Combination of the neutrophil to lymphocyte ratio and the platelet to lymphocyte ratio as a useful predictor for recurrence following radiofrequency ablation of hepatocellular carcinoma

KAI CHEN[1,2*], MEI-XIAO ZHAN[2*], BAO-SHAN HU[2*], YONG LI[2], XU HE[2], SI-RUI FU[2], YONG-JIE XIN[2] and LI-GONG LU[1,2]

[1]The Second Clinical Medical College, Southern Medical University, Guangzhou, Guangdong 510515; [2]Department of Interventional Radiology, Cancer Center, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong 510080, P.R. China

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Abstract. The aim of the present study was to investigate the prognostic potential of a novel inflammation-based system, the combination of the neutrophil to lymphocyte ratio (NLR) and the platelet to lymphocyte ratio (PLR) (CNP), for predicting the survival time of patients with hepatocellular carcinoma (HCC) who had received radiofrequency ablation (RFA). A total of 287 HCC patients treated with RFA were enrolled in the study. Patients with an elevated NLR (>2.58) and an elevated PLR (>131.78) were allocated a score of 2, and patients exhibiting one or neither of these characteristics were allocated a score of 1 or 0, respectively. The association between the CNP and various HCC clinicopathological factors, patterns of recurrence and prognoses were analyzed. The CNP was associated with liver cirrhosis (P=0.015), Child-Pugh class (P=0.024), total bilirubin level (P=0.028), neutrophil count (P<0.001), lymphocyte count (P<0.001) and platelet count (P<0.001). Compared with their low-CNP counterparts, patients with an elevated CNP were more likely to develop distant intrahepatic recurrence [52.3% (CNP 2) vs. 33.9% (CNP 0) and 34.6% (CNP 1), P=0.015; CNP 0 vs. CNP 1, P=0.922; CNP 1 vs. CNP 2, P=0.020] and extrahepatic metastasis [25.0% (CNP 2) vs. 7.6% (CNP 0) and 18.5% (CNP 1), P=0.003; CNP 0 vs. CNP 1, P=0.020; CNP 1 vs. CNP 2, P=0.309], and had shorter overall survival (OS) time (CNP 0 vs. CNP 1, P<0.001; CNP 1 vs. CNP 2, P<0.001) and recurrence-free survival (RFS; CNP 0 vs. CNP 1, P=0.012; CNP 1 vs. CNP 2, P=0.004). Moreover, multivariate analysis revealed that the CNP was superior to the NLR and the PLR as an independent prognostic marker of OS and RFS. Therefore, it was concluded that the CNP may represent a useful predictor for recurrence and prognosis in patients with HCC treated with RFA.

Correspondence to: Professor Li-Gong Lu, Department of Interventional Radiology, Cancer Center, Guangdong General Hospital, Guangdong Academy of Medical Sciences, 96 Dongchuan Road, Weilun Building, Guangzhou, Guangdong 510080, P.R. China
E-mail: luligong1969@126.com

*Contributed equally

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third leading cause of cancer-related mortality in the world (1). Only 20% of HCC patients are candidates for resection (2) due to multifocal disease or poor liver function reserve as a result of the underlying cirrhosis. Liver transplantation is an alternative curative treatment for early HCC, but is often unfeasible due to the shortage of liver transplantation donors. Therefore, various non-surgical therapies have been introduced. Among these, local ablative therapies have been developed for the management of small HCC (3). Currently, local ablative therapies compete with resection and liver transplantation as curative treatments for small HCC.

Percutaneous radiofrequency ablation (RFA) is one of the most widely used local ablation therapies for HCC, with a high complete ablation rate of >85% for solitary tumors <5 cm in diameter or up to 3 tumors with a maximum diameter of 3 cm (4,5). Two previous randomized controlled trials have suggested that RFA is as effective as resection in terms of overall survival (OS) and disease-free survival times (6,7). Increasing evidence demonstrates that RFA for small HCC (<3 cm) may result in comparable survival time with partial hepatectomy (7,8). However, RFA is associated with a high incidence of postoperative recurrence. It has been reported that the cumulative 5-year recurrence rate of patients undergoing RFA is >70% (8,9).

Previous studies have demonstrated that the systemic inflammatory response (SIR) serves an important role in the progression of cancer (10,11), including the proliferation, invasion, recurrence and metastasis of tumors. SIR is associated with OS and postoperative survival times in patients of several

cancer types (12-14). As typical representatives of inflammatory factors, the neutrophil to lymphocyte ratio (NLR) and the platelet to lymphocyte ratio (PLR) have become a research focus for a number of malignancies. Published data also suggest that an elevated NLR may be associated with a worse prognosis in patients with HCC who have undergone resection, orthotopic liver transplantation or transcatheter arterial chemoembolization (TACE) (15-18). Several clinical studies in advanced HCC have demonstrated that an elevated NLR or PLR reflects an inflammatory process elicited by cancer cells, and is associated with unfavorable clinicopathological features (15,17,19).

To the best of our knowledge, few studies have evaluated the association between the NLR, the PLR and the prognosis of HCC patients following RFA treatment. Therefore, the present study evaluated the value of a novel inflammation-based prognostic system, the combination of the neutrophil to lymphocyte ratio and the platelet to lymphocyte ratio (CNP), in patients undergoing RFA for HCC.

Materials and methods

Patients and samples. A retrospective analysis was conducted of 287 patients with HCC who had undergone RFA at Guangdong General Hospital (Guangzhou, Guangdong, China) between January 2010 and December 2014. Written informed consent was obtained from patients prior to treatment and the study was approved by the Ethics Committee of Guangdong General Hospital. A diagnosis of HCC was based on the criteria of the American Association of the Study of Liver Disease (20). The inclusion criteria were as follows: i) Patient age of 18-75 years; ii) a solitary HCC tumor ≤5.0 cm in diameter or multiple HCC lesions each ≤3 in diameter; iii) an Eastern Cooperative Oncology Group Performance Status (ECOG-PS) (21) of 0; iv) liver function Child-Pugh (22) class A or B cirrhosis; v) RFA as the first-line anticancer treatment for HCC; vi) preoperative NLR and PLR data obtained <1 week prior to RFA; vii) no other malignancies that may determine the prognosis; and viii) no extrahepatic metastases. The exclusion criteria were as follows: i) Radiological evidence of invasion into the major portal/hepatic vein branches; ii) the presence of extrahepatic metastases; iii) previous chemotherapy and/or radiotherapy; iv) previous anti-inflammatory medicines within 1 week; v) active infection at the time of blood sampling to establish NLR and PLR; vi) severe coagulation disorders; and vii) loss to follow-up within 3 months post-treatment.

RFA procedure. RFA was performed on an inpatient basis using an RFA system (RITA Medical Systems Inc., Mountain View, CA, USA). The pathological features (size, number, shape and border) of the tumors were identified prior to surgery and the access routes were determined by contrast-enhanced computed tomography (CT) or ultrasound. All procedures were performed percutaneously, under general or local anesthetic, by two qualified interventional radiologists with the guidance of real-time ultrasonography or X-ray. Each ablation cycle lasted between 5 and 12 min. For tumors ≤3.0 cm, a single ablation was performed. For tumors >3.0 cm, multiple overlapping ablations were performed. The range of ablation was extended 0.5-1.0 cm into the surrounding non-cancerous tissues to ensure complete coverage.

RFA was deemed successful based on the following CT observations: i) The ablation zone was beyond the original tumor borders; ii) the margin of the ablation zone was clear and smooth; and iii) no arterial enhancement or abnormal wash-out was detected within or around the tumor.

Follow-up. Dual-phase spiral CT was performed 4-6 weeks post-treatment. Short-term response was assessed using the modified Response Evaluation Criteria In Solid Tumors (m-RECIST) (23), based on the CT images acquired 1 month after RFA. Residual viable tumor tissue was considered to be present if enhancement areas were observed within the tumor at either the arterial or the portal venous phase. In these cases, further RFA treatment was administered.

All patients were followed up in the Oncology Clinic of the Gangdong General Hospital. Follow-up involved physical examination, blood tests, including liver functions tests and α-fetoprotein (AFP) level assessment, and abdominal CT/magnetic resonance imaging (MRI) every 3 months for the first 2 years, every 4-6 months for the subsequent 3 years and annually thereafter. If extrahepatic recurrence was suspected (on the basis of clinical symptoms or an unexplained elevation in AFP level), chest CT, brain MRI and whole-body bone scintigraphy were also performed. The patients were censored on the date of mortality or the date of last follow-up if tumor recurrence was not diagnosed. The last follow-up date for the present study was December 2015.

OS and recurrence-free survival (RFS) times were assessed. The OS time was defined as the time between termination of RFA and the date of mortality or the last follow-up. The RFS time was defined as the time between termination of RFA and the first recording of disease recurrence or the date of mortality in patients without evidence of disease recurrence. Recurrence included local recurrence, distant intrahepatic recurrence and extrahepatic metastasis. Local recurrence was defined as tumor recurrence within or at the periphery of the ablated lesion on CT/MRI after complete ablation had been confirmed on the first post-ablation CT scan; distant intrahepatic recurrence was defined as a separate new lesion in the liver >2 cm away from the primary lesion and extrahepatic metastasis was defined as any tumor lesion outside of the liver.

When recurrent tumors were diagnosed, patients received appropriate management, including repeated RFA, percutaneous ethanol injection therapy, TACE, resection surgery, liver transplantation, chemotherapy, radiotherapy or supportive treatment.

CNP evaluation. Data on preoperative blood cell counts were extracted retrospectively from the medical records. All white blood cell and differential counts were taken within 1-3 days prior to RFA. The NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count and PLR was defined as the absolute platelet count divided by the absolute lymphocyte count. The recommended cut-off values of the preoperative NLR and PLR were determined using receiver operating characteristic (ROC) curve analysis.

Statistical analysis. Statistical analyses were performed using SPSS 21.0 statistical software (IBM Corp., Armonk, NY, USA). Baseline continuous variables were expressed as the

mean ± standard deviation or the median and were compared using one-way analysis of variance with post-hoc Bonferroni's correction. Categorical data were presented as frequencies and were analyzed using the Pearson χ^2 test or Fisher's exact test. Correlation analysis was performed using Pearson's and Spearman's correlation analyses. Survival curves were calculated using Kaplan-Meier analysis, and the difference in survival rates between the groups were compared using the log-rank test. The primary endpoint was OS and the secondary endpoint was RFS. Univariate analysis was used to assess significant differences in the clinicopathological characteristics that influence survival following RFA. Multivariate analysis was performed using Cox's regression analysis for significant variables identified by univariate analysis. Risk ratios with a 95% confidence interval were used to quantify the strength of the association between predictors and survival. All statistical tests were two-sided, and P<0.05 was considered to indicate a statistically significant difference. Bonferroni's correction was performed on the survival curve data, so P<0.017 was considered to indicate a statistically significant difference in survival curve analysis.

Results

CNP evaluation. The recommended cut-off values of the preoperative NLR and PLR were determined using ROC curve analysis (Fig. 1). The recommended cut-off value for the NLR was based on the most prominent point on the ROC curve for sensitivity and specificity (0.674 and 0.604, respectively). These two parameters indicated a cut-off value of 2.58, and the area under the ROC curve (AUC) was 0.663. Similarly, the ROC curve for PLR recommended a cut-off value of 131.78 for sensitivity and specificity (0.601 and 0.792, respectively), and the AUC was 0.703.

The CNP was calculated as follows: Patients with an elevated NLR (>2.58) and an elevated PLR (>131.78) were allocated a score of 2, and patients exhibiting one or neither of these factors were allocated a score of 1 or 0, respectively.

Patient characteristics. A total of 287 patients were enrolled in the present study [male:female, 215 (74.9%):72 (25.1%)]. The mean age was 56.1±14.2 years, with an age range of 27-75 years. Table I presents the clinical background characteristics of the patients in the 3 groups divided according to their CNP. The present study demonstrated that CNP was associated with liver cirrhosis (P=0.015) and Child-Pugh class (P=0.024). No significant differences were observed between the CNP and the hepatitis B surface antigen (HBsAg), the number of tumors, the tumor diameter or the AFP level (P>0.05). In addition, there was a positive correlation between the PLR and the NLR (r=0.534; P<0.001; Fig. 2).

Association between CNP and various clinicopathological characteristics of HCC. The associations between CNP and different clinicopathological features of HCC were analyzed (Table II). Significant differences were identified among the groups with regard to the absolute neutrophil count (CNP 0 vs. CNP 1, P<0.001; CNP 1 vs. CNP 2, P=0.139), the absolute lymphocyte count (CNP 0 vs. CNP 1, P<0.001; CNP 1 vs. CNP 2, P<0.001), the total platelet count (CNP 0 vs. CNP 1, P=0.120; CNP 1 vs. CNP 2, P<0.001), the total bilirubin level (mol/l) (CNP 0 vs. CNP 1, P=0.043; CNP 1 vs. CNP 2, P=0.012), the NLR (CNP 0 vs. CNP 1, P<0.001; CNP 1 vs. CNP 2, P<0.001) and the PLR (CNP 0 vs. CNP 1, P<0.001; CNP 1 vs. CNP 2, P<0.001).

Table I. Associations between general clinical variables and CNP in hepatocellular carcinoma patients undergoing radiofrequency ablation.

Variables	CNP0 (n=118)	CNP1 (n=81)	CNP2 (n=88)	P-value
Sex				0.258
Male	89	65	61	
Female	29	16	27	
Age, years				0.352
≤55	51	43	39	
>55	67	38	49	
HBsAg				0.606
Positive	72	53	51	
Negative	46	28	37	
Liver cirrhosis				0.015
Presence	79	63	74	
Absence	39	18	14	
Number of tumors				0.798
1	87	58	67	
≥2	31	23	21	
Tumor diameter, cm				0.824
≤3	76	55	56	
3-5	42	26	32	
Tumor location near intrahepatic vessels				0.761
Yes	41	30	35	
No	77	51	53	
Child-Pugh class				0.024
A grade	92	61	54	
B grade	26	20	34	
AFP, ng/ml				0.690
<400	43	34	36	
≥400	75	47	52	
Prothrombin time prolongation, sec				0.163
≤3	71	48	42	
>3	47	33	46	

CNP, combination of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio; HBsAg, hepatitis B surface antigen; AFP, α-fetoprotein.

Pattern of recurrence in patients with HCC following RFA. The present follow-up study demonstrated that 179/287 (62.4%) patients developed recurrence. Among these patients, 151 (52.6%) developed intrahepatic recurrence (37 with local recurrence and 114 with distant intrahepatic recurrence). A

Table II. Correlations between CNP and clinicolaboratory variables.

Variable	CNP0 (n=118)	CNP1 (n=81)	CNP2 (n=88)	P-value (0 vs. 1)	P-value (1 vs. 2)	P-value
ALT, U/l	38.7±30.5	45.3±33.9	41.2±36.4	-	-	0.627
AST, U/l	41.6±33.8	47.2±35.7	49.8±41.6	-	-	0.483
Absolute neutrophil count, n	3.72±1.43	4.87±2.48	5.29±1.70	<0.001	0.139	<0.001
Absolute lymphocyte count, n	2.05±0.61	1.59±0.73	1.22±0.40	<0.001	<0.001	<0.001
γ-glutamyltransferase, U/l	70.7±45.7	65.8±49.3	75.1±40.8	-	-	0.207
Total platelets, n	169.90±62.35	186.42±80.55	243.13±80.00	0.120	<0.001	<0.001
Albumin, g/dl	3.78±0.66	4.12±0.81	3.91±0.73	-	-	0.774
Total bilirubin, mol/l	16.6±9.1	18.9±8.3	22.1±11.4	0.043	0.012	0.028
NLR	1.92±0.83	3.11±1.00	4.64±2.14	<0.001	<0.001	<0.001
PLR	84.63±23.09	124.39±52.55	206.22±63.30	<0.001	<0.001	<0.001

NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; CNP, combination of NLR and PLR; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

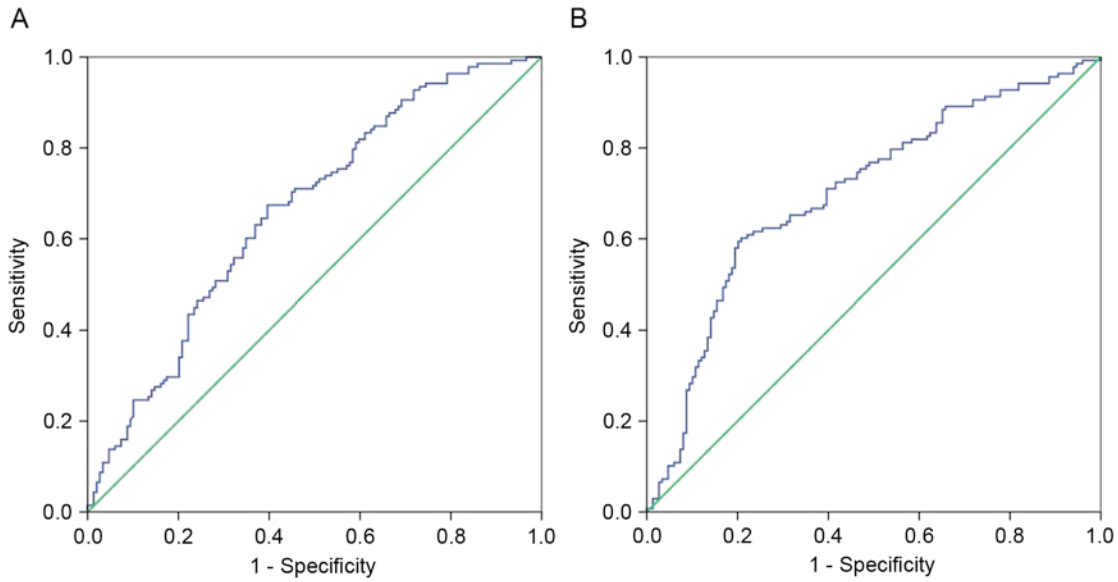

Figure 1. ROC curves for (A) NLR and (B) PLR. ROC curves for overall survival prediction were plotted to verify the optimum cut-off point for NLR and PLR, which was 2.58 and 131.78, respectively. (A) The area under the ROC curve for NLR was 0.663, with a sensitivity of 0.674 and a specificity of 0.604. (B) The area under the ROC curve for PLR was 0.703, with a sensitivity of 0.601 and a specificity of 0.792. ROC, receiver operating characteristic; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

total of 46 patients (16.0%) developed extrahepatic metastasis, including 18 cases of concurrent intrahepatic and extrahepatic recurrences.

To further evaluate the association between CNP and tumor recurrence in these patients, the pattern of recurrence was analyzed and the results are presented in Table III. The present study demonstrated that, although no significant differences were identified in the local recurrence rate among the three groups, the patients with an elevated CNP had significantly higher distant intrahepatic recurrence (52.3, 34.6 and 33.9% for CNP groups 2, 1 and 0, respectively; CNP 0 vs. CNP 1, P=0.922; CNP 1 vs. CNP 2, P=0.020) and extrahepatic recurrence rates (25.0, 18.5 and 7.6% for CNP groups 2, 1 and 0, respectively; CNP 0 vs. CNP 1, P=0.020; CNP 1 vs. CNP 2, P=0.309) compared with their low-CNP counterparts. In addition, 3, 6, and 9 patients had concurrent intrahepatic and extrahepatic recurrences in the CNP 0, CNP 1 and CNP 2 groups, respectively (data not shown).

OS and RFS. Kaplan-Meier analysis and log-rank tests demonstrated a significant difference in the median OS time among the three groups (23.2, 50.0 and 62.8 months for CNP groups 2, 1 and 0, respectively; CNP 0 vs. CNP 1, P<0.001; CNP 1 vs. CNP 2, P<0.001) (Fig. 3A). In addition, there were also significant differences in the RFS time among the three groups (18.8, 32.9 and 40.2 months for CNP groups 2, 1 and 0, respectively; CNP 0 vs. CNP 1, P=0.012; CNP 1 vs. CNP 2, P=0.004) (Fig. 3B). Thus, the CNP was able to clearly classify patients into three independent groups.

Table III. Correlation between pattern of recurrence and CNP in patients with hepatocellular carcinoma.

Recurrence	CNP 0, n (%)	CNP 1, n (%)	CNP 2, n (%)	P-value (0 vs. 1)	P-value (1 vs. 2)	P-value
Type of recurrence						
Local recurrence	18 (15.3)	13 (16.0)	6 (6.8)	-	-	0.123
Distant intrahepatic recurrence	40 (33.9)	28 (34.6)	46 (52.3)	0.922	0.020	0.015
Extrahepatic recurrence	9 (7.6)	15 (18.5)	22 (25.0)	0.020	0.309	0.003
Total patients	64 (54.2)	50 (61.7)	65 (73.9)	-	-	0.016
Treatment for recurrence						
Repeat RFA	22	13	25	-	-	0.105
Others	42	37	40	-	-	0.241

CNP, combination of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio; RFA, radiofrequency ablation.

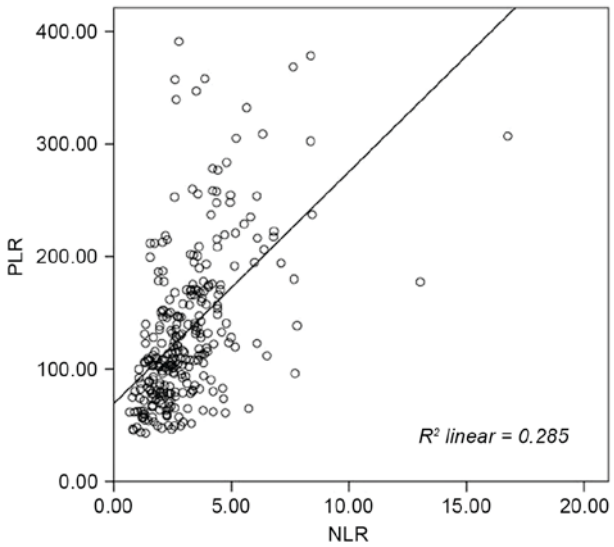

Figure 2. Correlation between NLR and PLR. There was a positive correlation between NLR and PLR (r=0.534, P<0.001). NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

Prognostic factors associated with the OS time of HCC patients undergoing RFA. Univariate and multivariate analyses were next performed to assess the association between clinical characteristics and the OS, the results of which are represented in Table IV. Univariate analysis demonstrated that the OS was significantly associated with high HBsAg (P=0.041), presence of liver cirrhosis (P=0.024), large tumor diameter (P=0.011), high Child-Pugh class (P=0.017), an elevated NLR (P=0.018), an elevated PLR (P=0.012) and an elevated CNP (P<0.001).

Multivariate analysis was performed with the Cox proportional hazards model using the clinical characteristics revealed to be significantly associated with the OS (P<0.05) by univariate analysis (Table IV). The results indicate that the high Child-Pugh class (P=0.019), large tumor diameter (P=0.005), an elevated CNP (P<0.001) and PLR (P<0.001) were independent prognostic factors of OS. In addition, the results of the present study revealed that CNP (RR, 2.183; P<0.001) is superior to PLR (RR, 1.732; P=0.024) as a predictive factor in patients with HCC (Table IV).

Prognostic factors associated with the RFS of patients with HCC undergoing RFA. Univariate and multivariate analyses were performed to assess the association between clinical characteristics and the RFS (Table V). Univariate analysis revealed that the RFS was significantly associated with high number of tumors (P=0.024), high HBsAg (P=0.046), presence of liver cirrhosis (P=0.031), location near intrahepatic vessels (P=0.021), an elevated NLR (P=0.033), an elevated PLR (P=0.028) and an elevated CNP (P<0.001).

Multivariate analysis was performed with the Cox proportional hazards model using the characteristics revealed to have statistical significance (P<0.05) by univariate analysis (Table V). The results indicated that high number of tumors, location near intrahepatic vessels and an elevated CNP were statistically significant independent prognostic factors of RFS (P=0.008, P=0.017 and P<0.001, respectively).

Discussion

There is a strong association between inflammation and cancer. SIR is associated with overall or postoperative survival in patients of several cancer types (12-14,24). The change in tumor-associated inflammatory cells reflects the degree of inflammatory response to the tumor, with a higher inflammatory response often indicating a poor prognosis (25). Due to the convenient and economically viable nature of blood sampling, neutrophils, platelets and lymphocytes are common inflammatory markers that form the composite indices NLR and PLR, and reflect the host inflammatory status. There is increasing evidence that the NLR or the PLR may be used as clinical indicators of the host inflammatory response and immune status, and an elevated NLR or PLR has been revealed to be a strong predictor of poor survival in certain types of malignancies (17,26-30). Previous studies have investigated the prognostic value of combining the blood routine indices in patients with esophageal squamous cell carcinoma or colorectal cancer (31,32), These studies established a novel inflammation-based system, named CNP (the combination of NLR and PLR) or COP-NLR (the combination of platelet count and NLR), and found that CNP and COP-NLR were useful predictors of postoperative survival in cancer patients. The present study analyzed the potential prognostic value of

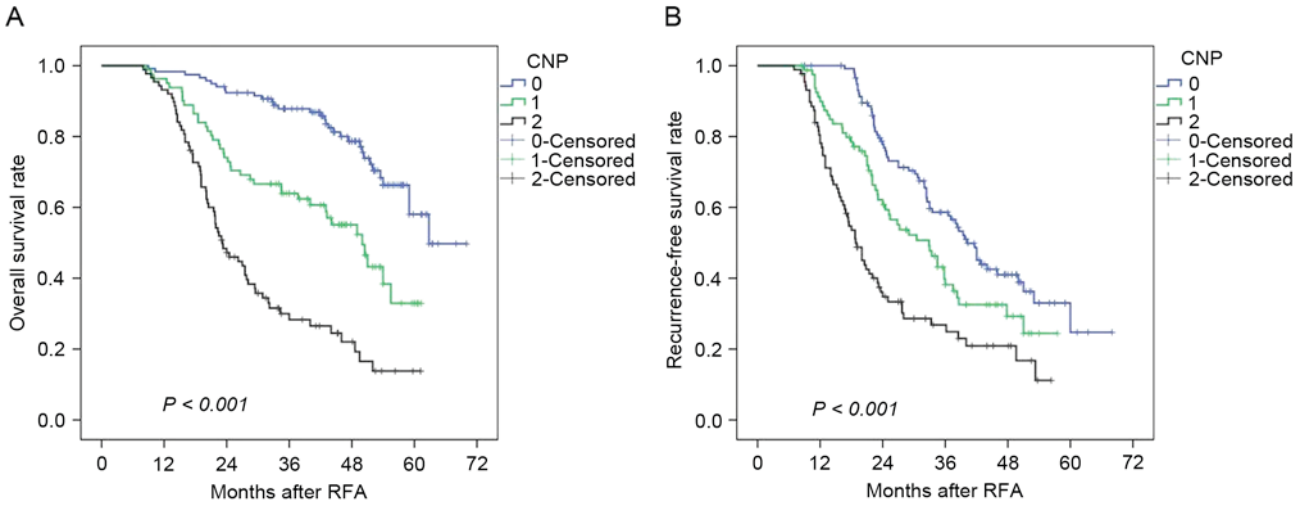

Figure 3. Kaplan-Meier survival curve of (A) OS and (B) RFS for patients with hepatocellular carcinoma accepting RFA. Kaplan-Meier analysis and log-rank test demonstrated a significant difference in OS and RFS among the three groups (P<0.001). OS, overall survival; RFS, recurrence-free survival. CNP, combination of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio; RFA, radiofrequency ablation.

CNP (the combination of NLR and PLR) in HCC patients who had received RFA. To the best of our knowledge, this is the first study to examine the prognostic value of CNP for predicting the prognosis of patients with HCC following treatment with RFA. The present study demonstrated that CNP is associated with tumor progression and thus, can be regarded as an independent prognostic biomarker of poor prognosis in patients who have undergone treatment with RFA for HCC.

A complex tumor microenvironment is one of the most important factors in a cancer prognosis. Several studies have confirmed that the interactions between the tumor itself and the SIR will lead to tumorigenesis (33). The main features of a tumor-associated inflammatory response are the infiltration of leukocytes, the production of cytokines, the remodeling of tissue and angiogenesis (34). Due to the high incidence of hepatitis B in China, the majority of HCC patients are also infected with hepatitis B, the persistent inflammation of which affects the development of HCC (35).

A change of NLR and PLR could be interpreted as a relative increase in the number of neutrophils and the platelet count, or a relative decrease in the number of lymphocytes. There are a number of generally accepted explanations for this imbalance. Firstly, neutrophils can promote tumor growth and invasion by releasing vascular endothelial growth factor (VEGF), an important factor in promoting tumor angiogenesis (36), and there is a significant negative correlation between tumor angiogenic ability and the prognosis of the patient (37). Additionally, inflammatory cells and tumor cells can release a series of inflammatory mediators, including cell growth factor (CXCL8), matrix metalloproteinase 8 and the anti-apoptotic factor, nuclear factor-κB, to promote the growth, invasion and metastasis of the tumor (38), and to induce the involvement of more inflammatory cells. The excessive release of inflammatory mediators leads to oxidative damage, DNA mutation and an altered tumor microenvironment, which further promote cell transformation, and tumor cell growth and reproduction (10).

Secondly, clinical and experimental studies have revealed that malignancy is often associated with thrombocytosis. Platelets can secrete several types of growth factor, including platelet-derived growth factor, platelet factor 4, transforming growth factor-β, thrombospondin-1 and VEGF, which stimulate the proliferation and differentiation of tumor cells and the degradation of the extracellular matrix. Furthermore, platelets can form an adhesion bridge, enabling tumor cells to spread to other locations, including the capillaries, thereby promoting the growth, invasion and metastasis of the tumor (39). Additionally, certain pro-inflammatory cytokines [including interleukin (IL)-1 and IL-6] may promote the proliferation of megakaryocytes and stimulate the differentiation of megakaryocytes to platelets in the bone marrow, leading to further thrombocytosis (40). As their numbers increase, platelets will release more growth factors to stimulate the growth and proliferation of the tumor, thereby aggravating the disease, reducing the survival and the efficacy of treatment in cancer patients. For this reason, elevated levels of neutrophils and platelets may lead to a worse prognosis in cancer patients. The present study not only confirmed that the OS time of patients with an elevated CNP is significantly shorter than that of their low-CNP counterparts, but also observed that patients with an elevated CNP are more likely to develop distant intrahepatic recurrence and extrahepatic recurrence.

Thirdly, lymphocytes are one of the most important components of antitumor immunity. A reduced number of lymphocytes is suggestive of abnormal immune mechanisms and a decline in antitumor immunity, and also creates an environment that enables tumor invasion and metastasis (41). In addition to promoting tumor growth and diffusion, a low number of lymphocytes results in an increased CNP, which is consistent with the results of the present study confirming that CNP is associated with survival times and that a higher CNP was associated with a worse prognosis.

Under normal conditions, the NLR and the PLR maintain a relative dynamic balance. The increase in the NLR and PLR does not indicate the imbalance of any single indicator among neutrophils, platelets or lymphocytes. NLR and PLR can comprehensively reflect the tumor inflammation and immune status in the body, and once this dynamic balance is broken (for example by the relative increase of neutrophils and platelets, or a relative reduction in lymphocytes), the balance between

Table IV. Univariate and multivariate analysis of different factors associated with overall survival in hepatocellular carcinoma patients treated with radiofrequency ablation.

Factors	Univariate P-value	Multivariate analysis		
		Risk ratio	95% CI	P-value
Sex (male/female)	0.785	-	-	-
Age (≤55/>55 years)	0.466	-	-	-
Number of tumors (1/≥2)	0.487	-	-	-
HBsAg (positive/negative)	0.041	-	-	-
Liver cirrhosis (presence/absence)	0.024	-	-	-
Tumor diameter (≤3/3-5 cm)	0.011	1.543	1.032-2.588	0.005
Location near intrahepatic vessels (yes/no)	0.127	-	-	-
Total bilirubin (≤17.1/>17.1 μmol/l)	0.543	-	-	-
Child-Pugh class (A/B)	0.017	1.693	1.078-2.796	0.019
NLR (≤2.58/>2.58)	0.018	-	-	-
PLR (≤131.78/131.78)	0.012	1.732	1.093-2.956	0.024
CNP (0/1/2)	<0.001	2.183	1.251-3.564	<0.001

CI, confidence interval; HBsAg, hepatitis B surface antigen; NLR, neutrophil to leukocyte ratio; PLR, platelet to lymphocyte ratio; CNP, combination of NLR and PLR.

Table V. Univariate and multivariate analysis of prognostic factors associated with recurrence-free survival in hepatocellular carcinoma patients treated with radiofrequency ablation.

Factors	Univariate P-value	Multivariate analysis		
		Risk ratio	95% CI	P-value
Sex (male/female)	0.965	-	-	-
Age (≤55/>55 years)	0.189	-	-	-
Number of tumors (1/≥2)	0.024	1.821	1.194-3.068	0.008
HBsAg (positive/negative)	0.046	-	-	-
Liver cirrhosis (presence/absence)	0.031	-	-	-
Tumor diameter (≤3/3-5 cm)	0.078	-	-	-
Location near intrahepatic vessels (yes/no)	0.021	1.642	1.084-2.765	0.017
Total bilirubin (≤17.1/>17.1 μmol/l)	0.061	-	-	-
Child-Pugh class (A/)B	0.685	-	-	-
NLR (≤2.58/>2.58)	0.033	-	-	-
PLR (≤131.78/131.78)	0.028	-	-	-
CNP (0/1/2)	<0.001	1.965	1.252-3.207	<0.001

CI, confidence interval; HBsAg, hepatitis B surface antigen; NLR, neutrophil to leukocyte ratio; PLR, platelet to lymphocyte ratio; CNP, combination of NLR and PLR.

the tumor inflammatory response and antitumor inflammation response will be destroyed, normal immune function will be impaired, and the patient's ability to fight the tumor will decline. This indicates that the host is in a state of antitumor immunosuppression and that the inflammatory response will develop towards the promotion of tumor progression, leading to poor patient prognosis.

In the present study, univariate analysis demonstrated that NLR, PLR and CNP were predictive of OS and RFS times in HCC patients who had undergone RFA. The Kaplan-Meier analysis and log-rank tests also demonstrated that CNP was able to clearly classify patients into three independent groups, results which assisted in illustrating that patients with a higher NLR and PLR had a worse prognosis. In addition to CNP, PLR was also revealed to be an independent prognostic indicator of OS by multivariate analysis. In addition, CNP is an independent prognostic indicator of RFS, but no prognostic value was demonstrated by PLR in the recurrence of HCC.

Furthermore, the present study emphasized the association between CNP and the pattern of recurrence in HCC patients following RFA, and CNP was revealed to be associated with distant intrahepatic recurrence and extrahepatic recurrence. Since the majority of the Chinese patients with liver cancer have hepatitis B infections, persistent inflammation has always been associated with the progression of the disease in HCC patients. Inflammatory mediators produced by tumor-associated inflammatory responses, as well as abnormalities in the immune mechanism and a reduction of antitumor immunity caused by the reduction in the number of lymphocytes, provide favorable conditions for tumor invasion and metastasis. This suggests that a higher inflammatory response always indicates a poor prognosis and that a patient with an elevated CNP is more likely to develop distant intrahepatic recurrence and extrahepatic recurrence.

Multivariate analysis also identified other factors, including the Child-Pugh class and the tumor diameter, as independent predictive factors for OS, and the number of tumors as an independent predictor of tumor recurrence, which was consistent with the results of previous studies (42-44). In particular, location near the intrahepatic vessels was revealed to be an imperative risk factor for HCC recurrence following RFA therapy. It has been demonstrated that blood flow promotes heat loss, which may account for the reduced effectiveness of RFA (45).

The present study is a retrospective analysis of a small patient population with a certain degree of heterogeneity. Therefore, future studies require larger sample sizes to further validate the prognostic capability of CNP in HCC patients who have undergone RFA treatment.

In conclusion, as a simple, readily available indicator, CNP has the potential to serve as a novel non-invasive circulating marker for monitoring HCC progression. Additionally, CNP can also be considered an effective biomarker for tracking tumor recurrence and predicting the prognosis of HCC patients following RFA therapy. Therefore, CNP not only appears capable of classifying HCC patients undergoing RFA into three independent groups, but also has potential as a novel independent unfavorable predictor of post-operative survival in such patients.

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