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Lymph node ratio is negatively correlated with the Crohn's-like lymphoid reaction to the tumor and tumor-infiltrating lymphocytes in colorectal cancers

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Abstract

Aim: Colorectal cancer is one of the most common cancers with increasing incidence. Lymph node ratio is reported as a prognostic factor in colorectal cancer patients. The tumor-infiltrating lymphocytes and lymphoid reaction around the tumor, indicating the immune response of the body, are reported to correlate with prognosis. This study aims to investigate the association between lymph node ratio, tumor-infiltrating lymphocyte, and lymphoid reaction in colorectal cancers.

Materials and Methods: A total of 119 patients with colorectal carcinoma were histopathologically reviewed. The lymph node ratio, the percentage of positive lymph node numbers to the total number of sampled lymph nodes was calculated. The lymph node ratio was compared to the histopathological and clinical parameters, particularly tumor budding, tumor-infiltrating lymphocytes, Crohn's-like lymphoid reaction, and overall survival.

Results: The tumors with a high number of tumor budding and a low number of tumor-infiltrating lymphocytes and Crohn's-like lymphoid reaction rate were significantly associated with increased lymph node ratio. There was no significant relationship between lymph node ratio and overall survival.

Conclusion: The lymph node ratio is closely correlated with tumor budding, tumor-infiltrating lymphocytes, and Crohn's-like lymphoid reaction in colorectal cancers. As they are all important prognostic factors in colon cancer, their relationship can offer insights into the disease's progression and potential outcomes.

Introduction

Colorectal cancers (CRC) are the third most common cancers worldwide [1]. The staging of CRCs is based on the tumor-node-metastasis (TNM) staging system. 'N' in the TNM staging system stands for the number of positive lymph nodes and is one of the most important prognostic factors [2]. The obtained number of lymph nodes after surgical resection depends on the surgical procedure, length of the specimen, tumor size, and the ability of grossing personnel. Recent studies have reported that lymph node ratio, as well as positive lymph node number, is also a prognostic indicator [3,4].

Tumor budding (TB) is single cells or small cell clusters up to 5 cancer cells at the invasive margin of the tumor. Due to TB being an independent prognostic factor, it is recommended to report in CRCs [5]. It is demonstrated that the number of positive lymph nodes increases as the TB score increases [6].

Tumor infiltrating lymphocytes (TILs) and the Crohn's-like lymphoid reaction to the tumor (CLR) are protective anticancer immune responses of the host against cancer and they are prognostic indicators for disease-free and overall survival [7]. They are reported to be prognostic parameters in CRCs [7,8]. As far as we know there is no study focusing on the relationship of LNR and TILs or CLR in the literature.

This study aims to investigate the correlation between LNR and other clinicopathological parameters particularly

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TB, TILs, and CLR in surgical specimens of CRCs.

Materials and Methods

Approval for this study was obtained from the Ankara Bilkent City Hospital Clinical Research Ethics Committee (Approval number: TABED 1-24-513).

Patient selection

The archives of the Department of Pathology at Ankara Bilkent City Hospital were retrospectively searched and 119 patients with colorectal resection due to CRC were selected. The patients who were treated with neoadjuvant chemotherapy were excluded.

The demographic data was noted. The hematoxylin-eosin stained slides were reviewed and histopathological and clinical parameters were determined as follows: tumor localization, tumor size, histopathological type, differentiation of tumor, invasion level of the tumor, presence of lymphovascular invasion and perineural invasion, length of the specimen, the number of positive lymph node, TB score, TILs, and CLR, clinical stage and survival.

Determination of LNR, TILs, and CLR

Lymph node ratio is defined as the ratio of positive lymph nodes to the total number of lymph nodes examined. Patients were categorized further into four subcategories according to LNR as follows: LNR1: LNR< 0.07; LNR2: 0.07-0.25; LNR3: 0.25-0.50 and LNR4: 0.50-1.0 as proposed by Wang et al. [9].

According to the reporting guidelines recommended by the Federation of Turkish Pathology Societies, tumor-infiltrating lymphocytes were evaluated as absent, mild/moderate (0-2 lymphocyte per high power field), and marked (≥3 lymphocytes per high power field); CLR were evaluated as absent, mild/moderate and marked (including lymphoid follicles) in advancing edge of the tumor.

The correlation between LNR and TB, TILs, and CLR was investigated.

Statistical analysis

The data were analyzed statistically using SPSS 22.0 for Windows (SPSS, Inc.; Chicago, IL. USA). The normal distribution of data was evaluated using histogram, q-q plot, and Shapiro-Wilk's test. For parametric variables, the Independent t-test was performed; for nonparametric variables, the Kruskal-Wallis Test was used to evaluate multiple study groups, and Mann-Whitney U tests were utilized for pairwise comparisons. The Bonferroni correction were applied to adjust significance level to control the overall probability of a Type I error. The relationship between the variables was assessed using the Spearman Correlation test. Statistics were deemed significant if $p<0.05$ at the 95% confidence interval. The overall survival (OS) was evaluated using a Kaplan-Meier analysis.

Results

The study included 119 patients (56 females and 63 males) with a mean age of 63.7 ranging from 32 to 89. The vast majority of the tumors (55.5%) were located in the left colon, particularly the sigmoid colon (42%). Descriptive data was summarized in Table 1.

Table 1. General characteristics of the cases.

Parameters		Number [min-max] or (%)
Age		64 (54-73)
Gender	Male	63 (52.9%)
	Female	56 (47.7%)
Resection Length		28 cm [20-40]
Tumor location	Sigmoid colon	50 (42%)
	Caecum	24 (20.2%)
	Hepatik flexura	13 (10.9%)
	Ascending colon	11 (9.2%)
	Descending colon	9 (7.6%)
	Splenic flexura	7 (5.9%)
	Transiverse colon	5 (4.2%)
Tumor stage	T2	1 (0.8%)
	T3	56 (47.1%)
	T4A	56 (47.1%)
	T4B	5 (5%)
LVI	Present	42 (35.3)
	Absent	77 (64.7)
PNI	Present	34 (28.6%)
	Absent	85 (71.4%)
Clinical stage	Stage 2	63 (52.9%)
	Stage 3	56 (47.1%)
CLR	Absent	12 (10.1%)
	Mild/moderate	93 (78.2%)
	Marked	14 (11.8%)
TIL	Absent	46 (38.7%)
	Mild/moderate	28 (23.5%)
	Marked	45 (37.8%)
TB	Low	70 (58.8%)
	Moderate	32 (26.9%)
	High	17 (14.3%)
LNR	LNR1	93 (78.2%)
	LNR2	15 (12.6%)
	LNR3	6 (5%)
	LNR4	5 (4.2%)
Survival	Alive	61 (51.3%)
	Deceased	58 (48.7%)

CLR: Crohn's-like lymphoid reaction; TIL: Tumor-infiltrating lymphocytes; LNR: Lymph node ratio; LVI: Lymphovascular invasion; PNI: Perineural invasion.

The mean number of resected lymph nodes was 22.8 (±15.6). The lymph node count was higher in longer resection specimens (26.04±19.2) than in shorter ones (20.06±11.2), but the difference was statistically insignificant. The total number of sampled lymph nodes was significantly higher in young patients than in elderly patients ($p=0.04$). The median number of total lymph nodes in younger patients was 23 (IQR:17-35) and 18 (IQR: 14-24) in older patients.

The specimen's length and the patient's age were not sig-

Table 2. Relationship of lymph node ratio and tumor budding, tumor-infiltrating lymphocytes, and Crohn's-like lymphoid reaction.

	LNR Median [min-max]	P value
TB		
Low	0.0 [0-0.500]	
Moderate	0.011 [0-0.556]	<0.001*
High	0.066 [0-0.650]	
TILs		
Absent	0.0 [0-0.650]	
Mild/moderate	0.0386 [0-0.500]	0.09
High	0.0 [0-0.552]	
CLR		
Absent	0.051 [0-0.619]	
Mild/moderate	0.0 [0-0.650] 0	0.03*
High	.00 [0.0-0.62]	

LNR: Lymph node ratio; TB:Tumor budding; TILs: Tumor-infiltrating lymphocytes; CLR: Crohn's-like lymphoid reaction; *statistically significant.

Table 3. The pairwise comparisons of lymph node ratio values between study groups.

	P values for LNR
TB	
Low-Moderate	0.352
Low-High	< 0.001*
Moderate-High	0.036*
TILs	
Absent vs Mild/moderate	0.981
Absent vs High	0.056
Mild/moderate vs High	0.056
CLR	
Absent vs Mild/moderate	0.859
Absent vs High	0.045*
Mild/moderate vs High	0.083

LNR: Lymph node ratio; TB:Tumor budding; TILs: Tumor-infiltrating lymphocytes; CLR: Crohn's-like lymphoid reaction; *statistically significant.

nificantly correlated with LNR.

The mean number of positive lymph nodes was 1.37 (±2.743) and the mean number of LNR was 0.0065 (±0.132). The ninety-three (78.2 %) cases were categorized as LNR1; 15 cases (12.6%) were as LNR2; 6 cases (5%) as LNR3, and 5 cases (4.2%) were as LNR4. Seventy patients (58.8%) had a low TB score, 32 patients (26.9%) had a moderate TB score and 17 patients (14.3%) had a high TB score. TILs were absent in 46 cases (38.7%), mild in 28 cases (23.5%), and marked in 45 cases (37.8%). CLR was absent in 12 cases (10.1%), mild in 93 cases (78.2%), and marked in 14 cases (11.8%) (Table 1).

The mean follow-up was 73.5 months ranging from 0 to 170 months. The fifty-eight patients were deceased in the follow-up period (48.7 %).

Table 4. Correlations between LNR, TB, TIL, and CLR.

	LNR	TB	TIL	CLR
LNR				
Correlation coefficient	1	0.343	-0.176	-0.230
p values		< 0.001*	0.055	0.012*
TB				
Correlation coefficient	0.343	1	-0.193	-0.059
p values	< 0.001*		0.036*	0.524
TIL				
Correlation coefficient	-0.176	-0.193	1	0.453
p values	0.055	0.036*		< 0.001*
CLR				
Correlation coefficient	-0.230	-0.059	0.453	1
p values	0.012*	0.524	< 0.001*	

LNR: Lymph node ratio; TB:Tumor budding; TILs: Tumor-infiltrating lymphocytes; CLR: Crohn's-like lymhphoid reaction;*statistically significant.

There was a statistically significant difference in LNR between TB groups with a medium effect size ($p<0.001$, $\varepsilon^2=0.127$) (Table 2). The LNR rate was significantly higher in cases with high TB than in cases with low TB with a large effect size ($p<0.001$, $\eta^2= 0.18$). The LNR also significantly increased in cases with high TB than in cases with moderate TB with a medium effect ($p= 0.036$, $\eta^2= 0.13$) (Table 3).

LNR was lower in cases with marked TILs, but this was not statistically significant ($p= 0.09$) (Table 2). The pairwise comparisons of TIL groups are given in Table 3. Figure 1 illustrates low and high TILs in CRC.

The LNR significantly differed between the CLR groups with a small effect ($p= 0.03$, $\varepsilon^2=0.04$) (Table 2). LNR was significantly lower in tumors with marked CLR than those without CLR with a large effect ($p= 0.04$, $\eta^2=0.24$) (Table 3). Figure 2 illustrates moderate and marked CLR in CRC.

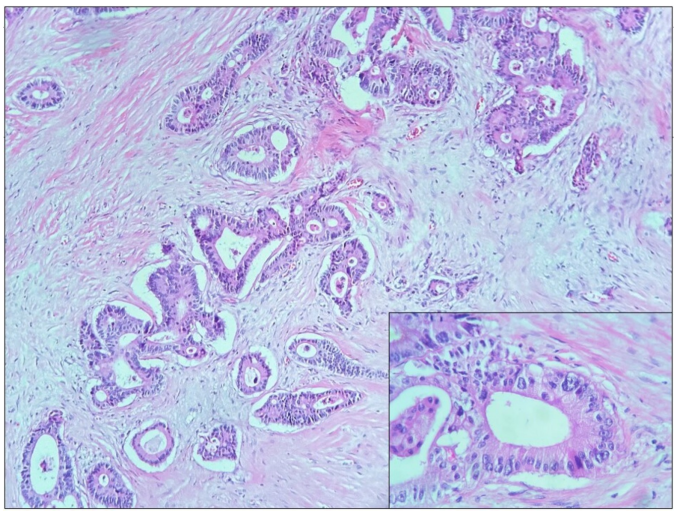
There was a significant positive correlation between LNR and TB and a slightly significant negative correlation between LNR and TILs and CLR (Table 4).

There was no significant difference in survival between various LNR groups.

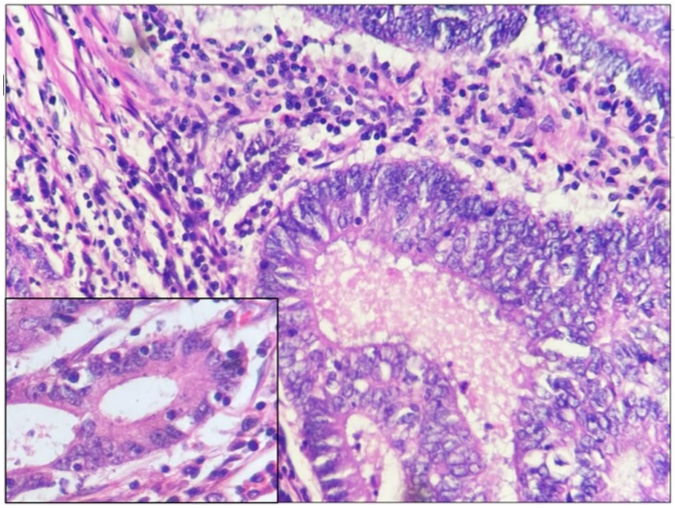
Discussion

The current study demonstrated that LNR is statistically higher in tumors with high TB and significantly lower in tumors with marked CLR. There was a statistically significant positive correlation between LNR and TB, and a significant negative correlation between LNR and TILs & CLR.

As the number of metastatic lymph nodes is a well-known prognostic factor in CRCs, optimal surgical resection and macroscopic evaluation of the lymph nodes are crucial.

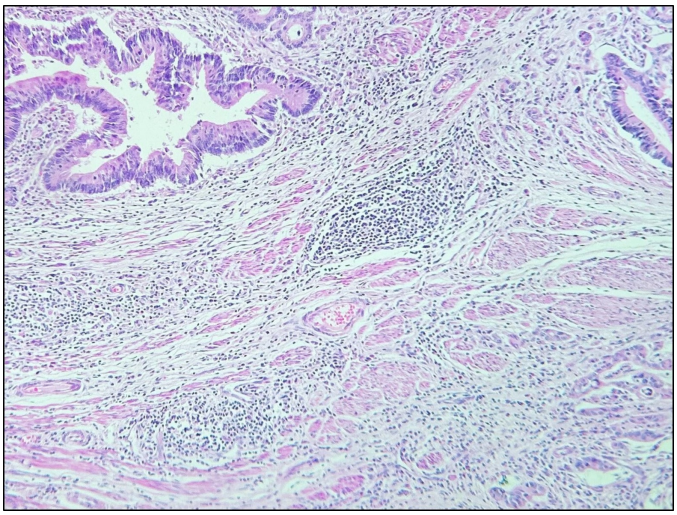


(a) A low density of tumor-infiltrating lymphocytes within the tumor (H&E,x100, insets x400).

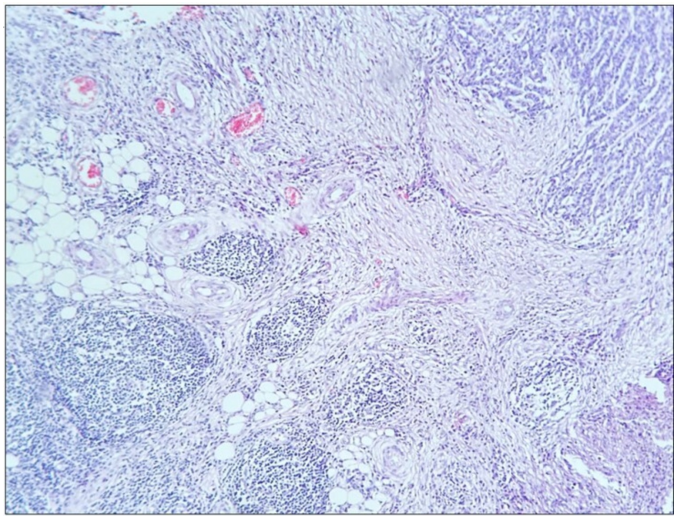


(b) A significant increase in tumor-infiltrating lymphocytes within the tumor (H&E, x400, insets x600).

Figure 1. Low and high TILs in CRC.



(a) A moderate lymphoid aggregation at the advancing edge of the tumor (H&E, x100).



(b) A marked increase in aggregates (including lymphoid follicles) was observed at the advancing edge of the tumor (H&E, x100).

Figure 2. Moderate and marked CLR in CRC.

The length of the sample shows substantial variability depending on the location of the tumor, margin status, and patient characteristics [10]. Although the longer resection materials had more lymph nodes than shorter ones, the total number of lymph nodes didn't differ significantly between long and short specimens in our study. Additionally, it is reported that the number of sampled lymph nodes was higher in younger patients [11]. Compatible with that, we found younger patients had a significantly higher number of lymph nodes than older patients.

The lymph node ratio is the percentage of metastatic lymph nodes to the total number of lymph nodes. As LNR predicts disease-free survival and overall survival, recent studies showed that LNR may be a better prognostic factor [4,12]. A higher LNR is associated with a poorer prognosis [13]. However, we couldn't find any statistically significant relationship between LNR and overall survival.

Tumor budding, single cells or small clusters at the invasive front of the tumor, are reported in a large variety of cancers. After the recommendation of the Tumor Budding Consensus Conference, TB is included in many guidelines. Growing evidence suggests that TB might be a morphologic mirror of epithelial-mesenchymal transition at the invasive front associated with a stem cell-like phenotype [14]. Therefore, the presence of TBs is tightly associated with poor prognostic factors such as lymph node metastasis, recurrence, and distant metastasis [15]. Supporting these findings, we found LNR is statistically higher in tumors with high TB rates and there is a statistically significant positive correlation between LNR and TB.

The tumor-infiltrating lymphocytes within and around the tumor and the presence of lymphoid aggregates, often with germinal centers, surrounding the tumor are indicators of our immune response to the cancer [16]. Despite some evidence indicating that some inflammatory cells might be recruited by tumors to avoid immune attacks, generalized TILs are reported to correlate with good prognosis in CRCs [17]. Many studies showed that TILs in primary tumors are associated with decreased metastasis to the local lymph nodes and spread of the tumor [7,18]. In compliance with the literature, we found that the tumors with a high ratio of TILs have a reduced number of LNR. How-

ever, we couldn't find any relationship between LNR and different rates of TILs.

Besides TILs, the presence of CLR is also reported to be related to better clinical outcomes [19]. An increased CLR is correlated with low local recurrence and distant metastasis [20]. Supportingly, the current study, as the first study in the literature, revealed that increased CLR was significantly related to low LNR. Despite the wide acceptance of the prognostic importance of TILs, and CLR, there are no precisely standardized evaluation criteria. Some studies showed that CLR size, count, and density have different significance levels in predicting survival in CRC [21]. Given the importance of these parameters in predicting prognosis, further studies might focus on a standardized and combined approach to evaluate them.

Conclusion

As far as we know, this is the first study in the literature comparing LNR, TILs, and CLR. Our findings exhibit that a high LNR combined with low TILs & CLR and a high TB indicates a poor prognosis, reflecting ineffective immune containment and increasing the metastatic capacity of the tumor. Understanding the relationship between these parameters can be helpful for risk stratification and tailoring treatment approaches in CRC patients.

Ethical approval

Approval for this study was obtained from the Ankara Bilkent City Hospital Clinical Research Ethics Committee (Approval number: TABED 1-24-513).

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