Virchows Archiv (2020) 477:807–816
https://doi.org/10.1007/s00428-020-02841-5

ORIGINAL ARTICLE

Prognostic effect of preoperative neutrophil-lymphocyte ratio is related with tumor necrosis and tumor-infiltrating lymphocytes in hepatocellular carcinoma

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Received: 11 March 2020 / Revised: 26 April 2020 / Accepted: 6 May 2020 / Published online: 1 June 2020

Abstract

Blood neutrophil-to-lymphocyte ratio (NLR) is one index representing systemic inflammation, and high preoperative NLR has been suggested as an independent prognostic factor in HCC. However, the NLR cutoff value with the highest prognostic significance is not consistent, and the mechanism of this phenomenon remains unclear. Preoperative NLR was calculated from complete blood counts obtained before 14 days from operation day in 234 patients who underwent curative resection for primary HCC. The presence of tumor necrosis and degree of tumor-infiltrating lymphocytes (TILs) was determined histologically. High preoperative NLR (≥ 2.5) was observed in 28 (12.0%) of 234 HCCs and was significantly associated with younger age, larger tumor size, high Edmonson grade, microvascular invasion, major portal invasion, advanced AJCC T or BCLC stage, and low albumin level. Patients with high preoperative NLR showed shorter disease-specific survival (DSS) ($p = 0.002$) and a tendency for shorter recurrence-free survival (RFS) ($p = 0.096$). High preoperative NLR was associated with presence of tumor necrosis and low TIL. On multivariable analysis, preoperative NLR was an independent prognostic factor for DSS (hazard ratio: 2.050 (95% confidence interval 1.139–3.691), $p = 0.017$). However, the independent prognostic effect of NLR for DSS disappeared when tumor necrosis and TILs were added as co-variables. High NLR is an independent prognostic factor in patients with HCC who undergo curative resection. The prognostic effect of high NLR might originate from the prognostic effect of tumor necrosis or TILs.

Keywords Hepatocellular carcinoma · Systemic inflammation · Neutrophil to lymphocyte ratio · Prognosis · Tumor-infiltrating lymphocytes · Necrosis

Introduction

Hepatocellular carcinoma (HCC) is a devastating malignancy that is the sixth most common cancer and the fourth leading cause of cancer death worldwide [1]. Although surgical resection is the treatment of choice in HCC, it is not curative for most patients because of the high recurrence rate [2]. Prediction of recurrence is important, and prevention with appropriate therapy will improve patient outcome [3].

The prognostic significance of preoperative neutrophil-lymphocyte ratio (NLR) has been suggested as an easily approachable indicator of systemic inflammation and has been highlighted as a biomarker for predicting prognosis in a variety of cancers, including HCC [4–10]. However, the cutoff value of NLR is not consistent, and the underlying mechanism of the prognostic effect of preoperative NLR is not yet fully understood.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00428-020-02841-5) contains supplementary material, which is available to authorized users.

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In this study, we evaluated the prognostic effect of preoperative NLR in 234 patients with HCC who underwent curative resection and had long-term follow-up. We also studied the association of preoperative NLR with histologic features such as tumor necrosis or tumor-infiltrating lymphocytes (TILs).

Materials and methods

Patient population and clinical information

Initially, a total of 291 patients who underwent curative resection for primary HCCs at Samsung Medical Center, Seoul, Korea from July 2000 to May 2006 were enrolled in this study. Among them, 57 patients were excluded as follows: 8 patients with preoperative treatment such as transarterial chemoembolization, radiofrequency ablation, or radiotherapy, 16 patients with evidence of systemic inflammation (WBC > 10,580/μL or segmented neutrophil > 73.5%) and 33 patients with no record of complete blood count (CBC) from 14 days before operating day. Finally, 234 patients were included in this study. The Institutional Review Board of Samsung Medical Center approved this study and waived informed consent.

Curative resection was defined as complete resection of all tumors with negative microscopic resection margins and no visible residual tumors on a computed tomography scan 1 month after surgery. All patients were classified as Child-Pugh class A. Medical records were reviewed for clinical parameters such as age, gender, alcohol history, and results of laboratory tests, including CBC, serology for hepatitis virus A, B, C, and D, serum alpha fetoprotein (AFP), and serum albumin. American Joint Committee on Cancer (AJCC) staging system and Barcelona Clinic Liver Cancer (BCLC) staging classification were used for staging [11, 12].

Patients were followed every 3 months after surgery. To detect tumor recurrence, three phase dynamic computed tomography scans or magnetic resonance imaging was performed. The median follow-up period was 120 months (range 14–151 months) for survivors. Recurrence-free survival (RFS) was defined as the interval from the date of surgery to detection of tumor recurrence. Disease-specific survival (DSS) was defined as the interval between date of surgery and date of HCC-related death, which was defined as follows: (1) tumor occupying more than 80% of the liver, (2) portal venous tumor thrombus proximal to second bifurcation, (3) obstructive jaundice due to tumor, (4) distant metastases, and (5) variceal hemorrhage with portal venous tumor thrombus proximal to first bifurcation [13].

Neutrophil-lymphocyte ratio

Preoperative (within 14 days from surgery) and postoperative (on first follow-up day after about 1 month from surgery) NLR was calculated based on CBC values. To identify the optimal cutoff value with highest statistical significance related to patient survival, X-Tile statistics package (Yale University, New Haven, CT, USA) [14] was used. Cases were dichotomized into low and high NLR groups based on the established cutoff value.

Histopathologic evaluation: tumor necrosis and tumor-infiltrating lymphocytes

Paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin. When the tumor was less than 3 cm, the entire tumor was sectioned and embedded. When the tumor was larger than 3 cm, at least four sections were obtained and the mean number of blocks was 1 per cm of tumor diameter. Two liver pathologists (SYH and CKP) reviewed hematoxylin- and eosin-stained slides and confirmed the diagnosis of HCC as well as histopathologic features of HCCs, such as differentiation, microvascular invasion, major portal vein invasion, intrahepatic metastasis, multicentric occurrence, and non-tumor liver pathology. Tumor differentiation was defined according to the criteria of Edmondson and Steiner [15]. Intrahepatic metastasis and multicentric occurrence were determined according to criteria of the Liver Cancer Study Group of Japan [16].

The presence of tumor necrosis and degree of lymphocytic infiltration in tumors were also evaluated. Tumor necrosis was defined as presence of microscopic coagulative necrosis, typified by homogeneous clusters of degenerating and dead tumor cells [17]. The percent of tumor necrosis area was recorded. The number of TILs was counted in 10 high power fields (HPFs) of hematoxylin- and eosin-stained slides. Areas with the most TILs in low power field were identified, and counting was initiated from that area. Specimens were classified into three groups according to the intensity of TILs following a modified method of Wada et al., as follows [18]: −, negative or almost no infiltration; +, mild to moderate infiltration (< 500 cells/10 HPFs); and ++, marked infiltration (> 500 cells/10 HPFs).

Statistical analysis

The association between NLR and clinicopathologic parameters was analyzed using the Chi-square test, Fisher's exact test, or Cochran Armitage test as appropriate. Mann-Whitney or Kruskal-Wallis test was used for comparison of the mean value of NLR, absolute neutrophil, or lymphocyte count. Kaplan-Meier method was used for survival analysis, and differences in survival rates were assessed by the log rank or Breslow test. To evaluate associations between clinicopathologic parameters and survival, the Cox proportional hazard regression model was used. Significant prognostic factors by univariate analysis were entered into multivariate analysis. Proportional hazard assumption was examined graphically. All statistical analyses were performed with SPSS software

Table 1 Association between neutrophil to lymphocyte (NLR) groups and clinicopathologic variables

Variables			Preoperative NLR		
		Total	< 2.5 $n = 206$	≥ 2.5 $n = 28$	p value
Gender					
	Female	38	33 (16.0)	5 (17.9)	0.787[a]
	Male	196	173 (84.0)	23 (82.1)	
Age					
	≤ 40	27	20 (9.7)	7 (25.0)	0.027[a]
	> 40	207	186 (90.3)	21 (75.0)	
Tumor size					
	≤ 5 cm	148	135 (65.5)	13 (46.4)	0.049
	> 5 cm	86	71 (34.5)	15 (53.6)	
Edmondson grade					
	I	25	23 (11.2)	2 (7.1)	0.026
	II	186	167 (81.1)	19 (67.9)	
	III	23	16 (7.8)	7 (25.0)	
Microvascular invasion					
	(−)	107	100 (48.5)	7 (25.0)	0.019
	(+)	127	106 (51.5)	21 (75.0)	
Major portal vein invasion					
	(−)	223	200 (97.1)	23 (82.1)	0.005[a]
	(+)	11	6 (2.9)	5 (17.9)	
Intrahepatic metastasis					
	(−)	183	165 (80.1)	18 (64.3)	0.057
	(+)	51	41 (19.9)	10 (35.7)	
Multicenteric occurrence					
	(−)	220	194 (94.2)	26 (92.9)	0.677[a]
	(+)	14	12 (5.8)	2 (7.1)	
AJCC T stage					
	1	99	93 (45.1)	6 (21.4)	0.024[b]
	2	97	83 (40.3)	14 (50.0)	
	3	33	27 (13.1)	6 (21.4)	
	4	5	3 (1.5)	2 (7.1)	
BCLC stage					
	0,A	141	130 (63.1)	11 (39.3)	0.004[b]
	B	81	69 (33.5)	12 (42.9)	
	C	12	7 (3.4)	5 (17.9)	
Albumin level, g/dL					
	> 3.5	216	196 (95.1)	20 (71.4)	< 0.001[a]
	≤ 3.5	18	19 (4.9)	8 (28.6)	
AFP level, ng/mL					
	≤ 200	140	128 (62.1)	12 (42.9)	0.051
	> 200	94	78 (37.9)	16 (57.1)	
Etiology					
	Non-viral	33	29 (14.1)	4 (14.3)	0.726
	HBV	175	154 (74.8)	21 (75.0)	
	HCV	22	20 (9.7)	2 (7.1)	
	HBV and HCV	4	3 (1.5)	1 (3.6)	

Table 1 (continued)

Variables		Total	Preoperative NLR		
			< 2.5 $n = 206$	≥ 2.5 $n = 28$	p value
Liver cirrhosis					
	(−)	115	100 (48.5)	15 (53.6)	0.618
	(+)	119	106 (51.5)	13 (46.4)	
Tumor necrosis					
	(−)	172	160 (77.7)	12 (42.9)	< 0.001
	(+)	62	46 (22.3)	16 (57.1)	
Tumor-infiltrating lymphocytes					
	(−)	122	100 (48.5)	22 (78.6)	0.009[a]
	(+)	29	27 (13.1)	2 (7.1)	
	(++)	83	79 (38.3)	4 (14.3)	

NLR neutrophil-to-lymphocyte ratio, *AJCC* The American Joint Committee on Cancer, *BCLC* Barcelona Clinic Liver Cancer, *AFP* alpha fetoprotein

[a] By Fisher's exact test

[b] By Cochran Armitage test, otherwise by Chi-square test

(SPSS Inc., Chicago, IL, USA) or R software (version 3.03). *P* values less than 0.05 were considered statistically significant.

Results

Preoperative NLR in patients with HCC and its relationship with clinicopathologic characteristics

The mean preoperative NLR was 1.67 (95% confidence interval, 1.57–1.76), and the median value was 1.50 (range 0.55–4.41). The optimal cutoff value with the highest statistical significance for patient survival, determined by X-Tile package, was 2.50. Preoperative NLR was graded as low (< 2.5) or high (≥ 2.5), and high NLR was observed in 28 (12.0%) of 234 HCCs.

The associations between preoperative NLR and clinicopathologic characteristics are summarized in Table 1. High preoperative NLR was significantly associated with younger age ($p = 0.027$), larger tumor size ($p = 0.049$), high Edmonson grade ($p = 0.026$), microvascular invasion ($p = 0.019$), major portal invasion ($p = 0.005$), advanced AJCC T ($p = 0.024$) or BCLC stage ($p = 0.004$), and low albumin level ($p < 0.001$). Similar results are found in comparison between absolute value of preoperative NLR, neutrophil or lymphocyte count, and clinicopathologic variables. Detailed information is provided in Supplementary Table 1.

Preoperative NLR as an independent prognostic factor for DSS

Patients with high preoperative NLR showed shorter DSS ($p = 0.002$) and a tendency for shorter RFS ($p = 0.096$) (Fig. 1). On multivariate analysis including co-variables (age, tumor size, Edmondson grade, microvascular invasion,

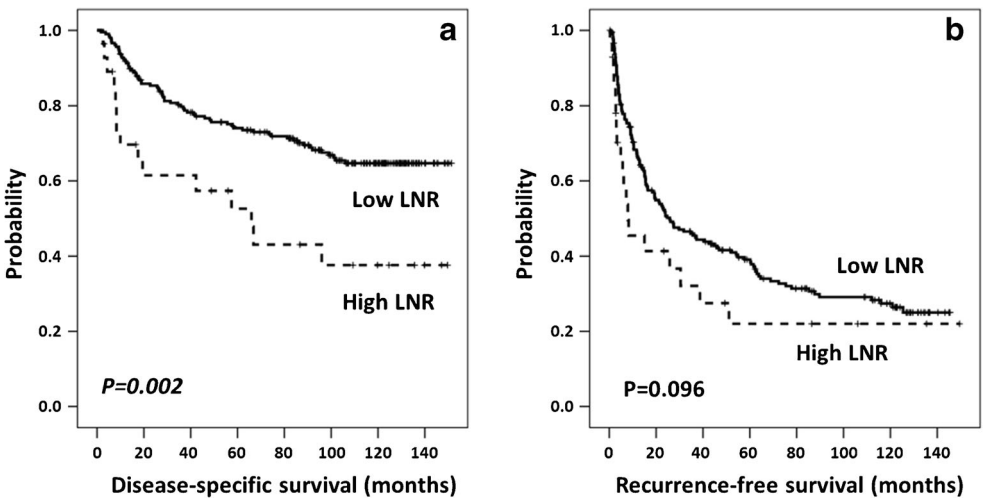

Fig. 1 Kaplan-Meir survival curves according to preoperative neutrophil to lymphocyte ratio. **a** Disease-specific survival. **b** Recurrence-free survival

Table 2 Multivariate analysis for recurrence-free survival and disease-specific survival

		Recurrence-free survival			Disease-specific survival		
		HR	95% CI	*p* value	HR	95% CI	*p* value
Age	≤ 40 years vs > 40 years				1.442	0.802–2.593	0.221
Tumor size	> 5 cm vs ≤ 5 cm	0.967	0.668–1.400	0.859	1.788	1.105–2.895	0.018
Edmondson grade	III vs I + II	1.391	0.834–2.321	0.206	1.319	0.705–2.467	0.387
Microvascular invasion	Yes vs no	1.370	0.933–2.013	0.108	1.571	0.852–2.900	0.148
Major portal vein invasion	Yes vs no	0.777	0.374–1.617	0.5	1.140	0.521–2.493	0.743
Intrahepatic metastasis	Yes vs no	3.528	2.431–5.119	< 0.001	3.984	2.436–6.516	< 0.001
Albumin level, g/dL	≤ 3.5 vs > 3.5	2.218	1.248–3.943	0.007	2.486	1.266–4.881	0.008
AFP level, ng/mL	> 200 vs ≤ 200	1.398	1.004–1.948	0.047	1.060	0.654–1.718	0.814
Etiology	Viral vs non-viral	1.942	1.92–3.454	0.024			
NLR	≥ 2.5 vs < 2.5	1.236	0.753–2.030	0.402	2.050	1.139–3.691	0.017

HR hazard ratio, *CI* confidence interval, *NLR* neutrophil-lymphocyte ratio

major portal vein invasion, intrahepatic metastasis, serum albumin level, and serum AFP level) with statistical significance in univariate analysis, preoperative NLR was an independent prognostic factor for DSS (hazard ratio: 2.050 (95% confidence interval 1.139–3.691, $p = 0.017$) (Table 2), not for RFS.

Preoperative NLR status was associated with tumor necrosis and tumor-infiltrating lymphocytes

Tumor necrosis was identified in 62 (26.5%) of 234 cases. TILs were observed in 112 (47.9%) cases, including 83 (35.5%) with mild to moderate infiltration and 29 (12.4%) with marked infiltration. Tumor necrosis was more frequently identified in the high NLR group than in the low group (57.1% vs 22.3%, $p < 0.001$), and there was a weak correlation between percent of tumor necrosis and NLR ($r = 0.169$, $p = 0.010$). However, TILs were less frequently found in the high NLR group ($p = 0.009$).

The mean value of preoperative NLR was higher in patients with tumor necrosis than in patients without tumor necrosis (Fig. 2a). This effect was mostly induced by differences in preoperative absolute neutrophil count between two groups according to tumor necrosis, not differences in absolute lymphocyte count (Fig. 2b, c). In contrast, the mean value of preoperative NLR was higher in patients without TILs than in patients with TILs (Fig. 2d). This effect was mostly induced by differences in preoperative absolute lymphocyte count between patient groups according to TILs, not differences in absolute lymphocyte count (Fig. 2e, f).

After curative resection, NLR in patients with tumor necrosis or without TILs was decreased, and postoperative NLR was not different in different patient groups according to tumor necrosis or TILs (Fig. 3a–d).

Effects of tumor necrosis or TILs on patient survival

Patients with tumor necrosis showed shorter DSS ($p < 0.001$) and RFS ($p < 0.001$) (Fig. 4a, b). Patients with no TILs showed shorter DSS ($p < 0.001$) and a tendency for shorter RFS ($p = 0.100$) (Fig. 4c, d).

On multivariate analysis including tumor necrosis and TILs as additional co-variables, preoperative NLR was not an independent prognostic factor for DSS, while tumor necrosis and TILs remained strong independent predictors for DSS (Table 3).

Discussion

In this study, we demonstrated the prognostic significance of preoperative NLR in patients undergoing curative resection for primary HCC and long-term follow-up. High preoperative NLR was significantly associated with aggressive tumor phenotype, such as large size, high Edmonson grade, microvascular invasion, major portal invasion, and advanced AJCC T or BCLC stage. High NLR was an independent adverse prognostic predictor for DSS. However, the independent prognostic effect of NLR for DSS disappeared when tumor necrosis and TILs, which were highly associated with NLR status, were added as co-variables.

The prognostic significance of preoperative NLR has been reported in many studies of a variety of cancers. In HCCs, most previously reported studies have highlighted preoperative NLR as a prognostic factor in the setting of curative hepatectomy, although the NLR cutoff value was not consistent and ranged from 2.31 to 5 (summarized in Table 4) [4–10]. While the underlying mechanism of the prognostic effect of preoperative NLR is not fully understood, some hypothetical

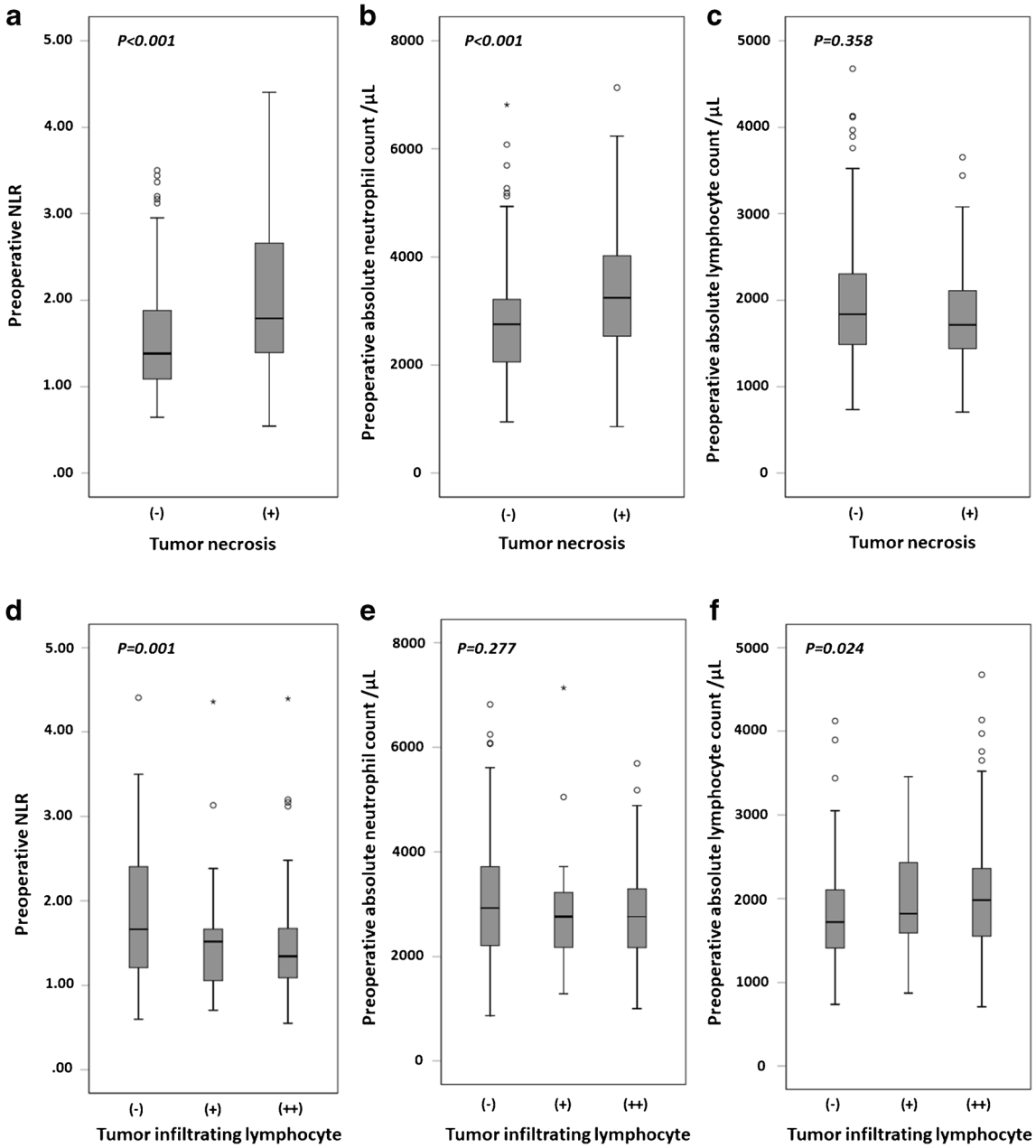

Fig. 2 Association between preoperative neutrophil to lymphocyte ratio (NLR) and tumor necrosis or tumor-infiltrating lymphocytes. **a**–**c** Mean value of preoperative NLR (**a**), absolute neutrophil count (**b**), and absolute lymphocyte count (**c**) in two groups according to presence of tumor necrosis. **e**, **f** Mean value of preoperative NLR (**d**), absolute neutrophil count (**e**), and absolute lymphocyte count (**f**) in two groups according to presence of tumor-infiltrating lymphocytes

explanations have been suggested. High NLR implies relative lymphocytopenia and neutrophilic leukocytosis. The former indicates a decrease in lymphocyte-mediated anti-cancer effects [19], and the latter indicates higher secretion of pro-angiogenic factors for tumor growth [20]. In a study of the largest cohort ($n = 958$) to date, Mano et al. showed that preoperative NLR of more than 2.81 was an independent predictor of survival after hepatectomy in patients with HCC [7]. They also demonstrated that the number of CD163-postive tumor-associated macrophages (TAM) was significantly higher in the group with high NLR than in the group with low NLR. TAMs express some cytokines, such as IL-4 and IL18, which may promote systemic neutrophilia. Montomura et al. suggested IL-17, a proinflammatory cytokine promoting neutrophil recruitment by CXC cytokines, as a key molecule involved in the relationship between NLR and HCC

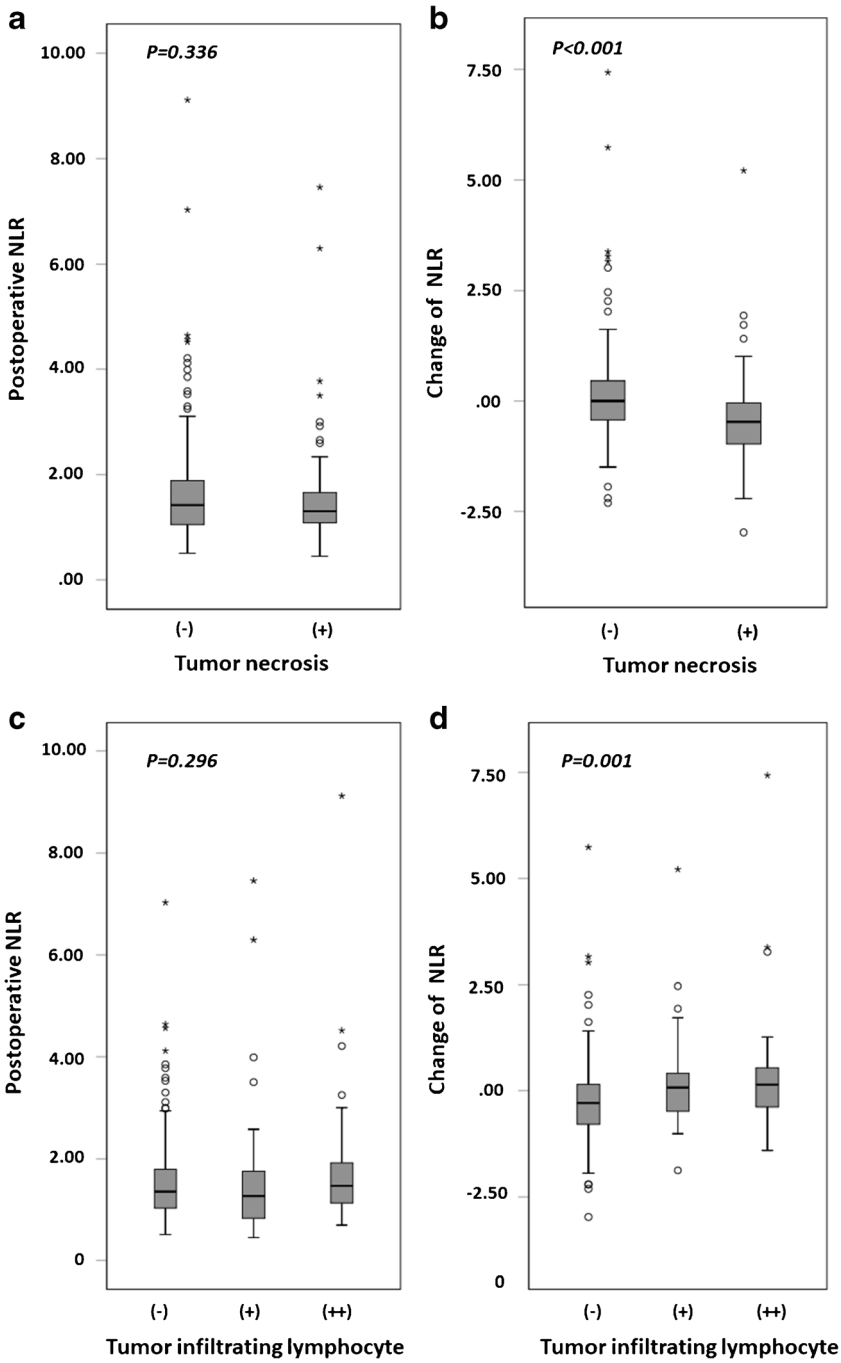

Fig. 3 Change of preoperative and postoperative neutrophil to lymphocyte ratio (NLR) according to presence of tumor necrosis (**a**, **b**) or tumor-infiltrating lymphocytes (**c**, **d**)

recurrence [21]. They showed that the number of peritumoral IL-17-producing cells and blood IL-17 levels were higher in high NLR groups than in low NLR groups.

In this study, we hypothesized that tumor necrosis and TILs are associated with NLR based on the following facts: (1) tumor necrosis could be a stimulus for acute inflammation, which induces neutrophil recruitment, and (2) high infiltration of TILs, which are generally associated with better prognosis in a variety of cancers including HCCs, could be associated with lymphocytosis in peripheral blood. Therefore, we evaluated the presence of tumor necrosis or TILs in HCC tissue and the association with NLR status. As a result, patients with tumor necrosis showed higher preoperative NLR than patients without tumor necrosis, and this effect was mostly induced by differences in preoperative absolute neutrophil count, not absolute lymphocyte count. In contrast, the mean value of preoperative NLR was higher in patients without TILs than in patients with TILs, and this effect was mostly induced by

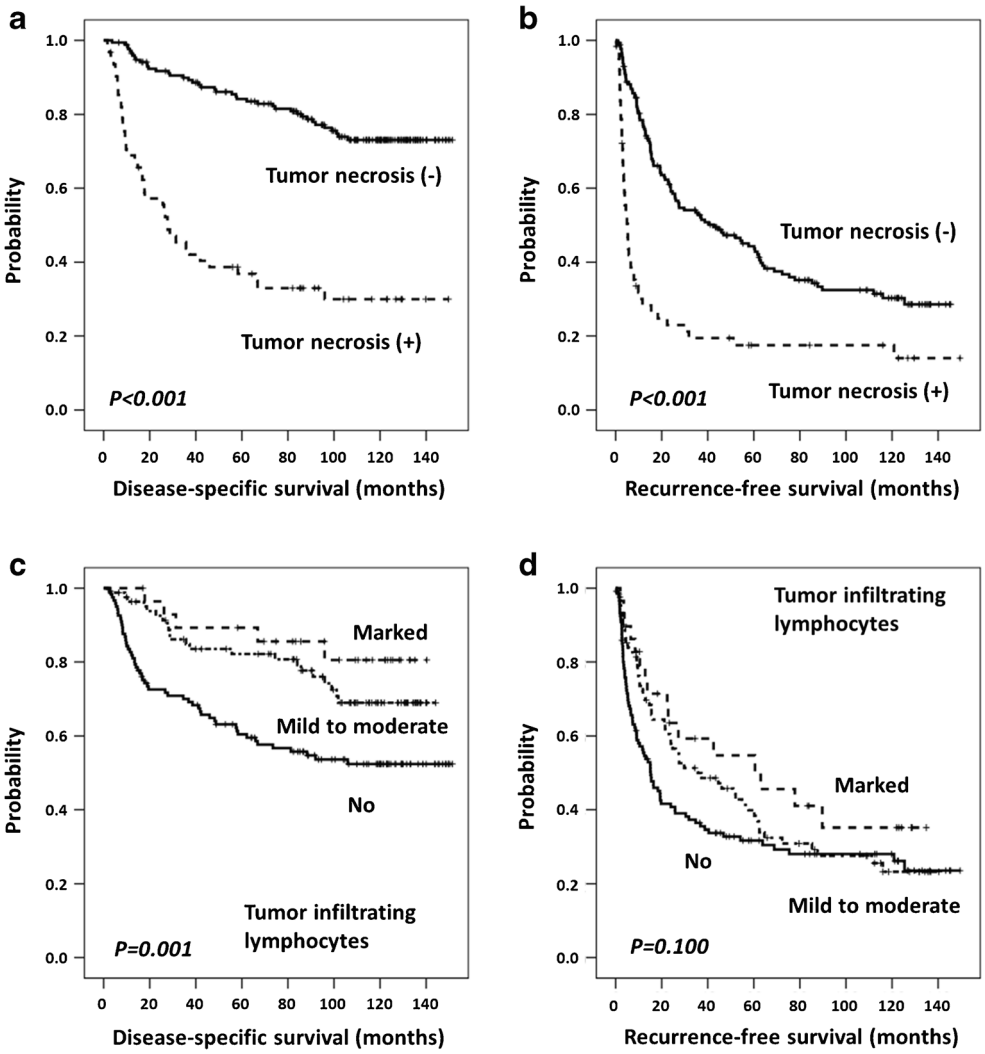

Fig. 4 Kaplan-Meier survival curves according to tumor necrosis or tumor-infiltrating lymphocytes. **a** Disease-specific survival for tumor necrosis, **b** recurrence-free survival for tumor necrosis, **c** disease-specific survival for tumor-infiltrating lymphocytes, **d** recurrence-free survival for tumor-infiltrating lymphocytes

Table 3 Multivariate analysis for recurrence-free survival and disease-specific survival, including tumor necrosis and TILs as additional co-variables

		Recurrence-free survival			Disease-specific survival		
		HR	95% CI	*p* value	HR	95% CI	*p* value
Age	≤ 40 years vs > 40 years				1.790	1.000–3.207	0.05
Tumor size	> 5 cm vs ≤ 5 cm	0.779	0.530–1.147	0.206	1.252	0.763–2.054	0.374
Edmondson grade	III vs I + II	1.364	0.825–2.256	0.227	1.190	0.639–2.215	0.584
Microvascular invasion	Yes vs no	1.032	0.670–1.590	0.888	1.104	0.575–2.117	0.767
Major portal vein invasion	Yes vs no	0.660	0.302–1.445	0.299	1.093	0.472–2.533	0.836
Intrahepatic metastasis	Yes vs no	3.287	2.254–4.795	< 0.001	4.234	2.663–6.730	< 0.001
Albumin level, g/dL	≤ 3.5 vs > 3.5	2.079	1.157–3.735	0.014	2.331	1.185–4.586	0.014
AFP level, ng/mL	> 200 vs ≤ 200	1.411	1.013–1.966	0.041	1.034	0.629–1.700	0.895
Etiology	Viral vs non-viral	1.846	1.037–3.286	0.037			
Necrosis	Yes vs no	1.735	1.253–2.404	0.001	2.882	1.824–4.552	< 0.001
TIL	Yes vs no	0.716	0.510–1.006	0.054	0.416	0.242–0.714	0.001
NLR	≥ 2.5 vs < 2.5	1.050	0.627–1.759	0.853	1.525	0.819–2.839	0.183

HR hazard ratio, *CI* confidence interval, *NLR* neutrophil-lymphocyte ratio, *TIL* tumor-infiltrating lymphocytes

Table 4 Summary of previous studies evaluating the prognostic role of neutrophil-lymphocyte ratio in patients with curative resection for hepatocellular carcinoma

Author	Year	Country	Patient number	Cutoff	Cutoff method	% of high NLR	Significant findings of patient survival
Gomez et al. [4]	2008	UK	96	5	Based on the cutoff in previous study in colorectal cancers	27.1%	High NLR is an independent predictor of poorer disease-free survival
Mano et al. [7]	2013	Japan	958	2.81	The best cutoff using ROC curve	24.8%	High NLR is an independent prognostic factor in overall and recurrence-free survival CD163-positive macrophage counts were higher in tumors with high NLR.
Liao at el. [6]	2014	China	256	2.31	The best cutoff using ROC curve	52.7%	High NLR is an independent predictor of disease free survival and overall survival.
Yamamura et al. [9]	2014	Japan	113	3	Based on the cutoff in previous study in colorectal cancers	24.8%	NLR is an independent predictor of recurrence free survival and superior to the other inflammation-based prognostic scores
Okamura et al. [8]	2015	Japan	256	2.81	Based on the cutoff in previous study in HCC by Mano et al.	19.1%	High NLR is an independent predictor of disease-free survival and overall survival.
Yang et el. [10]	2016	China	526	2.81	Based on the cutoff in previous study in HCC by Mano et al.	23.8%	High NLR is an indicator of poor DFS and OS. Among patients with preoperative high NLR, patients with postoperative decrease in NLR showed better survival than them with increase.
Hung et al. [5]	2017	Taiwan	672	2.5	the best cutoff by Youden index	32.3%	High NLR is an independent unfavorable prognostic factor. Subsequent change of NLR between liver resection and HCC recurrence could predict post-recurrent survival.
Current study	2019	Korea	234	2.5	Optimal cutoff value with level of the highest statistical significance related to patient survival	12.0%	High preoperative NLR was an independent adverse prognostic factor for disease-specific survival. High NLR was associated with presence of tumor necrosis and absence of tumor infiltrating TILs. The independent prognostic effect of NLR for disease-specific survival was disappeared as adding tumor necrosis and TILs as covariables.

NLR neutrophil-lymphocyte ratio, *HCC* hepatocellular carcinoma, *TIL* tumor-infiltrating lymphocytes

differences in preoperative absolute lymphocyte count, not absolute lymphocyte count. These results suggest that tumor factors may influence systemic inflammation represented by NLR status, and the prognostic effect of NLR may originate from proinflammatory conditions such as tumor necrosis or absence of TILs. In a study by Han et al., high pre-treatment NLR was significantly associated with high neutrophil infiltration and low CD3(+) T cell infiltration into tumors, consistent with the results of our study [22]. After curative resection, NLR in patients with tumor necrosis or without TILs was decreased more, and postoperative NLR was not different in patient groups according to tumor necrosis or TILs. We also showed that the prognostic effect of NLR was induced by the prognostic effect of tumor necrosis or TILs. These findings suggest that high NLR may be caused by a tumor factor, represented by the presence of tumor necrosis or low TILs.

HCC can be diagnosed by radiologic examination without histologic confirmation, and there are several treatment modalities for HCC. Especially, there has been controversy in the most effective first-line treatment modality for the patients with small solitary HCC: radiofrequency ablation, surgical hepatic resection, and liver transplantation [23]. Treatment modality is usually determined by tumor stage or the underlying liver parenchymal disease with function [23]. However, tumor biology predicting tumor aggressiveness, which can be represented by histologic features, is hardly to be evaluated before surgical resection. Based on the results of this study, preoperative NLR could represent tumor biology by predicting the presence of tumor necrosis or TIL and might be used as an ancillary marker for determining the treatment modality.

Conclusion

High NLR is an independent prognostic factor in patients with HCC undergoing curative resection. The prognostic effect of high NLR might originate from the prognostic effect of tumor necrosis or TILs.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval and consent to participate The Institutional Review Board of Samsung Medical Center approved this study and waived informed consent for this retrospective study.

References

1. Global Burden of Disease Liver Cancer C, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Furst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topor-Madry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C (2017) The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the Global Burden of Disease Study 2015. JAMA Oncol 3:1683–1691
2. Sherman M (2008) Recurrence of hepatocellular carcinoma. N Engl J Med 359:2045–2047
3. Portolani N, Coniglio A, Ghidoni S, Giovanelli M, Benetti A, Tiberio GA, Giulini SM (2006) Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. Ann Surg 243:229–235
4. Gomez D, Farid S, Malik HZ, Young AL, Toogood GJ, Lodge JP, Prasad KR (2008) Preoperative neutrophil-to-lymphocyte ratio as a prognostic predictor after curative resection for hepatocellular carcinoma. World J Surg 32:1757–1762
5. Hung HC, Lee JC, Cheng CH, Wu TH, Wang YC, Lee CF, Wu TJ, Chou HS, Chan KM, Lee WC (2017) Impact of neutrophil to lymphocyte ratio on survival for hepatocellular carcinoma after curative resection. J Hepatobiliary Pancreat Sci 24:559–569
6. Liao W, Zhang J, Zhu Q, Qin L, Yao W, Lei B, Shi W, Yuan S, Tahir SA, Jin J, He S (2014) Preoperative neutrophil-to-lymphocyte ratio as a new prognostic marker in hepatocellular carcinoma after curative resection. Transl Oncol 7:248–255
7. Mano Y, Shirabe K, Yamashita Y, Harimoto N, Tsujita E, Takeishi K, Aishima S, Ikegami T, Yoshizumi T, Yamanaka T, Maehara Y (2013) Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival after hepatectomy for hepatocellular carcinoma: a retrospective analysis. Ann Surg 258:301–305
8. Okamura Y, Ashida R, Ito T, Sugiura T, Mori K, Uesaka K (2015) Preoperative neutrophil to lymphocyte ratio and prognostic nutritional index predict overall survival after hepatectomy for hepatocellular carcinoma. World J Surg 39:1501–1509
9. Yamamura K, Sugimoto H, Kanda M, Yamada S, Nomoto S, Nakayama G, Fujii T, Koike M, Fujiwara M, Kodera Y (2014) Comparison of inflammation-based prognostic scores as predictors of tumor recurrence in patients with hepatocellular carcinoma after curative resection. J Hepatobiliary Pancreat Sci 21:682–688
10. Yang HJ, Guo Z, Yang YT, Jiang JH, Qi YP, Li JJ, Li LQ, Xiang BD (2016) Blood neutrophil-lymphocyte ratio predicts survival after hepatectomy for hepatocellular carcinoma: a propensity score-based analysis. World J Gastroenterol 22:5088–5095
11. Edge SB, Compton CC (2010) AJCC cancer staging manual, 7th edn. Springer, Chicago
12. Llovet JM, Bru C, Bruix J (1999) Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 19:329–338
13. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR (2008) Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med 359:1995–2004
14. Camp RL, Dolled-Filhart M, Rimm DL (2004) X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res 10:7252–7259
15. Edmondson HA, Steiner PE (1954) Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. Cancer 7:462–503
16. Japan LCSGo (2003) General rules for the clinical and pathological study of primary liver cancer, 2nd edn. Kanehara, Tokyo
17. Pichler M, Hutterer GC, Chromecki TF, Jesche J, Kampel-Kettner K, Rehak P, Pummer K, Zigeuner R (2012) Histologic tumor necrosis is an independent prognostic indicator for clear cell and papillary renal cell carcinoma. Am J Clin Pathol 137:283–289
18. Wada Y, Nakashima O, Kutami R, Yamamoto O, Kojiro M (1998) Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. Hepatology 27:407–414
19. Chew V, Tow C, Teo M, Wong HL, Chan J, Gehring A, Loh M, Bolze A, Quek R, Lee VK, Lee KH, Abastado JP, Toh HC, Nardin A (2010) Inflammatory tumour microenvironment is associated with superior survival in hepatocellular carcinoma patients. J Hepatol 52:370–379
20. Kusumanto YH, Dam WA, Hospers GA, Meijer C, Mulder NH (2003) Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. Angiogenesis 6:283–287
21. Motomura T, Shirabe K, Mano Y, Muto J, Toshima T, Umemoto Y, Fukuhara T, Uchiyama H, Ikegami T, Yoshizumi T, Soejima Y, Maehara Y (2013) Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. J Hepatol 58:58–64
22. Han S, Liu Y, Li Q, Li Z, Hou H, Wu A (2015) Pre-treatment neutrophil-to-lymphocyte ratio is associated with neutrophil and T-cell infiltration and predicts clinical outcome in patients with glioblastoma. BMC Cancer 15:617
23. Ahn KS, Kang KJ (2019) Appropriate treatment modality for solitary small hepatocellular carcinoma: radiofrequency ablation vs. resection vs. transplantation? Clin Mol Hepatol 25:354–359