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Original Article

Performance of the preoperative alkaline phosphatase-to-lymphocyte ratio index as an independent prognostic factor in patients with hepatocellular carcinoma

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Abstract: Few studies have systematically explored the prognostic value of the preoperative alkaline phosphatase (ALP)-to-lymphocyte ratio index (APLRI) for hepatocellular carcinoma (HCC). We sought to determine the prognostic value of the preoperative APLRI on HCC patients after hepatic resection and further compare its prognostic significance with the clinicopathologic features of HCC. A retrospective investigation and analysis were carried out in 232 patients who underwent surgery for HCC at Guilin Medical University between June 2000 and February 2007. Receiver operating characteristic (ROC) curve analysis was performed to determine the ideal cut-off value of the preoperative APLRI and calculate the area under curve (AUC) for the preoperative APLRI. A survival analysis was performed using the Kaplan-Meier method, and a Cox proportional hazards regression model was used for univariate and multivariate survival analyses of the predictive value of the preoperative APLRI for the prognosis of HCC patients after resection. ROC curves showed that the optimum cut-off value of the preoperative APLRI was 40.0 (AUC = 0.693 [95% confidence interval = 0.647-0.738], sensitivity = 61.3%, specificity = 71.1%). Our results showed significant correlations between the preoperative APLRI and serum AFP levels (P = 0.010), median size (P = 0.002), number of tumors (P = 0.002), clinical BCLC stage (P = 0.004), and serum AST levels (P = 0.007). And a higher preoperative APLRI prospectively conferred poor prognostic features for HCC patients after surgical tumor resection. Disease-free survival (DFS) and overall survival (OS) rates of HCC patients with a preoperative APLRI > 40 were significantly worse than those of HCC patients with a preoperative APLRI < 40. More importantly, preoperative APLRI showed its apparent prognostic values for various clinical HCC subgroups. In summary, the preoperative APLRI is an innovative index that is easily derived from routine tests and might be a novel prognostic biomarker in HCC after curative resection.

Keywords: Hepatocellular carcinoma, APLRI, prognosis, biomarker

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and has been ranked as the third leading cause of cancer-related mortality worldwide [1, 2]. Over the years, the global high morbidity rate of HCC has been largely due to chronic hepatitis virus infections, alcohol abuse, liver cirrhosis, and aflatoxin exposure coupled with chromosomal rearrangements and epigenetic alterations [3-5]. Despite advances in diagnosis and numerous treatments, the 5-year survival rate of HCC patients has still shown a steady decline, mainly because of the high relapse and distant metastasis rates [6-8]. Meanwhile, much effort has been spend on exploring the prognostic markers of HCC patients undergoing a hepatectomy, such as genetic markers, molecular biomarkers, and miRNAs. However, the applicability of these markers in routine clinical settings is still limited, largely because of the complexity

of the laboratory experiments. This necessitates the exploration of available noninvasive markers to determine the prognosis of HCC patients after liver resection, furthermore, to guide the best clinical treatment for improving the longer-term outcomes of HCC patients.

Hepatic function plays an important role in the prognosis of HCC patients who have undergone liver resection and can be easily inferred from serum liver-related enzyme activities, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), and alkaline phosphatase (ALP). Among these, ALP is a phosphate monoester hydrolase that can be found particularly localized in the liver and bones and in lesser amounts in the intestines, placenta kidney and leucocytes [9]. The increased levels of ALP can serve as a powerful prognostic indicator to predict recurrence in high-risk HCC patients [10, 11].

Growing evidence has demonstrated that dysregulated of the systemic inflammatory response results in the upregulation of proinflammatory cytokines and inflammatory mediators and in orchestrated HCC tumor cell proliferation, migration, invasion, and metastasis [12-14] via DNA damage repair, apoptosis inhibition, and angiogenesis promotion [15-17]. Generally speaking, patrolling and infiltrating lymphocytes play a crucial role in the subsequent inflammatory activity of anti-malignant neoplasms [18]. Elevated tumor-infiltrating lymphocytes in the tumor microenvironment were found to be related to better long-time survival rates and lower rates of disease recurrence in HCC patients [19].

In the current study, we presented a novel index, the preoperative ALP-to-lymphocyte ratio index (APLRI), and explored the prognostic power of this ratio index in HCC patients undergoing curative surgery. A broader understanding of the prognostic value of the preoperative APLRI could better predict disease outcome and effectively improve longer-term survival rates of HCC patients.

Materials and methods

Study population

Written informed consent was obtained from all patients enrolled in this study. The study was approved by the Human Research Ethics Committee of the hospital affiliated with Guilin Medical University, and it was in accordance with the ethical guidelines of the Declaration of Helsinki. A total of 232 patients suffering from HCC who underwent liver resection between June 2000 and February 2007 at the hospital affiliated with Guilin Medical University, Guangxi, China, were enrolled in this retrospective study. Patients who met any of the following criteria were excluded: 1) a confirmed diagnosed of cholangiocellular carcinoma (CCC) or a diagnosis that ruled out HCC by pathological examination; 2) the presence of tumor emboli in the main trunk of the portal vein or its branches; 3) the absence of extrahepatic distant metastases or lymph node metastasis; 4) death during the perioperative period; 5) incomplete clinical laboratory test data; 6) a confirmed diagnosed of infectious disease, lymphatic system disease, or hematological disorders with sufficient clinical evidence (medications can affect the result of a blood test); 7) unable to be reached during the follow-up period; or 8) a positive confirmation of HIV infection.

Clinical and laboratory parameters

Demographic and clinical data were obtained from all 232 HCC patients who met the inclusion criteria. Routine physical examinations and imaging assessments, including ultrasonography (US), computed tomography (CT) scans, and magnetic resonance imaging (MRI), were performed prior to surgery. Demographic variables and clinical data collected for this study included age, gender, family history, alcohol consumption, smoking, HBsAg, alpha-fetoprotein (AFP), median size, cirrhosis, number of tumors, Barcelona Clinic Liver Cancer (BCLC) stage, AST, and recurrence.

Follow-up visits

All 232 patients were regularly followed-up and underwent abdominal ultrasonography, chest radiography and serum AFP tests every 6 months for the first two years after the operation and every 3-6 months during the subsequent years. Tumor recurrences were diagnosed based on the combined findings of a typical CT or MRI appearance and elevated AFP levels. The mean postoperative follow-up period was 36.2 months (median, 21.0 months; range, 2.0 to 84.0 months). Disease-free sur-

Table 1. Correlation between the clinicopathologic variables and APLRI level in HCC

Clinical character	Clinical variable	No.of patients	APLRI		χ^2	*p* value
			≤ 40 n (%)	> 40 n (%)		
Age (years)	≤ 55	154	49 (31.8)	105 (68.2)	0.131	0.717
	> 55	78	23 (29.5)	55 (70.5)		
Gender	Female	33	10 (30.3)	23 (69.7)	0.010	0.922
	Male	199	62 (31.2)	137 (68.8)		
Family history	No	202	63 (31.2)	139 (68.8)	0.017	0.896
	Yes	30	9 (30.0)	21 (70.0)		
HBsAg	Negative	28	8 (28.6)	20 (71.4)	0.090	0.764
	Positive	204	64 (31.4)	140 (68.6)		
AFP (ng/ml)	≤ 100	106	42 (39.6)	64 (60.4)	6.726	0.010
	> 100	126	30 (23.8)	96 (76.2)		
Median size (cm)	≤ 5	101	42 (41.6)	59 (58.4)	9.301	0.002
	> 5	131	30 (22.9)	101 (77.1)		
Cirrhosis	No	14	5 (35.7)	9 (64.3)	0.152	0.696
	Yes	218	67 (30.7)	151 (69.3)		
Tumor number	Single	158	59 (37.3)	99 (62.7)	9.207	0.002
	Multiple	74	13 (17.6)	61 (82.4)		
Wine-drinking	No	109	34 (31.2)	75 (68.8)	0.002	0.961
	Yes	123	38 (30.9)	85 (69.1)		
Smoking	No	117	33 (28.2)	84 (71.8)	0.883	0.347
	Yes	115	39 (33.9)	76 (66.1)		
BCLC stage	0-A	160	59 (36.9)	101 (63.1)	8.217	0.004
	B-C	72	13 (18.1)	59 (81.9)		
AST (U/L)	≤ 40	124	48 (38.7)	76 (61.3)	7.331	0.007
	> 40	108	24 (22.2)	84 (77.8)		
Recurrence	No	137	39 (28.5)	98 (71.5)	1.030	0.310
	Yes	95	33 (34.7)	62 (65.3)		

APLRI, alkaline phosphatase-to-lymphocyte ratio index; n, number of patients; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; BCLC, barcelona-clinic liver cancer; AST, aspartate aminotransferase.

vival (DFS) was defined as the time interval between the operation and the first incidence of recurrence, metastasis, death or the most recent follow-up visit. Overall survival (OS) was defined as the interval between the operation and death or the most recent follow-up visit.

Calculation of preoperative APLRI

To calculate the preoperative APLRI, serum ALP activity and lymphocyte count were used, which were obtained from the standard established protocols of liver function tests and complete fluorescent flow cytometry. The normal serum range of ALP for healthy adults is 20 to 140 U/L [20]. The preoperative APLRI index was calculated according to the following formula: (ALP value/lymphocyte count) × 10^9/U.

Statistical analysis

All data were analyzed using SPSS 13.0 (SPSS Inc., Chicago, IL). Receiver operating characteristic (ROC) curve analysis was performed to determine the most appropriate cut-off value of the preoperative APLRI in patients with HCC. The cut-off value is associated with the probability of a true positive (sensitivity) and a true negative (specificity). Pearson's χ^2 test was performed to compare the categorical variables. Survival rates for HCC patients were plotted using the Kaplan-Meier method and tested using the log-rank test. The Cox proportional hazards model was used to assess the independent prognostic factors for DFS and OS on the basis of variables selected by univariate analysis. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. A *P* value of < 0.05 was deemed statistically significant.

Results

Baseline characteristics

The baseline demographic and clinicopathological characteristics of the 232 HCC patients who underwent liver resection are shown in **Table 1**. There were 199 male patients and 33 female patients. Of the patient group, 154 were ≤ 55 years old; and 78 were > 55 years old; 95 developed a recurrence during the follow-up period; 158 had one nodule and 74 had multiple nodules; and 101 had a tumor ≤ 5.0 cm in diameter and 131 had a tumor > 5.0 cm in diameter. With regard to tumor differentiation according to the BCLC staging system, 160 patients in our study population were stage 0-A and 72 were stage B-C. The hepatitis B surface antigen (HBsAg) was positive in 204 patients, and 218 patients had underlying hepatic cir-

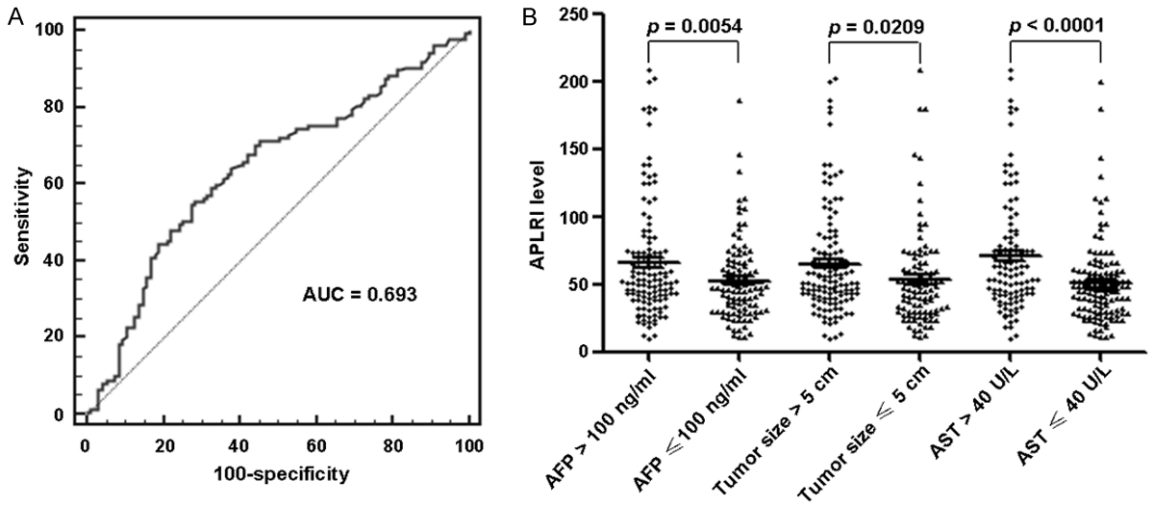

Figure 1. ROC curve and stratified analysis. A. Receiver operating characteristic (ROC) curve for evaluating the cut-off value of the preoperative APLRI in patients with HCC after hepatic resection. B. All HCC patients were stratified based on serum AFP level, tumor size, and serum AST level and compared based on preoperative APLRI in the HCC subgroups. The number of HCC individuals with an elevated preoperative APLRI along with an increased serum AFP level (> 100 ng/ml), tumor size > 5 cm, and an increased serum AST level (> 40 U/L) was much higher than those with a serum AFP level lower than 100 ng/ml, tumor size ≤ 5 cm, and serum AST level lower than 40 U/L (P = 0.0054, P = 0.0209, P < 0.0001, respectively).

rhosis. There were 126 patients with an AFP level of 100 ng/ml or higher, and the remainder had an AFP level lower than 100 ng/ml.

A best cut-off value for preoperative APLRI

A ROC curve was applied to estimate the optimal cut-off of the preoperative APLRI in predicting the prognosis of HCC patients. A cut-off value of 40.0 corresponded to the maximum joint sensitivity and specificity on the ROC plot. The area under curve (AUC) for survival status had the biggest area (0.693), coupled with a 95% CI of 0.647 to 0.738, a sensitivity of 61.3% and a specificity of 71.1% (**Figure 1A**).

Stratified analysis according to AFP level, tumor size, and AST level

A stratified analysis was performed with regard to the preoperative APLRI in subsets of HCC patients with a series of different clinical features, including serum AFP level, tumor size, and serum AST level. Our results demonstrated that the preoperative APLRI in patients with an increased serum AFP level (> 100 ng/ml) was significantly higher than those with a serum AFP level lower than 100 ng/ml (66.26 ± 3.838, 52.42 ± 2.844, respectively) (t = 2.807, P = 0.0054, **Figure 1B**). It is also worth noting that the preoperative APLRI in patients with a tumor > 5.0 cm increased significantly relative to those with a tumor ≤ 5.0 cm (64.98 ± 3.422, 53.39 ± 3.532, respectively) (t = 2.327, P = 0.0209, **Figure 1B**), and this general trend was also found in patients with an increased serum AST level (> 40 U/L) relative to those with a serum AST level lower than 40 U/L (70.98 ± 4.128, 50.32 ± 2.704, respectively) (t = 4.287, P < 0.0001, **Figure 1B**). According to the data shown above, we hypothesized that there is an inextricable relationship between the preoperative APLRI and serum AFP level, tumor size, and serum AST level of HCC patients.

Correlation of preoperative APLRI with clinicopathologic features

To investigate the correlation between the preoperative APLRI and clinicopathological parameters in 232 HCC patients, patients were divided into two groups: a low preoperative APLRI group (≤ 40, n = 72) and a high preoperative APLRI group (> 40, n = 160) as defined in the ROC curve. The results showed significant correlations between the preoperative APLRI and five parameters, including serum AFP levels (χ^2 = 6.726; P = 0.010), median size (χ^2 = 9.301; P = 0.002), number of tumors (χ^2 = 9.207; P = 0.002), clinical BCLC stage (χ^2 = 8.217; P = 0.004), and serum AST levels (χ^2 = 7.331; P = 0.007). No obvious correlation was found be-

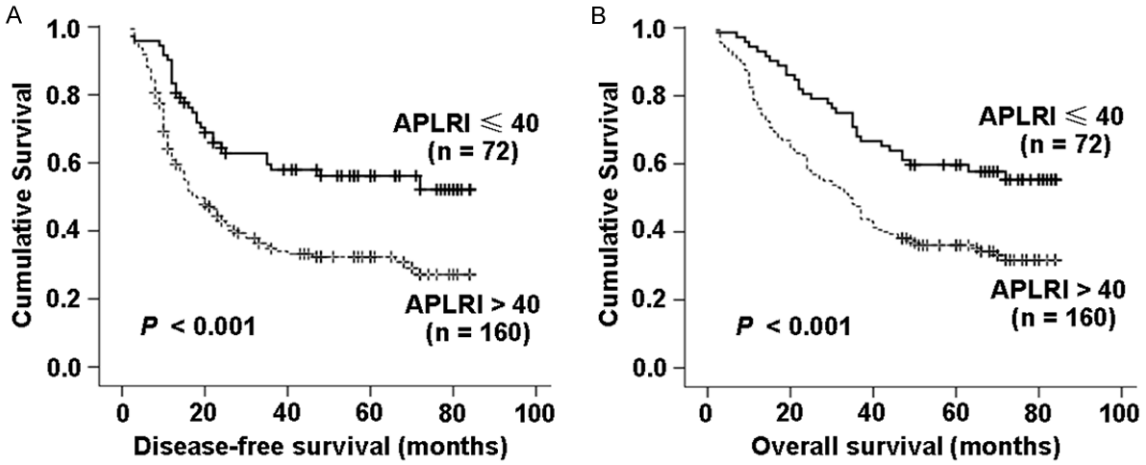

Figure 2. Kaplan-Meier survival curves of HCC patients after curative resection with APLRI ≤ 40 group and APLRI > 40 group. Patients were divided into two groups, APLRI ≤ 40 and > 40, by the optimal cut-off value of APLRI. Patients with an APLRI ≤ 40 had a better DFS compared to those with an APLRI > 40 (A). Patients with an APLR ≤ 40 had a better OS compared to those with an APLR > 40 (B).

Table 2. Association between APLRI level or clinical parameters and DFS/OS

Clinical character	Category	No.of patients	DFS (months)			OS (months)		
			Mean	95% CI	*p* value	Mean	95% CI	*p* value
APLRI	≤ 40	72	54.54	46.64-62.43	< 0.001	60.15	53.43-66.87	< 0.001
	> 40	160	36.22	30.98-41.46		42.98	38.10-47.87	
Age (years)	≤ 55	154	43.58	37.98-49.18	0.350	48.97	43.83-54.10	0.557
	> 55	78	38.87	31.34-46.39		47.09	40.36-53.84	
Gender	Female	33	47.19	34.44-59.95	0.435	53.58	42.41-64.76	0.284
	Male	199	41.17	36.37-45.98		47.48	43.09-51.87	
Family history	No	202	40.13	35.35-44.89	0.074	46.92	42.57-51.27	0.086
	Yes	30	54.02	41.39-66.65		57.73	46.22-69.23	
HBsAg	Negative	28	44.15	31.46-56.83	0.567	50.45	38.83-62.07	0.734
	Positive	204	41.57	36.76-46.39		48.03	43.66-52.40	
AFP (ng/ml)	≤ 100	106	44.31	37.66-50.96	0.333	51.18	45.31-57.05	0.273
	> 100	126	39.99	33.88-46.10		45.95	40.28-51.61	
Median size (cm)	≤ 5	101	55.23	48.69-61.78	< 0.001	61.62	56.15-67.09	< 0.001
	> 5	131	31.74	26.18-37.31		38.10	32.84-43.37	
Cirrhosis	No	14	46.33	27.64-65.03	0.843	51.09	34.36-67.82	0.841
	Yes	218	41.70	37.05-46.35		48.18	43.95-52.39	
Tumor number	Single	158	48.22	42.74-53.70	< 0.001	54.53	49.74-59.33	< 0.001
	Multiple	74	28.51	21.55-35.47		34.66	28.01-41.31	
Wine-drinking	No	109	40.39	33.83-46.95	0.505	47.61	41.65-53.58	0.717
	Yes	123	43.26	37.07-49.46		48.92	43.30-54.55	
Smoking	No	117	40.53	34.23-46.82	0.657	47.82	42.14-53.49	0.753
	Yes	115	43.12	36.67-49.58		48.81	42.90-54.71	
BCLC stage	0-A	160	47.80	42.35-53.25	< 0.001	54.26	49.49-59.02	< 0.001
	B-C	72	28.93	21.80-36.05		34.77	27.97-41.57	
AST (U/L)	≤ 40	124	50.31	44.11-56.51	< 0.001	56.53	51.16-61.91	< 0.001
	> 40	108	32.59	26.50-38.90		38.91	33.14-44.69	
Recurrence	No	137				42.78	37.03-48.54	0.010
	Yes	95				56.30	51.14-61.47	

Table 3. Cox multivariate proportional hazard model of independent predictors on DFS and OS

Variable	Hazard ratio (95% CI)	*P* value
Disease-free survival		
APLRI, (> 40 *vs* ≤ 40)	1.628 (1.148-2.435)	0.015
Tumor size, cm (> 5 *vs* ≤ 5)	1.910 (1.269-2.874)	0.002
Tumor number (multiple *vs* single)	1.470 (0.680-3.177)	0.328
BCLC stage (B-C *vs* 0-A)	1.150 (0.530-2.495)	0.723
AST, U/L (> 40 *vs* ≤ 40)	1.699 (1.202-2.402)	0.003
Overall survival		
APLRI, (> 40 *vs* ≤ 40)	1.699 (1.158-2.466)	0.011
Tumor size, cm (> 5 *vs* ≤ 5)	1.872 (1.241-2.825)	0.003
Tumor number (multiple *vs* single)	1.463 (0.628-3.407)	0.378
BCLC stage (B-C *vs* 0-A)	1.095 (0.471-2.544)	0.833
AST, U/L (> 40 *vs* ≤ 40)	1.763 (1.250-2.486)	0.001
Recurrence	1.531 (1.079-2.171)	0.017

tween the preoperative APLRI and age, gender, family history, HBsAg, cirrhosis, alcohol consumption, smoking, or recurrence (all P > 0.05, **Table 1**).

Relationship between preoperative APLRI and patient survival

After differentiating patients with high and low preoperative APLRI, we investigated the prognostic effect of the preoperative APLRI on HCC patients who underwent hepatic resection. In the Kaplan-Meier survival analysis, the median DFS time in patients with preoperative APLRI (> 40) was 36.22 months, which was significantly shorter than that in patients with APLRI (≤ 40) (54.54 months) (P < 0.001, **Figure 2A**). Moreover, the median OS time in the APLRI (> 40) group was 42.98 months, which was remarkably shorter than that in the APLRI (≤ 40) group (60.15 months) (P < 0.001, **Figure 2B**).

Univariate and multivariate analyses of the prognostic power of preoperative APLRI

A univariate analysis was then performed on 14 traditional clinicopathological variables that may affect HCC patients' survival time. The results indicated that a preoperative APLRI > 40 (P < 0.001), as well as median tumor size > 5 cm (P < 0.001), multiple tumors (P < 0.001), BCLC stage B-C (P < 0.001), increased serum AST level > 40 U/L (P < 0.001), were among the factors that affected the DFS of HCC patients. Additionally, the results demonstrated a significant impact of clinicopathologic prognostic features, including a preoperative APLRI > 40 (P < 0.001), median tumor size > 5 cm (P < 0.001), multiple tumors (P < 0.001), BCLC stage B-C (P < 0.001), increased serum AST level > 40 U/L (P < 0.001), and recurrence (P = 0.010) on the OS of HCC patients (**Table 2**).

Furthermore, the multivariate Cox proportional hazard regression analysis was used to evaluate the independent predictors for DFS and OS among the entire HCC cohort. The difference in predicting the prognosis was evaluated by calculating the HR, 95% CI, and *p* value for each parameter. The results indicated that the DFS of HCC patients could be predicted on the basis of significant prognostic factors, including a preoperative APLRI > 40 (HR, 1.628; 95% CI, 1.148-2.435; P = 0.015), as well as tumor size > 5 cm (HR, 1.910; 95% CI, 1.269-2.874; P = 0.002), and increased serum AST level > 40 U/L (HR, 1.699; 95% CI, 1.202-2.402; P = 0.003). The results also showed that the OS of HCC patients could be predicted on the basis of significant prognostic factors, including a preoperative APLRI > 40 (HR, 1.699; 95% CI, 1.158-2.466; P = 0.011), as well as tumor size > 5 cm (HR, 1.872; 95% CI, 1.241-2.825; P = 0.003), increased serum AST level > 40 U/L (HR, 1.762; 95% CI, 1.250-2.486; P = 0.001), and recurrence (HR, 1.531; 95% CI, 1.079-2.171; P = 0.017) (**Table 3**).

Prognostic values of preoperative APLRI in different HCC subgroups

To further demonstrate the role of the preoperative APLRI in predicting the survival of HCC patients, a validation cohort was performed in this study. The results indicated that a preoperative APLRI > 40 showed its predictive value in predicting poorer DFS (**Figure 3A**, P = 0.016) and OS (**Figure 4A**, P = 0.019) in HCC subgroups with median tumor size ≤ 5 cm, and this forecast value depends on HCC subgroups with a single tumor (**Figure 3B**, P = 0.002; **Figure 4B**, P = 0.003; respectively). Beyond that, a preoperative APLRI < 40 significantly correlated with a better DFS (**Figure 3C**, P = 0.002) and OS (**Figure 4C**, P = 0.002) and was also observed in patients with BCLC stage 0-A, and

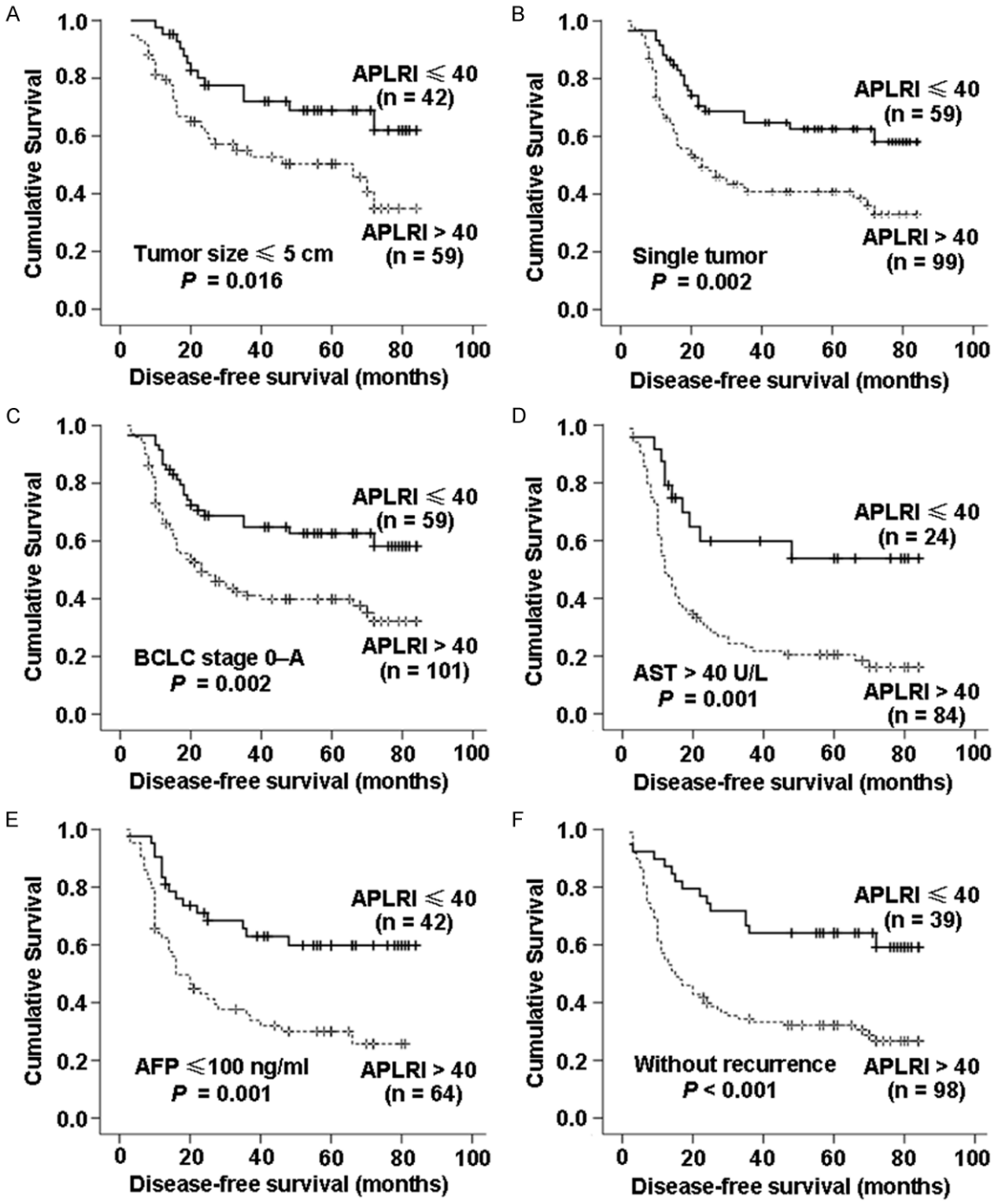

Figure 3. Kaplan-Meier survival analysis of DFS for APLRI in HCC subgroups patients. A preoperative APLRI > 40 predicted a poorer DFS in subgroups with median tumor size ≤ 5 cm (A), a single tumor (B), BCLC stage 0-A (C), an increased serum AST level > 40 U/L (D), a serum AFP level lower than 100 ng/ml (E), and no recurrence (F).

such a predictive role was also found in subgroups with an increased serum AST level > 40 U/L (**Figure 3D**, P = 0.001; **Figure 4D**, P = 0.001; respectively). In addition, for subgroups with a serum AFP level lower than 100 ng/ml, a preoperative APLRI > 40 was a significant favorable prognostic factor for DFS (**Figure 3E**, P = 0.001) and OS (**Figure 4E**, P = 0.001), and this significant trend was also observed in patients without recurrence (**Figure 3F**, P <

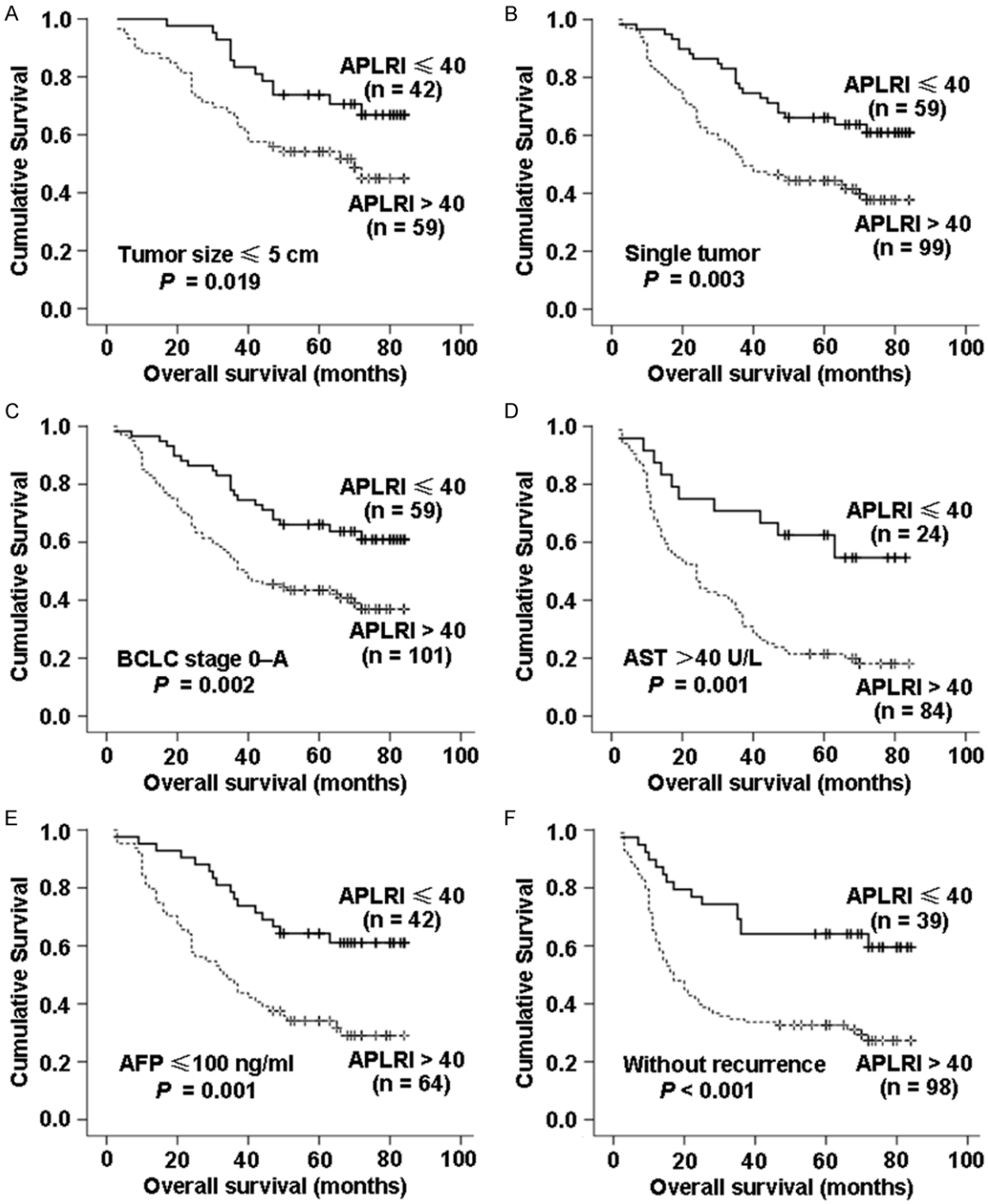

Figure 4. Kaplan-Meier survival analysis of OS for APLRI in HCC subgroups patients. A preoperative APLRI > 40 predicted a poorer OS in subgroups with median tumor size ≤ 5 cm (A), a single tumor (B), BCLC stage 0-A (C), an increased serum AST level > 40 U/L (D), a serum AFP level lower than 100 ng/ml (E), and no recurrence (F).

0.001; **Figure 4F**, P < 0.001; respectively). Therefore, these data provide compelling evidence that the preoperative APLRI may serve as a promising prognostic factor for different clinical HCC subgroups.

Discussion

The prognosis of HCC remains unsatisfactory due to the high risk of recurrence and distant metastasis, which are the most difficult points

in the clinical treatment. More importantly, tumor cell heterogeneity, genetic changes, and epigenetic alterations may resulted in prognostic variability of HCC individuals after hepatic resection. Thus, there is an urgent need for some new markers that can effectively evaluate the prognosis and further guide the best treatment of HCC individuals. In the current study, we researched and developed a novel index, preoperative APLRI, which was calculated from two routine biochemical laboratory tests and will be quite valuable as an inexpensive and noninvasive method for distinguishing an favorable or unfavorable prognoses in patients with HCC.

Liver function tests (LFT) is an available biochemical test for evaluating host liver function. As one of the important enzymes in the liver, ALP is widely present in prokaryotes and higher eukaryotes [21, 22], which are indispensable plasma membrane-bound glycoproteins [23]. Previous studies have reported that a high elevation of ALP in serum is significantly correlated with the blockage of bile ducts [20]. Despite that, ALP has been included as one of the parameters in the Chinese University Prognostic Index (CUPI) and was regarded as an adverse prognostic factor if patients had an ALP level higher than 200 U/L [24, 25]. Additionally, the albumin-to-alkaline phosphatase ratio index (AAPR) is considered an independent prognostic indicator for HCC patients [26]. Furthermore, the elevation of preoperative ALP could be used to predict recurrence and outcome of HCC individuals after resection [11].

Recently, numerous studies have demonstrated that systemic inflammation and its associated immunomodulation play a central role in the progression and development of various malignant neoplasms, including HCC [27, 28]. Systemic inflammation may increase the adhesive capacity of circulating tumor cells, predisposing the tumor cells to proliferate and metastasize to other sites [29]. As an important part of the immune system, lymphocytes have powerful tumor-fighting properties [18]. On the one hand, the liver is enriched in natural killer (NK) and NKT cells, which are the main population of the innate immune system and exhibit antitumor functions. Hepatic NK cells account for one-third of the intrahepatic lymphocytes [30], and they can directly kill infected cells and malignant cells by releasing cytokines [13, 31]. Even more importantly, hepatic NK cells significantly correlated not only with the survival rate after a hepatectomy but also with the depletion of tumor cells [32]. Hepatic NKT cells are a subpopulation of T lymphocytes, which account for 30% of all lymphocytes in the liver and play a crucial role in the response against neoplasm metastasis [13, 33]. On the other hand, as an integral component of the host adaptive immune response, CD4+ T helper type 1 lymphocyte (Th1) cells and CD8+ cytotoxic T lymphocytes (CTLs) have been shown to play key roles in orchestrating the development and prognosis of tumor cells. The combination of tumor necrosis factor α (TNFα) and interferon γ (IFNγ) released by activated CD4+ Th1 cells directly induces significant cancer cell growth arrest [34]. Crucially, CD4+ Th1 cells closely relate with the state of activation of CTLs [35], and once activated, the CTLs will cause a complete eradication or regression of the malignant tumor cells [36].

The above results inspired our interest in conducting an in-depth exploration of the combination of preoperative ALP and serum lymphocytes, which are both generally available in everyday practice, for predicting the prognosis of HCC patients. To our amazement, our studies clearly demonstrated that the preoperative APLRI was an independent prognostic marker for survival of the HCC cohort after liver resection. In addition, HCC individuals with a preoperative APLRI value > 40 had a significantly poorer DFS and OS when compared to those with a preoperative APLRI value ≤ 40.

There are several explanations for the association between an elevated preoperative APLRI and an unfavorable prognosis of HCC patients who underwent liver resection. As a hydrolase enzyme, a high ALP level can indicates damage to the liver cells and is statistically related to cancer cell proliferation or promotion [11, 20]. In the CUPI system, HCC patients with an ALP level higher than 200 U/L had a poor prognosis [24]. Conversely, patients with a high preoperative APLRI might have relative lymphocytopenia, which might compromise anti-tumor immunity via the reduced regulatory T cells [37]. CD4+ Th1 cells are an important effector of host immune regulation, which is involved in mediating the elimination of (pre)malignant

cells and further preventing oncogenesis [38]. The reduced number of CD4+ Th1 cells may not improve the anti-tumor effect in HCC lesions [13]. Moreover, the reduction in the number of CD4+ T lymphocyte cells was combined with an impaired functionality of dendritic cells (DCs), which may produce limited amounts of interleukin (IL)-12; however, IL-12 has been shown to play an important anti-tumor role *in vivo* [39, 40]. Of particular note, the lack of CD4+ T lymphocyte cells contributes to the defective function of CTLs [41] . There are also studies indicating that the depletion of CTLs leads to a rapid spread of tumor cells in visceral organs [42] , but the presence of CTLs is significantly associated with a favorable prognosis in cancer [43].

Serologic biomarker AFP is generally recommended in the clinic for the diagnosis and monitoring of HCC and also acts as a prognostic factor for HCC after tumor resection [44]. The clinical value of AFP, however, remains controversial because 30-40% of HCC patients without AFP elevation have a recurrence [45, 46]. Interestingly, we found that a preoperative APLRI > 40 exhibited potential value in discriminating poorer survival in subgroups with an AFP level lower than 100 ng/ml. Therefore, HCC patients with a lower AFP (≤ 100 ng/ml) but a higher preoperative APLRI should be closely monitored and followed-up with for an optimal response.

Early diagnosis and treatment are crucial to achieve a satisfactory prognosis of HCC patients [47]. However, to date, molecular biomarkers that could effectively predict the prognosis and further determine the best therapeutic strategies for HCC individuals in early stages are still substantially limited. We found that the preoperative APLRI has the power to effectively discriminate survival at relatively early stages (e.g., tumor size less than 5 cm, single tumor nodule, BCLC stage 0-A). In subgroups with tumor sizes smaller than 5 cm in diameter, a preoperative APLRI > 40 significantly correlated with a poorer DFS and OS, and this prognostic value also existed in subgroups with a single tumor nodule or HCC patients with BCLC stage 0-A. Thus, we believe that an elevated preoperative APLRI is related to a poor prognosis in early-stage HCC, which may be a reliable guiding principle for subsequent treatment.

Consistent with the previous results, univariate and multivariate analyses showed that tumors greater than 5 cm in diameter were significantly correlated with adverse outcomes in patients undergoing a hepatectomy in our current study. As is well known, tumor burden is one of the most important independent prognostic factors for HCC patients [48]. HCC patients with tumor size < 5 cm had a better survival rate compared with those with tumor size > 5 cm [49]. In addition, we also found that patients with multiple nodules had significantly worse survival rates. This is consistent with previous reports that multiple nodules of tumor was responsible for an inferior DFS and OS rate [50].

In conclusion, the preoperative APLRI, an innovative and available index, is closely related to clinicopathological parameters and survival outcomes in HCC patients. However, above all, it can be used to predict the prognosis for patients with early-stage HCC. An important limitation of our study was the retrospective nature. Therefore, a further comparative study in a larger population would allow us to better understand the predictive value of the preoperative APLRI.

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Disclosure of conflict of interest

None.

Abbreviations

HCC, hepatocellular carcinoma; ALP, alkaline phosphatase; APLRI, alkaline phosphatase-to-lymphocyte ratio index; ROC, receiver operating characteristic; HR, Hazard ratios; CI, confidence intervals; DFS, disease-free survival; OS, overall survival; BCLC, barcelona-clinic liver cancer; AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; AST, aspartate aminotransferase.

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