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Prognostic Value of Neutrophil-to-Lymphocyte Ratio in Therapy Selection for High-Risk Stage II Colon Cancer

Horia Teodor Cotan*, Cristina Maria Orlov-Slavu, Alexandra Lazar, Cornelia Nitipir

Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
Department of Oncology, Prof. Dr. Agrippa Ionescu Emergency Clinical Hospital, Bucharest, Romania

***Corresponding author:**
Horia Teodor Cotan, MD
Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
E-mail: horia-teodor.cotan@rez.umfcd.ro

Abbreviations:
MSS: microsatellite stable;
NLR: neutrophil-to-lymphocyte ratio;
DFS: disease-free survival;
ICD: immunogenic cell death;
CC: Colon cancer;
MSI-H: microsatellite instability-high;
MMR: mismatch repair;
DFS: disease-free survival;
OS: overall survival;
G-CSF: granulocyte colony-stimulating factor;
GM-CSF: granulocyte–macrophage colony-stimulating factor;
IL-6: interleukin-6;
VEGF: vascular endothelial growth factor.

Rezumat

Valoarea prognostică a raportului neutrofile/limfocite în alegerea terapiei pentru cancerul de colon în stadiul II cu risc crescut

Context/Obiective: Beneficiul chimioterapiei adjuvante în cancerul de colon stadiul II cu risc crescut şi stabilitate microsatelitară (MSS) rămâne controversat. Ghidurile actuale se bazează în principal pe factori de risc histopatologici, însă markerii inflamației sistemice, precum raportul neutrofile/limfocite (NLR), ar putea oferi informații prognostice suplimentare. Studiul şi-a propus să evalueze rolul NLR preoperator în predicția supraviețuirii fără boală (DFS) şi în ghidarea alegerii între regimurile cu oxaliplatină şi monoterapia cu fluoropirimidine.
Metode: Am analizat retrospectiv 57 de pacienți cu cancer de colon stadiul II MSS cu risc crescut, operați cu intenție curativă între 2013 şi 2023. Pacienții au fost stratificați în funcție de NLR preoperator (<3 vs. ≥3) şi au primit fie monoterapie cu fluoropirimidine, fie un regim pe bază de oxaliplatină (FOLFOX sau CAPEOX). Riscul de recidivă şi rezultatele terapeutice au fost evaluate prin regresie Cox multivariată şi analiză de supraviețuire Kaplan–Meier.
Rezultate: Un NLR ≥ 3 s-a asociat independent cu un risc crescut de recidivă (HR: 3,22; CI 95%: 1,97–4,57; p=0,001). La pacienții cu NLR crescut, cei tratați cu regimuri pe bază de oxaliplatină au avut o DFS mediană mai lungă decât cei tratați cu monoterapie (81 vs. 67 de luni). În schimb, pacienții cu NLR < 3 au avut rezultate excelente, indiferent de tratamentul ales. Nu au fost raportate toxicități de grad 4.
Concluzii: NLR preoperator este un marker prognostic puternic şi independent în cancerul de colon stadiul II MSS cu risc crescut. Pacienții cu NLR crescut par să beneficieze mai mult de chimioterapia adjuvantă cu oxaliplatină, posibil datorită efectelor imunogene ale acesteia. NLR ar putea reprezenta un instrument simplu şi cost-eficient pentru rafinarea deciziilor privind terapia adjuvantă la această categorie dificilă de pacienți.

Cuvinte cheie: raport neutrofile/limfocite, cancer de colon stadiul II, stabilitate microsatelitară, chimioterapie adjuvantă, oxaliplatină, moarte celulară imunogenă, biomarker prognostic, inflamație sistemică, CAPEOX, FOLFOX

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Abstract

Background/Objectives: The benefit of adjuvant chemotherapy in high-risk, microsatellite stable (MSS) stage II colon cancer remains controversial. Current guidelines rely primarily on histopathologic risk features, but systemic inflammatory markers such as the neutrophil-to-lymphocyte ratio (NLR) may provide additional prognostic insight. This study aimed to evaluate the role of preoperative NLR in predicting disease-free survival (DFS) and guiding treatment selection between oxaliplatin-containing and fluoropyrimidine monotherapy regimens.

Methods: We retrospectively analyzed 57 patients with high-risk stage II MSS colon cancer who underwent curative resection between 2013 and 2023. Patients were stratified by preoperative NLR (<3 vs. ≥3) and received either fluoropyrimidine monotherapy or an oxaliplatin-based regimen (FOLFOX or CAPEOX). Multivariate Cox regression and Kaplan–Meier survival analyses were performed to assess recurrence risk and treatment outcomes.

Results: An NLR ≥ 3 was independently associated with increased recurrence risk (HR: 3.22, 95% CI: 1.97–4.57, p=0.001). Among patients with high NLR, those treated with oxaliplatin-based regimens had a longer median DFS (81 vs. 67 months) compared to monotherapy. In contrast, NLR <3 patients showed excellent outcomes regardless of treatment choice. No grade 4 toxicities were reported.

Conclusions: Preoperative NLR is a strong and independent prognostic marker in high-risk stage II MSS colon cancer. Patients with high NLR appear to benefit more from oxaliplatin-containing adjuvant chemotherapy, potentially due to its immunogenic effects. NLR may serve as a simple, cost-effective tool for refining adjuvant therapy decisions in this challenging patient population.

Keywords: neutrophil-to-lymphocyte ratio, stage II colon cancer, microsatellite stable, adjuvant chemotherapy, oxaliplatin, immunogenic cell death, prognostic biomarker, systemic inflammation, CAPEOX, FOLFOX

Introduction

Colon cancer (CC) ranks as the fourth most common and the third deadliest cancer worldwide, with an annual incidence of 1,142,286 cases and a yearly mortality of 538,167 cases as of 2022 (1). Over one-third of colon cancer cases are diagnosed at a localized stage (stage I-II) (2). Stage II colon cancer is biologically and clinically heterogeneous. Tumors exhibiting microsatellite instability-high (MSI-H) in stage II are associated with significantly lower recurrence rates compared to MSS tumors (3). Notably, MSI-H tumors are more frequently found in stage II than in stage III disease (22% vs. 12%) (4), and their prevalence drops further to just 3.5% in stage IV cases (5). These findings suggest that MSI-H (or dMMR) tumors have a reduced potential for metastasis. Indeed, considerable evidence supports that, in stage II patients, deficiency in mismatch repair (MMR) protein expression or MSI-H status serves as a favorable prognostic marker (6,7).

The majority of published data also suggest that MMR status is an important predictor of benefit from fluoropyrimidine-based adjuvant chemotherapy (8). Most studies support the view that dMMR tumors are resistant to fluoropyrimidine chemotherapy but retain the benefit of oxaliplatin therapy among high risk tumors (i.e pT4 primary) (9,10).

Given the heterogeneity of stage II colon cancer, adjuvant chemotherapy is not routinely recommended for all patients. Clinical trials investigating adjuvant therapy in resected stage II disease have shown, at most, a modest improvement in disease-free survival (DFS) and a minimal overall survival (OS) benefit-generally no more than 5% at five years (11). Among patients with MSS stage II colon cancer, the use of adjuvant therapy is guided by the presence of specific pathological and clinical risk factors. While definitions of "high-risk" stage II disease differ slightly among guidelines from ASCO (12), the National Comprehensive Cancer Network (NCCN) (13), and the European Society for Medical Oncology (ESMO) (14), they consistently include factors such as T4 primary tumors, inadequate lymph node sampling (<12 nodes), poor or undifferentiated tumor grade, perforation, clinical obstruction, lymphovascular invasion (LVI), and perineural invasion (PNI) (*Table 1*).

The extent of risk associated with these factors in stage II disease especially the independent impact of obstruction and perforation remains difficult to accurately quantify based on existing literature (15,16).

The link between inflammation and carcinogenesis is well established (17,18). Inflammatory cytokines involved in cancer development stimulate the bone marrow to produce more neutrophils while suppressing

Table 1. High risk factors in stage II colon cancer

Risk Factor	ASCO	NCCN	ESMO
T4 primary tumor	+	+	+ (major)
Inadequately sampled nodes (<12)	+	+	+ (major)
Poorly differentiated or undifferentiated tumor	+	+	+ (minor)
Perforation	+	+	+ (major)
Clinical obstruction	+	+	+ (minor)
Lymphovascular invasion (LVI)	+	+	+ (minor)
Perineural invasion (PNI)	+	+	+ (minor)
Close/indeterminate or positive margins		+	
High preoperative serum CEA levels			+ (minor)
High tumor budding score (BD3, ≥10 buds)	+		

Abbreviations: ASCO: American Society of Clinical Oncology; NCCN: National Comprehensive Cancer Network; ESMO: European Society for Medical Oncology; LVI: Lymphovascular invasion; PNI: Perineural invasion; CEA: Carcinoembryonic antigen

circulating cytotoxic lymphocytes, resulting in an elevated NLR (19), a recognized marker of systemic inflammation in colorectal cancer. However, the potential role of NLR in guiding decisions about post-operative adjuvant chemotherapy for patients with stage II colorectal cancer has been rarely explored.

Inflammatory cytokines stimulate neutrophil proliferation in cancer through coordinated signaling networks that reprogram hematopoiesis and reshape systemic immunity. Tumor cells, cancer-associated fibroblasts, and infiltrating immune cells produce cytokines such as granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), interleukin-1ß, tumor necrosis factor-α, and chemokines including CXCL8. These mediators activate inflammatory transcriptional pathways - most prominently NF-κB and JAK/STAT signaling - which sustain chronic myeloid expansion and tumor progression (19).

At the bone marrow level, inflammatory cytokines induce emergency granulopoiesis, a stress-adapted hematopoietic program that accelerates neutrophil production. G-CSF signaling is particularly important in this context, as activation of the G-CSF receptor triggers STAT3-dependent transcriptional programs that regulate progenitor proliferation and differentiation (20).

Inflammatory cytokines further regulate the egress of neutrophils from the bone marrow and their recruitment to tumor sites. G-CSF-induced STAT3 signaling alters chemokine gradients and adhesion molecule expression, facilitating neutrophil mobilization (21).

Chemokines such as CXCL8 (IL-8) play a central role in neutrophil chemotaxis and have also been implicated in tumor proliferation and metastasis. Additionally, IL-17 promotes neutrophil expansion indirectly by inducing IL-6 and G-CSF production, thereby amplifying inflammatory circuits within the tumor microenvironment (22).

Functionally, cytokine-expanded neutrophils acquire immunosuppressive and tumor-supportive properties. These cells release reactive oxygen species, arginase-1, and pro-angiogenic factors such as vascular endothelial growth factor (VEGF), inhibiting T-cell proliferation and facilitating tumor progression. Tumor-associated neutrophils may also express immune checkpoint molecules such as PD-L1 under IL-6/STAT3 regulation, further promoting immune evasion (23).

Taken together, inflammatory cytokines stimulate neutrophil proliferation in cancer through integrated mechanisms involving accelerated granulopoiesis, altered marrow trafficking, and functional polarization toward immunosuppressive phenotypes. Experimental and clinical data consistently demonstrate that cytokine-driven neutrophilia is an active, tumor-orchestrated process that supports malignant progression and represents a potential therapeutic target

The primary aim of this study is to determine whether NLR can serve as a biomarker for guiding the selection of optimal adjuvant therapy and for informing decisions on therapy escalation or deescalation in MSS stage II colorectal cancer.

Materials and Methods

This study involved patients diagnosed with MSS stage II colon cancer who underwent surgical resection between 2013 and 2023 (*Fig. 1*). Following pathological evaluation, only those meeting high-risk criteria based on NCCN guidelines were included. Data collected included demographic details (age, sex), preoperative laboratory results, TNM staging, treatment strategies, as well as outcomes such as DFS and treatment-related toxicities. DFS was defined as the time interval during which no clinical signs of cancer recurrence were observed. All patients received fluoropyrimidine-based adjuvant chemotherapy, either 5-FU/capecitabine for 6 months or an oxaliplatin-containing regimen (CAPEOX for 3 months or FOLFOX for 3–6 months). Based on their pretreatment NLR, patients were divided into two groups: low NLR and high NLR. Within each NLR group, patients were further stratified into two treatment arms: Arm A, receiving a combination of oxaliplatin and a fluoropyrimidine (FOLFOX or CAPOX), and Arm B, receiving capecitabine monotherapy. The choice of chemotherapy regimen was made at the physician's discretion.

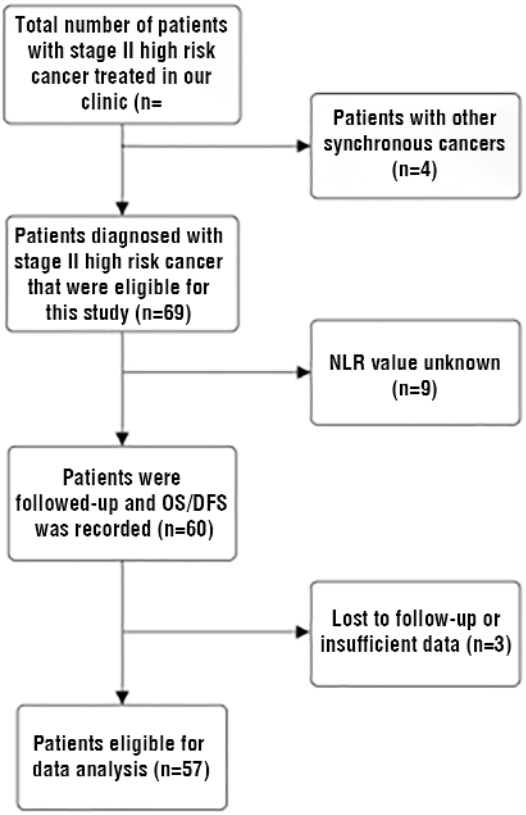

Figure 1. Study flow diagram

Inclusion Criteria

Inclusion criteria consisted of: histopathological and immunohistochemical confirmation of colon cancer (CRC), with accurate pathological and clinical staging. Pathological staging followed AJCC 8th edition (2017), and clinical staging included CT or MRI of the chest, abdomen, pelvis, and brain MRI if symptomatic. Preoperative NLR was calculated from complete blood count within 3 days before surgery as the ratio of absolute neutrophil count to absolute lymphocyte count. To reduce potential confounding related to systemic inflammatory conditions, patients with clinical or laboratory evidence of acute infection at the time of preoperative evaluation (e.g., fever, leukocytosis, or positive microbiological cultures) were excluded. Considering that the study period (2013-2023) overlapped with the COVID-19 pandemic, available medical records were reviewed for documented SARS-CoV-2 infection prior to surgery or during adjuvant chemotherapy. Patients with confirmed active infection at the time of neutrophil-to-lymphocyte ratio (NLR) determination were not included. However, due to the retrospective design and lack of systematic screening during earlier pandemic phases, asymptomatic or undocumented infections could not be completely excluded.

Comorbidities potentially influencing baseline inflammatory status, including diabetes mellitus, cardiovascular disease, chronic pulmonary disease, renal impairment, and other chronic conditions, were recorded when available. Overall comorbidity burden was retrospectively assessed using the Charlson Comorbidity Index (CCI). Exploratory analyses were performed to evaluate the potential impact of comorbidity burden on inflammatory biomarkers and oncologic outcomes by stratifying patients according to CCI score (≤ 3 vs >3).

Exclusion Criteria

Exclusion criteria consisted of: signs of infection (e.g., fever, leukocytosis, positive cultures), immunocompromised or autoimmune conditions, corticosteroid use, or presence of other synchronous cancers. Also taking into account that the study period from 2013 to 2023 also encompasses the COVID-19 pandemic, we excluded all patients with SARS-CoV-2 infection. Patients were screened for SARS-CoV-2 infection using RT-PCR testing.

Surgical procedures were selected according to tumor location and included transverse colectomy, extended right or left hemicolectomy, sigmoid resection, and anterior proctocolectomy, all with appropriate end-to-end anastomosis. All patients underwent postoperative abdominal or pelvic drainage. Surgeries were performed by experienced general surgeons with over five years of expertise in colorectal oncology, ensuring adherence to standard surgical protocols. No surgeries were performed in an emergency setting. The severity of postoperative complications was classified according to the Clavien-Dindo classification, providing a standardized assessment of their clinical impact.

Statistical Analysis

Statistical analyses were performed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). The optimal cut-off value for the neutrophil-to-lymphocyte ratio (NLR) was determined using receiver operating characteristic (ROC) curve analysis, with the threshold selected based on the maximum Youden index (sensitivity + specificity − 1).

The distribution of continuous variables was assessed for normality using the Shapiro–Wilk test. Normally distributed variables were presented as mean ± standard deviation, whereas non-normally

distributed variables were reported as median with interquartile range. Comparisons between groups were performed using the Student's t-test or Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate.

Survival outcomes were estimated using the Kaplan-Meier method and compared using the log-rank test. Multivariable analyses were conducted using Cox proportional hazards regression models to identify independent predictors of recurrence. Clinically relevant variables were included in the models, including pathological T stage, resection margin status, type of surgical intervention (elective versus emergency), and, where data completeness permitted, comorbidity burden as assessed by the Charlson Comorbidity Index. Results were expressed as hazard ratios (HRs) with corresponding 95% confidence intervals (CIs).

All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant.

Results

The study cohort consisted of 57 patients with high-risk stage II colon cancer. Of these, 29.8% were female (n = 17) and 70.2% were male (n = 40). In terms of tumor stage, 57.9% had T3 tumors (n = 33) and 42.1% had T4 tumors (n = 24).

Lymphovascular invasion (LVI) was present in 61.4% of patients (n = 35), while perineural invasion (PNI) was also observed in 61.4% (n = 35). Tumor budding was distributed as follows: Tb0 in 21.1% (n = 12), Tb1 in 29.8% (n = 17), Tb2 in 36.8% (n = 21), and Tb3 in 12.3% (n = 7).

Clear (R0) resection margins were achieved in 84.2% of patients (n = 48), whereas 15.8% (n = 9) had microscopic residual disease (R1). Tumor-related occlusion or perforation was observed in 14% of cases (n = 8). Specifically, seven patients presented with occlusion—five in the ascending colon and two in the descending colon—while one patient had perforation. Notably, all patients with R1 resection and tumor-related occlusion or perforation were classified as T4 stage.

Histologic grading showed that 14% (n = 8) of tumors were well-differentiated (G1), 63.2% (n = 36) moderately differentiated (G2), and 22.8% (n = 13) poorly differentiated (G3). Adequate lymph node sampling (>12 nodes) was achieved in 77.2% (n = 44), while 22.8% (n = 13) had fewer than 12 nodes examined.

Preoperative carcinoembryonic antigen (CEA) levels were elevated (>ULN) in 50.9% of patients (n = 29), while 49.1% (n = 28) had normal levels. In terms of combined high-risk features, 56.1% of patients (n = 32) had only one risk factor, while 43.9% (n = 25) had two or more.

Tumor location was evenly distributed, with 49.1% (n = 28) located in the left colon and 50.9% (n = 29) in the right colon. As for adjuvant chemotherapy regimens, 49.1% (n = 28) received CAPEOX or FOLFOX, and 50.9% (n = 29) received fluoropyrimidine monotherapy (5-FU or capecitabine).

Among the 27 patients for whom toxicity data were available (47.4%), the most frequently reported adverse events were neuropathy (22.2%, n = 6), hand-foot syndrome (14.8%, n = 4), neutropenia (14.8%, n = 4), emesis (14.8%, n = 4), and nausea (11.1%, n = 3). Less common events included diarrhea (7.4%, n = 2), anemia (11.1%, n = 3), and allergic reactions (3.7%, n = 1). According to CTCAE grading, most toxicities were grade 1 (40.7%, n = 11) or grade 2 (51.9%, n = 14), while only two patients (7.4%) experienced grade 3 toxicity. No grade 4 or life-threatening events were recorded.

The study cohort showed a relatively low comorbidity burden, with the majority of patients having a Charlson Comorbidity Index (CCI) score ≤ 3, accounting for 43 patients (75.4%), while 14 patients (24.5%) had a CCI score >3.

Regarding postoperative outcomes, according to the Clavien-Dindo classification, most patients experienced no or only minor complications. Specifically, 32 patients (56%) had no postoperative complications (Grade 0), 17 patients (30%) developed Grade I complications, and 8 patients (14%) had Grade II complications. No severe complications (Grade III–V) were observed in this cohort, indicating an overall favorable postoperative course. This data is shown in *Table 2*.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive performance of the neutrophil-to-lymphocyte ratio (NLR) for tumor recurrence. The area under the curve (AUC) was 0.874, indicating a high level of discriminative ability. This suggests that NLR is a strong predictor of recurrence in high-risk stage II colon cancer patients. It is noted that at least one tie was present between the positive and negative recurrence groups, which may introduce slight bias in the statistical estimation; however, the overall result remains robust.

Analysis of the ROC curve coordinates showed that the optimal cutoff was approximately 3, which provided a sensitivity of 90% and a 1-specificity of 25%. The corresponding Youden index was 0.65 (*Fig. 2*).

An analysis of survival time stratified by NLR levels and treatment regimens revealed notable differences in DFS. For patients with NLR < 3, the

Table 2. Clincal characteristics of patients

Variable	Category	Frequency (n, %)
Age (median)	<70	31 (54.4%)
	≥ 70	26 (45.6%)
Sex, n (%)	Female	17 (29.8%)
	Male	40 (70.2)
Tumor Stage, n (%)	T3	33 (57.9)
	T4	24 (42.1)
Lymphovascular Invasion (LVI), n (%)	Present	35 (61.4)
	Absent	22 (38.6)
Perineural Invasion (PNI), n (%)	Present	35 (61.4)
	Absent	22 (38.6)
Tumor Budding, n (%)	Tb0	12 (21.1)
	Tb1	17 (29.8)
	Tb2	21 (36.8)
	Tb3	7 (12.3)
Surgical Margins, n (%)	R0	48 (84.2)
	R1	9 (15.8)
Occlusion/Perforation, n (%)	Yes	8 (14.0)
	No	49 (86.0)
Tumor Grade, n (%)	G1	8 (14.0)
	G2	36 (63.2)
	G3	13 (22.8)
Lymph Nodes Examined, n (%)	< 12 nodes	13 (22.8)
	≥ 12 nodes	44 (77.2)
CEA Level, n (%)	< ULN	28 (49.1)
	> ULN	29 (50.9)
Number of Risk Factors, n (%)	One	32 (56.1)
	Two or more	25 (43.9)
Tumor Location, n (%)	Left colon	28 (49.1)
	Right colon	29 (50.9)
Treatment Regimen, n (%)	CAPEOX/FOLFOX	28 (49.1)
	5-FU/Capecitabine	29 (50.9)
Toxicity Type, n (%)	Diarrhea	2 (7.4)
	Emesis	4 (14.8)
	Hand-foot syndrome	4 (14.8)
	Neutropenia	4 (14.8)
	Neuropathy	6 (22.2)
	Allergic reaction	1 (3.7)
	Anemia	3 (11.1)
	Nausea	3 (11.1)
CTCAE Grade, n (%)	Grade 1	11 (40.7)
	Grade 2	14 (51.9)
	Grade 3	2 (7.4)
NLR Group, n (%)	< 3	29 (50.9)
	≥ 3	28 (49.1)
Charlson Comorbidity Index (CCI), n (%)	≥ 3	43 (75.4)
	>3	14 (24.5)
Clavien-Dindo Grade, n (%)	0	32 (56)
	I	17 (30)
	II	8 (14)

Abbreviations: CEA: carcinoembryonic antigen; ULN: upper limit of normal; LVI: lymphovascular invasion; PNI: perineural invasion; Tb: tumor budding; R0/R1: resection margin status (R0 indicates negative/clear margins, R1 indicates positive/microscopic residual disease); G1/G2/G3: tumor grade (well, moderately, and poorly differentiated, respectively); CAPEOX: capecitabine and oxaliplatin; FOLFOX: 5-fluorouracil, leucovorin, and oxaliplatin; 5-FU: 5-fluorouracil; CTCAE: Common Terminology Criteria for Adverse Events; NLR: neutrophil-to-lymphocyte ratio; CCI: Charlson Comorbidity Index.

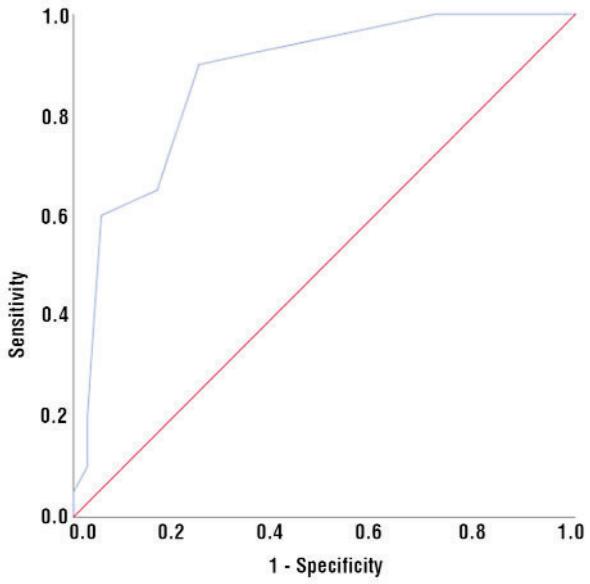

Figure 2. Receiver operating characteristic (ROC) curve for NLR as a predictor of tumor recurrence in high-risk stage II colon cancer

median survival time was not reached, regardless of treatment regimen, indicating longer survival in this group (*Fig. 3A*). In contrast, patients with NLR ≥ 3 exhibited shorter DFS times. Those treated with CAPEOX or FOLFOX had a median survival of 81.00 months (95% CI: 70.60-91.40). The 5-FU/Capecitabine subgroup within the same NLR category had a median survival of 67.00 months (95% CI: 58.32-75.68) (*Fig. 3B*).

Univariate and multivariate Cox regression analyses were conducted to identify factors associated with recurrence. Age ≥70 years was associated with an increased risk of recurrence in both univariate (HR: 1.732; 95% CI: 1.123–2.567; p=0.236) and multivariate

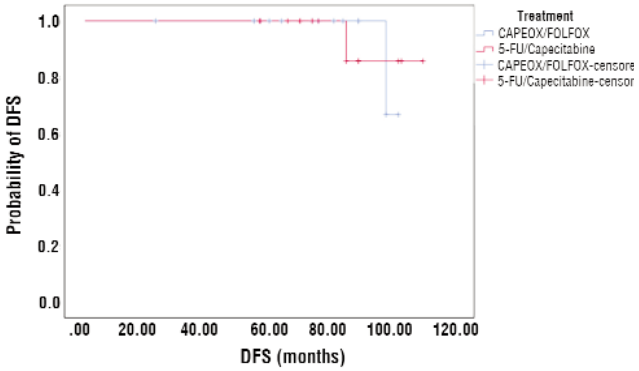

Figure 3A. Kaplan-Meier curves comparing DFS by treatment regimens in NLR<3 patients.

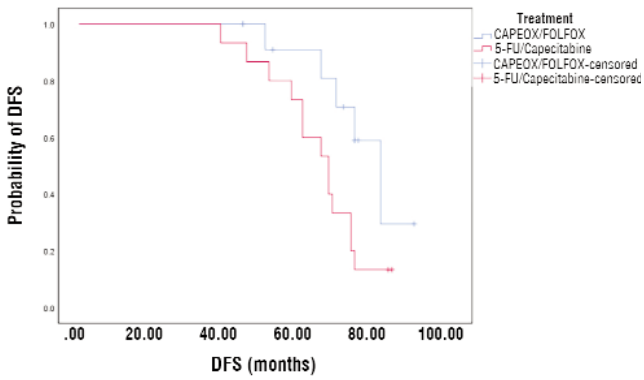

Figure 3B. Kaplan-Meier curves comparing DFS by treatment regimens in NLR ≥ 3 patients.

models (HR: 1.564; 95% CI: 1.098–2.341; p=0.301), though without statistical significance. Gender did not significantly impact recurrence risk. Lymphovascular invasion was significantly associated with recurrence in both analyses (multivariate HR: 2.100; 95% CI: 1.305-4.021; p=0.040). Perineural invasion was not a significant predictor.

Positive surgical margins (R1) were associated with a higher recurrence risk (HR: 1.893; 95% CI: 1.243-2.951; p=0.012). Tumor stage T4 was also significantly associated with recurrence compared to T3 (HR: 2.045; 95% CI: 1.483–3.734; p=0.030). Elevated CEA levels (>ULN) were not significantly correlated with recurrence in either analysis. Fewer than 12 lymph nodes examined was a strong independent predictor (HR: 2.545; 95% CI: 1.325-3.541; p<0.001).

Clinical presentation with occlusion or perforation was significantly associated with recurrence (HR: 3.757; 95% CI: 1.045-4.967; p=0.001). Although most recurrences presented as disseminated metastases or peritoneal carcinomatosis.

High tumor budding (Tb3) showed a non-significant trend toward increased recurrence (HR: 1.671; 95% CI: 0.845–3.782; p=0.341). A neutrophil-to-lymphocyte ratio (NLR) >3 was a strong and independent predictor of recurrence (HR: 3.22; 95% CI: 1.971–4.566; p=0.001). Lastly, high-grade tumors (G3) demonstrated a non-significant trend toward elevated recurrence risk (HR: 1.429; 95% CI: 0.893–2.374; p=0.103). This data is shown in *Table 3*.

Overall survival (OS) was not included as an endpoint in this study due to the limited number of death events and the relatively short follow-up for some patients, which precluded meaningful statistical analysis.

Discussion

The optimal management of stage II colon cancer, particularly in microsatellite stable (MSS) patients with high-risk features, remains an area of clinical ambiguity. Our study adds to the growing body of evidence supporting the use of the neutrophil-to-lymphocyte ratio (NLR) as a prognostic biomarker in this subset of patients. We demonstrated that a pre-operative NLR ≥3 was a strong, independent predictor of disease recurrence (HR=3.22), consistent with prior findings linking elevated NLR to poor outcomes in colorectal cancer (24). This reflects the central role of systemic inflammation in carcinogenesis, wherein a neutrophil-dominant immune profile suppresses cyto-

Table 3. Univariate and multivariate Cox regression analyses to identify predictors for increased risk of progression in high-risk stage II colon cancer patients

Dependent variable: Recurrence			Univariate analysis		Multivariate analysis	
	Tested	Reference	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	≥70 years	<70 years	1.732 (1.123-2.567)	0.236	1.564 (1.098-2.341)	0.301
Gender	Male	Female	1.289 (0.876-1.899)	0.176	1.145 (0.789-1.862)	0.249
Lymphovascular Invasion	Yes	No	1.975 (1.225-3.771)	0.055	1.502 (0.156–14.428)	0.072
Perineural Invasion (PNI)	Yes	No	1.244 (0.715-1.973)	0.129	1.115 (0.531-1.669)	0.256
Surgical margins	R1	R0	2.145 (1.452-3.127)	0.005	1.893 (1.243-2.951)	0.012
Tumor stage	T4	T3	2.923 (2.115-4.321)	0.032	2.045 (1.483-3.734)	0.030
CEA	>ULN	<ULN	1.612 (1.087-2.245)	0.472	1.612 (0.985-2.558)	0.578
Lymph nodes examined	<12	≥ 12 nodes	1.995 (1.302-2.789	0.009	2.545 (1.325-3.541)	<0.001
Occlusion/Perforation	Yes	No	3.812 (1.087-4.245)	0.001	3.757 (1.045-4.967)	0.001
Tumor budding	Tb3	Tb1-2	1.904 (1.023-3.123)	0.223	1.671 (0.845-3.782)	0.341
NLR	>3	≤ 3	3.571 (1.754)	0.001	3.220 (1.971-4.566)	0.001
Grade	G3	G1-G2	1.564 (1.025-2.663)	0.085	1.429 (0.893-2.374)	0.103

Abbreviations: HR: hazard ratio; CI: confidence interval; NLR: neutrophil-to-lymphocyte ratio; LVI: lymphovascular invasion; PNI: perineural invasion; CEA: carcinoembryonic antigen; ULN: upper limit of normal; R0: negative surgical margin; R1: positive surgical margin; Tb: tumor budding; G1–G3: tumor grade (G1: well-differentiated, G2: moderately differentiated, G3: poorly differentiated).

toxic lymphocyte function, fostering a tumor-promoting microenvironment (18,19).

The addition of oxaliplatin to fluoropyrimidine-based adjuvant chemotherapy (e.g., FOLFOX or CAPEOX) has shown selective benefit in high-risk stage II patients, particularly those with T4 tumors, inadequate nodal sampling, or other adverse histopathologic features (14). Data from the MOSAIC trial revealed that while the absolute disease-free survival (DFS) benefit of oxaliplatin in unselected stage II patients was modest (~2%), the benefit was more pronounced in high-risk subsets (10). Similary in the NSABP C-07 trial, which randomized over 2200 patients with stage II or III disease to receive fluorouracil/leucovorin (FU/LV) with or without oxaliplatin (FLOX), the overall cohort experienced improved 4-year disease-free survival (DFS) with oxaliplatin (73% vs. 67%, HR 0.80) (25). However, no DFS or overall survival (OS) benefit was observed in the stage II subgroup (HR 0.94 and 1.04, respectively) (26).

A pooled analysis of the MOSAIC and NSABP C-07 trials evaluating 1595 patients with resected stage II colon cancer similarly showed no OS benefit from oxaliplatin in either low- or high-risk patients (HR 1.17 and 0.86, respectively). However, in high-risk subgroups (T4, perforation, or inadequate nodal staging), a non-significant trend toward improved DFS and time to recurrence was noted (HR 0.75 for both) (27).

Furthermore, a comprehensive analysis of the ACCENT database, encompassing over 12,000 patients from five major trials, revealed that oxaliplatin modestly reduced recurrence risk within the first 14 months in stage II patients, though no long-term OS benefit was identified (28). These trials largely predated routine MMR testing, and most included patients likely had proficient MMR (pMMR), potentially explaining the limited oxaliplatin effect.

Beyond its cytotoxicity, oxaliplatin's immunomodulatory effects may play a crucial role in patients with MSS tumors. Unlike microsatellite instability-high (MSI-H) tumors, which have high mutational loads and are naturally immunogenic, MSS tumors typically elicit weaker immune responses. However, oxaliplatin induces immunogenic cell death (ICD) -a process whereby dying tumor cells release damage-associated molecular patterns (DAMPs), such as calreticulin, HMGB1, and ATP (29). These signals facilitate dendritic cell maturation and cross-presentation of tumor antigens to CD8+ T cells, effectively "vaccinating" the host against residual disease (30). In preclinical models, oxaliplatin - but not other agents like cisplatin - has been shown to activate this immunogenic pathway, making it a unique agent for potentiating antitumor immunity in otherwise poorly immunogenic tumors (31).

In this context, the prognostic significance of NLR may reflect the interplay between tumor-driven inflammation and host immunity. Elevated NLR signifies a predominance of neutrophils, which can suppress T-cell proliferation and function through secretion of reactive oxygen species, arginase, and other immunosuppressive factors (23).

Simultaneously, lymphopenia in high NLR patients compromises adaptive immunity, including the cytotoxic response necessary to clear micrometastatic disease post-resection or in response to chemotherapy-induced ICD (32). Therefore, the efficacy of oxaliplatin in patients with elevated NLR may stem from its ability to reengage immune responses that would otherwise remain dormant (33). This hypothesis is indirectly supported by our finding that patients with NLR $\geq$3 derived more DFS benefit from oxali-platin-containing regimens than from fluoropyrimidine monotherapy (81 vs. 67 months median DFS), whereas patients with NLR <3 fared well regardless of regimen.

These findings have practical implications. In patients with low NLR, fluoropyrimidine monotherapy may be sufficient, thereby avoiding the neurotoxicity and cumulative side effects of oxaliplatin. Conversely, patients with elevated NLR may benefit disproportionately from oxaliplatin due to both their increased baseline risk and their reliance on chemotherapy to stimulate an otherwise ineffective immune surveillance system. This supports a model of biologically informed treatment selection, integrating both classical histopathologic risk factors and systemic inflammatory markers like NLR.

However, the prognostic role of NLR remains subject to ongoing debate. While several meta-analyses (34,35) have demonstrated that elevated NLR is associated with worse overall and disease-free survival across gastrointestinal malignancies, other studies have highlighted significant heterogeneity and inconsistent prognostic performance (36).

Importantly, NLR is influenced not only by tumor biology but also by host-related factors, particularly comorbid conditions. Chronic diseases such as cardiovascular disorders, diabetes mellitus, and chronic pulmonary disease are associated with persistent low-grade systemic inflammation, which may elevate baseline neutrophil counts and suppress lymphocyte levels. In our cohort, the majority of patients had a low comorbidity burden; however, patients with higher comorbidity indices may present with elevated NLR values independent of tumor aggressiveness. This represents a potential confounding factor and may

partly explain the variability observed across studies. Similar observations have been reported in large retrospective cohorts, such as the study by Lee et al. (37), where systemic inflammatory markers were influenced by both oncologic and non-oncologic factors.

In addition, perioperative factors and surgical outcomes may further influence inflammatory markers and their interpretation. Surgical trauma itself induces a transient inflammatory response, and postoperative complications - particularly infectious events or anastomotic leakage - can significantly alter leukocyte dynamics. Xu et al. (38) demonstrated that elevated preoperative NLR is associated not only with oncologic outcomes but also with an increased risk of postoperative complications, including anastomotic leakage, highlighting the bidirectional relationship between inflammation and surgical outcomes. In our cohort, the absence of severe postoperative complications suggests that the observed prognostic value of NLR is less likely to be confounded by postoperative inflammatory events. However, in real-world settings, where complication rates may be higher and surgical approaches more heterogeneous, this interaction warrants careful consideration.

Our study has limitations inherent to its retrospective design and modest sample size. Additionally, incomplete adjustment for comorbidities and lack of detailed data on surgical approach or perioperative inflammatory dynamics may introduce residual confounding. Nonetheless, the strong association between NLR and recurrence risk, coupled with the observed interaction between NLR and oxaliplatin benefit, warrants further prospective validation. In particular, future trials should consider stratifying stage II MSS colon cancer patients by NLR while also integrating comorbidity burden and perioperative factors. Moreover, combining systemic inflammatory markers with immune profiling and molecular biomarkers may provide a more comprehensive and clinically applicable risk stratification model.

Conclusions

This study demonstrates that the neutrophil-to-lymphocyte ratio (NLR) is a meaningful and independent prognostic biomarker in patients with high-risk stage II microsatellite stable colon cancer. An elevated preoperative NLR (≥3) was strongly associated with increased recurrence risk and identified a subgroup of patients who derived greater benefit from oxaliplatin-based adjuvant chemotherapy. In contrast, patients with low NLR exhibited favorable outcomes regardless of treatment intensity, suggesting that deescalation strategies may be appropriate in this population.

Importantly, our findings highlight that NLR should not be interpreted in isolation, but rather within the broader clinical context, including comorbidity burden, surgical outcomes, and established pathological risk factors. The interaction observed between systemic inflammation and chemotherapy efficacy supports the hypothesis that host immune status plays a critical role in modulating treatment response.

Overall, NLR represents a simple, cost-effective, and widely accessible biomarker that may enhance current risk stratification models and support more individualized treatment decisions in stage II colon cancer. Prospective validation in larger, well-characterized cohorts is warranted to confirm its predictive value and to establish standardized thresholds for clinical implementation.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Statement

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee. Owing to the retrospective design of the study, the requirement for informed consent was waived by the Ethics Committee. All patient data were anonymized prior to analysis.

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