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Original Article

Preoperative neutrophil-to-lymphocyte ratio as a prognostic marker in patients with gallbladder carcinoma

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Abstract: *Background and aim:* Primary gallbladder cancer is one of the most common malignant tumors. Neutrophil and lymphocyte counts and the neutrophil-to-lymphocyte ratio (NLR) are markers of systemic inflammation. We here aimed to evaluate haemogram parameters of our patients with gallbladder carcinoma. *Methods:* The study included 127 patients diagnosed with gallbladder carcinoma in our hospital from 2005 to 2013. NLR values were categorized into two groups: <1.94 and ≥1.94. The correlation of clinical data, including tumor differentiation, Nevin stage, TNM stage, operation margin, operation mode, NLR, albumin, C reactive protein (CRP), carcinoembryonic antigen (CEA), and carbohydrate antigen 199 (CA199) with median survival period of patients was analyzed by univariate survival analysis. The multivariate prognosis analysis was performed to select the independent prognostic factors. *Results:* Compared with low NLR group, the 5-year survival rates in high NLR group were reduced (P<0.05). The degree of tumor differentiation, Nevin stage, TNM stage, operation margin, operation mode, NLR, albumin, CRP, CEA, and CA199 were associated with the median survival period of patients (P<0.01). The multivariate prognosis analysis showed that NLR, Nevin stage, operation mode and TNM stage were independent prognostic factors (P<0.05). *Conclusion:* Preoperative NLR were closely related to prognosis of patients with GBC and might be useful for the evaluation of prognosis of patients with GBC.

Keywords: Neutrophil-to-lymphocyte ratio, prognosis, gallbladder cancer

Introduction

Primary gallbladder cancer (GBC) is one of the most common malignant tumors, accounting for about 1% of all malignant tumors in China [1]. Gallbladder stones in patients with gallbladder cancer are 7-10 times without gallbladder stones [2]. The infection of some bacteria (such as salmonella typhus, and helicobacter pylori) is also significantly associated with gallbladder cancer [3]. The changes of inflammatory response and immune status in the body system are closely related to tumor initiation, thus affecting the patient's prognosis [4]. Studies have shown that inflammatory responses are closely associated with gallbladder cancer [5]. Preoperative peripheral blood neutrophil-to-lymphocyte ratio (NLR) is associated with inflammation, a commonly used indicator of systemic inflammation. The study found that NLR was associated with prognosis in certain solid tumors such as non-small cell lung cancer, prostate cancer, cervical cancer, breast cancer and liver cancer [6-9]. So the cutoff value of NLR in patients who underwent curative resection of GBC should be optimized; otherwise, it is difficult to evaluate the clinical value of NLR and to compare different studies. Our study was designed to determine the optimal value of NLR and to evaluate the correlation of preoperative NLR with clinicopathologic features and prognosis in patients with GBC who underwent curative resection.

Materials and methods

Patients

A total of 211 patients with GBC that underwent operation treatments by the surgeons were collected in the Jinhua Central Hospital from 2005

Table 1. Clinical pathological characteristic of patients in the low (NLR<1.94) and high (NLR≥1.94) NLR group

Variables	Classification	<1.94 (n=73, 57.48%)		≥1.94 (n=54, 42.52%)		*P*#
		n	%	n	%	
Gender	Female	47	64.4	28	51.9	0.156*
	Male	26	35.6	26	48.1	
Pathological types	Adenocarcinoma	67	91.8	51	94.4	0.764*
	Squamous carcinoma	3	4.1	1	1.9	
	Adenosquamous carcinoma	3	4.1	2	3.7	
Degree of differentiation	Poorly differentiated	25	34.2	26	48.1	0.066*
	Moderately differentiated	37	50.7	26	48.1	
	Well-differentiated	11	15.1	2	3.7	
Nevin stage	1	0	0	0	0	0.007*
	2	24	32.9	6	11.1	
	3	19	26	12	22.2	
	4	8	11	5	9.3	
	5	22	30.1	31	57.4	
TNM stage	1	22	30.1	6	11.1	0.039*
	2	23	31.5	22	40.7	
	3	18	24.7	12	22.2	
	4	10	13.7	14	25.9	
Resection margin	Negative	60	82.2	35	64.8	0.026*
	Positive	13	17.8	19	35.2	
Operation modes	Radical cholecystectomy	54	74	34	63	0.036*
	Expand cholecystectomy	9	12.3	3	5.6	
	Palliative cholecystectomy	10	13.7	17	31.5	
Age		63.8 ± 10.5		65.4 ± 12.2		0.403
Total bilirubin		39.4 ± 3.5		36.6 ± 4.9		<0.001
Albumin		39.4 ± 3.5		36.6 ± 4.9		<0.001
Platelets		250.5 ± 88.8		238.5 ± 79.9		0.389
CA199		85.9 ± 68.2		211.1 ± 151.9		<0.001
CEA		8.1 ± 20.8		11.5 ± 26.6		0.167
AFP		4.6 ± 9.5		6.6 ± 14.9		0.087
CRP		19.6 ± 6.2		49.1 ± 18.4		<0.001

NLR neutrophil-to-lymphocyte ratio, CA199 carbohydrate antigen 199, CRP C reactive protein, CEA carcinoembryonic antigen, AFP α-fetoprotein #Mann-Whitney *U* test, *Fisher's exact test or χ^2 test.

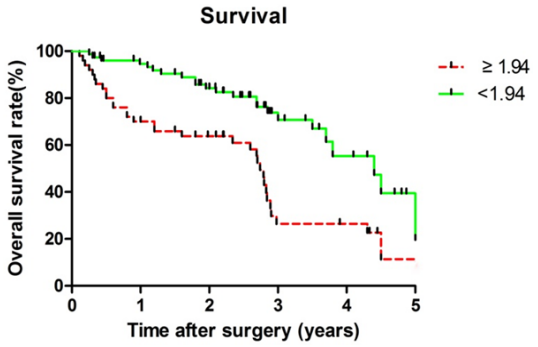

Figure 1. The univariate survival analysis for the survival time of patients in the low (NLR<1.94) and high (NLR≥1.94) NLR group by Kaplan-Meier.

to 2013. Among these 211 patients, 127 patients with complete clinical data and follow-up data were included in the current study. The exclusive criteria were as follows: (1) the patients died during perioperative period; (2) the patients died of non-cancer diseases; (3) the patients had autoimmune diseases; (4) the patients were combined with other tumors.

Study grouping and data collection

According to an article published by Zhang [10], we used 1.94 as the threshold of NLR. All cases were divided into low NLR group (NLR<1.94) and high NLR group (NLR≥1.94).

Table 2. Univariable analysis of clinicopathological factors and overall survival following GBC surgery

Variables	Classification	n	Median survival time (years)	*P*	(95% for CI)	
Genders	Female	75	3.8	0.524	2.242	5.358
	Male	52	2.98			
Pathological types	Adenocarcinoma	118	3.7	0.1		
	Squamous carcinoma	4	1.95			
	Adenosquamous carcinoma	5	1.5			
Degree of differentiation	Poorly differentiated	51	5	<0.001		
	Moderately differentiated	63	3.8			
	Well-differentiated	13	2.6			
Nevin stage	1	0	5	<0.0001		
	2	30	4.8			
	3	31	3.7			
	4	13	2.15			
	5	53	0.7			
TNM stage	1	28	5	<0.0001		
	2	45	2.98			
	3	30	2.35			
	4	24	0.6			
Resection margin	Negative	95	3.8	<0.001	2.985	4.615
	Positive	32	2.69			
Operation modes	Radical cholecystectomy	88	4.3	<0.0001		
	Expand cholecystectomy	12	2.5			
	Palliative cholecystectomy	27	0.8			
NLR	<1.94	73	4.4	<0.0001	3.445	5.355
	≥1.94	54	2.74			
Age	<70	86	3.7	0.213	2.448	4.952
	≥70	41	2.83			
Total bilirubin (μmol/L)	<30	74	3.8	0.179	2.082	5.518
	≥30	52	3			
Albumin (g/L)	<37	46	2.9	0.037	1.743	4.057
	≥37	81	3.8			
CA199	<30	32	4.5	0.03	4.383	4.617
	≥30	95	2.9			
Platelets ($\times 10^9$/L)	<200	31	4.5	0.545	3.578	5.422
	≥200	96	3			
CEA	<4	80	4.3	0.014	3.487	5.113
	≥4	47	2.74			
AFP	<4	91	3.7	0.08	2.461	4.939
	≥4	36	2.84			
CRP	<10	53	2.81	<0.0001	2.134	4.561
	≥10	74	0.6			

NLR neutrophil-to-lymphocyte ratio, CA199 carbohydrate antigen 199, CRP C reactive protein, CEA carcinoembryonic antigen, AFP α-fetoprotein.

Clinical characteristic of patients, including age, gender, pathological types, degree of differentiation, Nevin stage [11], tumor-node-metastasis (TNM) stage, incisal margin and operation modes were evaluated (**Table 1**). Operation pathological and clinical results were

Table 3. Multivariable analysis of clinicopathological factors and overall survival following GBC surgery

	Standard error	Wald	*P*	RR	95% CI for RR	
					Lower line	Upper line
NLR	0.272	13.246	<0.0001	2.694	1.58	4.591
Nevin stages	0.146	37.759	<0.0001	2.45	1.841	3.261
Operation modes	0.171	30.515	<0.0001	2.577	1.842	3.607
TNM stages	0.156	56.315	<0.0001	3.217	2.371	4.366

evaluated according to the 2010 version the seventh American Joint committee on Cancer staging system [12].

Laboratory measurements

Peripheral venous blood samples of 127 patients with GBC were collected within one week before operation and then used to detect the content of granulocytes, leukocytes, platelet, albumin, total bilirubin, C-reactive protein (CRP), carbohydrate antigen 199 (CA199), α-fetoprotein (AFP) and carcinoembryonic antigen (CEA) (**Table 1**).

Statistical analysis

The SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A value of $P<0.05$ was regarded as significantly different. Count data were analyzed by Chi square test. Measurement data were expressed as the mean ± standard deviation (S.D). The Kaplan-Meier method was used to analyze the 5-year survival rate. The log-rank was employed in univariate survival analysis. Cox proportional hazard model was used for multivariate factor analysis.

Results

Clinical pathological factors of patients with GBC in the NLR group

The results demonstrated that there was no significant difference in age, gender, pathological type, differentiated degree, CEA, platelet count, AFP and other aspects of the patients between the high and low NLR groups (P>0.05). In contrast, CRP, total bilirubin, albumin, and CA199 in the high NLR group were significant difference between the high and low NLR groups (P<0.05, **Table 1**). In addition, significant differences were found in TNM stage, margin, operation mode, and Nevin stage between the high and low NLR groups (P<0.05, **Table 1**).

Survival analysis

The 5-year survival rates in the high NLR group and low NLR group were 11.6 and 19.8% (**Figure 1**). Moreover, the 5-year survival rates in the high NLR groups were lower than that in the low NLR groups (P<0.05, **Figure 1**). The univariate survival analysis showed that the degree of tumor differentiation (P<0.001), Nevin stage (P<0.0001), TNM stage (P<0.001), resection margin (P<0.0001), operation mode (P<0.0001), albumin (P=0.037), NLR (P< 0.0001), CRP (P<0.0001), CEA (P=0.014) and CA199 (P=0.03) were significantly related to the median survival period (**Table 2**). Therefore, the above factors were associated with the survival of patients with GBC. The multivariate prognosis analysis showed that Nevin stage (P<0.0001), TNM stage (P<0.0001), operation mode (P<0.0001) and NLR (P <0.0001) were independent prognostic factors in the patients with GBC (**Table 3**).

Discussion

Gallbladder carcinoma is the most common malignancy of biliary tract, accounting for the 5th position of digestive tract tumor [13]. At present, the specific pathogenesis of gallbladder cancer is not clear, so there is no good preventive and early diagnosis method in clinic. Gallbladder disease in addition to related to age, gender, geographic distribution, known risk factors have gallstones [14], obesity [15], many child birth, smoking [16], gallbladder chronic infection [17], the integration of a special chemicals contact, bile pancreatic duct abnormalities, genetic predisposition, and typhoid carrier state. The pathology of gallbladder carcinoma is divided into adenocarcinoma and squamous carcinoma.

The relationship between systemic inflammation and cancer has been previously shown. Inflammation is known to promote tumor devel-

opment and angiogenesis, and inhibit apoptosis [18]. With regard to its mechanism, on the one hand, direct infiltration of the tumor or lymphocyte infiltration in the tumor microenvironment can promote cancer patients to produce a series of inflammatory mediators, cytokines [18]. On the other hand, inflammatory mediators such as interleukin-6 (IL-6), tumor necrosis factor (TNF), neutrophils and inflammatory cells in systemic inflammatory response can play a role in tumorigenesis, invasion and metastasis by promoting angiogenesis and tissue infiltration [19, 20]. Recent studies have demonstrated that inflammation occurs earlier than the malignant transformation of tissue cells during the development of solid malignancies, and systemic inflammation provides a suitable microenvironment for tumorigenesis, progression and migration [21, 22]. The body of this inflammatory reaction can be through the serum of some inflammatory cells and inflammatory protein reflected, such as platelets, neutrophils, lymphocytes, high sensitivity C-reactive protein and albumin. It has been suggested that neutrophils, as well as T and B lymphocytes play a prominent role in tumor inflammation and immunology [23, 24].

Neutrophils are the most common leukocyte subtype in human blood and have important biological functions. What is the mechanism by which neutrophils are involved in the development of malignant tumors is not yet fully understood. Maybe through the following channels: infiltration in the tumor around the neutrophils in autologous way to produce a large number of vascular endothelial growth factor (VEGF) [25]; A large number of VEGF can provide a suitable environment for tumor growth and proliferation and promote the formation of tumor blood vessels, thereby speeding up tumor growth [26]. Lymphocytes involved in the destruction of tumor cells and apoptosis process, which is the main member of anti-tumor immunity. Reduction of lymphocytes suggests a reduction in anti-tumor immune function, which in turn affects the prognosis of cancer patients. It has been reported that lymphopenia will contribute to tumor cell growth and migration, which is associated with poor prognosis [27].

In our study, the 5-year survival rate of patients with GBC in the high NLR group was significantly lower than that in the low NLR group. What is more, the NLR was found to be an important independent prognostic factor of patient with GBC. Therefore, we speculated that the evaluation of preoperative NLR might serve as an effective and simple method for predicting the survival and prognosis of the patients with GBC.

NLR as a peripheral blood neutrophils and lymphocyte ratio, can reflect the body tumor inflammation and anti-tumor immune balance between the state, and has been confirmed with a variety of solid tumors related to the prognosis. Zhang et al [10] retrospectively analyzed the clinical and pathological data of 145 patients with cholecystectomy, the threshold of NLR was 1.94, and the 5-year survival rate of patients with high NLR was significantly lower than that of low NLR group. As an independent risk factor for gallbladder cancer patients.

In conclusion, this study showed that elevated preoperative serum NLR represents a negative prognostic factor in patients who undergo surgery for GBC. NLR measurement is routine and repeatable, and may be a useful adjunct to conventional TNM staging for future clinical trial enrollment and individualized treatments.

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Disclosure of conflict of interest

None.

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