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Beyond the Node Count: Metastatic Lymph Node Ratio as a Prognostic Marker in Locally Advanced Colorectal Cancer

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Abstract:

Objective: Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, and staging based on the number of metastatic nodes may not fully reflect nodal tumor burden. The metastatic lymph node ratio (mLNR) has been proposed as a more informative prognostic marker, but its role in locally advanced CRC is unclear. This study aimed to evaluate the prognostic value of mLNR in patients with locally advanced (T3–T4), non-metastatic CRC and to identify an optimal cut-off for recurrence prediction.

Methods: We retrospectively analysed 225 patients with T3–T4, non-metastatic CRC who underwent curative resection between 2015 and 2019. Disease-free survival (DFS) and overall survival (OS) were estimated using Kaplan–Meier analysis. Associations between mLNR, recurrence, stage and survival were examined with standard statistical tests. An optimal mLNR cut-off for recurrence was derived by receiver operating characteristic (ROC) analysis.

Results: Patients with recurrence had significantly higher mLNR than recurrence-free patients. mLNR correlated inversely with DFS but not with OS. Higher mLNR was associated with T4 tumors and with higher AJCC stage. ROC analysis identified an mLNR cut-off of 0.1846 (area under the curve 0.643). Patients with mLNR ≥ 0.1846 had significantly shorter DFS and OS. A lymph node yield ≥12 was associated with longer DFS but not OS.

Conclusion: mLNR is associated with recurrence, tumor stage and survival in locally advanced CRC, and a threshold around 0.18 may improve risk stratification beyond conventional node counts.

Keywords: Colorectal Cancer, Disease-Free Survival, Lymph Node Yield, Metastatic Lymph Node Ratio, Overall Survival

Colorectal cancer (CRC) is one of the most common malignancies worldwide and remains a leading cause of cancer-related mortality despite improvements in screening, diagnosis, and treatment. This substantial global burden highlights the need for robust staging systems that provide accurate prognostic information and guide therapeutic decision-making for optimal treatment strategies. Recent comprehensive reviews have similarly emphasized the necessity of improved prognostic stratification in colorectal cancer, underscoring the central role of nodal status within contemporary

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staging approaches [1-3]. Through proper staging, high-risk individuals can be identified and advised tailored adjuvant therapies, thereby enabling individualized treatments.

The eighth edition of the American Joint Committee on Cancer (AJCC) TNM classification continues to serve as the principal framework for CRC staging, with lymph node status-defined by the number of metastatic lymph nodes (pN)-representing a key determinant for stage assignment and the selection of adjuvant chemotherapy; however, additional tumor- and patient-specific markers have emerged to refine prognostic precision [4].

The number of lymph nodes (LNs) retrieved and examined after surgery varies widely due to factors related to surgical technique, pathological evaluation, host immune response, and tumor biology [5, 6]. This variability may lead to stage migration and reduce the accuracy of prognostic assessment. Although current guidelines recommend evaluating at least 12 LNs to ensure adequate nodal staging [7], a fixed numerical threshold may not fully capture underlying tumor behavior or reliably predict outcomes, particularly in patients with locally advanced disease [8-11].

To address these limitations, the metastatic lymph node ratio (mLNR) - the proportion of metastatic nodes among the total examined - has been proposed as a more refined metric of nodal burden. By integrating both the extent of nodal involvement and LN yield, mLNR offers a biologically meaningful parameter that accounts for differences in surgical practice and pathological processing [12, 13]. Its prognostic significance has been demonstrated in several solid tumors, including gastric, pancreatic, and breast cancers, and increasing evidence supports its relevance in CRC [14-16]. Several studies so far have reported that mLNR provides more encouraging prognostic precision than absolute lymph node counts in estimating survival outcomes of CRC [17]. Moreover, even in early-stage colorectal cancer, the prognostic impact of mLNR has been clearly demonstrated [17-18]. Nonetheless, its clinical utility in CRC remains uncertain, largely due to heterogeneous cut-off values and the absence of standardized incorporation into current staging systems [12-15]. Despite the well-established relevance of nodal involvement in CRC, the mLNR has not yet been incorporated into standardized staging criteria such as the AJCC/UICC TNM system, indicating that it remains an underutilized prognostic parameter.

This study aimed to evaluate the prognostic value of mLNR in patients with locally advanced (T3–T4), non-metastatic CRC and to identify an optimal cut-off for predicting recurrence. Additionally, the study explored the potential contribution of mLNR as a complementary marker to the established TNM classification.

METHODS

This retrospective cohort study was conducted in the Department of General Surgery, Faculty of Medicine, Trakya University, and included colorectal cancer patients that were operated between January 2015 and December 2019. Ethical approval was obtained from the Ethics Committee of Trakya University Faculty of Medicine (approval number: TUTF-BAEK 2025/490, date: November 3, 2025).

Clinical and demographic data were retrieved from institutional patient files and the hospital's electronic medical record system. Initially, 693 patients who underwent surgical resection for histologically confirmed colorectal cancer were screened. Patients were excluded if they had early-stage tumors (pT1 or pT2), evidence of distant metastasis at diagnosis, incomplete postoperative follow-up, in-hospital death before discharge, or emergency surgery due to bowel obstruction or tumor perforation. Finally, 225 eligible patients were included in the final analysis (Figure 1). Missing or incomplete data were verified and supplemented, when available, through the national electronic health record platform "e-Nabız" [19].

Demographic and clinicopathological characteristics were systematically recorded for each patient. Histopathology reports were reviewed to determine tumor (T) and nodal (N) categories according to the AJCC 8th edition TNM classification (2017). Histopathological data, including lymph node assessment, were obtained from routine institutional pathology reports generated during standard clinical practice in a tertiary academic center. Owing to the retrospective design and routine reporting workflow, lymph node assessment was not performed under a

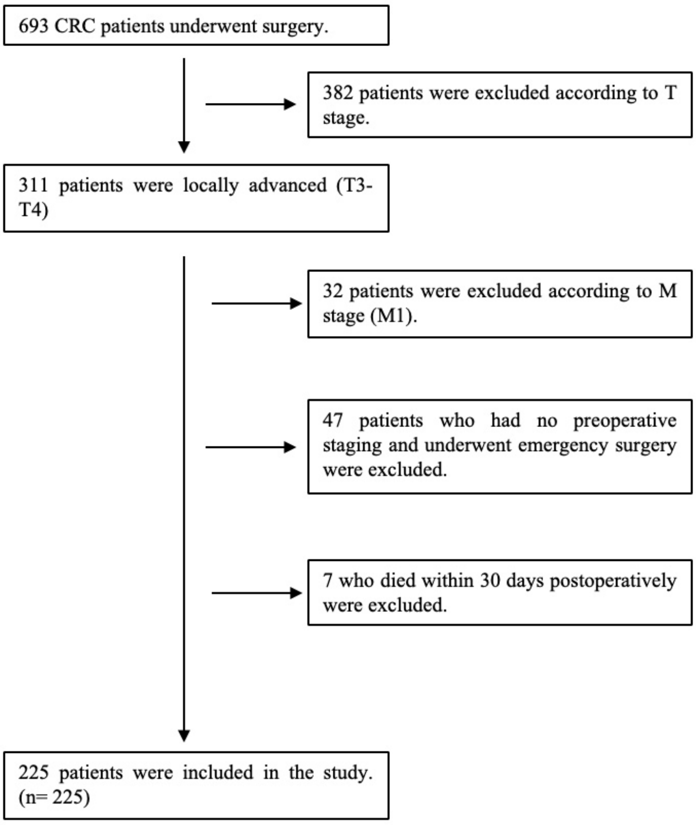

FIGURE 1. Patient selection and exclusion flowchart for the retrospective colorectal cancer cohort.

single-pathologist or centralized re-review protocol. For each case, the total number of dissected lymph nodes and the number of metastatic nodes were documented. The metastatic lymph node ratio (mLNR) was calculated as the number of metastatic nodes divided by the total number of examined nodes.

Postoperative surveillance was scheduled every three months during the first two years and every six months thereafter to complete five years observation period. Disease-free survival (DFS) and overall survival (OS) were defined as the intervals from the date of surgery to the date of recurrence/death or death, respectively, or last follow-up in censored cases. The prognostic impact of mLNR and its association with tumor stage were evaluated. In addition, patients were stratified according to lymph node yield (<12 vs. ≥12 examined nodes) to assess the influence of nodal harvest on recurrence and DFS.

Statistical Analysis

Data were analyzed using SPSS software (version 28.0; IBM Corp., Armonk, NY, USA) and the jamovi statistical platform (version 2.4.8; The Jamovi Project, Sydney, Australia). An a priori power analysis for non parametric two sample mean comparison (Mann Whitney, effect size $d = 0.5$, $\alpha = 0.05$, power $[1 - \beta] = 0.80$, $N2/N1 = 1$) showed a sample size of 67 for each group and a total sample size of 134 patients. The power calculation was performed using G*Power version 3.1.2. Categorical variables were summarized as counts and percentages. Continuous variables were described as means with standard errors (SE) or medians with interquartile ranges (IQR), depending on distribution. Normality of continuous variables was assessed with the Shapiro–Wilk test. For comparisons of continuous variables between two groups, the independent-samples t test was used when normality was satisfied; otherwise, the Mann–Whitney U test was applied. Associations between categorical variables were evaluated using Pearson's chi-square test. DFS and OS were estimated using the Kaplan–Meier method, and survival curves were compared using the log-rank test. The prognostic performance of mLNR for predicting tumor recurrence was examined by receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC), sensitivity, specificity, and corresponding 95% confidence intervals (CI) were calculated. The cut-off point with the best balance between sensitivity and specificity was selected as the optimal threshold. A P-value < 0.05 was considered statistically significant in all analyses.

RESULTS

A total of 225 patients with locally advanced (T3-T4), non-metastatic colorectal cancer were included in the final cohort. The median age was 66 years (IQR = 16), and 56.9% of patients were male. Tumors were staged according to the AJCC 8th edition classification: 39.1% were stage IIa, 14.7% stage IIb, 23.1% stage IIIb, and 23.1% stage IIIc. No patients were classified as stage IIc or IIIa. Demographic and clinicopathological characteristics are summarized in Table 1. The median number of harvested lymph nodes per patient was 11 (IQR = 5). The median number of metastatic nodes was 0 (IQR = 3), indicating that node-negative disease was slightly more frequent in the study population. The median metastatic lymph node ratio (mLNR) was 0.00 (IQR = 0.222), reflecting substantial variability in nodal involvement across the cohort.

In the cohort, 45 (20%) patients had T3N0 disease with adequate nodal evaluation (≥12 examined lymph nodes) and were followed without immediate postoperative adjuvant chemotherapy. All remaining patients received postoperative adjuvant chemotherapy according to the institutional treatment approach.

Association Between mLNR, Recurrence, and Survival

The relationship between mLNR and recurrence was evaluated by comparing 144 patients who developed tumor relapse with 81 patients who remained recurrence-free during the 60-month follow-up. Median mLNR was significantly higher in the recurrence group (Mann–Whitney U test, P<0.001), indicating a strong association between increased nodal metastatic burden and relapse risk (Figure 2). Spearman's rank correlation analysis revealed a significant inverse correlation between mLNR and DFS (r = −0.205, P=0.013), suggesting that higher mLNR is associated with shorter disease-free intervals. In contrast, no significant correlation was observed between mLNR and OS (r = −0.041, P=0.529) (Figure 3).

Association Between mLNR and Disease Stage

Subgroup analysis comparing patients with T3 (n=152) and T4 (n= 3) tumors showed that the T4 group had a significantly higher median mLNR (0.154 vs. 0.00; P=0.022, Mann–Whitney U test), indicating that deeper primary tumor invasion is associated with a greater metastatic nodal burden (Figure 4). When patients were stratified by AJCC stage-IIa (n=88, 39.1%), IIb (n=33, 14.7%), IIIb (n=52, 23.1%), and IIIc (n=52, 23.1%)-a progressive increase in median mLNR was observed with advancing stage: 0.000

TABLE 1. Baseline Demographic and Clinicopathological Characteristics of the Study Cohort

Variables	Data (n=225)
Age, median (IQR)	66 (IQR:16)
Gender, n (%)	
Female	97 (43.1%)
Male	128 (56.9%)
Tumor location, n (%)	
Right colon	65 (28.9%)
Left colon	23 (10.2%)
Sigmoid colon	90 (40%)
Rectum	47 (20.9%)
T classification, n (%)	
T3	152 (67.6%)
T4	73 (32.4%)
N classification, n (%)	
N_0	121 (53.8%)
N_1	77 (34.2%)
N_2	27 (12%)
Stage (AJCC 2017), n (%)	
IIa	88 (39.1%)
IIb	33 (14.7%)
IIIb	52 (23.1%)
IIIc	52 (23.1%)

AJCC, American Joint Committee on Cancer; IQR, interquartile range; N, nodal; T, tumor.

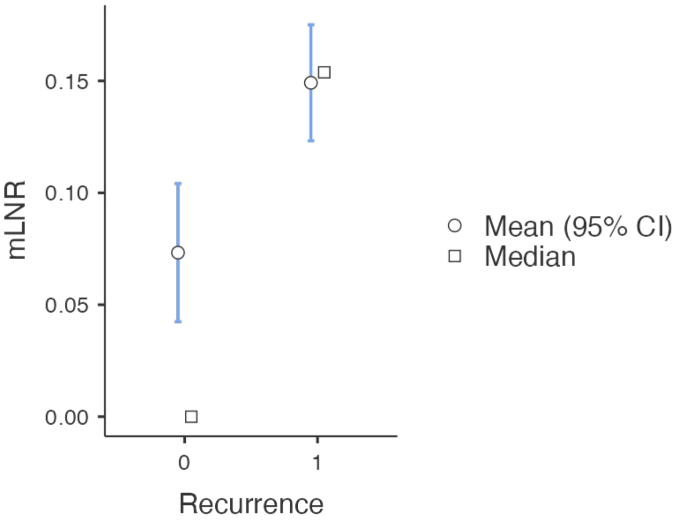

FIGURE 2. Metastatic lymph node ratio (mLNR) by recurrence status. Patients without recurrence were classified as Group 0, and those with recurrence as Group 1. Statistical analysis: Mann–Whitney U test, (P<0.001).

(IQR 0.000) for both stage IIa and IIb, 0.167 (IQR 0.250) for stage IIIb, and 0.500 (IQR 0.333) for stage IIIc. The Kruskal-Wallis test demonstrated a statistically significant difference in mLNR across stages (P<0.001), confirming a positive association between higher pathological stage and nodal metastatic burden (Figure 5).

Cut-Off Value for mLNR

ROC curve analysis was used to identify the most informative mLNR threshold for predicting tumor recurrence. The optimal cut-off was 0.1846, corresponding to a sensitivity of 40.4% and a false-positive rate (1-specificity) of 17.7% (specificity 82.3%). The AUC was 0.643 (95% CI: 0.569-0.717), indicating moderate discriminative ability (Figure 6). Based on this cut-off, patients were categorized into two groups: mLNR < 0.1846 and mLNR ≥ 0.1846. Kaplan–Meier analysis showed that patients with mLNR ≥ 0.1846 had significantly shorter DFS, with a median of 20.0 months compared with 48.0 months in the lower mLNR group (p < 0.001, log-rank test; Figure 7). This divergence in DFS curves was most pronounced during the early and mid-term follow-up. Overall survival was also significantly reduced in the high-mLNR group (P<0.001, log-rank test; Figure 8).

Association Between Lymph Node Yield and Survival

Using the guideline-recommended threshold for adequate nodal evaluation, patients were stratified into two groups: 108 patients (48.0%) with ≥12 examined lymph nodes and 117 patients (52.0%) with <12 nodes. Patients with ≥12 examined lymph nodes had significantly longer DFS compared with those with <12 nodes (median DFS: 24.0 vs. 17.0 months, respectively; P=0.017; Figure 9). In contrast, no significant differences were observed between the groups in terms of OS (P=0.445) or mLNR values (P=0.292).

DISCUSSION

In this retrospective cohort of patients with locally advanced (T3-T4), non-metastatic CRC undergoing elective curative resection, we found that the metastatic lymph node ratio (mLNR) was strongly associated with recurrence, disease-free survival, and overall survival. Using ROC analysis, we identified an

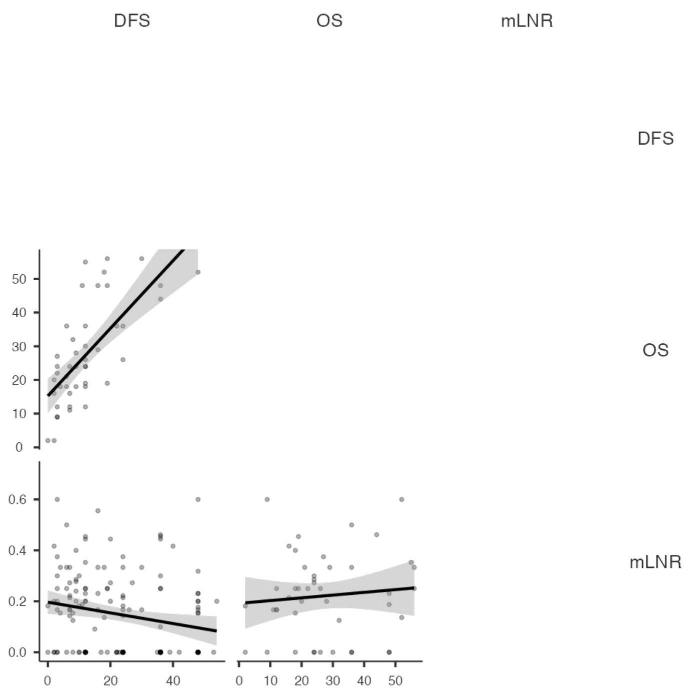

FIGURE 3. FIGURE 3. Correlation matrix for mLNR, disease-free survival (DFS), and overall survival (OS). Pearson correlation: Significant negative correlation between mLNR and DFS (r = –0.205, P=0.013).

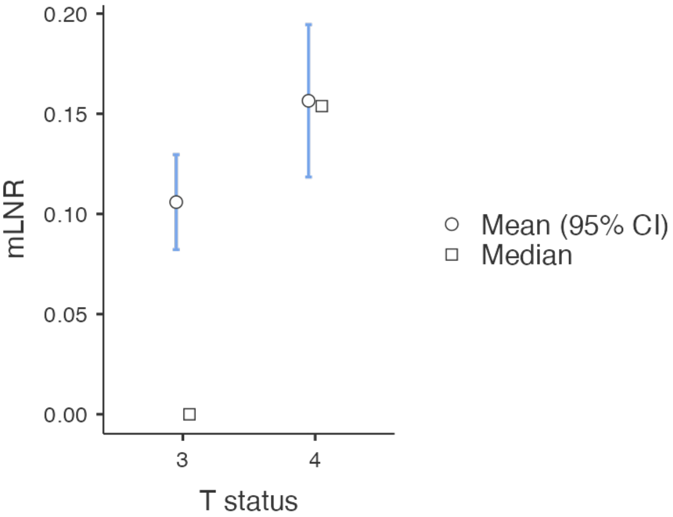

FIGURE 4. **Metastatic lymph node ratio (mLNR) by T stage. (AJCC 8th edition T3 and T4 groups. Statistical analysis: Mann–Whitney U test, P=0.022.**

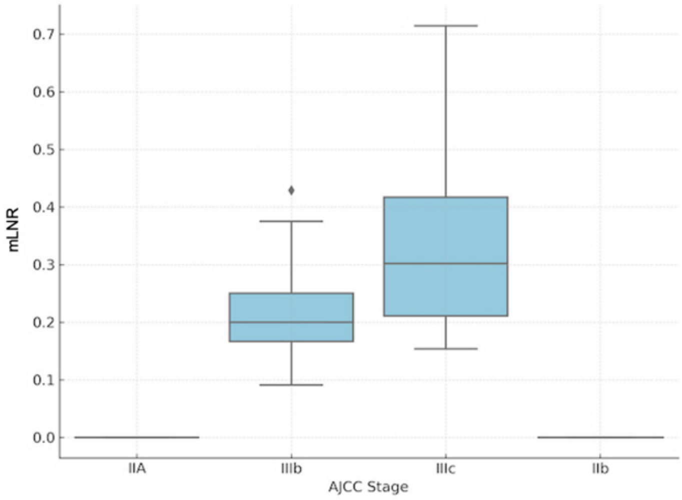

FIGURE 5. **Distribution of mLNR across pathological AJCC stage groups. Statistical analysis: Kruskal–Wallis test, P<0.001.**

mLNR cut-off of 0.1846, above which patients experienced significantly shorter DFS and OS, suggesting that this threshold may help refine risk stratification beyond conventional node counts. Although the prognostic relevance of mLNR in colorectal cancer has been investigated previously, studies focusing specifically on relatively homogeneous cohorts with locally advanced (T3–T4), non-metastatic disease remain limited. In this context, our study provides additional focused evidence by evaluating the association of mLNR with oncologic outcomes and by exploring a recurrence-oriented ROC-derived cut-off within this selected population. The stage distribution in our cohort further reflects the deliberate focus on advanced local disease: the absence of stage IIIa (T1–2N1) was expected given the exclusion of early-stage tumors (T1–T2), while the

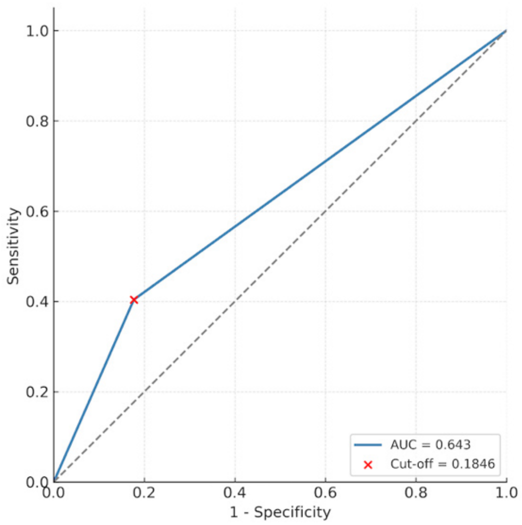

FIGURE 6. **ROC curve analysis of mLNR for predicting disease recurrence. Optimal cut-off value was 0.1846, yielding a sensitivity of 40.4% and a specificity of 82.3%. AUC = 0.643, 95% CI: 0.569–0.717.**

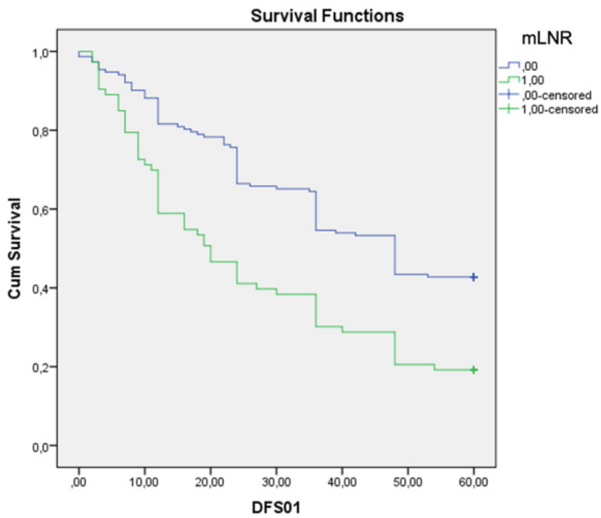

FIGURE 7. **Kaplan-Meier curves for disease-free survival based on optimal mLNR cut-off value. Kaplan–Meier analysis of disease-free survival (DFS) based on mLNR-based risk groups. Patients were stratified by an mLNR cut-off value of 0.1846. Statistical comparison using Log-rank test; P<0.001.**

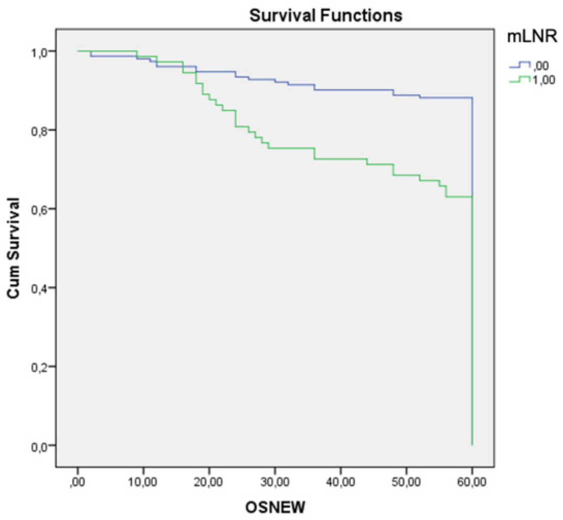

FIGURE 8. **Kaplan-Meier curves for overall survival based on mLNR < 0.1846 vs. ≥ 0.1846. Patients were categorized into two groups based on an mLNR cut-off value of 0.1846. Survival comparison was performed using the Log-rank test; P<0.001.**

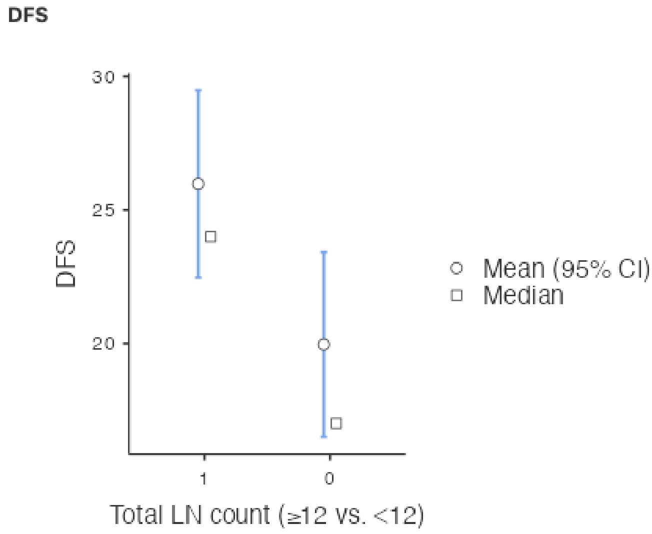

FIGURE 9. **Disease-free survival by total lymph node yield (<12 vs. ≥12). Group 0: patients with <12 retrieved lymph nodes; Group 1: patients with ≥12 nodes. Median DFS: 17.0 vs. 24.0 months, respectively. Mann–Whitney U test, P=0.017.**

lack of stage IIc (T4bN0) likely relates to the relative rarity of tumors with adjacent organ invasion in the absence of nodal involvement.

The recurrence burden observed in the present cohort should also be interpreted cautiously, given that the study population consisted exclusively of locally advanced (T3–T4), non-metastatic cases and the analysis was based on retrospective single-center data. In the present analysis of patients with locally advanced (T3–T4, M0) colorectal cancer, the metastatic lymph node ratio (mLNR) was inversely correlated with disease-free survival. In contrast, the correlation between mLNR and overall survival did not reach statistical significance, suggesting that mLNR may better reflect tumor biology related to recurrence risk than overall mortality in this cohort. Similarly, Sabbagh *et al*. [17] observed that mLNR predicted recurrence risk but did not show a statistically meaningful association with overall survival in their multicenter evaluation of patients with stage III colon cancer. Conversely, other studies, such as that by İmamoğlu *et al*. [20] involving 156 stage III CRC patients, found that elevated mLNR was independently predictive of worse OS. These discrepancies likely reflect variations in patient selection, mLNR cut-off thresholds, follow-up durations, and adjuvant therapy protocols. Taken together, such variability underscores that the prognostic role of mLNR-especially for OS-may be modulated by contextual and methodological factors. In addition, the lack of a significant correlation between continuous mLNR values and overall survival, despite the significant difference observed after dichotomization at the ROC-derived cut-off, may reflect a threshold-dependent relationship. At the same time, categorizing a continuous variable may accentuate between-group differences and should therefore be interpreted with caution. This point further supports viewing the identified cut-off as exploratory rather than definitive.

However, the optimal mLNR cut-off identified in this study to predict recurrence was also associated with overall survival, as well as disease-free survival. Patients with mLNR ≥ 0.1846 had significantly shorter DFS and OS. Currently, one of the main barriers to integrating mLNR into routine clinical practice is the absence of a universally accepted cut-off value. Various studies have proposed different thresholds based on their specific cohorts and analytical methods. For instance, Pyo *et al*. [22] tested cut-offs of 0.1, 0.2, 0.3, and 0.4 in colorectal cancer and found that all these values distinguished survival groups, but a cut-off of 0.2 performed consistently across tumor sites (colon and rectum). Supporting this, Costi *et al*. [23], in a study analyzing 761 node-positive colorectal cancer cases, found that an mLNR cut-off of 15% (0.15) significantly improved prognostic stratification compared with traditional TNM staging. A Turkish cohort study by Kamali *et al*. [24] identified an mLNR cut-off of 0.028 as the value correlating with overall survival (sensitivity 42%, specificity 71%). More recently, Aslan *et al*. [25] proposed a cut-off of 0.125 and reported that patients with mLNR ≥ 0.125 had significantly shorter DFS. An mLNR threshold around 0.18 identified a subgroup with less favorable outcomes in this cohort; however, this threshold should be considered exploratory given the modest discriminatory performance observed. Therefore, mLNR may serve as a complementary metric alongside conventional staging, but further validation is required before routine clinical implementation can be recommended. Nevertheless, variability in population characteristics, surgical technique, lymph node yield, follow-up duration, and adjuvant therapy regimens underscores the need for large-scale prospective and multicenter studies to validate and establish mLNR thresholds before widespread clinical adoption.

The subgroup analysis in our study demonstrated that mLNR was higher among individuals with T4 tumors. Moreover, our data show a statistically significant correlation between higher mLNR values and advanced AJCC stages, indicating that mLNR increases in parallel with overall disease stage. This relationship supports the notion that mLNR reflects cumulative tumor burden, encompassing both depth of invasion and the extent of nodal metastasis. Consistent with our results, several studies have reported that the prognostic impact of mLNR is intensified with increasing T stage [26, 27]. These findings across diverse cohorts strengthen the rationale for incorporating mLNR into staging systems, particularly in patients with more advanced primary tumors.

In our study, a lymph node yield ≥12 was associated with longer DFS but not OS, in line with current oncologic guidelines that recommend examination of at least twelve lymph nodes for reliable pathological staging and as a benchmark of surgical quality in CRC [16, 28, 29]. However, accumulating evidence suggests that this fixed threshold does not uniformly predict survival across all disease stages or risk groups. Li Destri *et al*. [29] reported that although survival tended to be lower when <12 nodes were retrieved in stage II–III CRC, node count alone did not ensure adequate prognostic accuracy, whereas mLNR provided more robust risk stratification. Similarly, Li *et al*. [30] found no survival difference by lymph node yield in T1 CRC, and Baxter *et al*. [31] observed that applying the twelve-node benchmark in stage II colon cancer did not translate into distinct OS outcomes. Many authors have also noted that harvesting ≥12 nodes is not consistently achievable outside high-volume or specialized centers [32-34]. In our cohort, the median lymph node yield was 11, slightly below the guideline-recommended threshold. This finding should be interpreted within the context of routine clinical practice, as lymph node yield is influenced by both modifiable factors, such as surgical technique, extent of resection, and pathological examination processes, and non-modifiable factors, including age, immune response, tumor biology, and anatomical variability [22]. Therefore, a relatively low nodal yield should not be viewed as an isolated observation unique to a single cohort, but rather as part of a broader and well-recognized challenge in colorectal cancer care. Nevertheless, limited nodal retrieval may affect both pathological staging accuracy and the calculation of mLNR, and this should be taken into account when interpreting the present findings. Our findings, together with these reports, support the notion that ratio-based metrics such as mLNR may offer more nuanced prognostic information than absolute node counts alone.

Strengths and Limitations

One notable advantage of the present research is its emphasis on a uniform patient cohort characterized by locally advanced (T3–T4, M0) colorectal cancer. By excluding early-stage cases, this approach minimizes potential confounding and enables a more accurate assessment of prognostic variables, including the metastatic-to-examined lymph node ratio (mLNR). Furthermore, the use of ROC curve analysis to determine an optimal mLNR cut-off enhances the robustness and clinical relevance of the findings. Nevertheless, the retrospective nature and single-center scope of the study may limit the broader applicability of the results to other clinical settings. Because the present analyses were predominantly univariate, the observed associations should not be interpreted as evidence of independent prognostic significance of mLNR beyond established clinicopathological factors such as T stage, nodal status, and lymph node yield. Another important consideration is adjuvant treatment. In our cohort, postoperative chemotherapy decisions were closely related to pathological risk features, particularly nodal status, T stage, and adequacy of lymph node harvest. Patients with nodal metastasis, T4 disease, or inadequate nodal evaluation generally received adjuvant chemotherapy, whereas patients with T3N0 disease and ≥12 examined lymph nodes were generally followed without immediate postoperative treatment. Although this reflects routine clinical practice, adjuvant treatment was not incorporated as a separate analytical variable and may therefore have influenced disease-free survival outcomes. Accordingly, the observed associations should be interpreted with appropriate caution. Additionally, potential variation in surgical techniques and pathological evaluations over time could have introduced bias. The absence of molecular or genetic tumor profiling also prevents exploration of possible biological mechanisms underlying the observed associations. Future prospective, multicenter studies incorporating molecular data are warranted to validate and expand upon these findings.

CONCLUSION

Our findings suggest that the metastatic lymph node ratio is associated with tumor burden and oncologic outcomes in patients with locally advanced (T3–T4, M0) colorectal cancer undergoing elective curative resection. An mLNR threshold around 0.18 identified a subgroup with less favorable recurrence and survival outcomes in this cohort; however, this threshold should be considered exploratory given the modest discriminatory performance observed. Thus, mLNR may serve as a complementary metric alongside conventional nodal counts and TNM staging rather than as a standalone prognostic tool. Given its straightforward derivation from routinely reported pathological data, mLNR may have practical value in postoperative risk assessment, but further validation is required before routine clinical implementation can be recommended. Nonetheless, before mLNR can be adopted in routine oncologic assessment, rigorously designed, multicenter prospective studies are needed to validate its prognostic impact across diverse settings, to define standardized threshold values, and to clarify how best to incorporate this parameter into contemporary staging and decision-making algorithms.

Ethics Approval and Consent to Participate

This study was approved by the Trakya University Faculty of Medicine Non-Interventional Scientific Research Ethics Committee. (Decision No: TUTF-BAEK 2025/490-20/32; date: 03/11/2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: YEA, OAÖ; Study Design: YEA, OAÖ; Supervision: N/A; Funding: N/A; Materials: N/A; Data Collection and/or Processing: YEA, OAÖ; Statistical Analysis and/or Data Interpretation: YEA; Literature Review: YEA, OAÖ; Manuscript Preparation: YEA, OAÖ; and Critical Review: YEA, OAÖ.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

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Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

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