

Preoperative Neutrophil-to-Lymphocyte Ratio Predicts Clinical Outcome in Patients with Stage II and III Colon Cancer

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Abstract. *Aim/Background: The neutrophil-to-lymphocyte ratio (NLR), a combined indicator of inflammation and immunology, is as yet unidentified regarding the clinical outcome of stage II and III colon cancer patients. We evaluated the effect of NLR on time-to-recurrence (TTR) and overall survival (OS) in selected patients. Patients and Methods: A total of 504 patients with stage II and III colon cancer were included in this retrospective study. Preoperative NLR with a cut-off level of 4 was associated with TTR and OS. Results: In univariate analysis, elevated NLR was significantly associated with decreased TTR ($p=0.001$) and remained significant in multivariate analysis ($p=0.006$). Patients with $NLR >4$ showed a median TTR of 62.2 months. In contrast, patients with $NLR \leq 4$ had a median TTR of 92.6 months. Conclusion: This study suggests that preoperative NLR may be an independent prognostic marker for TTR in stage II and III colon cancer patients.*

Colorectal cancer is the second leading cause of cancer-related death in Europe and the United States. Approximately 50% of patients with colon cancer develop metastases with a 5-year survival rate of less than 10% (1, 2). Current clinical guidelines recommend adjuvant chemotherapy for patients with stage III and those with high-risk stage II colon cancer in order to prevent tumor recurrence after curative surgery. However, the majority of colon cancer patients do not benefit from adjuvant treatment, either because they were cured by

surgery-alone or because they will relapse despite adjuvant treatment. There is an intense interest in the elucidation of prognostic and predictive biomarkers for colon cancer that would improve outcome through patient classification.

Recent data indicate that inflammatory cells that accumulate around neoplasms play a crucial role in tumor progression (3, 4). Patients with a high density of lymphocytes in the stroma of tumors were reported to have increased clinical outcome compared to those with low density of lymphocytes, whereas high density of neutrophils was associated with decreased clinical outcome (5, 6). The systemic inflammatory response also causes changes in the levels of circulating white blood cells, including neutrophils and lymphocytes. However, day-dependent fluctuations in the number of neutrophils are not always in line with those of lymphocytes (7). Hence, the relative value of a combined index using neutrophils and lymphocytes as a neutrophil-to-lymphocyte ratio (NLR) can more accurately reflect fluctuations between neutrophils and lymphocytes in order to more precisely reflect the antitumor efficacy of the host immune system.

A combined index using the NLR has been already associated with clinical outcome in various cancer entities including cervical carcinoma, kidney cancer, gastrointestinal cancers and lung cancer (8-13). The purpose of the present study was to evaluate the effect of the preoperative NLR on TTR and OS in patients with stage II and III colon cancer.

Patients and Methods

Eligible patients. A total of 504 treated patients from year 2002 to 2011, with histologically-confirmed stage II and III colon cancer, were included in this retrospective study. All patients were treated at the Division of Clinical Oncology, Department of Medicine, Medical University of Graz. Patients with stage III and those with high-risk stage II were treated with adjuvant 5-FU-based chemotherapy. High-risk stage II colon cancer patients were defined if they presented with at least one of the following features: lymph

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node sampling <12; poorly-differentiated tumor; vascular, lymphatic or perineural invasion; tumor presentation with obstruction or perforation and pT4. Patients were included in the colon cancer surveillance program providing history and physical examination and carcino-embryonal antigene (CEA) determination every 3 months for 3 years, every 6 months at years 4 and 5, and annually up to 10 years after surgery. Colonoscopy was performed at year 1 and thereafter every 3-5 years and ultrasound or CT scan of abdomen and chest X-ray every 6 months for the first 5 years and then annually.

Preoperative neutrophil-to-lymphocyte count was defined as the absolute count of neutrophils divided by the absolute count of lymphocytes, measured from the routinely-performed blood count within 3 days before surgery. The study has been approved by the Institutional Review Board of the Medical University of Graz (no. 25-137 ex 12/13).

Statistical analysis. The primary end-point of the study was time-to-recurrence (TTR) and secondary end-point was overall survival (OS). TTR was calculated from the date of diagnosis of colon cancer to the date of tumor recurrence and was censored at time of death or at the last follow-up if the patients remained tumor-free at that time. OS was calculated from time of diagnoses to the date of death by any cause. A NLR cut-off level of 4 was selected for the evaluation of early-stage colon cancer patients (10).

The distribution of the NLR across baseline demographic, clinical and pathological characteristics was examined using the Fisher's exact test. The association of the NLR with TTR and OS was analyzed using Kaplan-Meier curves and log-rank test. In the multivariate Cox-regression analysis, the model was adjusted for sex, tumor size, number of resected lymph nodes, histological grade, clinical stage and adjuvant chemotherapy. Hazard ratios (HRs) estimated from Cox regression analysis were reported as relative risks with corresponding 95% confidence intervals (CIs). All analyses were performed using the SPSS 20 statistical software package (SPSS Inc., Sunnyvale, California, USA).

Results

The median age at the time of diagnosis was 65 years (range 27-95 years), the median follow-up time was 45 months (range 1-108 month). The preoperative NLR was available for 302 patients (59.9%). The main reason for missing NLR data was the predefined narrow time frame of preoperative blood sampling within 3 days before surgery. We found a NLR≤4 in 173 (57.3%) patients, whereas 129 (42.7%) patients showed a NLR>4. The baseline patients' characteristics and their association with TTR and OS are summarized in Tables I and II.

A NLR >4 was significantly-associated with T4 tumors (p=0.03). None of the other clinicopathological parameters were associated with a NLR >4 (data not shown). In univariate analysis, the elevated NLR was significantly associated with decreased TTR (HR=2.27; 95%CI=1.42-3.62; p=0.001) (Figure 1) and remained significant in multivariate analysis including the factors of sex, tumor size, number of resected lymph nodes, histological grade, clinical stage and adjuvant chemotherapy (HR=1.95; 95% CI=1.21-

Table I. Baseline patients' characteristics.

	N	%
Gender		
Male	293	58.1
Female	211	41.9
T stage		
T1	10	2.0
T2	23	4.6
T3	358	71.0
T4	113	22.4
N lymph nodes		
N0	194	38.5
N1	203	40.3
N2	105	20.8
Missing	2	0.4
Number of resected lymph nodes		
<12	72	14.3
>12	431	85.5
Missing	1	0.2
Grading		
G1	36	7.1
G2	332	65.9
G3	135	26.8
Missing	1	0.2
Stage		
II 190	37.7	
III	312	61.9
Missing	2	0.4
Adjuvant treatment		
5-FU-based chemotherapy	337	66.9
No adjuvant chemotherapy	166	32.9
Missing	1	0.2

3.13, p=0.006). Patients with NLR ≤4 had a median TTR of 92.6 months. In contrast, patients with NLR >4 showed a median TTR of 62.2 months. In univariate analysis, the elevated NLR was also significantly associated with decreased OS (HR=2.05, 95% CI=1.06-3.95, p=0.033) (Figure 2). Patients with preoperative NLR ≤4 had a median OS of 101.3 months, whereas patients with NLR >4 showed a median OS of 83.4 months. In multivariate analysis the elevated NLR lost its statistical significance with a trend to decreased OS (HR=1.81, 95% CI=0.91-3.58, p=0.091).

Discussion

In the present study we identified preoperative NLR as a potential prognostic marker in patients with stage II and III colon cancer. Inflammation plays a critical role in the pathogenesis and progression of cancer. Chronic inflammatory bowel diseases are risk factors for colorectal cancer, whereas the regular use of non-steroidal, anti-rheumatic drugs decrease colorectal cancer risk (14). The host's response to stress or to critical illness activates several

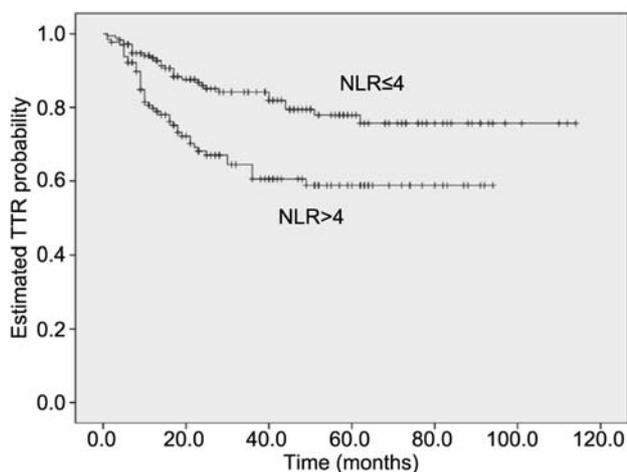


Figure 1. Elevated NLR >4 was significantly associated with decreased TTR.

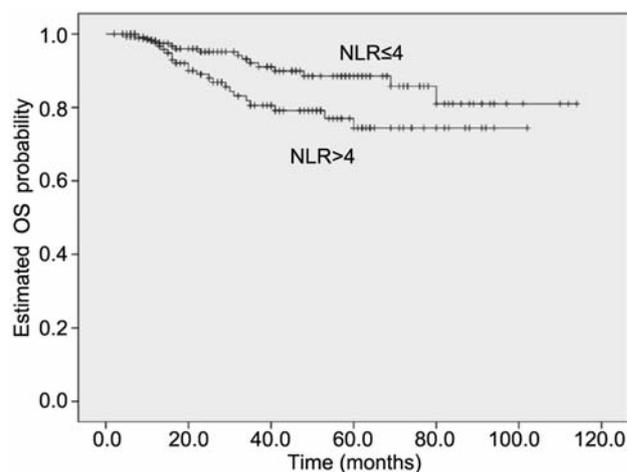


Figure 2. Elevated NLR ≤4 was significantly associated with decreased OS.

Table II. Association of clinicopathological features with time-to-recurrence (TTR) and overall survival (OS).

	TTR			OS		
	HR	CI	p-Value	HR	CI	p-Value
Gender (male vs. female)	0.879	0.615-1.255	0.476	1.089	0.517-2.294	0.822
T stage (1/2 vs. 3/4)	2.275	1.628-3.179	0.000	2.845	1.820-4.447	0.000
N (0 vs. 1/2)	1.000	0.999-1.001	0.889	1.000	0.999-1.001	0.911
Surgically-resected lymph nodes (≤12 vs. >12)	0.780	0.497-1.226	0.282	0.971	0.380-2.481	0.952
Grade (1/2 vs. 3)	1.236	0.898-1.701	0.194	2.105	1.068-4.148	0.31
Stage (II vs. III)	2.210	1.473-3.314	0.000	2.284	0.732-7.129	0.155
Adj. CTX (no vs. yes)	0.694	0.155-0.694	0.155	0.649	0.279-1.508	0.315

CI: Confidence interval, HR: hazard ratio.

immune pathways. Zahorec *et al.* showed that neutrophilia and lymphopenia are general innate immune responses in cancer patients treated at the intensive care unit (15). The prognostic role of NLR in cancer may be due to this physiological response of the host to tumor infiltration and metastases. Increased tumor-infiltrating lymphocytes have been recognized as a predictor of good prognosis in colorectal cancer (16), whereas decreased lymphocyte infiltration in tumor margins has been associated with worse prognosis (17). Elevated absolute lymphocyte counts have been shown to be an independent prognostic marker for increased OS in newly-diagnosed multiple myeloma and in breast cancer patients with curative resection (18, 19). Patients with high NLR have relative lymphocytopenia and therefore poor immune response against tumors, which could be due to the role of lymphocytes in cytotoxic cell death and cytokine production that inhibit proliferation and metastatic activity of tumor cells (19). Elevated neutrophil counts, however, may reflect tumor progression by providing an

adequate environment for its growth. Neutrophils are major contributors of tumor-related angiogenesis by providing angiogenesis-promoting chemokines (20). Kusumanto *et al.* showed that activation of neutrophils resulted in pro-angiogenic activity due to release of chemokines (interleukin-8) and growth factors (VEGF) (21). Taken these hypotheses together, the ratio of neutrophils and lymphocytes may reflect the balance of inflammatory status with immune response against tumor (lymphocytes) and pro-tumor activity of neutrophils.

Several translational research studies demonstrated an effect of NLR on the clinical outcome in various malignancies (9-11, 18, 22, 23). Walsh *et al.* found an association between preoperative NLR >5 and decreased OS in colon cancer patients of all clinical stages (12). In a retrospective analysis including 3,857 colorectal cancer patients, a preoperative NLR >3 was associated with larger tumor size, elevated CEA and an increased risk of cancer progression and decreased survival. Interestingly, no significant associations were found in the

subgroup of rectal cancer (22). In stage II colon cancer with surgery-alone a preoperative NLR >4 was significantly associated with decreased recurrence-free survival rates (10), which is in line with our results. Furthermore, in our study the association between NLR >4 and TTR was independent of clinical stage and adjuvant chemotherapy.

Some limitations of our study have to be taken into account; due to its retrospective design, a selection bias cannot be fully excluded. The preoperative blood count was pre-defined within 3 days before surgery to avoid misinterpretation for systemic inflammation or other confounding factors, therefore the number of available NLR was significantly decreased.

In conclusion our study suggests that preoperative NLR may be an independent prognostic marker for TTR in stage II and III colon cancer patients. Larger prospective trials are warranted to validate our findings.

Conflicts of Interest

None declared.

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