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Clinicopathologic correlates of tumor deposits, lymph node harvest, lymph node metastasis, and lymph node ratio in colorectal cancer: The importance of mucinous tumor histology

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Abstract

Introduction: The presence of tumor deposits (TDs), number of harvested lymph nodes (LNs), LN metastasis, and LN ratio (LNR) are well-known prognostic factors in colorectal carcinoma.

Materials and methods: The relationship between clinicopathologic parameters and the presence of TDs, number of harvested LNs, LN metastasis, and LNR was investigated in 278 consecutive patients who underwent surgery for colorectal adenocarcinoma.

Results: Multivariate logistic regression analysis revealed that mucinous tumor histology and lymphovascular invasion (LVI) were independent risk factors for the presence of TD (p=0.016 and p=0.003, respectively). Age, tumor localization, and pT stage were statistically significant parameters affecting harvested LN number (p=0.002, p=0.007, and p=0.028, respectively). Only LVI was an independent risk factor for LN metastasis (p=0.001), whereas advanced age, mucinous tumor histology, and LVI were independent risk factors for higher LNR (p=0.033, p=0.042, and p=0.001, respectively).

Conclusions: Our results revealed that mucinous tumor histology was an independent risk factor for the presence of TDs and higher LNR. In addition, LVI - which is a well-known prognostic factor- was also an independent risk factor for the presence of TD and higher LNR. Older age was found to be an independent risk factor for higher LNR. We found that the number of harvested lymph nodes was increased when the patients were younger, the tumor was located on the right colon, and the pT stage was advanced. These results have to be proven in larger cohorts with follow-up data.

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Introduction

Colorectal cancer (CRC) is the third most common cancer in both men and women, with an estimated 1.80 million new cases and the fourth main cause of cancer death with 881,000 deaths in 2018 [1]. The outcome is primarily dependent on the tumor stage [2], which is based on the assessment of the anatomic extent of the disease at the time of diagnosis [3], as well as other prognostic factors, which are not included in the tumor-node-metastasis (TNM) staging [4].

The presence of tumor deposits (TDs) is associated with a poor prognosis [5], and its presence is defined by a specific TNM sub classification. In the clinical setting, they are considered as the transition between lymph node involvement and metastatic disease development [2]. Although studies on the mechanisms of occurrence of TDs and their morphological relations (i.e.: lymphovascular and perineural invasions) have been carried out to date [5], we could not find a study on whether TDs are associated with a particular histological subtype in the English literature we searched.

A certain number of harvested lymph nodes (LNs) is crucial for the accurate staging of CRC patients. The total number of harvested LNs is affected by several factors [6], and the number of metastatic LNs is an important parameter in decision making for adjuvant chemotherapy or radiotherapy [7]. The LN ratio (LNR), which is the ratio of metastatic LNs divided by total examined LNs, combines these two parameters and is a valuable independent prognostic indicator for several solid tumors [8]. In the two last decades, several studies have shown that information about the number of positive LNs and its relation to harvested lymph nodes would allow better stratification allowing to tailor adjuvant therapy or the intensity of follow-up [7,8]. However, the histopathological parameters affecting LNR have not been clearly revealed yet.

In this study, we investigated the relationship between clinicopathologic parameters and TDs, number of harvested LNs, LN metastasis and LNR in 278 patients with colorectal carcinoma and aimed to search which histopathological parameters would affect them as an independent factor.

Materials and methods

Ethical approval

According to the ethical standards in Turkey, ethics committee approval and informed patient consent were not necessary due to the retrospective design of the study.

Patients

This study included 278 consecutive patients aged 28–94 years who had undergone surgery for CRC at two institutions (Institution A: January 2011-January 2016, and Institution B: February 2016-December 2018; Table 1). The exclusion criteria for resections were inflammatory bowel disease, familial polyposis syndrome, non-invasive malignancies, other carcinoma types (e.g., neuroendocrine, medullary, hepatoid, adenosquamous, and squamous), and stromal tumors. The data including basic demographic and clinicopathologic information (age, gender, neoadjuvant chemotherapy, surgical technique, length of resected specimen, status of mesorectum, tumor localization [right-left], tumor size, pT stage, LN metastasis, tumor histology, tumor grade, tumor multicentricity, and number of dissected LNs) were extracted from the database of both pathology clinics and institutions.

Pathologic evaluation

All surgical resection specimens were fixed in formaldehyde for a minimum of 2 days. The gross examination and lymph node harvest were performed following the same protocol and under the supervision of our expert pathologist at the two centers. The size of the carcinoma was represented by the largest dimension measured during gross examination [9]. For tumors smaller than 2 cm, the entire tumor was sampled. For larger tumors, at least 1 section per cm with deepest invasion was assessed [7]. Tumors were considered multicentric if normal colonic mucosa was present between tumor masses. The completeness of the mesorectal resection was evaluated according to CAP protocols [10]. The LNs were mainly retrieved by palpation by pathology residents or pathologists. Second and third LN searches were performed after alcohol fixation if the number of harvested LNs was < 12. The sections were processed and embedded in paraffin using standard techniques. The tumor was diagnosed as mucinous carcinoma (MC) if more than 50% of the tumor volume was composed of mucin [11]. The histological tumor grade was evaluated based on the proportion of gland formation and solid components [4]. Tumor necrosis was defined as the presence of microscopic coagulative tumor necrosis. TDs were defined as discrete lesions in pericolic or perirectal fat away from the leading edge of the tumor that showed no evidence of residual LN tissue but were within the lymphatic drainage of the carcinoma [12]. Peritumoral lymphocytic reaction and Crohn's-like lymphoid reaction were evaluated according to the literature [13-15]. Lymphovascular invasion (LVI) was considered tumor involvement of thin-walled structures lined by endothelium including lymphatics, capillaries, and postcapillary venules [16]. Perineural invasion (PNI) was considered present when there were tumor cells within any layer of the nerve sheath [17]. The LNR was defined as the ratio of positive nodes to total number of examined nodes [8].

Immunohistochemistry (IHC)

For the MMR protein IHC, only one representative block of tumor tissue was used. A section of 4 µm were cut from paraffin-embedded blocks and stained with the following antibodies using the Leica BOND-MAX Detection System (Leica Biosystems, Wetzlar, Germany) according to the manufacturer's instructions: MLH1 (Novocastra, clone ES505, 1:50), PMS2 (Novocastra, clone MOR4G, 1:100), MSH2 (Novocastra, clone 25D12, undiluted), and MSH6 (Novocastra, clone PU29, 1:100). Immunostaining procedure was performed on a (Leica Bond-Max) device after slides were incubated at 80°C for 3 hours. Bond-Dewak solution was applied for 10 minutes at 60°C, slides were then deparaffinized and rehydrated through graded ethanol solutions. Antibody retrieval was carried out by applying Epitope Retrieval Solution 2 at 98°C for 20 minutes, followed by H2O2 blocking for 10 minutes at room temperature. The primary antibody was applied for 30 minutes, then it was washed and secondary antibody was applied for 8 minutes at room temperature. DAB was used as a chromogen and hematoxylin was used for counterstaining.

The IHC stained slides were assessed by two of the participants in the study (G.K., R.B.G.) separately. Cases showing a disagreement were reviewed by the 2 pathologists using a double-headed microscope to reach a consensus. For MMR proteins (MLH1, PMS2, MSH2, MSH6), loss of expression in tumor cells was defined as complete absence of nuclear staining with positive nuclear staining of external and internal nonneoplastic controls (stromal, inflammatory, or nonneoplastic epithelial cells) [18].

Statistical analysis

We used the statistical software IBM SPSS Statistics 22 (IBM SPSS, Turkey) for statistical analysis. To compare qualitative/quantitative parameters, the Student's *t*-test, Fisher's exact test, χ^2 test with Yates' correction for continuity, and Fisher–Freeman–Halton test were performed. $P < 0.05$ were considered statistically significant. The end points of the current study were the presence of TDs, LN metastasis, harvested LN number and LNR. Multivariate logistic regression models were used to assess the relationship between clinicopathologic factors and outcomes. Only N(+) cases were selected for LNR evaluation.

Results

A total of 278 patients comprising 161 males (57.9%) and 117 females (42.1%) with a mean age of 64.5 (range: 28–94) years were included in this study. A standard open surgical technique was applied for 263 patients (94.6%), whereas 15 patients (5.4%) underwent laparoscopic surgery. The mean size of length of specimen (LOS) was 28 cm (range: 3.7–167 cm). We subdivided all specimens into two subgroups according to their length: ≤28 cm and >28 cm. The mesorectal resection was complete in 99 cases (93%) and incomplete in 8 cases (7%). Nine cases (3.2%) were pT1, 15 cases (5.3%) were pT2, 210 cases (75.5%) were pT3, and 44 cases (15.8%) were pT4. Tumor localization was divided into two groups: right colon 32% (n= 89), and left colon 68% (n=189). Median tumor size was 5 cm (mean: 5.2, range 0.8–17). A total of 11 cases (4%) were multicentric, 33 cases (11.9%) were MC, and 245 cases (88.1%) were invasive adenocarcinoma not otherwise specified (NOS). Among all adenocarcinoma NOS cases, 25 (10.2%) were well differentiated and 13 (5.3%) were poorly differentiated. Peritumoral and Crohn's-like lymphoid reaction were absent in the majority of cases (138 [49.6%] and 215 [77.3%], respectively). Tumor necrosis was present in 159 cases (57.4%), LVI was present in 100 cases (36%), and perineural invasion was present in 113 cases (40.6%). Concurrent loss of MLH1 and PMS2 was detected immunohistochemically in 18 cases (6.5%). Other isolated and combinations of loss of expression were detected in nine cases (six cases with concurrent loss of MSH2 and MSH6, one case with isolated loss of MLH1, one case with isolated loss of PMS2, and one case with isolated loss of MSH6). Thirty patients (10.8%) received neoadjuvant chemotherapy. TDs were present in 56 cases (20%). A lower limit of 12 LNs harvested in the colon cancer is recommended for accurate staging [6]. We harvested ≥ 12 LNs in 244 cases (88%). The maximum number of harvested LNs was 80 and the minimum number was 3. There was at least one metastatic LN in 118 cases. We analyzed the ratio of positive LNs to total examined nodes. The mean value was 0.22. We studied the LNR as a categorical variable rather than a continuous one and categorized the LNR groups as <0.22 and ≥0.22.

Comparison of the two institutions regarding practice of surgery and pathology

There were no statistically significant differences between the two institutions regarding the practice of surgery or pathology (Table 1).

Comparison of mucinous and non-mucinous tumors in terms of clinicopathologic parameters

We compared the clinicopathologic features of mucinous tumors versus non-mucinous tumors (Table 2). We observed that mucinous tumors were more commonly located in the right colon ($p = 0.011$) and were larger compared with non-mucinous tumors ($p = 0.035$). In addition, we saw tumor necrosis more often in non-mucinous tumors than in MCs ($p = 0.001$). Loss of MMR proteins, on the other hand, was more common in mucinous tumors than in non-mucinous tumors ($p = 0.042$), and TDs had a higher frequency in MC than in adenocarcinoma NOS ($p = 0.013$). We also noted a LNR ≥ 0.22 more often in MC than in adenocarcinoma NOS, but the difference was only marginally significant ($p = 0.067$).

Relationship of clinicopathologic parameters with the presence of TDs

On univariate analysis (Table 3), pT stage, the mucinous tumor histology and LVI were significantly associated with the presence of TDs (p=0.049, p=0.025 and p=0.001, respectively). There was no significant correlation between age, gender, type of surgery, LOS, mesorectum status, tumor localization, tumor size, tumor multicentricity, tumor grade, peritumoral lymphocytic reaction, Crohn's-like lymphoid reaction, tumor necrosis, PNI, MMR protein status, neoadjuvant chemotherapy, and the presence of TD. The multivariate logistic regression analysis revealed that mucinous tumor histology and LVI were independent risk factors for the presence of TDs (odds ratio [OR]=2.693, 95% confidence interval [CI]=1.199-6.047, p=0.016 and OR=2.546, 95% CI=1.377–4.707, p= 0.003, respectively). Advanced pT stage was not an independent risk factor for the presence of TD (OR=4.127, 95% CI=0.530-32.129, p=0.176, Table 3).

Assessment of clinicopathologic parameters affecting the number of harvested LNs

On univariate analysis (Table 4), age, LOS, tumor localization, pT stage, and tumor size were significantly related to the number of harvested LNs. Patients <65 years had a higher number of harvested LNs (p=0.009). The LOS >28 cm and advanced pT stage were significantly correlated with ≥12 harvested LNs (p=0.005 and p=0.001, respectively). The number of harvested LNs was significantly higher in right-sided colon tumors than in left-sided colon tumors ($p = 0.001$). Compared with patients who had a tumor diameter <5cm, more patients with a tumor diameter ≥5 cm also had ≥ 12 harvested LNs (p=0.001). In all cases with loss of at least one MMR protein, ≥12 LNs were harvested. In MSS tumors, ≥12 LNs were harvested in 217 cases (86.4%) although the difference was not statistically significant. Gender, type of surgery, mesorectum status, tumor grade, tumor multicentricity, tumor histology, tumor grade, peritumoral lymphocytic reaction, Crohn's-like lymphoid reaction, tumor necrosis, LVI, PNI, and neoadjuvant chemotherapy were not significantly correlated with the number of harvested LNs. On multivariate logistic regression analysis age, tumor localization, and pT stage were statistically significant parameters affecting harvested LN number (OR=3.951, 95% CI=1.690-9.237, p=0.002; OR=7.667, 95% CI=1.723–34.111, p=0.007; and OR=3.381, 95% CI=1.141-10.018, p=0.028, respectively). LOS and tumor size were not independent risk factors for harvested LN number, but showed marginal significance (p=0.069, p=0.065, respectively; Table 4).

Table 1: Comparison of two institutions regarding practice of surgery and pathology.

		Institution		*p*
		A	B	
		n (%)	n (%)	
Age group (range: 28-94, mean: 64.5)	<65	83 (51.6%)	61 (52.1%)	[2]0.923
	≥65	78 (48.4%)	56 (47.9%)	
Gender	Male	97 (60.2%)	64 (54.7%)	[2]0.355
	Female	64(39.8%)	53 (45.3%)	
Type of surgery	Open	155 (96.3%)	108 (92.3%)	[1]0.149
	Laparoscopic	6 (3.7%)	9 (7.7%)	
LOS group (range: 3.7-167, mean: 28)	≤28	99 (61.5%)	73 (62.9%)	[2]0.840
	>28	62 (38.5%)	43 (37.1%)	
Mesorectum	Complete	59 (92.2%)	40 (93%)	[1]0.872
	Incomplete	5 (7.8%)	3 (7%)	
pT stage	≤2	12 (7.5%)	11 (9.4%)	[1]0.560
	>2	149 (92.5%)	106 (90.6%)	
Tumor multicentricity	Absent	157 (97.5%)	110 (94%)	[1]0.140
	Present	4 (2.5%)	7 (6%)	
Tumor deposit	Absent	128 (79.5%)	94 (80.3%)	[1]0.863
	Present	33 (20.5%)	23 (19.7%)	
Number of harvested LNs	<12	20 (12.4%)	14 (12%)	[2]0.909
	≥12	141 (87.6%)	103 (88%)	
LN metastasis	N0	90 (55.9%)	70 (59.8%)	[2]0.513
	N (+)	71 (44.1%)	47 (40.2%)	
LNR group	< 0.22	41 (57.7%)	34 (72.3%)	[2]0.107
	≥ 0.22	30 (42.3%)	13 (27.7%)	

*[1]Fisher's Exact Test [2]Chi-Square Test *p<0.05, LOS: Length of specimen , LN: Lymph node,LNR: Lymph node ratio*

Table 2: Relationship between clinicopathologic parameters and tumor histology.

		Tumor histology		
		Invasive adenocarcinoma NOS	Mucinous carcinoma	*p*
		n (%)	n (%)	
Age group (range: 28-94, mean: 64.5)	<65	129 (52.6%)	15 (45.4%)	[2]0.437
	≥65	116 (47.3%)	18 (54.5%)	
Gender	Male	142 (57.9%)	19 (57.5%)	[2]0.967
	Female	103 (42%)	14 (42.4%)	
Type of surgery	Open	230 (93.8%)	33 (100%)	[1]0.144
	Laparoscopic	15 (6.1%)	0 (0%)	
LOS group (range: 3.7-167, mean: 28)	≤28	153 (62.4%)	17 (51.5%)	[2]0.308
	>28	92 (37.5%)	15 (45.4%)	
Mesorectum	Complete	89 (91.7%)	10 (100%)	[1]0.345
	Incomplete	8 (8.2%)	0 (0%)	
Tumor localization	Right colon	72 (29.3%)	17 (51.5%)	***[2]0.011****
	Left colon	173 (70.6%)	16 (48.4%)	

pT Stage	≤2	21 (8.5%)	2 (6.1%)	[2]0.623
	>2	224 (91.4%)	31 (93.9%)	
Tumor size (range: 0.8-17, median:5)	<5	122 (49.7%)	10 (30.3%)	*[2]0.035**
	≥5	123 (50.2%)	23 (69.6%)	
Tumor multicentricity	Absent	235 (95.9%)	32 (96.9%)	[1]0.771
	Present	10 (4%)	1 (3%)	
Peritumoral lymphocytic reaction	Absent	117 (47.7%)	21 (63.6%)	[3]0.270
	Mild	56(22.8%)	4 (12.1%)	
	Moderate	66 (26.9%)	8 (24.2%)	
	Marked	6 (2.4%)	0 (0%)	
Crohn's-like lymphocytic reaction	Absent	188 (76.7%)	27 (81.8%)	[3]0.460
	Mild	23 (9.3%)	3 (9%)	
	Moderate	32 (13%)	2 (6%)	
	Marked	2 (0.8%)	1 (3%)	
Tumor necrosis	Absent	89 (36.3%)	29 (87.8%)	*[2]0.001**
	Present	155 (63.2%)	4 (12.1%)	
LVI	Absent	157 (64%)	21 (63.6%)	[2]0.960
	Present	88 (35.9%)	12 (36.3%)	
PNI	Absent	141 (57.5%)	24 (72.7%)	[2]0.096
	Present	104 (42.4%)	9 (27.2%)	
MMR Proteins	No Loss	225 (91.8%)	26 (78.7%)	*[3]0.042**
	Loss of MLH1 and PMS 2	14 (5.7%)	4 (12.1%)	
	Others	6 (2.4%)	3 (9%)	
Neoadjuvant theraphy	Absent	218 (88.9%)	30 (90.9%)	[4]0.737
	Present	27 (11%)	3 (9%)	
Tumor deposit	Absent	201 (82%)	21 (63.6%)	*[4]0.013**
	Present	44 (17.9%)	12 (36.3%)	
Number of harvested LNs	<12	30 (12.1%)	4 (12.1%)	[2]0.984
	≥12	215 (87.7%)	29 (87.8%)	
LN metastasis	N0	145 (59.1%)	15 (45.4%)	[2]0.134
	N (+)	100 (40.8%)	18 (54.5%)	
LNR group	< 0.22	67 (67%)	8 (44.4%)	[2]0.067
	≥0.22	33 (33%)	10 (55.5%)	

*[1]Fisher's Exact Test- [2]Chi Square Test- [3]Fisher Freeman Halton Test- [4]Continuity (Yates) Correction *p<0.05*
LOS: Length of specimen, LVI: Lymphovascular invasion, PNI: Perineural invasion, MMR: Mismatch Repair LN: lymph node LNR: Lymph node ratio

Table 3: Univariate and multivariate analyzes of relationship between clinicopathologic parameters and the presence of tumor deposits.

		Tumor Deposit		p1+	p2++	OR+++	95 CI%++++
		Absent	Present				
		n (%)	n (%)				
Age group (range: 28-94, mean: 64.5)	<65	116 (52.3%)	28 (50%)	[2]0,763	-**	--	--
	≥65	106 (47.7%)	28 (%50)				

Gender	Male	133 (59.9%)	28 (50%)	[2]0.179	--**	--	--
	Female	89 (40.1%)	28 (50%)				
Type of surgery	Open	210 (94.6%)	53 (94.6%)	[1]1.000	--**	--	--
	Laparoscopic	12 (5.4%)	3 (5.4%)				
LOS group (range: 3.7-167, mean: 28)	≤28	137 (61.7%)	35 (63.6%)	[2]0.792	--**	--	--
	>28	85 (38.3%)	20 (36.4%)				
Mesorectum	Complete	78 (90.7%)	21 (100%)	[1]0.351	--**	--	--
	Incomplete	8 (9.3%)	0 (0%)				
Tumor localization	Right colon	71 (31.9%)	18 (32.1%)	[2]0.982	--**	--	--
	Left colon	151 (68%)	38 (67.8%)				
pT Stage	≤2	22 (9.9%)	1 (1.8%)	*[2]0.049**	0.176	4.127	0.530-32.129
	>2	200 (90.1%)	55 (98.2%)				
Tumor size (range: 0.8-17, median:5)	<5	106 (47.7%)	26 (46.4%)	[2]0.860	--**	--	--
	≥5	116 (52.3%)	30 (53.6%)				
Tumor multicentricity	Absent	214 (96.4%)	53 (94.6%)	[1]0.467	--**	--	--
	Present	8 (3.6%)	3 (5.4%)				
Tumor histology	Invasive adenocarcinoma NOS	201 (90.5%)	44 (78.6%)	*[4]0.025**	*0.016**	2.693	1.199-6.047
	Mucinous carcinoma	21 (9.5%)	12 (21.4%)				
Tumor grade	Well differentiated	18 (9%)	7 (15.9%)	[2]0.270	--**	--	--
	Moderately differentiated	173 (86.1%)	34 (77.3%)				
	Poorly differentiated	10 (5%)	3 (6.8%)				
Peritumoral lymphocytic reaction	Absent	107 (48.2%)	31 (55.4%)	[3]0.515	--**	--	--
	Mild	47 (21.2%)	13 (23.2%)				
	Moderate	62 (27.9%)	12 (21.4%)				
	Marked	6 (3%)	0 (0%)				
Crohn's-like lymphocytic reaction	Absent	168 (75.7%)	47 (83.9%)	[3]0.542	--**	--	--
	Mild	21 (9.5%)	5 (8.9%)				
	Moderate	30 (13.5%)	4 (7.1%)				
	Marked	3 (1.4%)	0 (0%)				
Tumor necrosis	Absent	96 (43.4%)	22 (39.3%)	[2]0.575	--**	--	--
	Present	125 (56.6%)	34 (60.7%)				
LVI	Absent	153 (68.9%)	25 (45%)	*[2]0.001**	*0.003**	2.546	1.377-4.707
	Present	69 (31.1%)	31 (55.4%)				
PNI	Absent	134 (60.4%)	31 (55.4%)	[2]0.496	--**	--	--
	Present	88 (39.6%)	25 (44.6%)				
MMR Proteins	No Loss	200 (90.1%)	51 (91.1%)	[3]0.789	--**	--	--
	Loss of MLH1 and PMS 2	14 (6.3%)	4 (7.1%)				
	Others	8 (3.6%)	1 (1.8%)				
Neoadjuvant theraphy	Absent	200 (90.1%)	48 (85.7%)	[4]0.483	--**	--	--
	Present	22 (9.9%)	8 (14.3%)				

*[1]Fisher's Exact Test [2]Chi Square Test, [3]Fisher Freeman Halton Test, [4]Continuity (Yates) Correction, *p<0.05 **for p values >0.05 obtained on univariate analysis; multivariate logistic regression models were not applied LOS: Length of specimen, LVI: Lymphovascular invasion, PNI: Perineural invasion, MMR: Mismatch Repair.*

[+] p1:Univariate analysis significance value, [++] p2:Multivariate logistic regression analysis significance value, [+++]OR: Odds ratio, [++++] CI: Confidence interval.

Table 4: Univariate and multivariate analyzes of relationship of clinicopathologic parameters with the number of harvested lymph nodes.

		Harvested lymph node		p1[+]	p2[++]	OR[+++]	95 CI%[++++]
		<12	≥12				
		n (%)	n (%)				
Age group (range: 28-94, mean: 64.5)	<65	10(29.4%)	134(54.9%)	[2]*0.009**	*0.002**	3.951	1.690-9.237
	≥ 65	24(70.6%)	110(45.1%)				
Gender	Male	25 (73.5%)	136 (55.7%)	[2]0.075	--**	--	--
	Female	9 (26.5%)	108 (44.3%)				
Type of surgery	Open	31 (91.2%)	232 (95.1%)	[1]0.407	--**	--	--
	Laparoscopic	3 (8.8%)	12 (4.9%)				
LOS (range: 3.7-167, mean: 28)	≤28	29 (85.3%)	143 (58.8%)	[2]*0.005**	0.069	2.672	0.927-7.701
	>28	5 (14.7%)	100 (41.2%)				
Mesorectum	Complete	16 (88.9%)	83 (93.3%)	[1]0.619	--**	--	--
	Incomplete	2 (11.1%)	6 (6.7%)				
Tumor localization	Right colon	2 (5.8%)	87 (35.6%)	[4]*0.001**	*0.007**	7.667	1.723-34.111
	Left colon	32 (94.1%)	157 (64.3%)				
pT stage	≤2	8 (23.5%)	15 (6.1%)	[1]*0.001**	*0.028**	3.381	1.141-10.018
	>2	26 (76.5%)	229 (93.6%)				
Tumor size (range: 0.8-17, median:5)	<5	25 (73.5%)	107 (43.9%)	[2]*0.001**	0.065	2.298	0.948-5.567
	≥5	9 (26.5%)	137 (56.1%)				
Tumor multicentricity	Absent	33 (97.1%)	234 (95.9%)	[1]1.000	--**	--	--
	Present	1 (2.9%)	10 (4.1%)				
Tumor histology	Invasive adenocarcinoma NOS	30 (88.2%)	215 (88.1%)	[1]1.000	--**	--	--
	Mucinous carcinoma	4 (11.8%)	29 (11.9%)				
Tumor grade	Well differentiated	2 (6.7%)	23 (10.7%)	[3]0.916	--**	--	--
	Moderately differentiated	27 (90%)	180 (83.7%)				
	Poorly differentiated	1 (3.3%)	12 (5.6%)				
Peritumoral lymphocytic reaction	Absent	17 (50%)	121 (49.6%)	[4]0.704	--**	--	--
	Mild	9 (26.5%)	51 (20.9%)				
	Moderate	8 (23.5%)	66 (27%)				
	Marked	0 (0%)	6 (2.5%)				
Crohn's-like lymphocytic reaction	Absent	31 (91.2%)	184 (75.4%)	[3]0.279	--*	--	--
	Mild	1 (2.9%)	25 (10.2%)				
	Moderate	2 (5.9%)	32 (13.1%)				
	Marked	0 (0%)	3 (1.2%)				
Tumor necrosis	Absent	16 (47.1%)	102 (42%)	[2]0.707	--**	--	--
	Present	18 (52.9%)	141 (58%)				
LVI	Absent	23 (67.6%)	155 (63.5%)	[2]0.781	--**	--	--
	Present	11 (32.4%)	89 (36.5%)				
PNI	Absent	21 (61.8%)	144 (59%)	[2]0.905	--**	--	--
	Present	13 (38.2%)	100 (41%)				

MMR Proteins	No Loss	34 (100%)	217 (88.9%)	[3]0.202	--**	--	--
	Loss of MLH1 and PMS 2	0 (0%)	18 (7.4%)				
	Others	0 (0%)	9 (3.7%)				
Neoadjuvant therapy	Absent	27 (79.4%)	221 (90.6%)	[1]0.071	--**	--	--
	Present	7 (20.6%)	23 (9.5%)				

*[1]Fisher's Exact Test [2]Continuity (Yates) Correctio [3]Fisher Freeman Halton Test [4]Chi Square Test *p<0.05 **for p values >0.05 obtained on univariate analysis; multivariate logistic regression models were not applied LOS: Length of specimen. LVI: Lymphovascular invasion. PNI: Perineural invasion. MMR: Mismatch Repair.*

[+]p1:Univariate analysis significance value, [++]p2:Multivariate logistic regression analysis significance value, [+++] OR: Odds Ratio, [++++] CI: Confidence interval.

Relationship between clinicopathologic parameters and LN metastasis

Univariate analysis revealed that male gender, advanced pT stage, the presence of LVI, and PNI were significantly correlated with LN metastasis (p=0.033, p=0.001, p=0.001, and p=0.001, respectively; Table 5). Although not statistically significant, 18 of 33 patients (54%) with MC were in the N(+) group, whereas 100 of 245 patients (40.8%) with invasive adenocarcinoma NOS were in the N(+) group (p=0.190). There was no significant correlation between age, type of surgery, LOS, mesorectum status, tumor localization, tumor size, tumor multicentricity, tumor histology, tumor grade, peritumoral lymphocytic reaction, Crohn's-like lymphoid reaction, tumor necrosis, MMR protein status, and LN metastasis. Multivariate logistic regression analysis revealed that only presence of LVI was an independent risk factor for LN metastasis (OR=14.664, 95% CI=7.697-27.934, p=0.001). The presence of PNI was not statistically significant parameter for LN metastasis (p=0.125). There was marginal significance between male gender, advanced pT stage and LN metastasis (OR=1.770, 95% CI=0.947-3.307, p=0.073; OR=3.926, 95% CI=0.799-19.295, p=0.092, respectively; Table 5).

Relationship between clinicopathologic parameters and LNR group

On univariate analysis, we found significant relationships among age, mucinous tumor histology, LVI, and LNR group (p=0.018, p=0.022, p=0.003, respectively; Table 6). Gender, type of of surgery, LOS, mesorectum status, pT stage, tumor localization, tumor size, tumor multicentricity, tumor grade, peritumoral lymphocytic reaction, Crohn's-like lymphoid reaction, tumor necrosis, PNI, MMR protein status, and neoadjuvant chemotherapy did not significantly correlate with LNR. Multivariate logistic regression analysis revealed that advanced age, mucinous tumor histology, and presence of LVI were independent risk factors for higher LNR (OR= 2.449, 95% CI= 1.074–5.585, p=0.033; OR=3.335, 95% CI=1.045-10.767, p= 0.042; OR=6.190, 95% CI= 2.013-19.031, p= 0.001, respectively; Table 6).

Table 5: Univariate and multivariate analyzes of relationship between clinicopathologic parameters and lymph node metastasis.

		N Stage		p1[+]	p2[++]	OR[+++]	95 CI%[++++]
		N0	N(+)				
		n (%)	n (%)				
Age group (range: 28-94, mean: 64.5)	<65	79 (49.4%)	65 (55.1%)	[2]0,346	--**	--	--
	≥65	81 (50.6%)	53 (44.9%)				
Gender	Male	84 (52.5%)	77 (65.3%)	*[2]0.033**	0.073	1.770	0.947-3.307
	Female	76 (47.5%)	41 (34.7%)				
Type of surgery	Open	148 (92.5%)	115 (97.5%)	[1]0.124	--**	--	--
	Laparoscopic	12 (7.5%)	3 (2.5%)				
LOS group (range: 3.7-167, mean: 28)	≤28	101 (63.1%)	71 (60.7%)	[2]0.679	--**	--	--
	>28	59 (36.9%)	46 (39.3%)				
Mesorectum	Complete	55 (91.7%)	44 (93.6%)	[3]1.000	--**	--	--
	Incomplete	5 (8.3%)	3 (6.4%)				
Tumor localization	Right colon	51 (57.3%)	38 (23.2%)	[1]0.954	--**	--	--
	Left colon	38 (42.7%)	80 (67.8%)				
pT Stage	≤2	21 (13.1%)	2 (1.7%)	*[3]0.001**	0.092	3.926	0.799-19.295
	>2	139 (86.9%)	116 (98.3%)				
Tumor size (range: 0.8-17, median:5)	<5	79 (49.4%)	53 (44.9%)	[2]0.462	--**	--	--
	≥5	81 (50.6%)	65 (55.1%)				

Tumor multicentricity	Absent	154 (96.3%)	113 (95.8%)	[3]1.000	--**	--	--
	Present	6 (3.8%)	5 (4.2%)				
Tumor histology	İnvasive adenocarcinoma NOS	145 (90.6%)	100 (84.7%)	[1]0.190	--**	--	--
	Mucinous carcinoma	15 (9.4%)	18 (15.3%)				
Tumor grade	Well differentiated	14 (9.7%)	11 (11%)	[2]0.565	--**	--	--
	Moderately differentiated	125 (86.2%)	82 (82%)				
	Poorly differentiated	6 (4.1%)	7 (7%)				
Peritumoral lymphocytic reaction	Absent	77 (48.1%)	61 (51.7%)	[4]0.894	--**	--	--
	Mild	36 (22.5%)	24 (20.3%)				
	Moderate	44 (27.5%)	30 (25.4%)				
	Marked	3 (1.9%)	3 (2.5%)				
Crohn's-like lymphocytic reaction	Absent	116 (72.5%)	99 (83.9%)	[4]0.098	--**	--	--
	Mild	17 (10.6%)	9 (7.6%)				
	Moderate	24 (15%)	10 (8.5%)				
	Marked	3 (1.9%)	0 (0%)				
Tumor necrosis	Absent	66 (41.3%)	52 (44.4%)	[2]0.595	--**	--	--
	Present	94 (58.8%)	65 (55.6%)				
LVI	Absent	142 (88.8%)	36 (30.5%)	***[1]0.001****	***0.001****	14.664	7.697-27.934
	Present	18 (11.3%)	82 (69.5%)				
PNI	Absent	110 (68.8%)	55 (46.6%)	***[2]0.001****	**0.125**	1.542	0.824-2.886
	Present	50 (31.3%)	63 (53.4%)				
MMR Proteins	No Loss	141 (%88,1)	110 (%93,2)	[2]0.314	--**	--	--
	Loss of MLH1 and PMS 2	12 (7.5%)	6 (5.1%)				
	Others	7 (4.4%)	2 (1.7%)				
Neoadjuvant therapy	Absent	143 (89.4%)	105 (89%)	[1]1.000	--**	--	--
	Present	17 (10.6%)	13 (11%)				

*[1]Continuity (Yates) Correction [2] Chi Square Test [3]Fisher's Exact Test [4]Fisher Freeman Halton Test *p<0.05 **for p values >0.05 obtained on univariate analysis; multivariate logistic regression models were not applied LOS: Length of specimen, LVI: Lymphovascular invasion, PNI: Perineural invasion, MMR: Mismatch Repair.*

[+]p1:Univariate analysis significance value, [++]p2:Multivariate logistic regression analysis significance value, [+++] OR: Odds Ratio, [++++] CI: Confidence interval.

Table 6: Univariate and multivariate analyzes of relationship between clinicopathologic parameters and lymph node ratio.

		LNR		***p1*[+]**	***p2*[++]**	**OR[+++]**	***95 CI%[++++]***
		<0.22	**≥0.22**				
		n (%)	**n (%)**				
Age group (range: 28-94, mean: 64.5)	<65 ≥65	49 (63.6%) 28 (36.4%)	16 (39%) 25 (61%)	*[2]0.018**	*0.033**	2.449	1.074-5.585
Gender	Male	50 (64.9%)	27 (65.9%)	[2]1.000	--**	--	--
	Female	27 (35.1%)	14 (34.15)				
Type of surgery	Open Laparoscopic	74 (96.1%) 3 (3.9%)	41 (100%) 0 (0%)	[1]0.551	--**	--	--
LOS (range: 3.7-167, mean: 28)	≤28	44 (57.1%)	27 (67.5%)	[2]0.374	--**	--	--
	>28	33 (42.9%)	13 (32.5%)				
Mesorectum	Complete	30 (93.8%)	14 (93.3%)	[1]1.000	--**	--	--
	Incomplete	2 (6.3%)	1 (6.7%)				
Tumor localization	Right colon	25 (33.3%)	13 (30.2%)	[1]0.729	--**	--	--
	Left colon	50 (66.7%)	30 (69.8%)				
pT Stage	≤2	1 (1.3%)	1 (2.3%)	[3]0.688	--**	--	--
	>2	74 (98.7%)	42 (97.7%)				

Tumor size (range: 0.8-17, median:5)	<5	37 (48.1%)	16 (39%)	[2]0.457	--**	--	--
	≥5	40 (51.9%)	25 (61%)				
Tumor multicentricity	Absent	72 (93.5%)	41 (100%)	[1]0.162	--**	--	--
	Present	5 (6.5%)	0 (0%)				
Tumor histology	Invasive Adenocarcinoma NOS	70 (90.9%)	30 (73.2%)	*[2]0.022**	*0.042**	3.355	1.045-10.767
	Mucinous carcinoma	7 (9.1%)	11 (26.8%)				
Tumor grade	Well differentiated	8 (11.4%)	3 (10%)	[3]0.704	--**	--	--
	Moderately differentiated	58 (82.9%)	24 (80%)				
	Poorly differentiated	4 (5.7%)	3 (10%)				
Peritumoral lymphocytic reaction	Absent	42 (54.5%)	19 (46.3%)	[3]0.854	--**	--	--
	Mild	14 (18.2%)	10 (24.4%)				
	Moderate	19 (24.7%)	11 (26.8%)				
	Marked	2 (2.6%)	1 (2.4%)				
Crohn's-like lymphocytic reaction	Absent	64 (83.1%)	35 (85.4%)	[3]0.739	--**	--	--
	Mild	7 (9.1%)	2 (4.9%)				
	Moderate	6 (7.8%)	4 (9.8%)				
Tumor necrosis	Absent	32 (%42,1)	20 (%48,8)	[4]0.488	--**	--	--
	Present	44 (57.9%)	21 (51.2%)				
LVI	Absent	31 (40.3%)	5 (12.2%)	*[2]0.003**	*0.001**	6.190	2.013-19.031
	Present	46 (59.7%)	36 (87.8%)				
PNI	Absent	39 (50.6%)	16 (%39)	[2]0.312	--**	--	--
	Present	38 (49.4%)	25 (%61)				
MMR Proteins	No loss	71 (92.2%)	39 (95.1%)	[3]0.846	--**	--	--
	Loss of MLH1 and PMS 2	4 (5.2%)	2 (4.9%)				
	Others	2 (2.6%)	0 (0%)				
Neoadjuvant therapy	Absent	67 (87%)	38 (92.7%)	[1]0.538	--**	--	--
	Present	10 (13%)	3 (7.3%)				

*[1]Fisher's Exact Test [2]Continuity (Yates) Correctio [3]Fisher Freeman Halton Test[4]Chi-Square Test *p<0.05 **for p values >0.05 obtained on univariate analysis; multivariate logistic regression models were not applied LNR: Lymph node ratio, LOS: Length of specimen, LVI: Lymphovascular invasion, PNI: Perineural invasion, MMR: Mismatch Repair*

[+]p1:Univariate analysis significance value,[++]p2:Multivariate logistic regression analysis significance value, [+++] OR: Odds Ratio, [++++] CI: Confidence interval

Discussion

Although a new era has begun with the molecular data obtained from CRCs, the pathologic assessment of resected specimens still remains the strongest predictor of survival for patients [11]. We performed this study to identify the relationships between clinicopathologic parameters and factors with prognostic implications on outcome such as TDs, number of harvested LNs, LN metastasis, and LNR.

Although the definition has been changed over the years, TD is a well-known factor for a poor prognosis in colorectal carcinomas [5]. Many studies have confirmed its prognostic significance. Bouquot *et al.* suggested that patients with TDs should be included among high-risk patients for whom adjuvant chemotherapy should be considered [19]. Al Sahaf *et al.* proposed that TDs should be in the metastasis category [20]. According to our research of the English literature only one study has assessed the relationship between clinicopathologic parameters and TDs. Puppa *et al.* found that tumor grade, pT stage, pN stage, number of positive LNs, vascular invasion, and perineural invasion were significantly correlated with the presence of TDs [3]. Among all of the MCs included in their study, TDs were present in the majority of cases but because the total number of MCs was low, the authors might have failed to notice a possible significance [3]. In the current study, TDs were more frequently observed in MC than in adenocarcinoma NOS. Our statistical analysis revealed that the mucinous tumor histology and presence of LVI were independent risk factors for the presence of TDs. MC of the colon is described as a distinct entity with a histomorphologic appearance and genetic background [19-22]. The clinical and prognostic significance of this subtype still remains the subject of debate [22-28]. Our results showed that mucinous tumor histology was significantly correlated with TDs, which was well recognized as a poor prognostic factor in a large series [5]. To the best of our knowledge, the current study is the first to reveal the significance of mucinous tumor histology with the presence of TDs. LVI is an already known risk factor of TD formation [3,5,26]. Yamano *et al.* showed the statistically significant relationship between LVI and TDs [26]. Our results also revealed that LVI was an independent predictor for TD.

An adequate number of LNs must be harvested from a resection specimen for accurate staging [7,11,29]. The key quality measurements for colon cancer care in the United States are the presence of at least 12 LNs in surgical resection [7]. Several studies have shown that the number of harvested LNs is affected on a large scale by age. LN number recovered from younger patients was significantly higher than that from the older age group [6,7,9,11,29]. Our results on multivariate analysis also

showed that age was an independent variable that can influence the harvested LN number. Tekkis *et al.* proposed the reduced immunologic response and the inflammatory reaction as the possible reason for the decreased number of harvested LNs in older individuals [30].

Tumor localization is a factor that affects surgical procedures, and the longest specimens are those resected for transverse colon tumors. It is possible that a long-resected colon with a totally resected mesorectum and more fatty tissue will be removed. One would expect that a higher number of LNs could be found if a longer segment is resected. When a tumor is located in the right side of the colon, the specimen often contains more LNs [6]. In this study, LOS had marginal significance with the number of harvested LNs. Similar data were found in studies investigating the factors affecting the total number of harvested LNs [6,7]. Our data indicated that pT stage was independently correlated with the number of LNs retrieved. A deeper tumor may cause a more prominent antigenic immune reaction. Mekenkamp *et al.* also found a similar result among factors affecting the LN retrieval. The authors showed that the number of harvested LNs was correlated with invasion depth [29]. Tsai *et al.* also demonstrated that pT stage was independently associated with the number of harvested LNs [11].

LN metastasis is the most powerful prognostic factor and progresses disease stage directly from II to III regardless of pT stage [2,26]. The predictivity of LN metastasis increase with the number of examined LN number [9]. Our results revealed that LVI was an independent risk factor for LN metastasis. On the other hand, gender and pT stage showed marginal significance with LN metastasis on multivariate logistic regression analysis. Gender, whether by genetics and/or sex-hormones, is a well-known factor that affects immune responses [31,32]. Our univariate analyses revealed that LN metastasis was more frequent in males. In multivariate logistic regression analysis, the *p* value lost significance, but there was still marginal significance in favor of females for N status. These results may have been due to the limited number of cases of the current study; thus, a larger cohort may show a possible relationship between gender and LN metastasis. The current study revealed a marginal significance between pT stage and LN metastasis on multivariate logistic regression analysis. Shen *et al.* found that LN metastasis was strongly correlated with advancing tumor stages [7]. Rössler *et al.* also showed that increasing pT stage was related to LN metastasis [17].

The LNR is highly significant for patient survival in the literature [29]. In some studies, the total number of positive LNs no longer represents an independent prognostic indicator when the LNR is included in the regression model. The LNR provides superior prognostic significance compared to the number of positive LNs [8]. According to our results, LNR was affected independently by age, LVI, and mucinous tumor histology. Many studies have evaluated the prognostic significance of LNR, but only one investigated the relationship between clinicopathologic parameters including systemic inflammatory response and LNR [6,8,33,34]. Dolan *et al.* showed that higher pT stage and poorer tumor differentiation were independently related with LNR [34]. The current study is one of the first studies to examine the relationship between clinicopathologic parameters and the LNR.

This study had a couple of limitations. It was a retrospective study consisting of cohorts from two institutions. It also involved two surgeons that may have affected the surgeon-dependent factors for the harvest of lymph nodes.

Conclusion

Our results were interesting in terms of mucinous tumor histology. Mucinous tumor histology was an independent risk factor for the presence of TD and higher LNR, but this has to be proven in larger cohorts. According to our research of the English literature, this is the first study to show the significance of mucinous tumor histology with the presence of TD and higher LNR. Pathologists should be aware of this relationship and examine carefully colorectal resection specimens of MCs in terms of TDs. In addition, also LVI influenced independently the presence of TD and LNR, which are well-known prognostic factors. Older age was an independent risk factor for higher LNR. Young age, right tumor location, and advanced pT stage were factors which increased the number of harvested LNs. These results have to be proven in larger cohorts with long-term follow-up data.

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References

1. Araghi M, Soerjomataram I, Jenkins M, Brierley J, Morris E, Bray F, et al. Global trends in colorectal cancer mortality: projections to the year 2035. International journal of cancer. 2019; 144: 2992-3000.
2. Amin MB, Edge SB. American Joint Committee on Cancer. AJCC cancer staging manual. 8th ed. New York: Springer International Publishing. 2017.
3. Puppa G, Maisonneuve P, Sonzogni A, Masullo M, Capelli P, Chilosi M, et al. Pathological assessment of pericolonic tumor deposits in advanced colonic carcinoma: relevance to prognosis and tumor staging. Mod Pathol. 2007; 20: 843-55.
4. Bosman FT, Carneiro F, Hruban RH, et al. WHO classification of tumours of the Digestive system. 4th ed. Lyon: WHO Press. 2010.
5. Sarioglu S. Tumor Deposits. Cham: Springer International Publishing; 2018.
6. Stanisavljević L, Søndenaa K, Storli KE, Leh S, Nesvik I, Gudlaugsson E, et al. The total number of lymph nodes in resected colon cancer specimens is affected by several factors but the lymph node ratio is independent of these. APMIS. 2014; 122: 490-8.
7. Shen SS, Haupt BX, Ro JY, Zhu J, Bailey HR, Schwartz MR. Number of lymph nodes examined and associated clinicopathologic factors in colorectal carcinoma. Arch Pathol Lab Med. 2009; 133: 781-6.
8. Ceelen W, Van Nieuwenhove Y, Pattyn P. Prognostic Value of the Lymph Node Ratio in Stage III Colorectal Cancer: A Systematic Review. Ann Surg Oncol. 2010; 17: 2847–55.
9. Ng SK, Lu CT, Pakneshan S, Leung M, Siu S, Lam AK. Harvest of lymph nodes in colorectal cancer depends on demographic and clinical characteristics of the patients. Int J Colorectal Dis. 2018;33:19-22.
10. Kapiteijn E, Marijnen CA, Nagtegaal ID, Putter H, Steup WH, Wiggers T, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med. 2001; 345: 638-46.
11. Tsai HL, Huang CW, Yeh YS, Ma CJ, Chen CW, Lu CY, et al. Factors

affecting number of lymph nodes harvested and the impact of examining a minimum of 12 lymph nodes in stage I-III colorectal cancer patients: A retrospective single institution cohort study of 1167 consecutive patients. BMC Surg. 2016; 16: 17.

12. Egner JR. AJCC Cancer Staging Manual. JAMA. 2010; 304: 1726.

13. Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clin Cancer Res. 2009; 15: 6412-20.

14. Klintrup K, Mäkinen JM, Kauppila S, Väre PO, Melkko J, Tuominen H, et al. Inflammation and prognosis in colorectal cancer. Eur J Cancer. 2005; 41: 2645-54.

15. Väyrynen JP, Sajanti SA, Klintrup K, Mäkelä J, Herzig KH, Karttunen TJ, et al. Characteristics and significance of colorectal cancer associated lymphoid reaction. Int J Cancer 2014; 134: 2126-35.

16. Lim SB, Yu CS, Jang SJ, Kim TW, Kim JH, Kim JC. Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. Dis Colon Rectum. 2010; 53: 377-84.

17. Rössler O, Betge J, Harbaum L, Mrak K, Tschmelitsch J, Langner C. Tumor size, tumor location, and antitumor inflammatory response are associated with lymph node size in colorectal cancer patients. Mod Pathol. 2017; 30: 897-904.

18. Bartley AN, Hamilton SR, Alsabeh R, Ambinder EP, Berman M, Collins E, et al; Members of the Cancer Biomarker Reporting Workgroup, College of American Pathologists. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the colon and rectum. Arch Pathol Lab Med. 2014; 138: 166-70.

19. Bouquot M, Creavin B, Goasguen N, Chafai N, Tiret E, André T, et al. Prognostic value and characteristics of N1c colorectal cancer. Colorectal Dis. 2018; 20: O248-O255.

20. Al Sahaf O, Myers E, Jawad M, Browne TJ, Winter DC, Redmond HP. The prognostic significance of extramural deposits and extracapsular lymph node invasion in colon cancer. Dis Colon Rectum. 2011; 54: 982-8.

21. Ott C, Gerken M, Hirsch D, Fest P, Fichtner-Feigl S, Munker S, et al. Advanced Mucinous Colorectal Cancer: Epidemiology, Prognosis and Efficacy of Chemotherapeutic Treatment. Digestion. 2018; 98: 143-152.

22. Song GA, Deng G, Bell I, Kakar S, Sleisenger MH, Kim YS. Mucinous carcinomas of the colorectum have distinct molecular genetic characteristics. Int J Oncol. 2005; 26: 745-50.

23. Gonzalez RS, Cates JMM, Washington K. Associations among histological characteristics and patient outcomes in colorectal carcinoma with a mucinous component. Histopathology. 2019; 74: 406-14.

24. Zhang H, Evertsson S, Sun X. Clinicopathological and genetic characteristics of mucinous carcinomas in the colorectum. Int J Oncol. 1999; 14: 1057–1118.

25. Verhulst J, Ferdinande L, Demetter P, Ceelen W. Mucinous subtype as prognostic factor in colorectal cancer: a systematic review and meta-analysis. J Clin Pathol 2012; 65: 381-8.

26. Y Yamano T, Semba S, Noda M, Yoshimura M, Kobayashi M, Hamanaka M, et al. Prognostic significance of classified extramural tumor deposits and extracapsular lymph node invasion in T3-4 colorectal cancer: a retrospective single-center study. BMC Cancer. 2015; 15: 859.

27. Mekenkamp LJ, Heesterbeek KJ, Koopman M, Tol J, Teerenstra S, Venderbosch S, Punt CJ, et al. Mucinous adenocarcinomas: poor prognosis in metastatic colorectal cancer. Eur J Cancer. 2012; 48: 501-9.

28. Park JS, Huh JW, Park YA, Cho YB, Yun SH, Kim HC, et al. Prognostic comparison between mucinous and nonmucinous adenocarcinoma in colorectal cancer. Medicine. 2015; 94: e658.

29. Mekenkamp LJ, van Krieken JH, Marijnen CA, van de Velde CJ, Nagtegaal ID. Pathology Review Committee and the Co-operative Clinical Investigators. Lymph node retrieval in rectal cancer is dependent on many factors--the role of the tumor, the patient, the surgeon, the radiotherapist, and the pathologist. Am J Surg Pathol. 2009; 33: 1547-53.

30. Tekkis PP, Smith JJ, Heriot AG, Darzi AW, Thompson MR, Stamatakis JD; Association of Coloproctology of Great Britain and Ireland. A national study on lymph node retrieval in resectional surgery for colorectal cancer. Dis Colon Rectum. 2006; 49: 1673-83.

31. Trigunaite A, Dimo J, Jørgensen TN. Suppressive effects of androgens on the immune system. Cell Immunol. 2015; 294: 87-94.

32. Cook MB, Dawsey SM, Freedman ND, Inskip PD, Wichner SM, Quraishi SM, et al. Sex disparities in cancer incidence by period and age. Cancer Epidemiol Biomarkers Prev. 2009; 18: 1174-82.

33. Storli KE, Søndenaa K, Bukholm IR, Nesvik I, Bru T, Furnes B, et al. Overall survival after resection for colon cancer in a national cohort study was adversely affected by TNM stage, lymph node ratio, gender, and old age. Int J Colorectal Dis. 2011; 26: 1299-307.

34. Dolan RD, McSorley ST, Horgan PG, McMillan DC. Determinants of lymph node count and positivity in patients undergoing surgery for colon cancer. Medicine. 2018; 97: e0185.