angiogenesis [5,23].

Table 6. Univariate and multivariate analyses of overall survival (OS).

Variable	Univariate analysis			Multivariate analysis		
	p-Value	Hazard ratio (HR)	95% confidence interval (CI)	p-Value	Hazard ratio (HR)	95% confidence interval (CI)
Age (years) (≤50 *vs.* >50)	**0.006**	**0.409**	**0.217–0.774**	**0.013**	**0.446**	**0.235–0.845**
Gender (Female *vs.* Male)	0.750	0.860	0.339–2.178			
HBsAg (yes *vs.* no)	0.225	2.060	0.640–6.632			
Cirrhosis (yes *vs.* no)	0.937	0.935	0.523–1.819			
Transfusion (yes *vs.* no)	0.514	1.210	0.683–2.142			
Child-Pugh grade (B/A)	0.382	0.531	0.129–2.191			
Tumor thrombus (yes *vs.* no)	**0.046**	**2.268**	**1.015–5.070**	–		
Tumor diameter (cm) (>5 *vs.* ≤5)	**0.002**	**2.497**	**1.397–4.466**	–		
Tumor number (multiple *vs.* single)	0.335	1.344	0.737–2.451			
AFP (≥400 *vs.* <400)	**0.013**	**2.070**	**1.169–3.667**	**0.021**	**1.969**	**1.108–3.498**
Vascular invasion (yes *vs.* no)	**0.003**	**2.350**	**1.327–4.161**	–		
Differentiation (poor *vs.* moderate/well)	0.140	1.908	0.809–4.496			
BCLC stage (C/B/A/0)	**0.002**	**1.656**	**1.213–2.263**	–		
ALT (>37 *vs.* ≤37)	**0.045**	**1.897**	**1.015–3.543**	–		
AST (>39 *vs.* ≤39)	**0.039**	**1.832**	**1.032–3.253**	–		
ALP (>98 *vs.* ≤98)	**0.003**	**2.360**	**1.327–4.196**	–		
Fib (>3.35 *vs.* ≤3.35)	**0.001**	**2.697**	**1.518–4.791**	–		
PA (>168 *vs.* ≤168)	0.654	0.878	0.497–1.550			
ALB (>38.9 *vs.* ≤38.9)	0.258	1.424	0.772–2.628			
GGT (>64 *vs.* ≤64)	**0.003**	**2.844**	**1.416–5.710**	**0.014**	**2.439**	**1.202–4.952**
NLR (>2.47 *vs.* ≤2.47)	**0.001**	**2.624**	**1.475–4.667**	–		
PLR (>102.53 *vs.* ≤102.53)	**0.002**	**2.656**	**1.452–4.860**	–		
MLR (>0.31 *vs.* ≤0.31)	**0.001**	**2.666**	**1.491–4.767**	–		
F-NLR (2/1/0)	**<0.001**	**2.187**	**1.523–3.141**	**<0.001**	**2.011**	**1.390–2.909**

OS – overall survival; AFP – alpha fetoprotein; BCLC – Barcelona-Clinic Liver Cancer staging system; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; Fib – fibrinogen; PA – prealbumin; ALB – albumin; GGT – gamma-glutamyl transpeptidase; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MLR – monocyte-to-lymphocyte ratio.

The neutrophil-to-lymphocyte ratio (NLR) is a marker of human cancer as lymphocytes play a significant role in immune editing and immune surveillance, and can also inhibit the invasion, migration, and proliferation of tumor cells [4]. Activation of T lymphocytes can destroy tumor cells and induce tumor cell apoptosis [24]. Increased circulating lymphocyte counts are favorable prognostic indicators in patients with HCC [24]. Also, circulating neutrophils can produce cytokines involved in cancer cell proliferation, migration, and proliferation, including vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), and interleukins [25,26]. In 2017, Yuan et al. showed that an increased neutrophil count in the peripheral blood was an independent indicator of poor prognosis in patients with HCC treated with sorafenib [27]. Therefore, the neutrophil-to-lymphocyte

Table 7. Univariate and multivariate analysis of disease-free survival (DFS).

Variable	Univariate analysis			Multivariate analysis		
	p-Value	Hazard ratio (HR)	95% confidence interval (CI)	p-Value	Hazard ratio (HR)	95% confidence interval (CI)
Age (years) (≤50 *vs.* >50)	**0.011**	**0.636**	**0.448–0.902**	**0.015**	**0.645**	**0.453–0.920**
Gender (Female *vs.* Male)	0.114	0.594	0.312–1.133			
HBsAg (yes *vs.* no)	0.364	1.293	0.742–2.251			
Cirrhosis (yes *vs.* no)	0.468	0.873	0.605–1.260			
Transfusion (yes *vs.* no)	**0.002**	**1.702**	**1.207–2.398**	**0.016**	**1.536**	**1.083–2.178**
Child-Pugh grade (B/A)	0.957	0.982	0.515–1.874			
Tumor thrombus (yes *vs.* no)	**<0.001**	**3.071**	**1.938–4.867**	**0.006**	**1.991**	**1.223–3.239**
Tumor diameter (>5 *vs.* ≤5)	**<0.001**	**2.360**	**1.668–3.340**	-		
Tumor number (multiple *vs.* single)	**<0.001**	**1.914**	**1.348–2.719**	**0.007**	**1.637**	**1.145–2.341**
AFP (≥400 *vs.* <400)	**0.023**	**1.511**	**1.060–2.155**	-		
Vascular invasion (yes *vs.* no)	**<0.001**	**1.915**	**1.360–2.696**	-		
Differentiation (poor *vs.* moderate/well)	0.948	0.978	0.496–1.925			
BCLC stage (C/B/A/0)	**<0.001**	**1.565**	**1.304–1.878**	-		
ALT (>37 *vs.* ≤37)	**0.038**	**1.463**	**1.022–2.093**	-		
AST (>39 *vs.* ≤39)	**0.005**	**1.628**	**1.156–2.294**	-		
ALP (>98 *vs.* ≤98)	**<0.001**	**1.898**	**1.348–2.674**	**0.010**	**1.592**	**1.119–2.265**
Fib (>3.35 *vs.* ≤3.35)	**<0.001**	**1.957**	**1.390–2.756**	-		
PA (>168 *vs.* ≤168)	0.897	0.977	0.691–1.382			
ALB (>38.9 *vs.* ≤38.9)	0.143	0.774	0.548–1.091			
GGT (>64 *vs.* ≤64)	**0.003**	**1.734**	**1.198–2.508**	-		
NLR (>2.47 *vs.* ≤2.47)	**<0.001**	**2.381**	**1.679–3.378**	-		
PLR (>102.53 *vs.* ≤102.53)	**<0.001**	**1.935**	**1.364–2.743**	-		
MLR (>0.31 *vs.* ≤0.31)	**<0.001**	**2.274**	**1.610–3.213**	**0.019**	**1.602**	**1.080–2.377**
F-NLR (2/1/0)	**<0.001**	**1.899**	**1.519–2.374**	**0.002**	**1.496**	**1.161–1.929**

DFS – disease free survival; AFP – alpha fetoprotein; BCLC – Barcelona-Clinic Liver Cancer staging system; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; Fib – fibrinogen; PA – prealbumin; ALB – albumin; GGT – gamma-glutamyl transpeptidase; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MLR – monocyte-to-lymphocyte ratio.

ratio (NLR) has been used as a marker of the systemic inflammatory response in patients with HCC.

Hyperfibrinogenemia has also previously been shown to be associated with poor prognosis in many tumors, but the underlying mechanisms remain unclear. However, fibrinogen can facilitate the adhesion of cancer cells to platelets, leading to the formation of platelet aggregation around the cancer cells, which may prevent immune attack [28]. Fibrinogen can regulate the proliferation, invasion, and metastasis of tumor cells by binding to growth factors, including platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), and vascular endothelial growth factor (VEGF) [29–31]. Tumor progression is accompanied by an increased inflammatory response

with leukocyte infiltration in the tumor matrix, which convert fibrinogen to fibrin that promotes tumor angiogenesis [25]. Also, studies have shown that fibrinogen can facilitate epithelial-mesenchymal transition (EMT) of cancer cells and promote invasion and metastasis [14]. In this study, the novel preoperative prognostic index, the F-NLR score, was studied that combines fibrinogen, neutrophil, and lymphocyte counts. The F-NLR score may reflect the preoperative inflammatory response and changes in the tumor microenvironment in HCC.

The F-NLR score has previously been shown as an independent variable in the prognosis of patients with ovarian cancer, gastric cancer, non-small cell lung cancer (NSCLC), and esophageal cancer [18–22]. However, studies on the prognostic significance of the F-NLR score in patients with HCC undergoing radical hepatectomy have not been conducted previously. In this retrospective study, the prognostic significance of the F-NLR score in patients with HCC following hepatic resection showed a significant correlation with tumor thrombus, larger tumor diameter, vascular invasion, and an increased Barcelona Clinic Liver Cancer (BCLC) stage, which was supported by subgroup analysis. Univariate and multivariate regression analysis showed that the F-NLR score was an independent risk factor for prognosis in patients with HCC following liver resection.

Currently, the BCLC stage is an important predictive and prognostic method for the selection of treatment options for patients with HCC [3]. However, the BCLC stage in HCC mainly reflects the pathological behavior of the resected tumor. The findings from the present study showed that the preoperative F-NLR score represented a new prognostic biomarker for patients with HCC which does not require additional diagnostic costs as the results of routine laboratory investigations can be used. Also, the F-NLR score can be used to supplement the BCLC stage to identify patients at high risk of recurrence or spread for HCC.

This study had several limitations. This study was retrospective and was conducted at a single-center with a small sample size. Because some inflammatory indicators were not routinely tested at our center, there was no data to analyze, including for C-reactive protein. Also, this study included patients who underwent radical hepatic resection for HCC, and the findings may not apply to patients with HCC who have unresectable primary tumors. Therefore, the findings from this retrospective study require validation with large-scale, multicenter, controlled clinical studies.

Conclusions

This retrospective study aimed to investigate the prognostic role of the combined fibrinogen and neutrophil-to-lymphocyte ratio (F-NLR) in patients with resectable hepatocellular carcinoma (HCC). The combined F-NLR score, a new inflammatory indicator, was shown to be an independent predictor of prognosis in terms of overall survival (OS) and disease-free survival (DFS), according to the Barcelona Clinic Liver Cancer (BCLC) stage. Further prospective clinical studies are needed to determine whether the combined F-NLR score has a clinical role in identifying high-risk patients with HCC and whether this test can be used to benefit patients by part of the evaluation for individualized treatment.

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