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BRIEF ARTICLE

Neutrophil-lymphocyte ratio predicts the prognosis of patients with hepatocellular carcinoma after liver transplantation

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Abstract

AIM: To determine whether an elevated neutrophil-lymphocyte ratio (NLR) is negatively associated with tumor recurrence in patients with hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) after liver transplantation (LT), and to determine the optimal predictive NLR cut-off value.

METHODS: The data of HCC patients who had undergone LT came from the China Liver Transplant Registry database. We collected data from 326 liver cancer patients who had undergone LT at our medical center. We divided the patients into groups based on their NLRs (3, 4 or 5). We then compared the clinicopathological data and long-time survival between these groups. Meanwhile, we used receiver operating characteristic analysis to determine the optimal NLR cut-off.

RESULTS: Of 280 HCC patients included in this study, 263 were HBV positive. Patients with an NLR < 3 and patients with an NLR ⩾ 3 but < 4 showed no significant differences in overall survival (OS) (P = 0.212) or disease-free survival (DFS) (P = 0.601). Patients with an NLR ⩾ 4 but < 5 and patients with an NLR ⩾ 5 also showed no significant differences in OS (P = 0.208) or DFS (P = 0.618). The 1-, 3- and 5-year OS rates of patients with an NLR < 4 *vs* an NLR ⩾ 4 were 87.8%, 63.8% and 61.5% *vs* 73.9%, 36.7% and 30.3%, respectively (P < 0.001). The 1-, 3- and 5-year DFS rates of patients with an NLR < 4 *vs* NLR ⩾ 4 were 83.9%, 62.9% and 60.7% *vs* 64.9%, 30.1% and 30.1%, respectively (P < 0.001). Univariate and multivariate analyses demonstrated that three factors, including NLR ⩾ 4 (P = 0.002), were significant predictors of tumor recurrence in HCC patients after LT.

CONCLUSION: A preoperative elevated NLR significantly increased the risk for tumor recurrence in HCC patients after LT.

Key words: Hepatocellular carcinoma; Liver transplantation; Inflammatory reaction; Neutrophil-lymphocyte ratio; Hepatitis B virus

Core tip: Inflammation has been linked to the biological characteristics of tumors. The neutrophil-lymphocyte ratio (NLR) is a simple biomarker of inflammation. Several studies have reported that a preoperative elevated NLR (in peripheral blood) is negatively associated with the prognosis of patients with hepatocellular carcinoma (HCC) after liver transplantation (LT). However, the ideal cut-off value is controversial, with studies citing both

3 and 5 as the appropriate cut-off NLR. In this study, we report 326 HCC patients who had undergone LT at our center. We identify NLR = 4 as the cut-off point for predicting the prognosis of HCC patients after LT.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and is the third leading cause of cancer-related death. Every year, more than 500000 people are diagnosed with HCC, and most of these patients are in developing countries[1]. Liver transplantation (LT) is the ideal choice for HCC patients[2] because it completely removes tumors in the liver and also improves hepatic function. However, the outcome of HCC patients after LT was unsatisfactory, owing to a high tumor recurrence, until Mazzaferro *et al*[3] proposed the Milan criteria. Since the Milan criteria were adopted, the outcome of HCC after LT has significantly improved. Several LT centers have confirmed the satisfactory outcome of HCC patients within the Milan criteria after LT[4-7]. However, a large proportion of patients fall outside the Milan criteria when they are diagnosed with liver cancer. Thus, the LT criteria for HCC should be revised so that more people can become candidates for LT. Over the past 10 years, many centers have attempted to establish more suitable criteria for selecting HCC patients[8-12]. Yao *et al*[8] presented the University of California San Francisco (UCSF) criteria in 2001. The Milan and UCSF criteria are based on tumor number, tumor size and macro-vascular invasion, which are estimated by preoperative imaging.

However, preoperative radiological imaging is inaccurate, especially for patients with liver cirrhosis. Micro-vascular invasion and histological grade cannot be detected by imaging, and these two important factors greatly influence the recurrence of HCC after LT. Some studies have reported a recurrence rate of HCC after LT of nearly 15%-20% in patients who were within Milan or UCSF criteria[13,14]. This condition prompted us to identify better predictors of the recurrence of HCC after LT.

Several studies have investigated the effect of inflammation on carcinogenesis because the cytokines and mediators released by inflammatory cells can promote angiogenesis and tumor cell metastasis[15-17]. Several inflammatory markers, such as C reactive protein, have been suggested as surrogates for biological characteristics in some types of tumors[18,19]. The neutrophil-lymphocyte ratio (NLR) is a simple biomarker of inflammation, and an elevated NLR has been linked to several malignancies[20-22]. Halazun *et al*[23] reported that patients with colorectal liver metastases with an elevated NLR had higher rates of recurrence after partial hepatic resection than patients with normal NLRs. Furthermore, studies have also shown that an elevated NLR has a negative impact on the prognosis of HCC patients after LT. However, these different studies have employed NLRs of 3, 4 and 5 as the cut-offs[24-28], and the NLR cut-offs are not unified. Our study aimed to calculate the optimal preoperative cut-off NLR for predicting the prognosis of HCC patients after LT.

MATERIALS AND METHODS

Patient selection and intra- and post-operative treatment

The data of HCC patients who had undergone LT came from the China Liver Transplant Registry database. We collected data from 326 liver cancer patients who had undergone LT at our medical center from August 2000 to January 2011. Preoperative demographic, clinical and laboratory data were recorded for these patients. A systemic plain/enhanced computed tomography (CT) scan or a magnetic resonance imaging scan was employed within one week before the surgery. Pathology was considered as the definite diagnosis for HCC. Pathological data were considered as the standard for tumor characteristics. Moreover, micro-vascular invasion and tumor differentiation were also assessed by pathology.

Blood cell testing is part of the routine work-up for HCC patients who have undergone LT. The absolute value of white blood cells and the differential counts were recorded within one week before surgery. The NLR was calculated by dividing the neutrophil count by the lymphocyte count. Patients with missing blood records; patients who had preoperative sepsis, hypersplenism, massive alimentary tract bleeding, hepatitis C virus (HCV) infection, cholangiocarcinoma or other neoplasms; and pediatric patients were excluded from this study.

The patients received LT at least one month after they had received preoperative adjuvant therapy when their blood test became normal. LT was performed using standard techniques without the use of veno-venous bypass, and a "piggy back" was used when necessary. After surgery, an immune-suppression regimen, including corticosteroids, cyclosporine or tacrolimus with or without azathioprine and mycophenolate, was administered. The steroids were withdrawn after 3-6 mo of post-operative treatment[29]. The e antigen status and HBV-DNA of several patients were positive, and the HBV-positive patients received anti-viral drugs, such as lamivudine, adefovir, telbivudine and entecavir, prior to and after transplantation[30].

Follow-up

After surgery, the patients underwent follow-up procedures. Plain/enhanced CT scans and α-fetoprotein (AFP) tests were performed every month for the first 6 mo. The above examinations were performed every 2 mo for the second 6 mo. In the following years, the patients received

Table 1 Comparison of demographic and clinicopathological data of patients with hepatocellular carcinoma classified by different neutrophil-lymphocyte ratios

Variable	NLR < 3 (*n* = 105)	3 ≤ NLR < 4 (*n* = 61)	4 ≤ NLR < 5 (*n* = 56)	NLR ≥ 5 (*n* = 58)	*P* (2-tailed)
Gender (F/M)	16/89	4/57	7/49	4/54	0.235
Age, yr (mean)	47.0	46.4	45.6	46.8	0.529
Age, yr (≥ 60/< 60)	13/92	5/56	6/50	7/51	0.859
Child-Pugh class (A/B/C)	59/39/7	31/26/4	31/21/4	25/22/11	0.083
BMI (mean)	23.3	22.3	22.2	22.9	0.899
AFP, g/L (< 400/≥ 400)	53/52	30/31	19/37	31/27	0.145
Preoperative adjuvant therapy (Y/N)	47/58	27/34	22/34	31/27	0.492
Tumor (≤ 3/> 3), *n*	90/15	49/12	41/15	39/19	0.036[2]
Largest tumor size, cm (≤ 5/5-9/> 9)	50/32/23	29/17/15	20/19/17	21/14/23	0.168
Total tumor size, cm (≤ 5/5-9/> 9)	41/31/33	24/16/21	13/17/26	15/10/33	0.257
Macro-vascular invasion (Y/N)	20/85	13/48	20/36	21/37	0.028[2]
Micro-vascular invasion (Y/N)	44/61	28/33	33/23	35/23	0.059
Differentiation (1-2/3-4)	44/17[1]	26/14[1]	29/15[1]	32/15[1]	0.866
HBV infection (-/+)	5/100	3/58	1/55	8/50	0.064
Donor (living/deceased)	30/75	13/48	16/40	7/51	0.083

[1]Differentiation was reported for 192 patients; [2]Significant *P* value. NLR: Neutrophil-lymphocyte ratios; BMI: Body mass index; AFP: α-fetoprotein; HBV: Hepatitis B virus.

examinations every 3-6 mo or when necessary. Suspicious lesions in the liver or lung were biopsied. Bone pain and progression of growth were observed. The date of tumor recurrence was regarded as the time that the AFP level began to rise once tumor recurrence had been confirmed.

Ethics

This study was approved by the Institutional Review Board of West China Hospital of Sichuan University in Sichuan Province. Written informed consent was obtained according to the Declaration of Helsinki of the World Medical Association.

Statistical analysis

SPSS v17.0 and MedCalc v11.3.0.0 were used to analyze the data. Receiver operating characteristic (ROC) analysis was used to determine the NLR cut-off value. Independent sample *t* test, Pearson's χ^2 test and Fisher's exact test were used to analyze the differences among HCC patients classified by different NLR values. Kaplan-Meier survival analysis was used to analyze overall survival (OS) and disease-free survival (DFS). Univariate analysis was performed to estimate the hazard ratio of the clinicopathological factors for the risk of tumor recurrence. The factors that had a significant impact on the outcome of HCC patients after LT were selected into multivariate Cox proportional hazards regression analysis to assess the hazard ratio for the risk of tumor recurrence in HCC patients after LT. The confidence interval quoted area was 95%, and significant differences were defined as $P < 0.05$.

RESULTS

Patient demographics and outcomes

Of 326 HCC patients who had undergone LT at our medical center from August 2000 to January 2011, 46 were excluded from the study: 10 for missing blood records, 2 pediatric patients, 3 for preoperative sepsis, 10 for hypersplenism, 2 for massive alimentary tract bleeding, 1 HCV-positive patient and 18 for the diagnosis of cholangiocarcinoma or other neoplasms by pathology. Thus, 280 patients were included in this study. Of these patients, 263 (93.9%) were HBV positive. The carcinogenic factor of 17 HCC patients may have been alcohol because they had a history of alcohol abuse. Among 280 patients, there were 31 (11.1%) women and 249 (88.9%) men. The mean age of the patients who had received LT was 46.5 years (range: 20.5-69.1 years, SD: 9.6 years). The median waiting times for living donor and deceased donor LT were 0.9 and 1.6 mo, respectively. The mean follow-up time was 2.63 years (range: 1.1-12.0 years). A total of 120 people died during follow-up. The 1-, 3- and 5-year OS rates of the patients in our study were 82.2%, 52.6% and 48.5%, respectively, and the 1-, 3- and 5-year DFS rates were 76.1%, 50.3% and 47.8%, respectively.

Comparison of variables between patients with different NLRs

Several studies have considered NLRs of 3, 4 and 5 as the cut-off points to predict the prognosis of HCC patients after LT[24-28]. Thus, we divided the HCC patients who had received LT at our hospital based on these three NLR cut-offs. There were 105 patients with an NLR < 3, 61 patients with NLRs between 3 and 4, 56 patients with NLRs between 4 and 5 and 58 patients with an NLR ≥ 5. We compared the demographic and clinicopathological data of HCC patients after LT. HCC patients classified based on their NLRs showed significant differences in tumor number > 3 ($P = 0.036$) and macro-vascular invasion ($P = 0.028$). There were no significant differences in the other variables among the groups with different NLRs (Table 1).

Table 2 Univariate analysis of the effects of clinicopathological factors on the disease-free survival of patients with hepatocellular carcinoma who underwent liver transplantation

Variable	χ^2	*P* value	HR	95%CI
Gender (F/M)	0.001	0.973	0.990	0.558-1.759
Age, yr (≥ 60/< 60)	4.573	0.032[1]	0.477	0.242-0.940
Child-Pugh class (A/B/C)				
A	-	-	-	-
B	0.291	0.590	1.106	0.766-1.598
C	0.287	0.592	0.827	0.412-1.658
AFP, g/L (< 400/ ≥ 400)	6.673	0.010[1]	1.600	1.120-2.287
NLR (< 4/ ≥ 4)	24.251	< 0.001[1]	2.424	1.704-3.440
Preoperative adjuvant therapy (Y/N)	0.019	0.890	1.025	0.721-1.457
Tumor No. (≤ 3/> 3)	23.518	< 0.001[1]	2.524	1.736-3.670
Largest tumor size, cm				
≤ 5	-	-	-	-
5-9	11.105	0.001[1]	2.195	1.382-3.487
> 9	36.829	< 0.001[1]	3.894	2.510-6.041
Total tumor size, cm				
≤ 5	-	-	-	-
5-9	6.590	0.010	2.123	1.195-3.771
> 9	45.107	< 0.001[1]	5.553	3.358-9.115
Macro-vascular invasion (Y/N)	33.195	< 0.001[1]	2.904	2.021-4.174
Micro-vascular invasion (Y/N)	31.135	< 0.001[1]	2.910	1.999-4.234
Differentiation (1-2/3-4)	3.136	0.077	1.435	0.962-2.140
HBV infection (Y/N)	0.012	0.912	1.048	0.461-2.382
Donor (living/deceased)	0.480	0.488	1.163	0.759-1.780

[1]Significant *P* value. NLR: Neutrophil-lymphocyte ratios; AFP: α-fetoprotein; HBV: Hepatitis B virus.

Outcome of different HCC categories divided by NLRs

Of the 105 patients with an NLR < 3, 12 died and 15 had tumor recurrence within 1 year. For these patients, the 1-, 3- and 5-year OS rates were 88.6%, 65.8% and 65.8%, respectively, and the 1-, 3- and 5-year DFS rates were 85.4%, 63.7% and 62.0%, respectively. For the 61 patients with NLRs between 3 and 4, their OS and DFS were not significantly different compared with patients having an NLR < 3 ($P = 0.212$ and $P = 0.601$, respectively). There were 56 patients with an NLR ≥ 4 but < 5 and 58 patients with an NLR ≥ 5. The outcome of these two categories was not significantly different, as shown in Figure 1. However, the outcome of patients with an NLR ≥ 3 but < 4 and an NLR ≥ 4 but < 5 revealed significant differences: the 1-, 3- and 5-year OS rates of patients with an NLR ≥ 3 but < 4 *vs* an NLR ≥ 4 but < 5 were 86.4%, 57.3% and 54.3% *vs* 79.7%, 35.5% and 31.1%, respectively ($P = 0.026$). The 1-, 3- and 5-year DFS rates of patients with an NLR ≥ 3 but < 4 *vs* an NLR ≥ 4 but < 5 were 81.2%, 61.6% and 58.6% *vs* 68.9%, 31.2% and 31.2%, respectively ($P = 0.005$) (Figure 1).

In addition, we used ROC curve analysis to determine the optimal NLR cut-off for HCC patients who received LT. The area under the ROC curve was 0.670 (Figure 2). When the NLR was 4.0634, the sensitivity was 56.3%, the specificity was 75.0%, and the sensitivity and specificity were highest. Therefore, we considered NLR = 4 as the cut-off. Patients with an NLR less than 3 and patients with an NLR ≥ 3 but < 4 were combined into one category, while the patients with an NLR ≥ 4 were considered as one category. We then compared the outcome of these two categories. As shown in Figure 3, the OS and DFS of these two categories were significantly different.

Table 3 Multivariate analysis of the effects of clinicopathological factors on the disease-free survival of patients with hepatocellular carcinoma who underwent liver transplantationpatients with hepatocellular carcinoma who underwent liver transplantation

Variable	χ^2	*P* value	HR	95%CI
Age, yr (≥ 60/< 60)	2.731	0.098	0.561	0.283-1.113
AFP, g/L (< 400/ ≥ 400)	0.397	0.529	1.128	0.776-1.640
NLR (< 4/ ≥ 4)	9.260	0.002[1]	1.758	1.222-2.527
Tumor No. (≤ 3/> 3)	1.450	0.229	1.301	0.848-1.997
Largest tumor size, > 5 cm	1.761	0.185	1.378	0.858-2.214
Total tumor size, > 9 cm	16.939	< 0.001[1]	2.725	1.691-3.393
Macro-vascular invasion (Y/N)	11.168	< 0.001[1]	2.013	1.336-3.035
Micro-vascular invasion (Y/N)	3.085	0.071	1.597	1.001-2.546

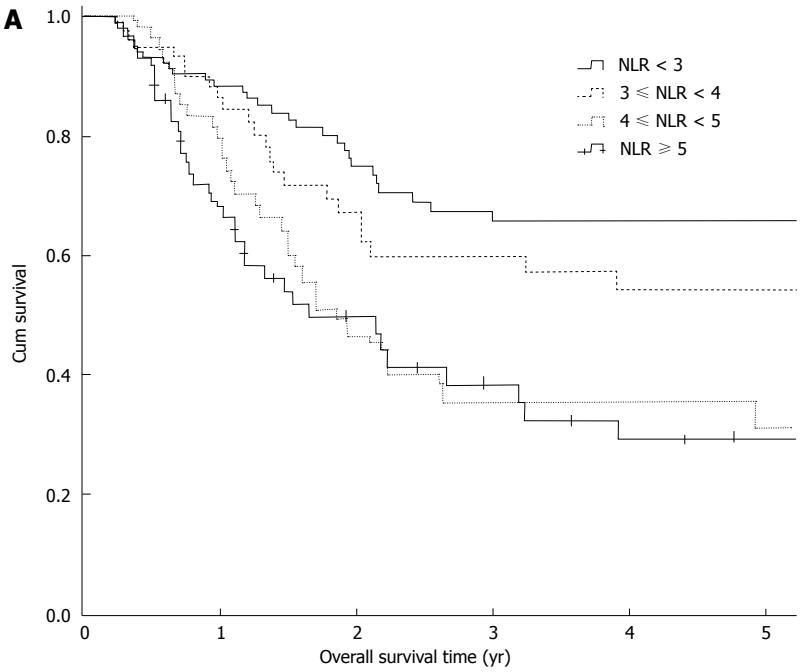
[1]Significant *P* value. NLR: Neutrophil-lymphocyte ratios; AFP: α-fetoprotein.

Predictors of prognosis of HCC patients after LT

The lists in Table 2 show 8 significant factors that affects the DFS of HCC patients after LT by univariate analysis: age ≥ 60 years, AFP ≥ 400 g/L, NLR ≥ 4, tumor number > 3, largest tumor size more than 5 cm, total tumor size more than 9 cm, macro-vascular invasion and micro-vascular invasion. The significant predictors were then utilized for a multivariate proportional hazard regression analysis. The result revealed that NLR ≥ 4 ($P = 0.002$, HR = 1.758, 95%CI: 1.222-2.527), total tumor size > 9 cm ($P < 0.001$, HR = 2.725, 95%CI: 1.691-3.393) and macro-vascular invasion ($P < 0.001$, HR = 2.013, 95%CI: 1.336-3.035) were independent predictors of DFS of HCC patients after LT (Table 3). We also performed univariate analysis and multivariate proportional hazard regression analysis to analyze the factors that affect the OS of HCC patients who underwent LT. The results showed that NLR ≥ 4 ($P = 0.006$, HR = 1.695, 95%CI: 1.164-2.466), total tumor size > 9 cm ($P < 0.001$, HR = 4.114, 95%CI: 2.438-6.940) and macro-vascular invasion ($P < 0.001$, HR = 2.049, 95%CI: 1.364-3.078) were independent predictors of OS of HCC patients after LT.

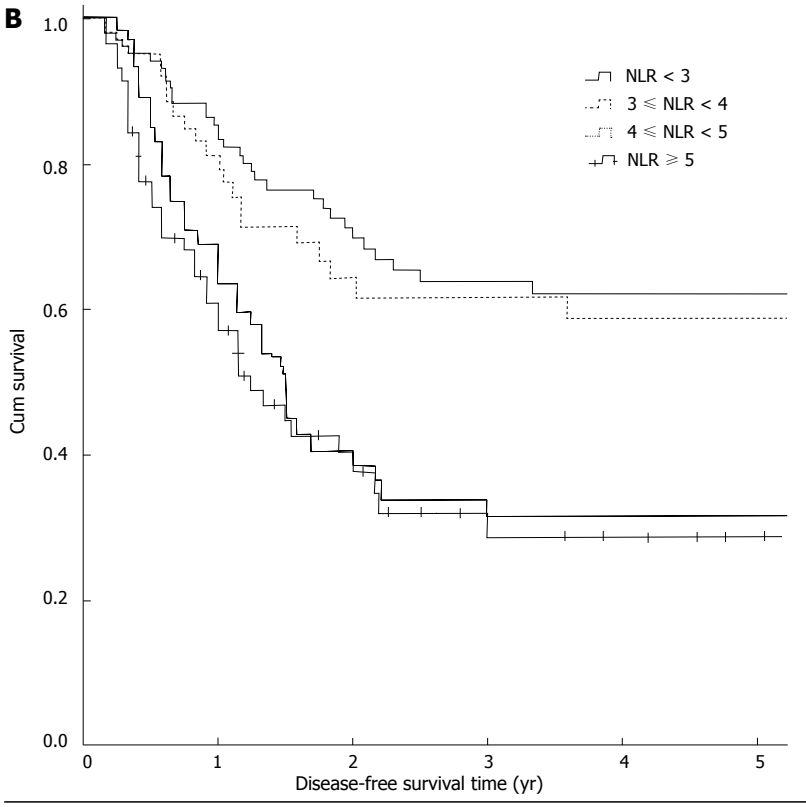
DISCUSSION

Since the Milan criteria were proposed by Mazzaferro *et al*[3] in 1996, many liver transplantation centers worldwide have reported excellent results after LT for patients with HCC who fall within the Milan criteria[6,29,31]. However, most HCC patients are outside the Milan criteria. To let those people receive corresponding treatment, revised LT criteria for HCC patients need to be established. Therefore, Yao *et al*[8] presented revised criteria and demonstrated that the outcomes of patients with HCC after LT outside the Milan criteria but within UCSF had no significant difference compared with the outcomes of patients within the Milan criteria. This result has been confirmed



	0-yr	1-yr	2-yr	3-yr	4-yr	5-yr	*P* value
NLR < 3	105	91 (88.6%)	57 (75.0%)	40 (65.8%)	40 (65.8%)	40 (65.8%)	-
3 ⩽ NLR < 4	61	47 (86.4%)	30 (67.4%)	22 (57.3%)	18 (54.3%)	18 (54.3%)	0.212
4 ⩽ NLR < 5	56	43 (79.7%)	21 (46.6%)	16 (35.5%)	7 (31.1%)	7 (31.1%)	0.026[1]
NLR ⩾ 5	58	38 (68.3%)	22 (49.7%)	12 (35.3%)	9 (29.2%)	9 (29.2%)	0.648

[1]Significant *P* value



	0-yr	1-yr	2-yr	3-yr	4-yr	5-yr	*P* value
NLR < 3	105	84 (85.4%)	52 (69.8%)	41 (63.7%)	36 (62.0%)	36 (62.0%)	-
3 ⩽ NLR < 4	61	44 (81.2%)	27 (64.2%)	23 (61.6%)	20 (58.6%)	20 (58.6%)	0.601
4 ⩽ NLR < 5	56	37 (68.9%)	17 (38.1%)	13 (31.2%)	13 (31.2%)	13 (31.2%)	0.005[1]
NLR ⩾ 5	58	32 (59.0%)	15 (37.6%)	9 (28.6%)	9 (28.6%)	9 (28.6%)	0.618

[1]Significant *P* value

Figure 1 Kaplan-Meier survival analysis curve. A: The overall survival for patients with hepatocellular carcinoma undergoing liver transplantation classified by different neutrophil-lymphocyte ratios (NLRs); B: The disease-free survival for patients with hepatocellular carcinoma undergoing liver transplantation by different NLRs.

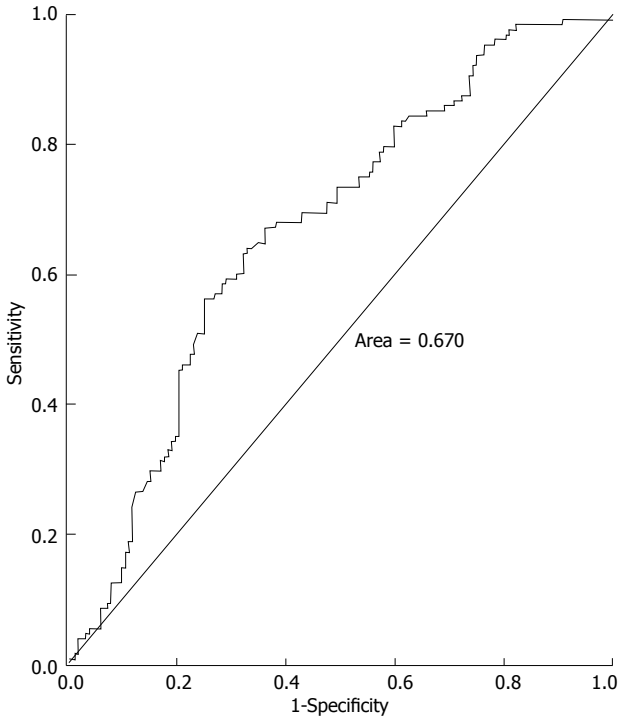


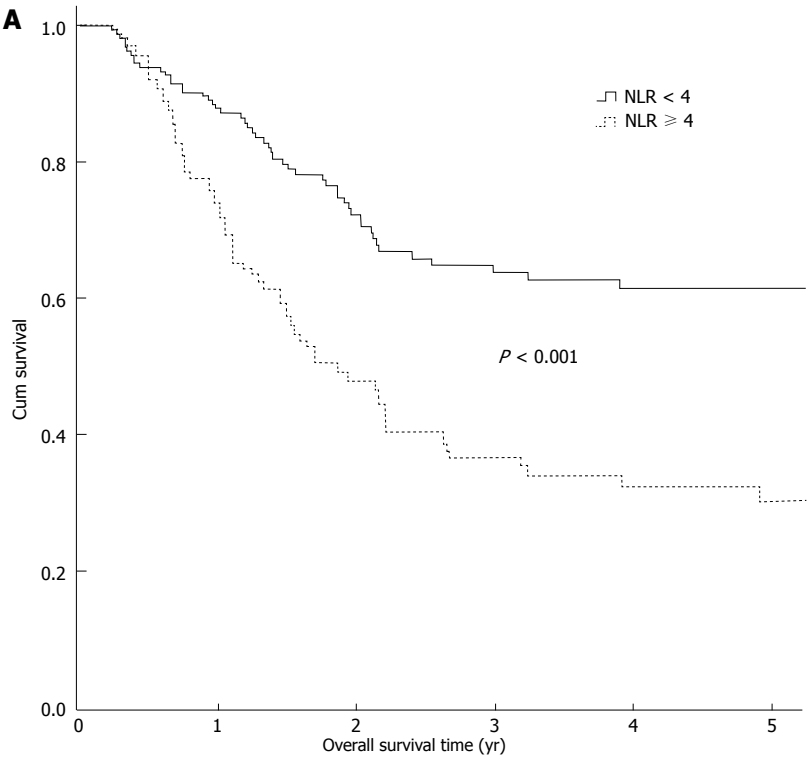
Figure 2 Receiver operating characteristic curve for the neutrophil-lymphocyte ratio cut-off value to predict tumor recurrence of hepatocellular carcinoma patients after liver transplantation.

by many LT centers[9,32]. With the revision of the LT criteria for HCC, another issue arises: organ shortages. Therefore, it is necessary to judge the biological characteristics of the tumor to exclude patients with negative tumor behavior. These patients will likely respond poorly to LT despite being within the Milan or UCSF criteria. Tumor size and number, as assessed by preoperative radiology, are used as surrogate markers of tumor biology. Some studies have reported that tumor size is related to the risk of recurrence and vascular invasion[13,33]. However, we found tumor number > 3 and largest tumor size > 5 cm were not independent predictors of the OS and DFS of patients with HCC after LT. Nevertheless, total tumor size > 9 cm was an independent factor that predicted OS and DFS (Table 2). In view of the inaccuracy of preoperative tumor assessments and the inconsistency of the effect of tumor number and size on the prognosis of HCC patients after LT, new non-invasive surrogates are needed to predict the outcome of HCC patients after partial hepatic resection or LT.

Several studies have reported that inflammation plays an important role in the development of malignant disease[15,17]. NLR was first linked to liver malignancy by Halazun *et al*[23]. NLR is a simple marker of inflammation and can be obtained easily by routine blood testing. However, the cut-off values of NLR are not unified. We found that the outcomes of patients with an NLR < 3 and patients with an NLR ≥ 3 but < 4 were not significantly different, nor were the outcomes of patients with an NLR ≥ 4 but < 5 and patients with an NLR ≥ 5. In our study, ROC analysis demonstrated that the sensitivity and specificity were highest when the NLR was 4.0634. Therefore, we considered patients with an NLR < 4 as one group and patients with an NLR ≥ 4 as another group. We then compared the outcomes of these two groups. We observed a marked and significant difference between these two groups in OS and DFS. Therefore, we consider NLR ≥ 4 as an elevated ratio. NLR ≥ 4 was also recognized as elevated by Shimada *et al*[21] in patients with gastric cancer. Halazun *et al*[25] considered NLR ≥ 5 to be elevated, and they reported the 1-, 3- and 5-year DFS of patients with an elevated NLR *vs* a normal NLR as 62%, 28% and 28% *vs* 88%, 74% and 64%, respectively ($P = 0.001$).

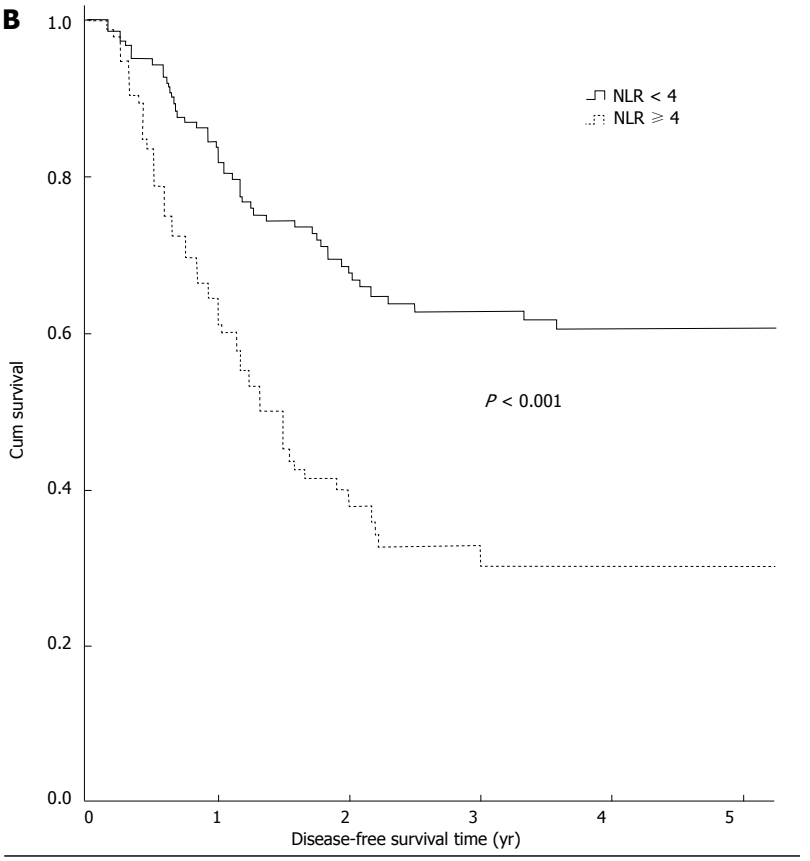
Although HCC patients with an elevated NLR have a poor prognosis, the mechanism through which the NLR effects tumor recurrence remains undefined. There are several hypotheses regarding the link between elevated NLR and tumor recurrence. First, neutrophils are the major source of vascular endothelial growth factor (VEGF), which promotes tumor angiogenesis and metastasis[34-37]. High levels of VEGF expression have been correlated with tumor recurrence in HCC[38]. Furthermore, some studies have reported that patients with elevated VEGF expression have increased vascular density in their tumor nodules[34,39]. Generally, the white blood cell counts of these patients were within the normal range, so patients with a higher NLR had higher neutrophil counts and higher VEGF expression. Second, the human immune system mostly depends on lymphocytes. However, lymphocyte counts are greatly reduced in patients with elevated NLRs, who are left unable to defend against the tumor malignancy. Several studies have demonstrated that patients with few lymphocytes infiltrating into the tumor margin have poor outcomes after treatment[39,40]. Patients with elevated NLRs have relative neutrophilia and lymphocytopenia, leading to an imbalance in the inflammatory cascade and immune response to malignant tumors. In this type of micro-environment, tumors proliferate and metastasize more easily. It remains unclear whether neutrophils or lymphocytes play a more important role in tumor recurrence of HCC after LT, and the mechanism has not been explored clearly. It is necessary to perform more clinical and basic studies.

Although univariate analysis of this study demonstrated that age ≥ 60 years, AFP ≥ 400 g/L, tumor number > 3, largest tumor size > 5 cm and micro-vascular invasion were preoperative predictors of DFS, none of these variables were independent factors for predicting tumor recurrence of HCC after LT. Some studies have reported tumor nodules > 3 as an independent predictor of tumor recurrence[9,24,41]. However, our results showed that the long-term survival of HCC patients with tumor nodules < 3 and those with tumor nodules > 3 was unchanged after LT, and our result is in agreement with the results of several other studies[25,42]. In this study, NLR ≥ 4 ($P = 0.002$, HR = 1.758, 95%CI: 1.222-2.527), total tumor size > 9 cm ($P < 0.001$, HR = 2.725, 95%CI: 1.691-3.393) and



	0-yr	1-yr	2-yr	3-yr	4-yr	5-yr	*P* value
NLR < 4	166	137 (87.8%)	85 (72.3%)	63 (63.8%)	51 (61.5%)	51 (61.5%)	-
NLR ⩾ 4	114	81 (73.9%)	41 (48.0%)	29 (36.7%)	21 (32.4%)	15 (30.3%)	< 0.001[1]

[1]Significant *P* value



	0-yr	1-yr	2-yr	3-yr	4-yr	5-yr	*P* value
NLR < 4	166	127 (83.9%)	76 (67.7%)	64 (62.9%)	55 (60.7%)	55 (60.7%)	-
NLR ⩾ 4	114	70 (64.9%)	32 (37.9%)	22 (30.1%)	22 (30.1%)	22 (30.1%)	< 0.001[1]

[1]Significant *P* value

Figure 3 Kaplan-Meier survival analysis curve. A: The overall survival for patients with hepatocellular carcinoma undergoing liver transplantation classified by the cut-off neutrophil-lymphocyte ratio (NLR) of 4; B: The disease-free survival for patients with hepatocellular carcinoma undergoing liver transplantation by the cut-off NLR of 4.

macro-vascular invasion ($P < 0.001$, HR = 2.013, 95%CI: 1.336-3.035) were independent factors that predict the prognosis of HCC patients after LT. HCC patients with a higher preoperative NLR had a higher tumor recurrence rate than those with a normal NLR after LT.

There are many limitations to this study. First, we know that the preoperative NLR is affected by many factors, such as unidentified sepsis, weight loss, massive hemorrhage and instrumental error, which make the NLR inaccurate. In addition, the majority of patients enrolled in our study had HBV infection, which may bias the result because hepatitis C is the most common cause of HCC in developed countries. Moreover, this is a retrospective study, and the number of patients included in our study is relatively small. More multi-center and prospective studies are needed to confirm and update the findings demonstrated in this study.

In summary, we have found that HCC patients with an elevated preoperative NLR have poorer OS and DFS after LT. This biomarker allows us to preoperatively identify patients with a high NLR, who have a poor prognosis and adverse tumor biology.

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COMMENTS

Background

Several studies have investigated the effect of inflammation on carcinogenesis because cytokines and mediators released by inflammatory cells can promote angiogenesis and tumor cell metastasis. Several inflammatory markers, such as C reactive protein, have been suggested as surrogates for biological characteristics in some types of tumors. The neutrophil-lymphocyte ratio (NLR) is a simple biomarker of inflammation, and an elevated NLR has been linked to several malignancies.

Research frontiers

Halazun *et al* reported that patients with colorectal liver metastases with an elevated NLR had higher rates of recurrence after partial hepatic resection than patients with normal NLRs. Furthermore, studies have also shown that an elevated NLR has a negative impact on the prognosis of hepatocellular carcinoma (HCC) patients after liver transplantation (LT).

Innovations and breakthroughs

Several studies have employed NLRs of 3, 4 and 5 as the cut-offs, and the NLR cut-offs are not unified. The study aims to calculate the optimal preoperative NLR cut-off for predicting the prognosis of HCC patients after LT.

Applications

A preoperative elevated NLR significantly increased the risk for tumor recurrence in HCC patients after LT. This biomarker allows them to preoperatively identify patients with a high NLR, who have a poor prognosis and adverse tumor biology.

Terminology

The NLR was calculated by dividing the neutrophil count by the lymphocyte count in peripheral blood.

Peer review

This is a very interesting paper on a prognostic factor for liver cancer recurrence after transplantation. The authors stated that NLR is a strong prognostic factor for outcome of liver transplantation for HCC from their experience. This manuscript is easy to understand and well organized.

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