Volume 17, No 2 | International Journal of Radiation Research, April 2019

The role of neutrophil-to-lymphocyte ratio (NLR) in predicting small bowel toxicity and outcome for rectal cancer patients who received chemoradiotherapy

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► ***Original article***

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Revised: April 2018
Accepted: June 2018

Int. J. Radiat. Res., April 2019; 17(2): 309-315

DOI: 10.18869/acadpub.ijrr.17.2.309

# Equal contribution

ABSTRACT

Background*:** In this study, we explored the relationship between neutrophil-to-lymphocyte ratio (NLR) and grade 3 or higher treatment related small bowel toxicity and treatment outcome of patients with rectal cancer undergoing capecitabine and concurrent intensity modulated radiotherapy (IMRT). ***Materials and Methods*:** From the year of 2012 to 2013, 117 rectal cancer patients who received concurrent chemoradiotherapy in our hospital were enrolled in this study. The association of baseline NLR level with grade 3 or higher treatment related small bowel toxicity and treatment outcome, including overall survival (OS) and progression free survival (PFS) were analyzed. ***Results: The optimal cut-off value of the NLR was determined to be 2.2 for the OS according to the receiver operating characteristic (ROC) analysis. A higher level of the baseline NLR was associated with hypoalbuminemia (P= 0.018). No relationship between NLR level and grade 3 or higher acute as well as late treatment related small bowel toxicity was found. A multivariate Cox model revealed that lymph node metastasis (p= 0.013), distant metastasis (p< 0.001), and high NLR level (p = 0.032) were significant predictors for poor OS. Nevertheless, a relationship between NLR level and PFS was not found. ***Conclusion:*** This study show that higher baseline NLR level could not predict treatment related small bowel toxicity of rectal cancer patients who received capecitabine and concurrent IMRT. It is very gratifying to see that NLR is a useful predictor for treatment outcome of these patients.

Keywords: *Neutrophil-to-lymphocyte ratio (NLR), rectal cancer, small Bowel toxicity, prognosis.*

INTRODUCTION

Rectal cancer is one of the most prevalent cancer in the world [1]. Preoperative and postoperative chemoradiotherapy play an essential role in the management of locally advanced rectal cancer [2-4]. However, chemoradiotherapy induced small bowel toxicity limits the usage of these therapeutic modalities. Several clinical and dosimetric predictors for small bowel toxicity have been investigated in previous studies [5-7]. In order to further accurately assess individual patient's risks of developing small bowel toxicity, there is still a need for more reliable and affordable markers for the prediction of this type of illness.

Inflammation is one of the hallmarks of cancer [8]. Recent studies have demonstrated that systemic inflammatory response or the host inflammatory background has a great impact on the prognosis of various types of cancer [9-12]. Additionally, as a representative indicator of systemic inflammation, neutrophil-to-lymphocyte ratio (NLR) has been established as

a prognostic factor for rectal cancer patients [13]. Year to date, there is no study exploring the role of NLR as a predictor for chemoradiotherapy induced small bowel toxicity in rectal cancer. Thus, we made a scientific hypothesis which was host inflammatory background as determined by NLR could be used as a predictor for small bowel toxicity in rectal cancer.

In this study, we assessed the association of pretreatment NLR status with the incidence of chemoradiotherapy induced small bowel toxicity in patients with locally advanced rectal cancer. The prognostic value of NLR in these patients was also evaluated.

MATERIALS AND METHODS

Patient population

Between January 2012 and December 2013, 117 consecutive patients with locally advanced rectal cancer who received neoadjuvant or adjuvant chemoradiotherapy at our hospital were enrolled in this study. Gender, age, stage of disease, and pathologic factors were obtained from electronic patient records retrospectively. Staging was determined according to the classification established by the American Joint Committee on Cancer (AJCC, 7th edition) [14]. Thirteen patients with coexistent autoimmune diseases, infectious diseases, and lacking baseline blood test records were excluded during the analysis. So, 104 patients were eligible for the final analysis. The study protocol was approved by the ethics committee of General Hospital of Ningxia Medical University (2016-199).

Treatment and follow-up

Pelvic magnetic resonance imaging (MRI) was used for pretreatment staging. All patients enrolled in this study were treated with intensity modulated radiotherapy (IMRT) concurrent with two cycles of oral capecitabine (1600 mg/m^2/d, twice daily from day 1–14 of radiotherapy, followed by a 7-day rest) before or after curative resection. The mean radiation dose was 50 Gy with daily fraction of 2Gy.

Acute treatment toxicity was scored according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE; version 4.0) and late toxicity was classified according to the Late Effects in Normal Tissue—Subjective, Objective, Management and Analytic (LENT-SOMA) system [15].

After the whole treatment procedure, all patients were complied with a follow-up every three months for the first two years, every six months for the next three years, and once every year thereafter. Each follow-up consisted of physical examinations, a routine blood test, serum carcinoembryonic antigen (CEA) and Cancer Antigen 19-9 (CA-199) level test. Chest and abdominal CT scans as well as total colonoscopy were performed annually except under suspicion of tumor recurrence.

Overall survival (OS) time was defined from the date of completion of treatment to death from any cause. Progression-free survival (PFS) time was positive as the time from the date of completion of therapy to the date of local recurrence or distant metastasis or death. Patient follow-up was lasted until death or the cutoff date of January 2017.

Definition of NLR

Blood sampling reports from each enrolled patient were obtained within seven days before treatment. White blood cell count, neutrophil, lymphocyte and platelet counts were examined. The NLR was calculated as the absolute neutrophil count divided by the absolute lymphocyte count using baseline blood test results.

Statistical analysis

The receiver operating characteristic (ROC) curve was used to determine the best cut-off value of NLR. Differences in clinicopathologic features between the higher and lower NLR groups were assessed by a chi-square test or Fisher's exact test where appropriate. The OS and PFS curves were made using the Kaplan-Meier method, and groups were compared using the log-rank test. For predictive or prognostic factors analysis, univariable and

multivariable Cox regression were used to identify the variable-independent influence on OS and PFS. All P values of less than 0.05 were considered statistically significant. Statistical analysis was undertaken using SPSS 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Patient characteristics and treatment outcome

The clinical features of enrolled patients are listed in table 1. The male-to-female ratio was 1.3:1. The median age was 60 years old, with a range from 26 to 85 years of age. According to the 7th edition AJCC TNM staging system, 50 (48.1%) patients had stage II disease and 54 (51.9%) patients in stage III disease. Of these patients, 18 (13.7%) patients received neoadjuvant chemoradiotherapy, and 86 (86.3%) patients received adjuvant chemoradiotherapy. Well tumor differentiation was reported in 39 (37.5%) patients. With a median follow-up interval of 47 months (range 4–60 months), 9 (8.7%) patients developed local recurrence, 26 (25.0%) patients developed distant metastasis and 23 (22.1%) patients had died by the time of last follow-up. According to CTCAE 4.0 system, grade 3 or higher acute small bowel toxicities were observed in 35 (28.3%) patients. In addition, 15 (4.9%) patients presented with grade 3 or higher late small bowel toxicities.

Table 1. Association between NLR and clinicopathologic factors of rectal cancer patients.

Characteristics	NLR, (n)		P value
	≥2.2	<2.2	
Age, years			
≥ 60	16	31	
< 60	13	44	0.203
Sex			
Male	18	41	
Female	11	34	0.494
Histology grade			
Well	13	26	
Moderate-poor	16	49	0.337
T stage			
T1-2	5	12	
T3-4	24	63	1.000
N stage			
N0-1	23	65	
N2	6	10	0.373
TNM stage			
II	13	37	
III	16	38	0.680
Treatment			
Pre-operative	4	14	
Post-operative	25	61	0.556
Local recurrence			
Yes	4	5	
No	25	70	0.261
Distant metastasis			
Yes	8	18	
No	21	57	0.705
Hb			
≥ 115 g/L	25	64	
< 115 g/L	4	11	1.000
Albumin			
≥ 40g/L	3	25	
< 40 g/L	26	50	0.018
Acute small bowel toxicity			
G1-2	20	49	
G3-4	9	26	0.725
Chronic small bowel toxicity			
G1-2	27	62	
G3-4	2	13	0.225

Abbreviations: NLR, neutrophil-lymphocyte ratio; Hb, Hemoglobin.

The predictive value of NLR in treatment related small bowel toxicity

For the whole study population, the median value of baseline NLR was 1.8 (range 0.8-4.1). According to the results of ROC analysis, we selected 2.2 as the optimal cut-off value for NLR to estimate the patients' survival. The patients' clinicopathological factors according to their NLR level are list in table 1. A high NLR level was only associated with hypoalbuminemia (albumin <40g/L). No other clinicopathological factors, including grade 3 or higher acute or chronic treatment related small bowel toxicity, were associated with NLR level.

An elevated baseline NLR was a poor prognostic factor for rectal cancer patients

To assess the prognostic role of NLR in rectal cancer patients who received chemoradiotherapy, Cox proportional hazard model was used for the analysis. The results of univariate analysis demonstrated that lymph node metastasis, TNM stage, local recurrence, distant metastasis, and NLR were significantly associated with OS. In the multivariate analysis, lymph node metastasis, distant metastasis, and high NLR level showed significant influence on the survival rate of rectal cancer patients. The 5-year OS of patients with lower NLR level were 83.1% and 60.0% in patients with higher NLR level (P = 0.036) (Figure 1). TNM stage was the only factor associated with PFS in multivariate analysis. The 5-year PFS in patients with lower NLR level was 71.2% as well as there was 65.0% in patients with higher NLR level (P = 0.457) (figure 1). The results of the Cox analysis for OS are shown in table 2, for PFS are shown in table 3.

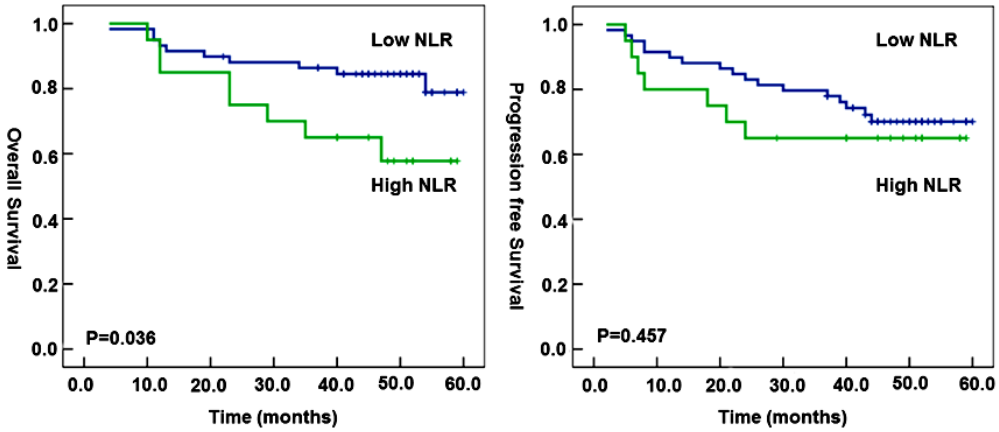

Figure 1. Kaplan–Meier survival curves for overall survival (OS) (left) and progression-free survival (PFS) (right) of rectal cancer patients with a high NLR and those with a low NLR.

Table 2. Univariate and multivariate Cox regression analyses of OS in patients with rectal cancer.

Clinicopathological factors		Univariable analysis			Multivariable analysis		
		Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	*p* value
Age, years	≥ 60 vs. < 60	1.912	0.827-4.419	0.130			
Gender	Female vs. ale	0.591	0.249-1.403	0.233			
Histology grade	Well vs. Poor	0.729	0.319-1.663	0.452			
T stage	T3-4 vs. T1-2	4.313	0.581-32.015	0.153			
N stage	N2 vs. N0-1	4.213	1.771-10.024	0.001	3.826	1.320-11.090	0.013
TNM stage	III vs. II	5.749	1.951-16.938	0.002	1.576	0.427-5.812	0.622
Treatment	Neoadjuvant vs. Adjuvant	0.997	0.339-2.934	0.996			
Local recurrence	No vs. Yes	0.233	0.086-0.631	0.004	0.469	0.118-1.858	0.336
Distant metastasis	No vs. Yes	0.087	0.034-0.222	0.000	0.067	0.021-0.214	0.000
Hb	< 115 g/L vs. ≥ 15 g/L	1.216	0.359-4.119	0.754			
Albumin	< 40 g/L vs. ≥ 0g/L	1.223	0.503-2.976	0.657			
NLR	≥ 2.2 vs. < 2.2	2.600	1.024-6.599	0.044	2.859	1.097-7.451	0.032
Acute small bowel toxicity	G1-2 vs. G3-4	1.203	0.520-2.780	0.666			
Chronic small bowel toxicity	G1-2 vs. G3-4	0.233	0.031-1.730	0.155			

Abbreviations: OS, overall survival; CI, confidence interval; Hb, hemoglobin; NLR, neutrophil-lymphocyte ratio.

Table 3. Univariate and multivariate Cox regression analyses of PFS in patients with rectal cancer.

Clinicopathological factors		Univariable analysis			Multivariable analysis		
		Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	*p* value
Age, years	≥ 60 vs. < 60	1.521	0.766-3.018	0.231			
Gender	Female vs. Male	0.756	0.376-1.522	0.434			
Histology grade	Well vs. Poor	0.886	0.441-1.782	0.753			
T stage	T3-4 vs. T1-2	3.208	0.767-13.411	0.110			
N stage	N2 vs. N0-1	2.412	1.085-5.363	0.031	1.212	0.527-2.762	0.651
TNM stage	III vs. I-II	4.839	2.096-11.169	0.000	4.580	1.910-10.980	0.001
Treatment	Neoadjuvant vs. Adjuvant	0.676	0.293-1.557	0.357			
Hb	< 115 g/L vs. ≥ 115 g/L	1.213	0.426-3.450	0.718			
Albumin	< 40 g/L vs. ≥ 40g/L	1.654	0.813-3.362	0.165			
NLR	≥ 2.2 vs. < 2.2	1.393	0.577-3.361	0.461			
Acute small bowel toxicity	G1-2 vs. G3-4	0.943	0.457-1.945	0.873			

DISCUSSION

Although the grade 3 or higher treatment related small bowel toxicity limits the application of chemoradiotherapy in rectal cancer patients, the predictors of this adverse effect have not been well documented. An increasing number of evidences suggest that NLR has a prognostic role in rectal cancer patients who received chemoradiotherapy [16-23]. Nevertheless, the value of NLR in grade 3 or higher treatment related toxicity prediction for rectal cancer has not been assessed. In the present study, we evaluated the relationship between baseline NLR level and the incidence of grade 3 or higher acute and late small bowel toxicity. The results demonstrated that baseline NLR could not predict the grade 3 or higher treatment related small bowel toxicity in current cohort. However, the same as other previous studies, the prognostic role of NLR was found in the subsequent analysis. The results showed that a higher baseline NLR level was associated with poor OS, but no association with PFS for rectal cancer patients who received capecitabine and concurrent IMRT.

Even so a relationship was found between baseline NLR level and poor outcome of rectal cancer patients in current study, the underlying mechanism remains unclear. The most possible reason is the host inflammatory response participates the initiation and progression of cancer [24]. As we know, neutrophils and lymphocytes are very important indicators for the host systemic inflammation. Neutrophils could secrete cytokines and chemokines mediate inflammatory cell recruitment, tumor growth, and angiogenesis. In addition, an elevated neutrophil could suppress the cytolytic activity of lymphocytes, natural killer cells, activated T cells, and adaptive immune response suppression. Furthermore, the pro-tumor effect of neutrophils has a huge impact on the tumor microenvironment, tumor initiation and progression [25]. On the other side, lymphocytes exert a critical role in cytotoxic cell death and cytokine production that reduce tumor infiltration. Elevated levels of lymphocytes in the peripheral blood and within the primary tumor have been linked with favorable prognosis in various cancer patients [26, 27]. Therefore, NLR can be considered as the balance between pro- and anti-tumor immune activities [28] as well as used as a simple laboratory marker for risk stratification in rectal cancer patients. There is no consensus on the cut-off value of NLR yet. A cut-off value of NLR ranges between 2 and 5 were reported in previous studies. Some researcher tried using the reported cut-off value of NLR in their own studies, these value could discriminate OS as well, but with less sensitivity [16]. The reasons of this phenomenon not only include heterogeneity of different studies, but also depend on the confounding factors for the explanation of the prognostic value of NLR. It must be noted that, as a marker of systemic

inflammation, NLR level can be influenced by concurrent diseases and conditions, such as infections and medications [29]. These limitations were not controlled or reported in detail of some published studies. Nevertheless, in the present study, patients with coexistent autoimmune diseases and infectious diseases were excluded. Finally, a value of 2.2, which was identified by ROC curve analysis, was selected as the cut-off value for the analysis.

As a retrospective design, there are some limitations in this study. First of all, the number of patients is relatively small and the inherent selection bias is unavoidable. Further analysis of a large population is needed to clarify the prognostic value of NLR status. Secondly, as indicated previously, the optimal cut-off value of NLR should be studied in the future. Furthermore, the data about the dynamic changes of NLR during the process of treatment was lacking.

In conclusion, this study indicated that higher baseline NLR level could not predict treatment related small bowel toxicity of rectal cancer patients who received capecitabine and concurrent IMRT. NLR still is a useful predictor for treatment outcome of these patients. Further prospective investigation is needed to assess the optimal NLR cut-off value and its prognostic implications.

ACKNOWLEDGEMENT

This work is supported by First Class Discipline (Clinical Medicine) Project of Ningxia (NXYLXK2017A05), Ningxia Science & Technology Supporting Plan Program (2012) and 312 talent plan of Ningxia (2017).

***Conflicts of interest*: Declared none.**

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