Chirurgia (2023) 118: 260-271
No. 3, May - June
Copyright© Celsius
http://dx.doi.org/10.21614/chirurgia.2023.v.118.i.3.p.260

Systemic Neutrophil-to-Lymphocyte Ratio as a Prognostic Biomarker for Colon Cancer

Cristian Ion Iaciu[1,2], Radu Andrei Emilescu[3,4], Horia-Teodor Cotan[1]*, Cornelia Nitipir[1,2]

[1]Department of Oncology, Elias Emergency University Hospital, Bucharest, Romania
[2]Department of Medical Oncology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[3]Department of Endocrinology, C.I. Parhon National Institute of Endocrinology, Bucharest, Romania
[4]Department of Endocrinology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

*Corresponding author:
Horia-Teodor Cotan, M.D.
Department of Oncology, Elias Emergency University Hospital
Bucharest, Romania
E-mail: horia-teodor.cotan@rez.umfcd.ro

Received: 15.05.2023
Accepted: 25.06.2023

Rezumat

Raportul neutrofile-limfocite ca factor de prognostic în cancerul colorectal

Context şi scop: Studiile anterioare au corelat un raport preoperator ridicat de neutrofile la limfocite (NLR) cu un prognostic mai slab la indivizii cu o varietate de afecțiuni maligne. Acest studiu şi-a propus să stabilească utilitatea prognostică şi predictivă a NLR preoperator la pacienții cu cancer colorectal (CRC) şi să identifice o valoare prag adecvată pentru NLR.
Metode: Am înrolat un total de 195 de pacienți care au fost operați pentru cancer colorectal în II şi III care au necesitat chimioterapie adjuvantă, precum şi pacienți cu cancer colorectal în stadiul IV operați cu intenție paliativă. Valoarea medie a NLR a fost 3,42 ± 2,27. Am folosit o curbă caracteristică de funcționare a receptorului pentru a estima valoarea prag optimă a NLR la 3. Pacienții cu o valoare NLR peste 3 au fost clasificați ca NLR ridicat, în timp ce pacienții cu un NLR sub 3 au fost clasificați ca NLR scăzut;
Rezultate: Supraviețuirea globală (OS) şi supraviețuirea fără progresie (PFS) au fost reduse semnificativ la pacienții cu NLR ridicat. Rata globală de răspuns (ORR) a fost, de asemenea, mai mică la pacienții cu NLR ridicat.
Concluzii: NLR preoperator este un biomarker inflamator care pare să aibă valoare prognostică şi predictivă la pacienții cu cancer colorectal.

Cuvinte cheie: raportul neutrofile-limfocite, cancer colorectal, biomarker

Abstract

Background and aim: Prior research linked a high preoperative neutrophil-to-lymphocyte ratio (NLR) to a worse prognosis in individuals with a variety of malignancies. This study aimed to establish the prognostic and predictive usefulness of preoperative NLR in colorectal cancer (CRC) patients and to identify an appropriate cut-off value for the NLR.

Methods: We enrolled a total of 195 patients that underwent surgery for stage II and III colorectal cancer that required adjuvant chemotherapy as well as stage IV colorectal cancer patients treated with palliative intent. The mean NLR value was 3.42 ± 2.27. We used a receiver operating characteristic curve to estimate the optimum NLR cut-off value at 3. Patients with a NLR value above 3 were classified as high-NLR, while patients with a NLR below 3 were classified as low-NLR;

Results: Overall survival (OS) and progression-free survival (PFS) were significantly reduced in high-NLR patients. The overall response rate (ORR) was also lower in high-NLR patients.

Conclusions: Preoperative NLR is an accessible measurement that seems to have prognostic and predictive value in patients with colorectal cancer.

Key words: neutrophil-to-lymphocyte ratio; colon cancer; biomarker

Introduction

In 2020 CRC had the third-highest incidence and the second-highest mortality with 2 million new cases and almost 1 million deaths per year. These figures are predicted to increase to 3.2 million new cases and 1.6 million deaths per year by 2040 (1). Despite advancements in surgical technique, screening, and constantly improving cancer therapies, CRC remains a substantial cause of mortality. While surgical resection is accepted as the only curative treatment for locoregional CRC, adjuvant therapy addresses micrometastatic disease and improves OS. The role of adjuvant treatment is established in stage III colon cancer, whereas the benefit of chemotherapy is uncertain in stage II, especially in dMMR/MSI-H tumors (2). In patients with stage IV CRC, the common therapeutic approach is a fluoropyrimidine (5-FU)-based regimen, and may include agents such as oxaliplatin (OX), irinotecan (IRI) as well as targeted therapy (anti-EGFR agents, anti-VEGF agents or immune checkpoint inhibitors). An ongoing area of research has been the development of tools and biomarkers that could better classify patients beyond stage-only prognostication (3,4).

The immune system's role in cancer pathogenesis and the antitumoral response has been the subject of extensive research. A link was established between inflammatory factors in the tumor microenvironment and tumor size, growth rate, and metastasis (5-8). The association between chronic inflammation and CRC susceptibility has been reported in patients with inflammatory bowel disease, a well-known risk factor for CRC, with a yearly risk increase of 0.5-1% (9). Cytokines, C-reactive protein (CRP), Glasgow Prognostic Score, modified Glasgow Prognostic Score, neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio are markers of inflammatory response that have been shown to have a prognostic impact in a variety solid tumors (10-12). It has been suggested that the systemic inflammatory response to malignancies manifests itself in peripheral white blood cells such as neutrophils and lymphocytes. Numerous studies have found NLR to be an independent prognostic factor for survival and recurrence (13-15). Despite its prognostic value, NLR has not, to date, been integrated

into any of the accepted predictive models.

This study aimed to evaluate the relationship between preoperative NLR and progression-free survival (PFS), overall survival (OS), and the overall rate of response (ORR).

Materials and Methods

All patients included in this retrospective study received treatment and follow-up at the Oncology Department of Elias Emergency University Hospital in Bucharest Romania between January 2013 and January 2023. All patients with high-risk stage II and stage III CRC received 5-FU-based adjuvant chemotherapy, while stage IV patients received 5-FU-based palliative chemotherapy as well as bevacizumab or an anti-EGFR agent based on RAS mutational status and primary tumor side (right colon vs. left colon). None of the stage IV patients included in this study received immuno-therapy.

High-risk stage II colon cancer was defined as MSS/pMMR (microsatellite stable) pT3 pN0 cM0 with the following high-risk factors for recurrence: poorly differentiated/undifferentiated histology; lymphatic/vascular invasion; bowel obstruction; <12 lymph nodes examined; perineural invasion (PNI); localized perforation; close, indeterminate or positive margins; high-tier tumor budding. High-risk stage II colon cancer also included pT4 pN0 cM0 MSS/pMMR CRC tumors. We also mention that all patients with stage IV disease underwent palliative surgery, and no metastasectomies were performed due to extensive disease at diagnosis.

Inclusion criteria consisted of a positive histopathological and immunohistochemical diagnosis of CRC as well as accurate pathological and clinical staging. Pathological staging was done by an experienced pathologist based on American Joint Committee on Cancer (AJCC) TNM Staging Classification for Colon Cancer 8th ed.,2017. The clinical staging consisted of a complete CT or MRI scan of the chest, abdomen, and pelvis as well as a brain MRI in symptomatic patients. We mention that for stage IV patients staging was based on clinical staging done by an experienced radiologist using CT or MRI scans. Preoperative NLR is defined as the NLR obtained before any type of surgery is performed on the patient. The preoperative NLR was calculated from the complete blood counts using a blood sample obtained no more than 3 days before curative or palliative surgery. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. Absolute neutrophil count (ANC) is a parameter calculated using the total number of white blood cells (WBC), percentage of neutrophils, and percentage of band cells. ANC was calculated by multiplying the total number of WBC by the percentage of neutrophils and dividing by 100, while the absolute lymphocyte count was calculated by multiplying the total number of white blood cells against the percentage of white blood cells which are lymphocytes.

Exclusion criteria consisted of any signs or symptoms of infection such as elevated procalcitonin, leukocytosis, fever, malaise, abnormal chest radiography, positive blood culture, urine culture, or pharyngeal exudate culture which could skew our final results. Patients with immunocompromised status or autoimmune pathologies were excluded as well. Patients treated with corticotherapy were also excluded. Patients with other synchronous cancers were also excluded.

Statistical Analysis

Statistical analysis was performed using SPSS software v26.0. We used a receiver operating characteristic (ROC) curve to establish an appropriate cut-off value for NLR. The point on the ROC curve with the highest sensitivity and specificity was chosen as a cut-off point. Survival probabilities were estimated by the Kaplan–Meier method and the log-rank test was performed to see if there was a difference between the variable levels in terms of survival. The Cox proportional hazard model was used to examine whether variables were independently associated with prognosis.

Overall survival was calculated from diagnosis until the time of death. Progression-free survival was calculated from treatment initiation until the first progression, which was certified using RECIST criteria. The overall response rate (ORR) is defined as the proportion of patients who have a partial or complete response to the first line of therapy. We considered partial response, complete response, stationary disease favorable responses, and progressive disease unfavorable. Continuous variables that had a normal distribution were presented as mean and standard deviation (SD) or median and quartiles for those that did not have a normal distribution. The qualitative variables were presented as a number of cases (n) and percentages (%). Results with $p<0.05$ were considered statistically significant.

Results

A total of 195 patients with stage II-IV colorectal cancer were included in this study. The mean preoperative NLR in 195 patients was 3.42 ± 2.27, with a median of 2.56 (range=1 to 11). After ROC curve analysis we established the best cut-off value to be 3 (sensitivity=81.9% and specificity=56.3%) (*Fig. 1*). We dichotomized the patients into high-NLR (≥ 3) and low-NLR (<3) groups to predict prognosis.

Based on the cut-off value we evaluated the correlations between NLR and clinicopathological parameters (*Table 1*), patients in the NLR-high group exhibited significant association with performance status, clinical stage, tumor depth, tumor location (left vs. right), tumor grade, and CEA values at diagnosis.

The mean NLR value increased with the tumor clinical stage with a mean±standard deviation (SD) of 2.05±2.12 in stage II to a value of 3.75±4.45 in stage IV (*Fig. 2*).

Out of the total of 195 patients, 98 (50.3%) progressed during follow-up while 67 (34.34%)

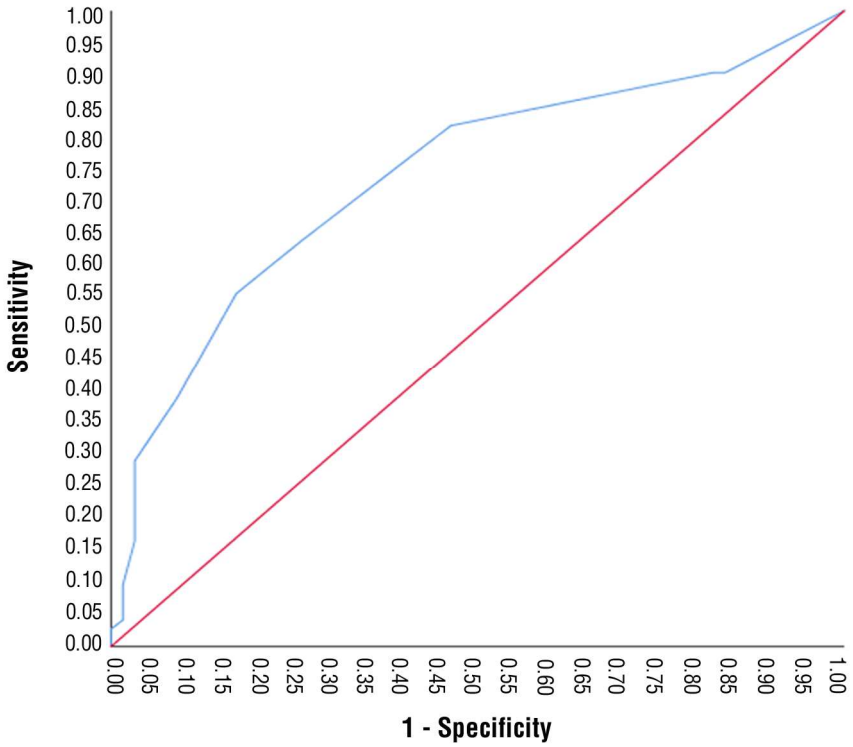


Figure 1. ROC curve analysis of the NLR in patients with colorectal cancer. Area under the curve=0.739, 95%CI 0.555- 0.727, p<0.001

Table 1. Correlations between NLR and clinicopathological characteristics in patients with colorectal cancer

	All patients (n=195)	NLR <3 (n=129)	NLR≥3 (n=66)	p-Value
Age, years, mean (SD)	67.3 (10.7)	66.7 (9.5)	68.2 (12.1)	0.155
Gender, n (%)				0.234
Male	89 (45.6%)	59 (45.73%)	30 (45.45%)	
Female	106 (54.35%)	70 (54.26%)	36 (54.54%)	
ECOG, n (%)				0.003
0	46 (23.58%)	37 (28.68%)	9 (13.63%)	
1	114 (58.46%)	83 (64.34%)	31 (46.96%)	
2	35 (17.94%)	9 (6.97%)	26 (39.39%)	
Chemotherapy, n (%)				0.06
No	22 (24.61%)	19 (62.8%)	3 (5.74%)	
Yes	173 (75.38%)	82 (37.2%)	91 (94.26%)	
TNM Stage, n (%)				<0.001
II	48 (5.38%)	41 (31.78%)	7 (8.04%)	
III	89(40.76%)	52 (40.31%)	37 (44.82%)	
IV	58 (53.84%)	36 (27.90%)	22 (47.12%)	
Tumor depth, n (%)				0.004
T 1-2	76 (39%)	56 (43.41%)	20 (30.30%)	
T 3-4	119 (61%)	73 (56.59%)	46 (69.69%)	
Location (left vs. right colon), n (%)				0.002
Right	76 (38.97%)	46 (35.65%)	30 (45.45%)	
Left	119 (61.03%)	83 (64.34%)	36 (54.54%)	
CEA value, n (%)				<0.001
< 5 ng/ml	106 (83.85%)	85 (65.11%)	21 (93.1%)	
≥ 5 ng/ml	89 (16.15%)	44 (34.88%)	45 (6.90%)	
CA 19-9 value, n (%)				0.105
< 37 U/ml	161 (83.85%)	120 (65.11%)	41 (93.1%)	
≥ 37 U/ml	34 (16.15%)	9 (34.88%)	25 (6.90%)	
Histology grade, n(%)				0.012
G1-G2	112 (57.43%)	89 (69%)	23 (34.84%)	
G3	83 (42.56%)	40 (31%)	43 (65.15%)	

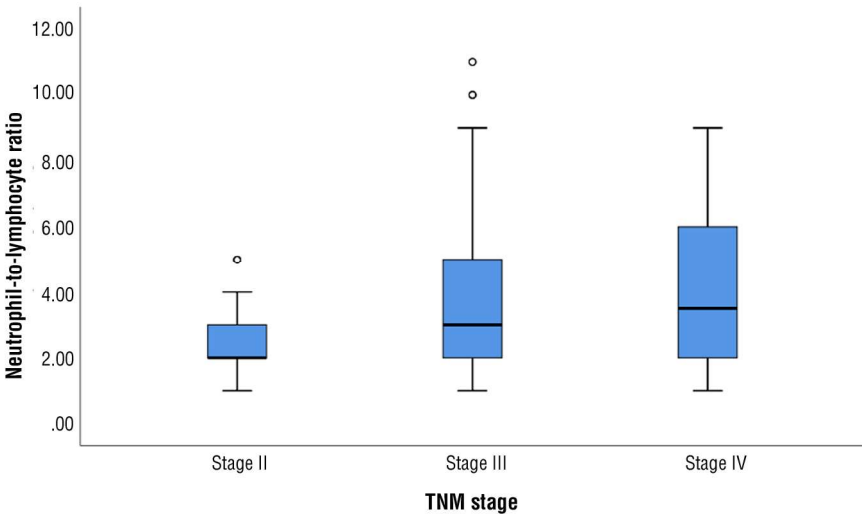


Figure 2. NLR stratified by TNM stage of colorectal cancer. Data is represented as mean ± standard deviation (SD)

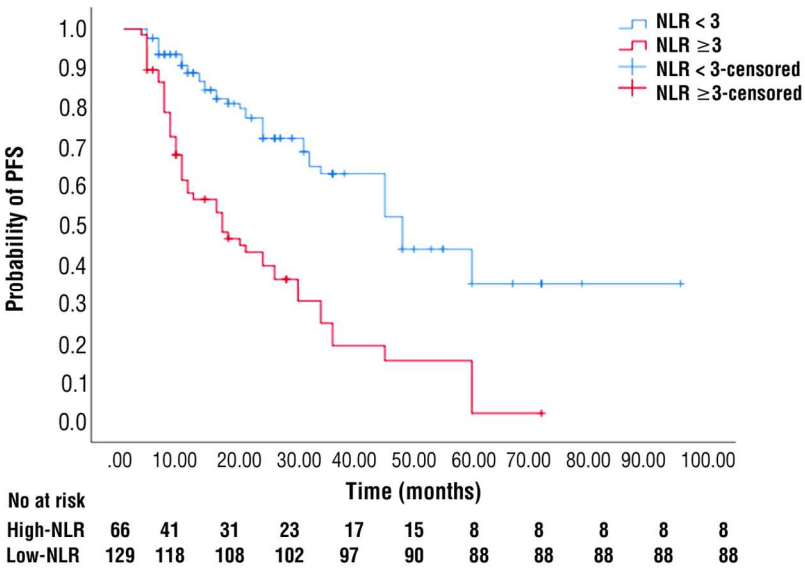


Figure 3. Kaplan-Meier curves comparing PFS for NLR<3 vs. NLR≥3 (p<0.001, HR=3.172, 95%CI 2.121- 4.745)

died by the end of the follow-up. To analyze if a high NLR was correlated with a higher rate of progression and a shorter overall survival rate we performed a Kaplan-Meier analysis and the log-rank test. Patients with high NLR had a significantly higher rate of progression (1 year PFS=56.6%; 3 years PFS= 18.3%; 5 years PFS=1.9%) with a median time until progression of 17 months, while patients with low NLR had a lower rate of progression (1 year PFS=88.8%; 3 years PFS= 63.6%; 5 years PFS=35.1%) with a median time until progression of 48 months (*Fig. 3*).

Similarly, patients with high NLR had a significantly lower survival rate (1 year OS=89.1%; 3 years OS= 66.7%; 5 years OS=34.9%) with a median time of survival of 78 months compared to low-NLR patients which had a higher rate of survival (1 year OS=93.2%; 3 years OS=79.1%; 5 years OS=67.9%) with a median time of survival of 49 months (*Fig. 4*).

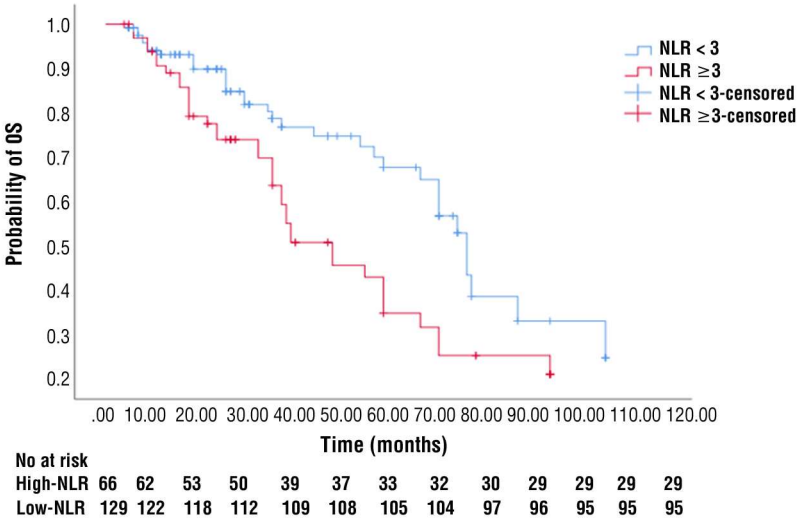


Figure 4. Kaplan-Meier curves comparing OS for NLR<3 vs. NLR≥3 (p=0.001, HR=1.701, 95%CI 1.048- 2.762)

Table 2. Univariate and multivariate Cox regression analyses to identify predictors for increased risk of death in colorectal cancer patients

Dependent variable: Death			Univariate analysis		Multivariate analysis	
	Tested	Reference	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	≥ 65 years	<65 years	1.236 (0.716-2.132)	0.486	1.188 (0.722-2.341)	0.734
Gender	Male	Female	1.854 (1.136- 3.026)	0.013	1.498 (1.068-3.423)	0.103
ECOG	ECOG 2	ECOG 0-1	2.481 (1.672-3.681)	<0.001	2.329 (1.522-3.422)	0.010
Tumor depth	T 3-4	T 1-2	2.231 (1.542-3.887)	0.002	2.045 (1.483-3.734)	0.030
Stage	IV	II-III	2.481 (1.672-3.681)	<0.001	2.190 (1.325-3.541)	<0.001
Location	Right	Left	1.940 (1.120-3.123)	0.028	1.620 (1.045-2.967)	0.088
CEA	≥ 5 ng/ml	< 5 ng/ml	1.935 (1.178-3.180)	0.055	1.475 (1.120-3.076)	0.077
Grade	G3	G1-2	1.562 (0.945-2.326)	0.038	1.326 (0.895-2.127)	0.067
NLR	≥ 3	<3	1.701 (1.048-2.762)	0.001	1.588 (0.975-2.689)	0.003

CI: confidence interval; CEA: carcinoembryonic antigen

We performed COX regression analysis to evaluate the correlation between overall survival and various clinicopathological factors which are shown in *Table 2*. According to univariate analysis, overall survival was significantly correlated with gender, performance status, tumor depth, TNM stage, tumor location, tumor grade, and NLR. However, in a multivariate analysis only performance status, tumor depth, TNM stage, and NLR were independent risk factors for survival. Multivariate analysis results are also displayed as a forest plot in *Fig. 5*.

We also analyzed the correlation between NLR and treatment response. Patients that were not eligible for chemotherapy treatment were excluded from this analysis. A favorable response rate was significantly better in patients with low NLR (p= 0.025), while an unfavorable response rate was higher among high-NLR patients (p=0.013) (*Table III*).

Discussion

This study investigated the usefulness of NLR as a prognostic factor for survival in patients diagnosed with CRC. A correlation between high preoperative NLR and a poor prognosis has been described in literature before (16-19). The NLR's value as a prognostic factor is based on it being a possible surrogate for both the inflammation associated with cancer as well as the immune status of cancer patients (20,21). The poor prognosis assigned to high

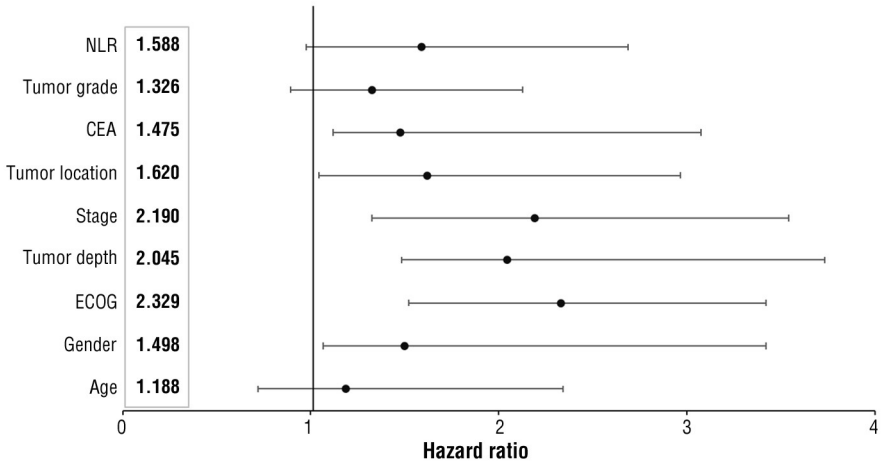


Figure 5. Forest plot of clinicopathological variables associated with OS.

Table 3. Treatment response according to preoperatory NLR

Response type	Patients treated with CHT	NLR		
	n=173	<3	≥ 3	p-value
Favorable response rate (CR+PR+SD), n(%)	130 (75.14%)	100 (76.92%)	30 (23.07%)	0.025
Unfavorable response rate (PD), n(%)	43 (24.85%)	19 (44.18%)	24 (55.81%)	0.013

CR=complete response; PR=partial response, SD=stable disease; PD=progressive disease; CHT=chemotherapy

NLR patients can be partially attributed to the difference in clinical stage, tumor depth, tumor grade, and performance status between high NLR and low NLR patients. High NLR patients usually exhibit more unfavorable clinicopathological characteristics such as higher tumor grade, T stage, clinical stage, and worse performance status at diagnosis (22). A retrospective study by Turhan et al. reports on the correlation between NLR and clinical stage as well as histological differentiation. Patients that exhibit an elevated NLR have a more advanced TNM stage as well as poorer tumor differentiation (23). This is compatible with the data in our study where we observed that high-NLR patients had worse clinicopathological characteristics compared to low-NLR patients. In our study, the majority of stage IV patients were included in the high-NLR cohort accounting for 47.12% of the total number of high-NLR patients. This is in stark contrast to patients with stage II CRC which represent only 8.04% of the total number of high-NLR patients. A retrospective series by Chiang et al. reported that an elevated NLR>3 was associated not only with an increased risk of disease progression but also with complicated presentation (obstruction, perforation, active bleeding or ischemia), larger tumor size, worse T-stage hypoalbuminemia and anemia (24).

Several other studies tried to answer the question of whether high NLR should be considered a risk factor for disease recurrence as well as overall survival after surgery in early-stage CRC. A retrospective study done by Jankova et al on early-stage CRC (stage I to III) patients, reported that preoperative NLR was weakly associated with poorer OS (HR=1.06, 95% CI 1.01-1.11, p = 0.013). No association between NLR and disease recurrence was found (HR=1.04, 95% CI 0.97-1.11, p = 0.241) (25). In another study that included only stage I CRC patients who underwent curative surgery the 5-year DFS (disease-free survival) and CSS (cancer-specific survival) rates were lower in patients with high NLR compared to those with a lower NLR (89.5% vs. 97.4%, p= .006 and 94.0% vs. 98.9%, p= 0.022, respectively). Cox multivariate analysis demonstrated that pre-operative NLR was independently associated both with DFS and CSS (26). The results mentioned in the aforementioned studies are similar to our own, with a 5-year OS rate of 67.9% for patients with low NLR compared to 34.9% in high NLR patients. The 5-year PFS rate follows the same trend with a 5-year PFS of 35.1% for low NLR patients compared to only 1.9% for high NLR patients.

A retrospective study by Kaneko et al. included 50 patients diagnosed with metastatic CRC (mCRC) and treated with 5-FU and oxaliplatin-based chemotherapy. This study evaluated the prognostic value of NLR, with a cut-off value fixed at 4. After univariate analysis, elevated NLR, performance status, and hypoalbuminemia were significantly associated with shorter OS and PFS. A multivariate analysis reported that elevated NLR (HR=4.39, 95% CI 1.82-10.7; p = 0.0013) was independently associated with OS (27). A retrospective study by Formica et al. which included 106 patients with unresectable mCRC that were treated with 5-fluorouracil, irinotecan, and bevacizumab (FOLFIRI and bevacizumab) reported on the prognostic value of baseline NLR. Lower NLR values were associated with a higher median OS time (41 months vs. 12 months, p<0.001) A low NLR value was also

predictive of longer PFS (p= .0002). Paradoxically this study also reported that patients with a stable or increasing NLR value after chemotherapy compared to NLR value at baseline had a more favorable ORR (28). The studies mentioned above suggest that NLR is a prognostic factor for both OS and PFS irrespective of clinical stage, results which are similar to those described in our study.

In our study clinical variables such as age, gender, ECOG, T stage, TNM stage, primary tumor location, CEA (carcinoembryonic antigen) values, and tumor grade were included in the statistical analysis. After adjusting for these variables in multivariate analysis, the NLR remained a statistically significant prognostic factor for OS. The role of NLR as an independent prognostic factor for survival in patients with colorectal cancer was also focused on in studies that compared its value in patients with the same tumor stage further suggesting its independent value as a prognostic factor (29).

After ROC curve analysis the resulting cut-off value for the NLR was 3. Previous studies reported similar values (3.0, 4.0, or 5.0) to ours. When we analyzed the Kaplan-Meier survival curves for both overall and progression-free survival patients with high NLR had a lower survival rate and a higher progression rate. Patients with high NLR also had a lower response rate, suggesting chemotherapy treatment is not as effective in this subgroup of patients, these findings are consistent with those reported in other studies (30).

The clear mechanism by which NLR can produce a prognostic effect is currently unknown. It was previously thought to be a measure of tumor-associated systemic inflammation, with a higher tumor burden being correlated to a higher NLR, currently, NLR is believed to reflect the dynamic balance between the innate and adaptative immune response (31). The antitumoral inflammatory response is distinguished by an increase in circulating neutrophil levels and a decrease in circulating lymphocyte levels. Because a high concentration of neutrophils is known to promote tumor progression and to suppress the antitumor effect of lymphocytes, the neutrophil-lymphocyte ratio (NLR) may be regarded as the balance between pro-tumor inflammatory status and antitumor immune status. Several studies suggested that an imbalance of neutrophils and lymphocytes in peripheral blood is correlated with the imbalance of the same cells in the tumor microenvironment and that this imbalance can be associated with tumor development (32, 33, 34). Interestingly, several studies have revealed that assessing local lymphocyte response, which is defined as a prominent lymphocytic reaction near the invasive boundary, is related to a lower NLR and thus a better prognosis (35). Although the NLR is an efficient inflammation-based biomarker that is associated with survival, other inflammation-based biomarkers such as the GPS/mGPS (Glasgow prognostic score/ modified Glasgow prognostic score) appear to be a better predictor of survival. In a large cohort study (Glasgow Inflammation Outcome Study), mGPS outperformed the NLR. Although an advantage of NLR is that it can distinguish between good and bad prognostic groups in a range of tumor types, including CRC (36). A retrospective study by Inamoto et al. showed that the combination of the NLR and GPS/mGPS into a novel prognostic model for CRC survival was superior to the NLR and GPS/mGPS alone (37). That being said we selected preoperative NLR as the main prognostic factor being analyzed in this study because of its ease of calculation and accessibility. We believe that the usefulness of the NLR as a prognostic factor resides in its accessibility and its reflection of the patient's antitumor immune response.

NLR has also been studied as a dynamic parameter with its normalization after surgery being associated with better OS time. In a retrospective study performed by Yasui et al. on 568 stage I-III CRC patients, patients were stratified by inflammation status: preoperative low NLR (normal group), preoperatively high NLR but normalized after surgery (normalized group), and persistently high NLR (elevated group). A superior OS time was

reported for the normal and normalized group compared to the elevated group (38). This study suggests that preoperative NLR reflects the patient's antitumoral response better than postoperative NLR because the immunomodulating effects of the tumor tissue are still in effect.

Research has clarified the relationship between inflammation and colorectal tumorigenesis (39,40). The components of the NLR (neutrophils and lymphocytes) have protumoral as well as antitumoral roles and their role may explain the mechanism by which the NLR can exhibit a prognostic effect. Tumor-associated neutrophils (TANs) can be distinguished according to their role in tumorigenesis. Circulating neutrophils that reach the tumor microenvironment are subject to the tumor's cytokines which facilitate the "polarization" of these cells into protumor and antitumor TANs. Antitumor neutrophils are classified as N1 while protumor neutrophils as N2. The main cytokine which facilitates the transformation of circulating neutrophils into N2 TANs is TGF-ß. N2 neutrophils play an essential role in tumor proliferation, invasion, and vascularisation through the secretion of various cytokines, therefore an increased number of neutrophils in the tumor microenvironment is conducive to tumoral growth and invasion. Additionally, N2 neutrophils can contribute to tumor proliferation through the inhibition of antitumoral immune response. N2 TANs recruit CD4+ Tregs (regulatory T lymphocytes), suppress CD8+ CTL (cytotoxic T lymphocytes), and cause the apoptosis of NK (natural killer) cells thus creating a perfect environment for tumor growth (41). Another study described tumor cytokine influence on circulatory neutrophils. It focused on TGF-ß which facilitates the transformation from high-density antitumoral neutrophils (HDNs) into low-density protumoral neutrophils (LDNs) (42).

Lymphocytes also play a key role in tumor control and are responsible for the cell-mediated antitumoral immune response. Their main role is to secrete cytokines that inhibit malignant cell growth and invasion and induce cytotoxic cell death. Similarly to neutrophils lymphocytes have protumoral and antitumoral roles, although CD8+ CTL are exclusively antitumoral (43). Tumor cells and N2 TANs facilitate the transformation of antitumoral lymphocytes into protumoral ones through cytokine secretion. Therefore a low number of lymphocytes as well as a protumoral lymphocyte phenotype impairs the host antitumor immune response.

Taking into account the role of both neutrophils and lymphocytes in antitumor immunity as well as in tumorigenesis and the fact that an imbalance of neutrophils and lymphocytes in peripheral blood is correlated with the imbalance of the same cells in the tumor microenvironment we hypothesize that NLR is an accessible biomarker that reflects the efficacy of the patient's antitumoral response. Although the NLR variability is a significant issue in the validation of this biomarkers prognostic value, an extensive review by Zahorec et al. describes the various cut-off ranges for NLR associated with different pathologies. The majority of meta-analyses that have analyzed the prognostic value of the NLR in solid tumors have established the cut-off value to be above 3 (IQR 2-5). Although NLR can be slightly elevated in other chronic diseases such as metabolic syndrome, auto-immune diseases, and psychiatric disorders a thorough selection of patients can exclude most non-malignant etiologies for NLR elevation (44).

Several studies also attempted to show the value of the NLR as a predictive marker for potential outcomes of chemotherapy in patients with colorectal cancer. Elevated NLR appears to be a strong predictor of poor response to chemotherapy in individuals with CRC, regardless of treatment. A retrospective study by Chua et al. evaluated the prognostic role of NLR in a study that included 349 patients with unresectable mCRC who were receiving first-line palliative care chemotherapy (45). Results from this study are confirmed by our results regarding ORR as well as similar studies present in the literature (46). Patients with a NLR <3 had a favorable ORR of 76.92%

compared to patients with a NLR ≥ 3 which had a favorable ORR of only 23.07%.

Due to its retrospective nature and single-institutional design, this study has some limitations, including the possibility of bias in the patient selection process and potential record-keeping errors. The findings of this study do however imply that preoperative NLR is an easy-to-perform assay that seems to have prognostic value in the survival of patients with CRC. In addition to TNM staging, this index may aid in determining the course of treatment for individuals with colorectal cancer.

Conclusions

In conclusion, our study suggests that NLR is an independent prognostic factor for both overall survival and progression-free survival with elevated values being associated with a worse prognosis. We also hypothesize that the NLR has predictive value, with patients with high NLR exhibiting lower rates of response to chemotherapy compared to low NLR patients.

Author's Contributions

Conceptualization, C.I.I, C.N.; Methodology, H.-T.C.,C.N.; Formal analysis, C.I.I, H.-T.C.; Investigation, H.-T.C, R.A.E.; Writing-original draft, C.I.I.; Writing-review and editing, C.N.; Supervision: C.N, H.-T.C. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Elias Emergency University Clinic Hospital (no 8125/20 April 2023).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement

Data supporting this study are included within the article and supporting materials.

References

1. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, et al. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN. Gut. 2023;72(2):338-344.
2. Chan GHJ, Chee CE. Making sense of adjuvant chemotherapy in colorectal cancer. J Gastrointest Oncol. 2019;10(6):1183-1192.
3. Galon J, Lanzi A. Immunoscore and its introduction in clinical practice. Q J Nucl Med Mol Imaging. 2020;64(2):152-161.
4. Felton J, Raufman JP. Is the ColDx assay a valid prognostic marker for stage II colon cancer? Transl Cancer Res 2016;5(Suppl 6):S1157-S1159.
5. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539-45.
6. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917): 860-7.
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
8. Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS, Pujari VB. Inflammation and cancer. Ann Afr Med. 2019;18(3):121-126.
9. Kim ER, Chang DK. Colorectal cancer in inflammatory bowel disease: the risk, pathogenesis, prevention and diagnosis. World J Gastroenterol. 2014; 20(29):\9872-81.
10. McMillan DC, Crozier JE, Canna K, Angerson WJ, McArdle CS. Evaluation of an inflammation-based prognostic score (GPS) in patients undergoing resection for colon and rectal cancer. Int J Colorectal Dis. 2007;22(8): 881-6.
11. Roxburgh CSD, Salmond JM, Horgan PG, Oien KA, McMillan DC. Comparison of the prognostic value of inflammation-based pathologic and biochemical criteria in patients undergoing potentially curative resection for colorectal cancer. Ann Surg. 2009;249(5):788-93.
12. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol. 2005; 91(3):181-4.
13. Absenger G, Szkandera J, Pichler M, Stotz M, Arminger F, Weissmueller M, et al. A derived neutrophil to lymphocyte ratio predicts clinical outcome in stage II and III colon cancer patients. Br J Cancer. 2013;109(2):395-400.
14. Galizia G, Lieto E, Zamboli A, De Vita F, Castellano P, Romano C, et al. Neutrophil to lymphocyte ratio is a strong predictor of tumor recurrence in early colon cancers: A propensity score-matched analysis. Surgery. 2015; 158(1):112-20.
15. Chua W, Charles KA, Baracos VE, Clarke SJ. Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer. Br J Cancer. 2011;104(8):1288-95.
16. Garcea G, Ladwa N, Neal CP, Metcalfe MS, Dennison AR, Berry DP. Preoperative neutrophil-to-lymphocyte ratio (NLR) is associated with

reduced disease-free survival following curative resection of pancreatic adenocarcinoma. World J Surg. 2011;35(4):868-72.
17. Sarraf KM, Belcher E, Raevsky E, Nicholson AG, Goldstraw P, Lim E. Neutrophil/lymphocyte ratio and its association with survival after completeresection in non-small cell lung cancer. J Thorac Cardiovasc Surg. 2009;137(2):425-8.
18. Yamanaka T, Matsumoto S, Teramukai S, Ishiwata R, Nagai Y, Fukushima M. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. Oncology. 2007;73(3-4):215-20.
19. Shibutani M, Maeda K, Nagahara H, Noda E, Ohtani H, Nishiguchi Y, et al. A high preoperative neutrophil-to-lymphocyte ratio is associated with poor survival in patients with colorectal cancer. Anticancer Res. 2013;33(8): 3291-4.
20. Kishi Y, Kopetz S, Chun YS, Palavecino M, Abdalla EK, Vauthey JN. Blood neutrophil-to-lymphocyte ratio predicts survival in patients with colorectal liver metastases treated with systemic chemotherapy. Ann Surg Oncol. 2009;16(3):614-22.
21. Kubo T, Ono S, Ueno H, Shinto E, Yamamoto J, Hase K. Impact of the perioperative neutrophil-to-lymphocyte ratio on the long-term survival following an elective resection of colorectal carcinoma. Int J Colorectal Dis. 2014;29(9):1091-9.
22. Mazaki J, Katsumata K, Kasahara K, Tago T, Wada T, Kuwabara H, et al. Neutrophil-to-lymphocyte ratio is a prognostic factor for colon cancer: a propensity score analysis. BMC Cancer. 2020;20(1):922.
23. Turhan VB, Ünsal A, Gök HF, Öztürk B, Öztürk D, Simsek GG, et al. Predictive Value of Preoperative Neutrophil-Lymphocyte and Platelet-Lymphocyte Ratio in Determining the Stage of Colon Tumors. Cureus. 2021;13(9): e18381.
24. Chiang SF, Hung HY, Tang R, Changchien CR, Chen JS, You YT, et al. Can neutrophil-to-lymphocyte ratio predict the survival of colorectal cancer patients who have received curative surgery electively? Int J Colorectal Dis. 2012;27(10):1347-1357
25. Jankova L, Dent OF, Chan C, Chapuis P, Clarke SJ. Preoperative neutrophil/lymphocyte ratio predicts overall survival but does not predict recurrence or cancer-specific survival after curative resection of node-positive colorectal cancer. BMC Cancer. 2013;13:442
26. Shin JS, Suh KW, Oh SY. Preoperative neutrophil to lymphocyte ratio predicts survival in patients with T1-2N0 colorectal cancer. J Surg Oncol. 2015;112(6):654-657.
27. Kaneko M, Nozawa H, Sasaki K et al. Elevated neutrophil to lymphocyte ratio predicts poor prognosis in advanced colorectal cancer patients receiving oxaliplatin-based chemotherapy. Oncology. 2012;82(5):261-268.
28. Formica V, Lucchetti J, Cunningham D, Smyth EC, Ferroni P, Nardecchia A, et al Systemic inflammation, as measured by the neutrophil/lymphocyte ratio, may have differential prognostic impact before and during treatment with fluorouracil, irinotecan and bevacizumab in metastatic colorectal cancer patients. Med Oncol. 2014;31(9):166.
29. Hung HY, Chen JS, Yeh CY, Changchien CR, Tang R, Hsieh PS, et al Effect of preoperative neutrophil-lymphocyte ratio on the surgical outcomes of stage II colon cancer patients who did not receive adjuvant chemotherapy. Int J Colorectal Dis. 2011;26(8):1059-65.
30. Wu Y, Li C, Zhao J, Yang L, Liu F, Zheng H, Wang Z, Xu Y. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios predict chemotherapy outcomes and prognosis in patients with colorectal cancer and synchronous liver metastasis. World J Surg Oncol. 2016;14(1):289.
31. Buonacera A, Stancanelli B, Colaci M, Malatino L. Neutrophil to Lymphocyte Ratio: An Emerging Marker of the Relationships between the Immune System and Diseases. Int J Mol Sci. 2022;23(7):3636.
32. Proctor MJ, McMillan DC, Morrison DS, Fletcher CD, Horgan PG, Clarke SJ. A derived neutrophil to lymphocyte ratio predicts survival in patients with cancer. Br J Cancer. 2012;107(4):695-699.
33. Zahorec R. Ratio of neutrophil to lymphocyte counts e rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy 2001;102(1):5e14
34. Pine JK, Morris E, Hutchins GG, West NP, Jayne DG, Quirke P, et al. Systemic neutrophil-to-lymphocyte ratio in colorectal cancer: the relationship to patient survival, tumour biology and local lymphocytic response to tumour. Br J Cancer. 2015;113(2):204-11.
35. Pine JK, Morris E, Hutchins GG, West NP, Jayne DG, Quirke P, et al. Systemic neutrophil-to-lymphocyte ratio in colorectal cancer: the relationship to patient survival, tumour biology and local lymphocytic response to tumour. Br J Cancer. 2015;113(2):204-11.
36. Proctor MJ, Morrison DS, Talwar D, Balmer SM, Fletcher CD, O'Reilly D, et al. A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. European Journal of Cancer 2011;47(17):2633–2641
37. Terzic J, Grivennikov S, Karin E and Karin M: Inflammation and colon cancer. Gastroenterology 138: 2101-2114, 2010.
38. Inamoto S, Kawada K, Okamura R, Hida K, Sakai Y. Prognostic impact of the combination of neutrophil-to-lymphocyte ratio and Glasgow prognostic score in colorectal cancer: a retrospective cohort study. Int J Colorectal Dis. 2019;34(7):1303-1315.
39. Yasui K, Shida D, Nakamura Y, Ahiko Y, Tsukamoto S, Kanemitsu Y. Postoperative, but not preoperative, inflammationbased prognostic markers are prognostic factors in stage III colorectal cancer patients. Br J Cancer. 2021;124(5):933-941.
40. Kraus S, Arber N. Inflammation and colorectal cancer. Curr Opin Pharmacol. 2009;9(4):405-10.
41. Masucci MT, Minopoli M, Carriero MV. Tumor Associated Neutrophils. Their Role in Tumorigenesis, Metastasis, Prognosis and Therapy. Front Oncol. 2019;9:1146.
42. Sagiv JY, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Rep. 2015;10(4):562-73.
43. Chraa D, Naim A, Olive D, Badou A. T lymphocyte subsets in cancer immunity: Friends or foes. J Leukoc Biol. 2019;105(2):243-255.
44. Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy. 2021;122(7):474-488.
45. Chua W, Charles KA, Baracos VE, Clarke SJ. Neutrophil/lymphocyte ratio predicts chemotherapy outcomes in patients with advanced colorectal cancer. Br J Cancer. 2011;104(8):1288-95.
46. Dell'Aquila E, Cremolini C, Zeppola T, Lonardi S, Bergamo F, Masi G, Stellato M, et al. Prognostic and predictive role of neutrophil/lymphocytes ratio in metastatic colorectal cancer: a retrospective analysis of the TRIBE study by GONO. Ann Oncol. 2018;29(4):924-930.