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Combined Detection of Preoperative Neutrophil to Lymphocyte Ratio and Interleukin-6 as an Independent Prognostic Factor for Patients with Non-Metastatic Colorectal Cancer

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Zhifeng Y, Yongjing L, Zhang K, Xuejie D, Shikuo R, Zhen W and these authors are contributed equally to this work.

1. Abstract

1.1. Purpose: To explore the value of neutrophil to lymphocyte ratio (NLR) and Interleukin-6 (IL-6) in predicting the prognosis in patients with non-metastatic Colorectal Cancer (CRC).

1.2. Methods: 88 surgical CRC patients were retrospectively analyzed. Receiver-operating characteristic (ROC) curve analysis was used to determine the patients' thresholds for NLR and IL-6. Kaplan–Meier curve and Cox regression models were used to assess the prognostic values.

1.3. Results: ROC analysis was used to calculate cut-off value of NLR. Area Under The Curve (AUC) of NLR was 0.739 (95% CI: 0.634 to 0.844) for Overall Survival (OS), and 0.799 (95% CI: 0.705 to 0.892) for Disease-Free Survival (DFS). The AUC of IL-6 was 0.773 (95% CI: 0.670 to 0.876) for OS, and 0.817 (95% CI: 0.728 to 0.906) for DFS. AUC of NLR+IL-6 was 0.805 (95% CI: 0.710 to 0.899) for OS and 0.853 (95% CI: 0.774 to 0.933) for DFS, which was higher than that of NLR or IL-6 alone for OS and DFS. In addition, high NLR and IL-6 was significantly correlated with tumor differentiation and TNM staging. NLR was positively correlated with IL-6 (r=0.481). The results of Kaplan-Meier method showed that high NLR+IL-6 was correlated with worse OS and DFS.

1.4. Conclusion: High NLR+IL-6 acts as a better independent prognostic biomarker of CRC than NLR or IL-6 alone, may be applied in clinical practice to identify high-risk patients.

2. Introduction

Colorectal cancer is one of the most common malignant tumors of the digestive system and is the fourth leading cause of cancer-related deaths worldwide [1]. The 5-year Overall Survival (OS) of CRC patients has been improved remarkably owing to the rapid development of surgical technology and other therapies such as chemotherapy, radiotherapy, targeted therapy and biotherapy [1]. However, approximately 45% of patients undergoing colorectal resection developed recurrences or died due to metastatic disease [2].

Inflammatory response plays a key role in the survival of cancer patients [3]. Neutrophil To Lymphocyte Ratio (NLR) is considered to be one of the markers of systemic inflammatory response which has been reported to be associated with the prognosis in patients with CRC [4, 5]. IL-6 is a pro-inflammatory factor which associated with the development and prognosis of CRC [6]. However, few studies have explored the prognostic value of NLR and IL-6 in resectable stage II and III primary CRC. Therefore, to explore

the effect of combined detection of NLR and IL6 on the prognosis of patients with CRC is of great clinical significance.

In the present study, we first evaluated the prognostic utility of NLR or IL-6 alone in patients with CRC after surgery, and then further clarify the clinical significance by exploring the prognostic value of NLR+IL-6 to better predicting the prognosis of patients with CRC.

3. Materials and Methods

3.1. Patients

We conducted a retrospective study of CRC patients who underwent surgery in Leshan People's Hospital between January 2010 to February 2016. The CRC patients in this study were pathologically confirmed stages II and III CRC without distant metastasis or local recurrence. The curative resection of all CRC patients were performed by the same surgical team of the same department. Patients underwent surgical treatment and had a pathological diagnosis of CRC. Patients with intact data of peripheral blood examination including blood routine, blood biochemistry and coagulation within 1 week before operation.

Patients who had systemic inflammation or infection, enterobrosis, evidence of hyperpyrexia, hematological diseases, onset of intestinal obstruction during hospitalization, or a history of other malignancies were excluded. This study was approved by the Ethics Committee of Leshan People's Hospital with the permit number of 20141124, and informed consent was obtained from all included patients.

3.2. Data Collection and Laboratory Methods

The patient's clinical parameters were collected, including: gender, age, tumor location, tumor size, operative adjuvant chemoradiotherapy, histological class, differentiation, tumor stage, and family histology. Tumor staging was performed according to the Tumor-Node-Metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC, 7th edition). Neutrophil count and lymphocyte count were measured within 3 days prior to the surgery in these patients. Blood was collected within 3 days before operation, neutrophil count and lymphocyte count were detected in Clinical laboratory, and then NLR was calculated. NLR was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes. At the same time, the IL-6 level was detected by ELISA assay according to the manufacturer's instructions (CSB-E04638h, CUSABIO, Wuhan, China).

3.3. Survival and Follow-Up

All 103 patients were followed up for 5 years. Among them, 22 patients lost follow-up due to various reasons. Recurrence of CRC patients was confirmed by a combination of CT, MRI imaging and tumor markers, and finally confirmed by pathological examination. Disease Free Survival (DFS) was measured from the date of surgery to the date of disease recurrence or the last follow-up of patients without recurrence. OS was calculated from the date of diagnosis to the date of death or the last follow-up of survivors.

3.4. Statistical Analysis

Receiver Operating Characteristic (ROC) curve analysis was performed to identify the optimal cut-off for the preoperative NLR and IL-6. According to these cut-off values, patients were divided into different groups according to the prognostic scores using NLR, IL-6, and NLR+IL-6. Categorical variables and Continuous variables were presented as number and mean ± SD, respectively. Chi-square test was performed to evaluate the differences in clinicopathological characteristics. Survival analysis was performed using the Kaplan-Meier survival curve. Cox proportional hazard regression model was used for univariate and multivariate analysis of clinical variables to determine the independent prognostic factors. Differences were estimated by log-rank test. P values < 0.05 were considered statistically significant. All statistical analyses were performed with SPSS software version 22.0 (IBM Corporation, NY, USA).

4. Results

4.1. Clinical Characteristics

A total of 88 patients (51.14% male and 48.86% female) with CRC were included in this study, ranging 25-81 years (<65, 46.59%, ≥65, 53.41%) and mean age was 58.31±12.22 years. The mean pre-treatment NLR and IL-6 were 2.94±0.76 and 219.20±38.25 (pg/mL), respectively.

4.2. ROC Curves of NLR and IL-6 For Both OS and DFS

ROC analysis was used to calculate cut-off value of NLR. Patients were divided into high NLR (≥2.99) group and low NLR (<2.99) group (Figure 1). Area under the curve (AUC) was 0.739 (95% CI: 0.634 to 0.844) for OS (Figure 1A), and 0.799 (95% CI: 0.705 to 0.892) for DFS (Figure 1B). Choose 2.99 as an optimal NLR value to evaluate OS and DFS. Similarly, based on ROC analysis, cut-off value of IL-6 in our study was 213.83 (pg/mL). The patients were divided into high IL-6 (≥213.83) group and low IL-6 (<213.83) group (Fig. 1). The AUC was 0.773 (95% CI: 0.670 to 0.876) for OS (Figure 1A), and 0.817 (95% CI: 0.728 to 0.906) for DFS (Figure 1B).

4.3. Correlations of NLR and IL-6 With Clinicopathological Factors

The correlation between NLR and IL-6 and various clinicopathological characteristics were analyzed. As shown in (Table 1), high NLR was significantly correlated with tumor differentiation and TNM staging. In addition, the high expression of IL-6 was correlated to tumor differentiation and TNM staging, However, there were no significant differences in the distribution of gender, age, tumor location, tumor size, and histologic class (table 1). In addition, the result of Spearman's rank correlation analysis was showed that NLR was positively correlated with IL-6 (r=0.481).

Table 1: Characteristics of the 88 patients grouped by NLR and IL-6

Variables	Sum(%)	NLR			IL-6(pg/mL)		
		≥2.99	<2.99	*P*	≥213.83	<213.83	*P*
Age (year)							
<65	41(46.59)	22	19	0.808	25	16	0.462
≥65	47(53.41)	24	23		25	22	
Gender							
Female	45(51.14)	21	24	0.673	20	25	0.286
Male	43(48.86)	22	21		24	19	
Cancer site							
Rectum	46(52.27)	25	21	0.393	28	18	0.212
Colon	42(47.73)	19	23		20	22	
Histologic class							
adenocarcinoma	40(45.45)	22	18	0.793	27	15	0.414
nonadenocarcinoma	48(54.55)	25	23		26	22	
Differentiation							
Well/moderate	68(77.27)	30	38	0.018	32	36	0.016
Poor	30(34.09)	21	9		22	8	
Tumor stage							
II	46(52.27)	17	29	0.005	19	27	0.05
III	40(45.45)	27	13		25	15	
Tumor size (cm)							
<5	53(60.23)	27	25	0.458	24	28	0.823
≥5	35(39.77)	21	14		17	18	

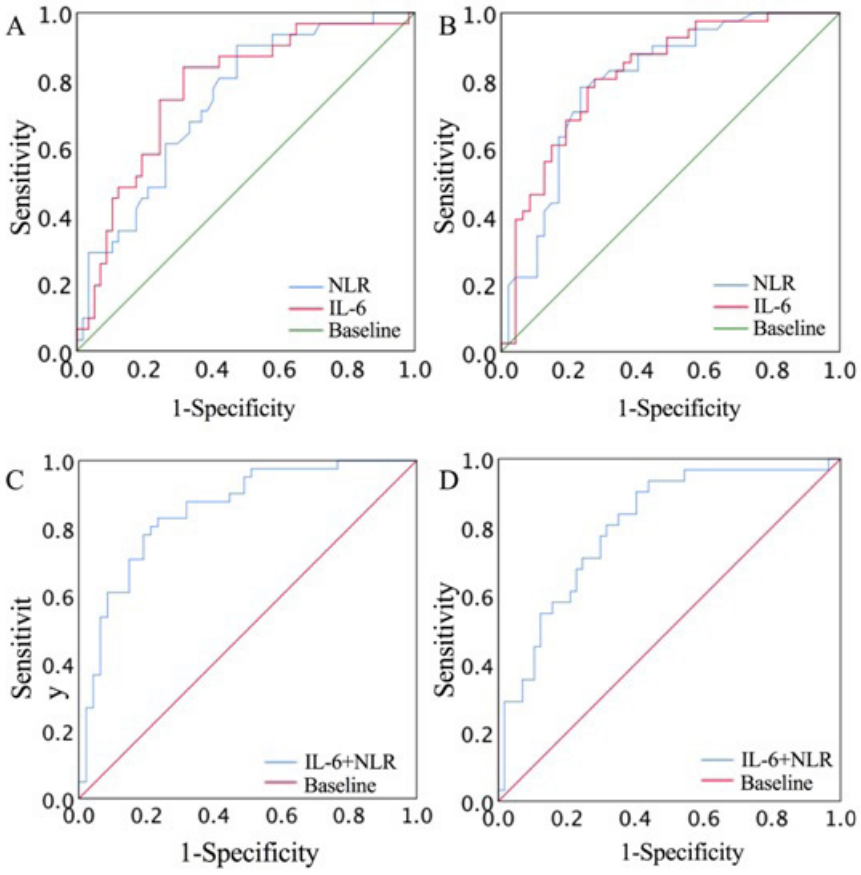

Figure 1: ROC curve analysis of NLR, IL-6 and NLR+IL-6 in CRC Patients ROC curve analysis of NLR and IL-6 for OS (A) and DFS (B). ROC curve analysis of NLR+IL-6 for OS (C) and DFS (D). Prognostic scores of NLR was assigned to 1 (≥2.99) or 0 (>2.99). Prognostic scores of IL-6 was assigned to 1 (≥213.83 pg/mL) or 0 (<213.83 pg/mL). Prognostic scores of combination of NLR and IL-6 was 2 (NLR≥2.99 and≥213.83 pg/mL), 1 (NLR≥2.99 or≥213.83 pg/mL), 0 (Neither NLR≥2.43 nor ≥213.83 pg/mL)

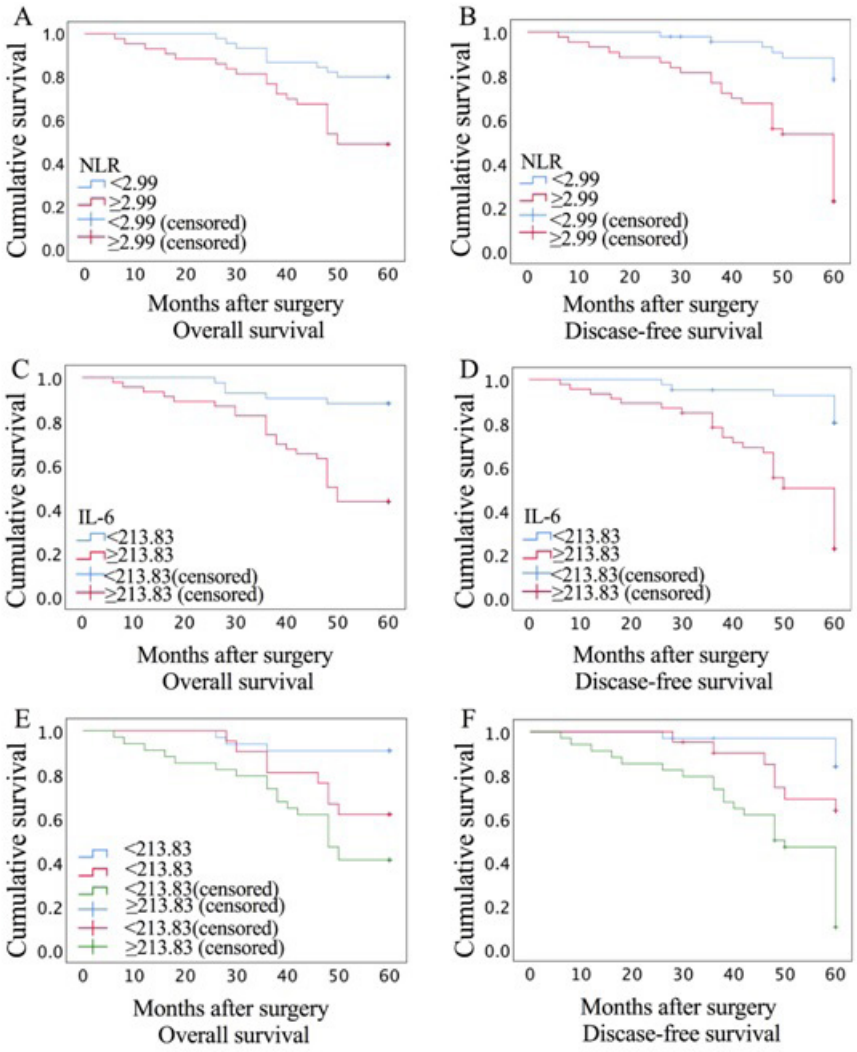

Figure 2: Kaplan-Meier survival curves for OS and DFS in CRC Patients (A) OS according to NLR. (B) DFS according to NLR. (C) OS according to IL-6 levels. (D) DFS according to IL-6 levels. (E) OS according to NLR+IL-6. (F) DFS according to NLR+IL-6. Prognostic scores of NLR was assigned to 1 (≥2.99) or 0 (<2.99). Prognostic scores of IL-6 was assigned to 1 (≥213.83 pg/mL) or 0 (<213.83 pg/mL). Prognostic scores of combination of NLR and IL-6 was 2 (NLR≥2.99 and≥213.83 pg/mL), 1 (NLR≥2.99 or≥213.83 pg/mL), 0 (Neither NLR≥2.43 nor ≥213.83 pg/mL).

4.4. NLR+IL-6 Is A Superior Prognostic Biomarker

As we showed above and other studies, NLR and IL-6 were independent prognostic biomarkers in CRC patients, but NLR+IL-6 had the same efficacy still remained unclear. Therefore, ROC and Kaplan-Meier method were used to assess the value of NLR+IL-6 in CRC patients. The result showed that AUC of NLR+IL-6 was 0.805 (95% CI: 0.710 to 0.899) for OS (Figure 1C) and 0.853 (95% CI: 0.774 to 0.933) for DFS (Figure 1D), which was higher than that of NLR or IL-6 for OS and DFS. In addition, the result of Kaplan-Meier method showed that high NLR+IL-6 was correlated with worse OS and DFS (Fig. 2). These results implied that NLR+IL-6 acts as a significant prognostic biomarker that can be superior to either NLR or IL-6 alone.

5. Discussion

In this study, high NLR+IL-6 was correlated with worse OS and DFS and significantly correlated with tumor differentiation and TNM staging. In addition, NLR was positively correlated with IL-6. Hence, these findings indicate that high NLR+IL-6 acts as a better independent prognostic biomarker of CRC than NLR or IL-6 alone, may be applied in clinical practice to identify high-risk patients.

Recently, many studies have proved that inflammation response affects the occurrence and development of CRC [7]. In the early immune response, inflammation may inhibit tumorigenesis, but persistent inflammatory stimulation can promote tumor progression [8]. Some inflammatory biomarkers are associated with poor clinical outcomes of CRC and have the ability to predict the prognosis of CRC [9, 10]. NLR which calculated by dividing the neutrophil count by the lymphocyte count has been proposed as an indicator of cancer-related inflammation [11]. Lymphocytes regulate the migration and proliferation of tumor cells and inhibit tumorigenesis [4, 12]. On the contrary, neutrophils promote tumor angiogenesis and metastasis [13]. The recruitment of neutrophils and lymphocytes plays a key role in the pathogenesis of CRC [14].

The increased ratio of neutrophils to lymphocytes in peripheral blood is associated with poor prognosis of CRC [15]. In our study, we demonstrated that high NLR was significantly correlated with tumor differentiation and TNM staging. CRC patients with high NLR had shorter OS and DFS than patients with low NLR. This is consistent with other studies [16-18]. These results suggest that high NLR values is positively correlated with the malignant degree of CRC and have a prognostic significance in CRC patients.

Inflammation is closely related to the development of cancer. Gastrointestinal cancer seems to be particularly sensitive to inflammation [19, 20]. Inflammation can trigger and accelerate the development of cancer, leading to the formation of malignant tumors [21]. IL-6 is a key regulator of prostate tumorigenesis, tumor growth, metastasis and chemotherapy resistance [22]. Serum IL-6 levels were associated with poor prognosis, tumor burden, survival rate and advanced stage of pancreatic cancer, lung cancer, esophageal cancer, breast cancer, ovarian cancer and renal cancer [23-27]. In addition, recent studies have shown that IL-6 may play a role in the occurrence and development of CRC. The high IL-6 levels in CRC patients has poor prognosis [28, 29]. In our study, the high expression of IL-6 was correlated to tumor differentiation and TNM staging. CRC patients with high IL-6 had shorter OS and DFS than patients with low IL-6. These results are consistent with other studies. These results suggest that high IL-6 promotes the malignant progression of CRC and have a prognostic significance in CRC patients.

Many studies have confirmed the prognostic value of NLR or IL-6 alone in CRC patients [6, 30]. However, the prognostic value of NLR combined with IL-6 in CRC has not been clarified. NLR and IL-6 was significantly correlated with advanced-stage disease and reduced overall survival OS of esophageal squamous cell carcinoma patients [17]. NLR was positively correlated with the expression of IL-6 in esophageal squamous cell carcinoma patients. Increased level of IL-6 was associated with NLR values in laryngeal cancer [4]. These results are in line with our results. In this study, we found that NLR was positively correlated with IL-6 in CRC. The result showed that high NLR+IL-6 was correlated with worse OS and DFS and higher than that of NLR or IL-6 alone. These results implied that NLR+IL-6 acts as a significant prognostic biomarker that can be superior to either NLR or IL-6 alone.

In our study, high NLR and IL-6 values is positively correlated with the malignant degree of CRC and have a prognostic significance in CRC patients. NLR+IL-6 acts as a significant prognostic biomarker that can be superior to either NLR or IL-6 alone, may be applied in clinical practice to identify high-risk patients.

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