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RESEARCH ARTICLE

The Effectiveness of the Serum Neutrophil-lymphocyte Ratio in the Determination of an Advanced Stage in Colorectal Cancers

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ABSTRACT

Aim: Patients with colorectal cancer (CRC) have a poor prognosis in the advanced stages. Although advanced imaging methods contribute to the diagnosis process, there is a need for potential biomarkers to help identify patients with poor prognoses. The neutrophil-lymphocyte ratio (NLR) is one of these potential biomarkers, and many studies have found that a high NLR is a predictive marker for a poor prognosis in various cancers. This study aims to determine whether there is a relationship between the NLR and the tumor stage in CRC patients.

Method: We retrospectively assessed patients who underwent elective curative resection for CRC between January 2016 and July 2019. The demographic information, preoperative NLR, and detailed pathological data were recorded and analyzed. The study's primary aim was to investigate whether there was a relationship between the pathological stage and the NLR. The secondary aim was to examine the relationship between the number of positive and removed lymph nodes, tumor biology, and the NLR.

Results: No statistically significant correlation existed between the tumor stage, the number of positive and removed lymph nodes, and the NLR. However, a statistically significant negative correlation was found between tumor differentiation and the NLR.

Conclusion: Although a relationship between the tumor stage and the NLR was shown in the authors' study, this did not reach the level of statistical significance. Likewise, there was no significant relationship between the lymph node involvement and the NLR; only the degree of differentiation of the tumor and the NLR were significantly correlated. Therefore, prospective randomized studies with large patient cohorts would be useful in evaluating the clinical use of the NLR in CRC.

Keywords: Colorectal cancer, neutrophil-lymphocyte ratio, stage, survival, differentiation, lymphatic infiltration, vascular invasion, perineural invasion

Introduction

Colorectal cancer (CRC) is one of the most common types of cancer worldwide.[1] Despite the advancement of new therapeutic strategies in surgery, chemotherapy, radiotherapy, and molecular therapy, the overall prognosis of advanced CRC is still poor, with a 5-year survival rate of less than 15%.[2] Although advanced imaging methods contribute to the diagnosis process, there is a need for potential biomarkers to help identify patients with poor prognoses.

Local immune response and systemic inflammation have been shown to play important roles in cancer progression and survival.[3] The clinical utility of inflammatory prognostic biomarkers has been described in many different forms of cancer.[4] The neutrophil-lymphocyte ratio (NLR), lymphocyte-C-reactive protein ratio, platelet-lymphocyte ratio (PLR), and lymphocyte-monocyte ratio are some of these biomarkers. Zhou et al.[5] showed that the NLR was significantly higher in patients with CRC compared with patients with colorectal polyps and healthy controls.

Many studies have found that a high NLR is a predictive marker for poor prognoses in various cancers, including gastric, esophageal, and colorectal.[6] Increased pretreatment

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NLR has been linked to disease progression, poor treatment response, and reduced chances of survival in CRC and other cancers.[6-9] Considering the importance of systemic inflammatory reactions in tumor development and treatment, it is thought that NLR levels might reflect systemic inflammatory reactions in CRC, and there may be a correlation between the tumor stage and the NLR.[10]

This study aims to determine whether there is a relationship between the NLR and the tumor stage in CRC patients by examining the preoperative neutrophil and lymphocyte values and postoperative pathological findings of patients operated on for CRC in the center's general surgery department.

Materials and Methods

The study protocol was approved by the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Ümraniye Training and Research Hospital (approval number: 142, date: 21.11.2018). The study was conducted in accordance with the 1975 Declaration of Helsinki, as revised in 2013.

We retrospectively assessed patients (age >18 years) who underwent elective curative resection for CRC between January 2016 and July 2019. Patients who underwent emergency surgery, had severe comorbid disease (American Society of Anesthesiologists score; >3), or had lost data were excluded from the study.

The patients' data were obtained from the hospital's data processing department. The patients' demographic information, preoperative neutrophil and lymphocyte counts, surgical procedure details, number of lymph nodes removed during surgery, tumor positive lymph node count, tumor differentiation rate, presence of lymphatic, vascular, and perineural invasion, and pathological stage were recorded and analyzed.

The study's primary aim was to investigate whether there is a relationship between the pathological stage and the NLR in CRC. The secondary aim was to examine the relationship between the number of positive lymph nodes and removed lymph nodes, the tumor biology (differentiation and lymphatic, vascular, and perineural invasion), and the NLR ratio.

The eighth edition of the American Joint Commission on Cancer's tumor-node-metastasis (TNM) staging system was used in the study.[11] For calculating the NLR, patients' complete blood counts obtained on the day of admission to the hospital were used. The NLR was calculated as neutrophil counts divided by lymphocyte counts. The reference values for neutrophils and lymphocytes were 2.00-7.00 10^3/uL and 0.80-4.00 10^3/uL, respectively.

Statistical Analysis

Statistical analyzes were performed using the SPSS® software (version 20.0, SPSS Inc., Chicago, IL, USA). The descriptive analyzes were presented as means ± standard deviations, medians and interquartile range (IQR), and percentages. The distribution normality was obtained using the graphical representation and the Kolmogorov-Smirnov test. While the ages of the patients were normally distributed in the Kolmogorov-Smirnov test ($p=0.200$), the number of lymph nodes removed ($p<0.001$), lymphocyte ($p<0.001$), and neutrophil ($p<0.002$) values were not normally distributed. In cases where the data were unsuitable for normal distribution, the Kruskal-Wallis H test, Mann-Whitney U test, and chi-squared test were used to measure the differences in discrete variables between the groups. The Spearman's rank correlation co-efficient was used to measure the relationship between the variables. Binary logistic regression was used if the dependent variable was binary discrete, and multinomial logistic regression was used if it was multiple. The results were evaluated at the 95% confidence interval at the $p<0.05$ significance level.

Results

Two hundred twenty-eight patients who met the inclusion criteria were included in this retrospective study. The mean age was 59.9±11.9, and 137 (60%) patients were male. No statistically significant difference was found in age according to gender ($p=0.16$).

The most common surgical procedure was low anterior resection (43.8%). Table 1 shows the patient characteristics and the surgical procedures performed. The median number of lymph nodes removed was 21 (IQR; 15-31). According to the TNM classification, most patients were in stage 2 in the postoperative pathological evaluation, while stages 1, 3, and 4 were 9.2%, 39.9%, and 7%, respectively.

While most tumors were moderately differentiated (85%), 4.9% were well differentiated, 8.8% were poorly differentiated, and 1.3% were undifferentiated. Lymphatic invasion was found in 33.7% of the patients, vascular invasion in 24.5%, and perineural invasion in 51.3%.

The median neutrophil value of the patients was 4.69 10^3/uL (IQR: 3.52-5.80), and the median lymphocyte value was 1.90 10^3/uL (IQR: 1.29-2.41). The median NLR was 2.23 (IQR: 1.59-3.56), 2.59 (IQR: 1.67-3.87), 2.78 (IQR: 1.89-3.81), and 2.40 (IQR: 1.79-3.86) in patients with stage 1, 2, 3, and 4, respectively. There was no statistically significant difference between the tumor stages regarding the NLR ($p=0.46$).

The relationship between the NLR and the tumor stage was investigated by multinominal regression. The variables were

statistically analyzable when the stage 1 reference category was taken (p>0.05; goodness-of-fit). Compared with stage 1, the NLR of stage 2 and stage 3 increased approximately 1 time, although it was not statistically significant (p>0.05). Compared with stage 1, the NLR of stage 4 decreased 0.88 times, although it was not statistically significant (p>0.05) (Table 2).

The relationship between the number of harvested and positive lymph nodes and the NLR was investigated, and no statistically significant correlation was found (r=0.032, r=0.06, p>0.05, respectively) (Table 3).

The relationship between the NLR and differentiation was investigated by multinominal regression. Patients with undifferentiated tumors were excluded from the analysis, and the analysis was continued with 225 patients. The variables were statistically analyzable when the group with poorly differentiated tumors was taken as the reference category (p>0.05; goodness-of-fit). Compared with the patients with poorly differentiated tumors, the NLR of patients with well-differentiated tumors decreased by 0.94 times, although it was not statistically significant (p>0.05), while the NLR of patients with moderately differentiated tumors was statistically significantly reduced by 0.88 times (p<0.05) (Table 4).

The relationship between the lymphatic, vascular, and perineural invasion and the NLR was investigated by logistic regression. Although not statistically significant, the NLR decreased approximately 0.95-fold in patients with lymphatic infiltration (p>0.05), while it increased 1-fold in patients with vascular and perineural invasion (p>0.05) (Table 5).

Table 1. Patient characteristics

Variable	n (%)
Age, mean (standard deviation)	59.9±11.9
Gender	
Female	91 (40)
Male	137 (60)
Neutrophil,10^3/uL, median (IQR)*	4.69 (3.52-5.80)
Lymphocyte, 10^3/uL, median (IQR)	1.90 (1.29-2.41)
Surgical procedure	
Low anterior resection	100 (43.8)
Right hemicolectomy	51 (22.4)
Left hemicolectomy	41 (17.9)
Anterior resection	25 (10.9)
Abdominoperineal resection	7 (0.3)
Total colectomy	4 (0.2)
Stage	
1	21 (9.2)
2	100 (43.8)
3	91 (39.9)
4	16 (7.1)
Presence of lymphatic invasion	77 (37.7)
Presence of vascular invasion	56 (24.5)
Presence of perineural invasion	117 (51.3)
Differentiation	
Well	11 (4.9)
Moderately	194 (85)
Poorly	20 (8.8)
Undifferentiated	3 (1.3)

*IQR: Interquartile range

Discussion

In the authors' study, patients who were operated on for CRC were evaluated, and it was investigated whether there was a correlation between the preoperative NLR and the pathological stage. Accordingly, the use of the NLR as a biomarker in patients with CRC was questioned. Although the results showed no significant relationship between the NLR and the pathological stage, the NLR was significantly higher in patients with poorly differentiated tumors than in patients with moderately differentiated tumors.

Biomarkers are important tools in early detection and predicting the prognosis, survival, and treatment response in CRC. Some previous studies have focused on the utility of inflammatory indices, such as the NLR and the PLR, in CRC patient prognosis or treatment response. The NLR has been proposed as a straightforward index of systemic inflammatory response. The cancer-associated systemic inflammatory response is often correlated with increased circulating neutrophil counts. Neutrophils secrete cytokines and chemokines, which play crucial roles in cancer progression. Moreover, lymphocytes can promote a cytotoxic immune response to cancer[4]. Simply, neutrophilia occurs during systemic inflammation, and lymphopenia is a marker of depressed cell-mediated immunity.[12]

In 2001 Zahorec[12] first described the role of the NLR in critically ill patients and found the NLR to be associated with the severity of the clinical condition. Walsh et al.[13] first reported a correlation between preoperatively elevated NLR and overall and cancer-specific survival in colon cancer. Proctor et al.[14] analyzed 12,118 patients, including 1,413 CRC patients, and indicated that the NLR was a significant marker for overall and cancer-specific survival.

Table 2. The relationship between the neutrophil-lymphocyte ratio and the tumor stage*

Stage	B	Standard error	Wald	p	Exp(B)	95% CI for exp(B)	
						Lower bound	Upper bound
2	0.081	0.101	0.638	0.424	1.084	0.889	1.323
3	0.072	0.102	0.494	0.482	1.074	0.88	1.312
4	-0.128	0.192	0.443	0.506	0.88	0.604	1.282

*Multinominal regression; stage 1 reference category. CI: Confidence interval

Table 3. The correlation between the number of harvested and positive lymph nodes and the neutrophil-lymphocyte ratio

	r*	p
Harvested lymph nodes	0.032	0.628
Positive lymph nodes	0.065	0.538

*Spearman's rank correlation coefficient

Table 4. The relationship between the neutrophil-lymphocyte ratio and differentiation*

Differentiation	B	Standard error	Wald	p	Exp(B)	95% CI for exp(B)	
						Lower bound	Upper bound
Well	-0.062	0.068	0.851	0.356	0.940	0.823	1.073
Moderately	-0.124	0.053	5.363	0.021	0.884	0.796	0.981

*Multinominal regression; poorly differentiated tumors reference category. CI: Confidence interval

Table 5. The relationship between the neutrophil-lymphocyte ratio and the lymphatic, vascular, and perineural invasion*

	B	Standard error	Wald	p	Exp(B)	95% CI for exp(B)	
						Lower bound	Upper bound
Lymphatic invasion	-0.049	0.049	1.002	0.317	0.952	0.865	1.048
Vascular invasion	0.013	0.044	0.084	0.772	1.013	0.930	1.103
Perineural invasion	0.053	0.043	1.496	0.221	1.054	0.969	1.148

*Logistic regression. CI: Confidence interval

Many studies have focused on the effects of the NLR on the prognosis of CRC.[4] Recently, a meta-analysis by Naszai et al.[15] found that high pretreatment blood NLR was associated with poor overall survival and surrogate endpoints in CRC patients. Jia et al.[10] retrospectively analyzed CRC patients for the relationship between the NLR, PLR, and tumor TNM stages. They observed that the levels of both markers were significantly higher in CRC patients than in healthy controls. Furthermore, the increase in the NLR and the PLR correlated with the TNM stages.[10] Pereira et al.[16] found that the NLR was significantly higher in patients with T3-T4 tumors than in T1-T2 tumors (5.8 vs 2.6, p<0.001). In a meta-analysis, Li et al.[6] presented data on the NLR and the TNM stages in three studies, showing that patients with a high NLR tended to the advanced TNM stage. Although there was no statistically significant relationship between the tumor stage and the NLR in the authors' study, a tendency for increased NLR was found in advanced-stage patients. It is thought that the inconsistency of these results with the literature may be due to the lack of homogeneity in the stage distribution of the cohort. Like the authors' study, Kwon et al.[17] did not find a significant relationship between the tumor stage and the NLR in 200 CRC patients.

There is a lack of studies in the literature that specifically examine the relationship between the NLR and the lymph node status. According to Caputo et al.[18], when the NLR's cut-off value was taken as 3.7, it showed that a high NLR was predictive of lymph node metastasis in patients with T1

CRC. Khan et al.[19] showed that a elevated NLR was associated with a positive nodal status in rectal cancer patients. The authors' study evaluated the relationship between the number of harvested and positive lymph nodes and other histopathological results with the NLR. No correlation was found between the number of harvested and positive lymph nodes and the NLR.

The patients with moderately differentiated tumors had a statistically lower NLR of 0.88 times compared with patients with poorly differentiated tumors ($p<0.05$). Four studies that examined the relationship between the NLR and tumor differentiation in the literature also support this finding.[20-23]

Study Limitations

This study inevitably has limitations due to its retrospective nature, single-center design, and the low number of patients. However, the NLR value and its relationship with the tumor stage, lymph node metastases, tumor differentiation, and vascular, lymphatic, and perineural invasion in patients with CRC have been thoroughly evaluated. Larger prospective studies will need to be performed for the clinical use of the NLR.

Concluison

In conclusion, most of the studies in the literature have shown a significant relationship between the NLR and the tumor TNM stage and prognosis. However, although a relationship between the tumor stage and the NLR is shown in the authors' study, this did not reach the level of statistical significance. Likewise, no significant relationship could be demonstrated between the lymph node involvement and the NLR; only the degree of differentiation of the tumor and the NLR were significantly correlated. Therefore, prospective randomized studies with large patient cohorts would be useful in evaluating the clinical use of the NLR in CRC.

Ethics

Ethics Committee Approval: The study protocol was approved by the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Ümraniye Training and Research Hospital (approval number: 142, date: 21.11.2018).

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Authorship Contributions

Surgical and Medical Practices: H.Ç., A.T., Ö.F.Ö., Ö.D., Concept: H.Ç., A.T., Ö.F.Ö., Design: H.Ç., Ö.D., Data Collection or Processing: H.Ç., Ö.F.Ö., Analysis or Interpretation: H.Ç., E.S., Literature Search: H.Ç., E.S., Ö.F.Ö., Writing: H.Ç., E.S.

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