

Surgical Resection Length and Harvested Lymph Nodes in Colon Cancer

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ABSTRACT

Objective: To determine the relationship between resected specimen length and tumor location, the number of LN harvested (LNh), and the positive LN ratio (LNR) in colon cancer.

Study Design: A descriptive study.

Place & Duration of Study: Department of General Surgery, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey, between January 2009 and December 2019.

Methodology: Colon specimens resected for colon cancer were evaluated retrospectively, in terms of tumor location, type of surgery, resected colon length, LNh and the number of metastatic LN, based on hospital records. Chi-square test, Kruskal-Wallis along with Dunn-Bonferroni post hoc tests were applied. The lymph node ratio (LNR) (= ratio of LN+ to LNh), the number of lymph nodes per unit distance (LNh/cm), the ratio of LNR to length of specimen (LNR/cm) were evaluated.

Results: The rate of ≥ 12 LNh in 644 colon cancer patients was 81.4%. The length of colon specimen and the number of harvested lymph nodes (LNh) were higher in patients, who underwent subtotal colectomy (StC), compared to patients who underwent right and left hemicolectomy (RhC and LhC, $p < 0.001$). Inadequate LNh was more common in LhC patients ($p < 0.001$). The ratio of the number of LNh to the length of the resected specimen (LNh/cm) was higher in RhC and LhC patients than StC patients ($p < 0.001$). LNR and LNR/cm were higher in LhC patients, though mean specimen length was shorter in LhC patients ($p < 0.05$). The number of LNh had a direct proportion with the resected specimen length; however, the LNh/cm and LNR/cm ratios decreased in > 50 cm length specimen patients ($p < 0.001$).

Conclusion: Central LN dissection and vascular high ligation, according to tumor site are more efficient than the length of the resected specimen for an adequate LN dissection in colon cancers.

Key Words: Colon cancer, Harvested lymph node, Specimen length, Adequate dissection

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INTRODUCTION

Colon cancer is one of the most common causes of cancer deaths in western countries, and surgery is still the gold standard curative treatment option. The most important prognostic factor for colon cancer is lymph node involvement during surgical treatment.^{1,2} The presence of nodal metastasis is an important marker for determining the postoperative adjuvant treatment decision and for disease-free survival.³ Therefore, correct nodal staging is essential to determine the proper treatment management in colon cancer patients.^{4,5}

The number of lymph nodes harvested from the resected colon segment has a key role in adequate nodal staging and is associated with many factors; such as, the patient's clinical features, surgeon's operative technique and the pathologist's microscopic evaluation.⁶⁻⁸ Over the years, the optimisation of some of the factors affecting them has been achieved with the oncological principles determined by surgeons in colorectal surgery and the chemical cleaning methods applied by pathologists in mesentery adipose tissue. The American Society of Clinical Oncology, the Cancer Commission of the American College of Surgeons, the American College of Pathologists, the National Comprehensive Cancer Network, and the National Cancer Institute have reported that at least 12 lymph nodes should be dissected with surgical specimens in colon cancer cases in order to standardise surgical and postoperative treatment.⁹⁻¹²

Considering that there may be a direct relationship between the resected colon specimen length and the mesentery size and the number of lymph nodes; the aim of this study was to evaluate the relationship between the length of resected colon and the number of dissected lymph nodes in colon cancer patients, in terms of the number of metastatic lymph nodes harvested and lymph node ratio.

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METHODOLOGY

Approval was obtained from the local ethics committee of our hospital (27.11.2017 / 43-24). This retrospective study included colon cancer patients, who underwent elective or emergency curative surgery between January 2009 and December 2019 at University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey. Patients who were operated due to benign diseases (ulcerative colitis, noninvasive colon polyps, familial polyposis syndrome, etc.), non-adenomatous colon neoplasm (neuroendocrine tumor, stromal tumor), synchronous colon tumors, and rectal tumors (tumors located up to 15 cm from the anal entrance), were excluded. Demographic data, type of surgery, location of the tumor, surgery and pathology reports of the patients were analyzed retrospectively from the database of this hospital. The histopathological findings, the degree of differentiation, the invasion depth of the tumor (T status), the presence of lymph node involvement (N status), the total number of lymph nodes harvested (LNh), the number of metastatic lymph nodes (LN +) and the length of the resected colon (cm) have been analysed from pathology reports. The lymph node ratio (LNR) (= ratio of LN+ to LNh) and the number of lymph nodes per unit distance (LNh/cm) were analysed. The average number of positive lymph nodes per unit distance were evaluated by the ratio of LNR to length of specimen (LNR/cm).

Neoplasm was evaluated according to the 8th American Joint Committee on Cancer TNM classification and removal of <12 lymph nodes was considered as an inadequate lymph node dissection.¹³ Cecum, ascending colon, hepatic flexure and transverse colon tumors were defined as right colon tumors, splenic flexure, descending colon and sigmoid colon tumors were defined as left colon tumors, and tumors located at the level of peritoneal reflection and rectosigmoid junction were defined as rectosigmoid tumors. Surgical resections were grouped as right hemicolectomy (RhC), left hemicolectomy (including sigmoidectomy, segmental colon resection) (LhC), and subtotal colectomy (StC). Although partial terminal ileum resection was included in RhC, the ileocecal junction was accepted as the proximal surgical margin. Cecal necrosis due to an obstructed mass located in the left colon, serosal abrasion in the cecum and a large-based proximal colon-located polyp accompanying the left colon tumor were defined as surgical indications of StC. Lymph node dissection was performed by resection of colon segment with mesentery containing vascular and lymphatic vessels together in one piece, according to the location of the tumor. The operations were performed by applying high ligation to the major vessels. The authors evaluated the relationship between the performed surgical techniques with the number of adequate harvested lymph nodes and the length of the colon segment that had to be resected to reach adequate lymph node dissection (≥ 12) for RhC, LhC and StC.

Data were analysed by using SPSS (SPSS Inc., an IBM Company, Chicago, IL) for Microsoft Windows 17.0. Chi-square test was used to evaluate qualitative data and were given as frequencies

and percentages (gender, staging, classification, etc.) as well as descriptive statistical methods (mean, standard deviation, median, 25th percentile and 75th percentile). Kruskal-Wallis along with Dunn-Bonferroni post hoc tests were applied because parametric test conditions could not be provided in quantitative data. The receiver operating characteristic (ROC) curve was used to determine the best cut-off for the amount of bowel to resect in order to achieve an adequate number of lymph nodes (minimum 12) according to the type of operation performed. The statistical significance level was taken as 0.05 in all tests.

RESULTS

The demographic data, tumor localisation, TNM classification, pathological diagnosis and LNh of 644 cases are listed in Table 1. The emergency cases were 29.8% of the patients, and all of them had an obstructive tumor. The presence of distant metastases was diagnosed by preoperative radiological imaging or intraoperative exploration. Histopathologically, most of the patients had moderately differentiated or NOS (not otherwise specified) tumors. The authors determined that 18.6% of the patients had inadequate LN dissection.

Table 1: Demographic characteristics of patients and neoplasms.

Variables	n (%)
Gender	
Male	397 (61.6%)
Female	247 (38.4%)
Age (years)	
<65	317 (49.2%)
≥ 65	327 (50.8%)
Elective/Emergency	
Emergency	192 (29.8%)
Elective	452 (70.2%)
Tumor Localisation	
Right	210 (32.6%)
Left	387 (60.1%)
Rectosigmoid	47 (7.3%)
Tumor	
T1	25 (3.9%)
T2	60 (9.3%)
T3	407 (63.2%)
T4	152 (23.6%)
Node	
N0	286 (44.4%)
N1	153 (23.8%)
N2	85 (13.2%)
Nx	120 (18.6%)
Metastasis	
M0	592 (91.9%)
M1	52 (8.1%)
Liver (M1a)	38 (5.9%)
Peritoneum (M1c)	5 (0.8%)
Liver+ Peritoneum(M1c)	9 (1.4%)
Histopathology	
NOS (not otherwise specified)	555 (86.2%)
Mucinous carcinoma	89 (13.8%)
Differentiation	
Well	81 (12.6%)
Moderate	484 (75.2%)
Poor	41 (6.4%)
Undefined	38 (5.9%)
Harvested Lymph Nodes (LNh)	
<12	120 (18.6%)
≥ 12	524 (81.4%)

*The neoplasms were pathologically classified according to the 8th AJCC TNM classification.

Table II: Comparison of surgical resections types.

	Right hemicolectomy (RhC)	Left hemicolectomy (LhC)	Subtotal colectomy (StC)	p
n (%)	210 (32.6%)	383 (59.5%)	51 (7.9%)	
Age mean±sd median (IQR 25%-75%)	63.68±12.06 65 (55-73)	63.77±11.81 64 (56-72)	65.24±10.72 67(58-74)	0.594
Gender				
Male	127 (60.5%)	238 (62.1%)	32 (62.7%)	0.918
Female	83 (39.5%)	145 (37.9%)	19 (37.3%)	
Elective/Emergency				
Emergency	47 (22.4%)	104 (27.2%)	41 (80.4%)	<0.001
Elective	163 (77.6%)	279 (72.8%)	10 (19.6%)	
Tumor Localisation				
Right	210 (100.0%)	0	0	<0.001
Left	0	341 (89.0%)	46 (90.2%)	
Rectosigmoid	0	42 (11.0%)	5 (9.8%)	
Tumor				
T1	4 (1.9%)	20 (5.2%)	1 (2.0%)	0.047
T2	19 (9.0%)	40 (10.4%)	1 (2.0%)	
T3	138 (65.7%)	239 (62.4%)	30 (58.8%)	
T4	49 (23.3%)	84 (21.9%)	19 (37.2%)	
Resected Colon Length^a(cm) mean±sd median (IQR 25%-75%)	29.07±15.53 25(19-34)	21.63±11.42 19(15-25)	97.67±23.37 100(80-110)	<0.001
Surgical Border (cm) mean±sd median (IQR 25%-75%)	7.29±5.13 6(4-9.35)	4.97±2.3 4.2(3-6.5)	11.93±9.81 10(4.5-16)	<0.001
LNh* (%)				
≥12	190 (90.5%)	284 (74.2%)	50 (98.0%)	<0.001
<12	20 (9.5%)	99 (25.8%)	1 (2.0%)	
LNh^b mean±sd median (IQR 25%-75%)	23.67±12.26 21(15-30)	18.12±10.39 16(11-23)	30.47±19.6 25(17-37)	<0.001
LNh/cm^c mean±sd median (IQR 25%-75%)	0.96±0.60 0.8(0.53-1.22)	0.93±0.57 0.82(0.54-1.22)	0.32±0.2 0.25(0.17-0.38)	<0.001
LN+ patients ** (%)	87 (41.4%)	156(40.7%)	21(41.2%)	
≥12	81 (93.1%)	115 (73.7%)	21 (100.0%)	<0.001
<12	6 (6.9%)	41 (26.3%)	0 (0%)	
LNR*** mean±sd median (IQR 25%-75%)	0.18±0.20 0.09(0.05-0.24)	0.25±0.25 0.16(0.08-0.3)	0.16±0.17 0.1(0.06-0.25)	0.003
LNR/cm mean±sd median (IQR 25%-75%)	0.0078±0.010 0.003(0.002-0.01)	0.013±0.014 0.008(0.004-0.016)	0.0017±0.013 0.001(0.0004-0.003)	<0.001

*LNh: The number of harvested lymph nodes. **LN+ patients: The number of metastatic lymph nodes. ***LNR: Metastatic lymph node ratio. ^a Comparison of the length of specimen between three groups was significant ($p < 0.001$). ^b Comparison of the number of LNh between three groups was significant ($p < 0.001$). ^c Comparison of LNh/cm between LhC and StC was significant ($p < 0.001$), between RhC and StC was significant ($p < 0.001$), but between RhC and LhC was not significant ($p > 0.05$) (Kruskal Wallis and post hoc Dunn-Bonferroni tests were used for all quantitative data).

LhC was the most performed surgery (59.5%), and no difference was found between resection types in terms of age and gender (p : 0.594 and 0.918). StC group was mostly

consisted of emergency cases ($p < 0.001$). In all three groups, most of the patients had T3 tumors.

Table III: Comparison of resected colon lengths.

	Resected Colon Length (cm)				P
	Group1: 0-10	Group2: 11-30	Group3: 31-50	Group4: >50	
n (%)	21 (3.3%)	455 (70.6%)	89 (13.8%)	79 (12.3%)	
Age mean±sd median (IQR 25%-75%)	66.29±10.57 66 (58.5-73.5)	63.98±11.95 65 (56-73)	62.65±12.0 64 (55-70)	63.67±11.17 64 (56-73)	0.656
Gender					
Male	12 (57.1%)	281 (61.8%)	55 (61.8%)	49 (62.0%)	0.979
Female	9 (42.9%)	174 (38.2%)	34 (38.2%)	30 (38.0%)	
Elective/Emergency(%)					
Emergency	5 (23.8%)	102 (22.4%)	32 (36.0%)	53 (67.1%)	<0.001
Elective	16 (76.2%)	353 (77.6%)	57 (64.0%)	26 (32.9%)	
Tumor Localisation					
Right	2 (9.5%)	135 (29.7%)	56 (62.9%)	17 (21.5%)	<0.001
Left	12 (57.1%)	287 (63.1%)	31 (34.8%)	57 (72.2%)	
Rectosigmoid	7 (33.3%)	33 (7.2%)	2 (2.2%)	5 (6.3%)	
T					
T1	2 (9.5%)	22 (4.8%)	0 (0%)	1 (1.3%)	0.003
T2	5 (23.8%)	43 (9.5%)	9 (10.1%)	3 (3.8%)	
T3	10 (47.6%)	295 (64.8%)	54 (60.7%)	48 (60.7%)	
T4	4 (19.0%)	95 (20.9%)	26 (29.2%)	27 (34.2%)	
Surgery					
RhC	2 (9.5%)	135 (29.7%)	56 (62.9%)	17 (21.5%)	<0.001
LhC	19 (90.5%)	320 (70.3%)	33 (37.1%)	11 (13.9%)	
StC	0 (0%)	0 (0%)	0 (0%)	51 (64.6%)	
Surgical Border (cm) mean±sd median (IQR 25%-75%)	2.51±0.25 2 (1.75-2.9)	5.0±2.5 4.5(3-6.5)	8.49±4.7 8.5(4.5-11.4)	12.13±9.42 10(5-16)	<0.001
LNh (%)					
≥12	10 (47.6%)	355 (78.0%)	82 (92.1%)	77 (97.5%)	<0.001
<12	11 (52.4%)	100 (22.0%)	7 (7.9%)	2 (2.5%)	
LNh* mean±sd median (IQR 25%-75%)	11.48±6.96 11(6.5-14.5)	19.24±10.72 17(12-24)	23.40±11.33 23(14-30)	30.24±18.24 25(18-37)	<0.001
LNh/cm* mean±sd median (IQR 25%-75%)	1.24±0.76 1.37(0.7-1.5)	1.01±0.59 0.88(0.62-1.27)	0.63±0.32 0.58(0.4-0.81)	0.36±0.23 0.33(0.2-0.43)	<0.001
LN+ patients** (%)	4(19.0%)	177(38.9%)	50(56.2%)	33(41.8%)	
≥12	2 (50.0%)	137 (77.4%)	46 (92.0%)	32 (97.0%)	<0.001
<12	2 (50.0%)	40 (22.6%)	4 (8.0%)	1 (3.0%)	
LNR*** mean±sd median (IQR 25%-75%)	0.17±0.12 0.12(0.1-0.3)	0.23±0.24 0.15(0.07-0.3)	0.20±0.22 0.095(0.06-0.3)	0.19±0.22 0.12(0.05-0.22)	0.435
LNR/cm^b mean±sd median (IQR 25%-75%)	0.02±0.01 0.012(0.01-0.03)	0.01±0.01 0.008(0.004-0.016)	0.007±0.011 0.003(0.002-0.008)	0.003±0.005 0.001(0.0005-0.003)	<0.001

*LNh: The number of harvested lymph nodes. **LN+ patients: The number of metastatic lymph nodes. ***LNR: Metastatic lymph node ratio. a Comparison of group 1 and 2 was not significant ($p>0.05$), but comparison of other groups was significant ($p<0.001$). b Comparison of group 1 with group 2 and group 3 was significant ($p<0.001$) (Kruskal Wallis and post hoc Dunn-Bonferroni tests were used for all quantitative data).

StC was performed in two early stage left colon tumors due to synchronous proximally located broad-based polypoid lesion, and pathology reported as T1 tumor in one case and T2 tumor in the other, villous adenoma in one

and tubulovillous adenoma with high grade dysplasia in the other. The length of the resected colon specimen was longer in the patients in the StC group than the other two groups ($p < 0.001$).^{*} However, the length of the specimen

resected in the RhC group was longer than in the LhC group ($p < 0.001$). The longest surgical margin length were in the StC group, the RhC group and the LhC group, respectively ($p < 0.001$).^{*} The ratio of adequate lymph node dissection in the RhC, LhC, and StC groups were 90.5%, 74.2% and 98.0%, respectively. The LNh obtained in the StC group were higher than the other two groups ($p < 0.001$),^{*} and the LNh obtained in the RhC group were higher than the LhC group ($p < 0.001$).

Metastatic LN was found 41% (264/644) of the patients, and among these patients inadequate lymph node dissection rate was the highest in the LhC group (26.3%) ($p < 0.001$).

The LNh/cm had the highest value in the RhC group and then in the LhC group, while the difference between the RhC and LhC groups and the StC group was significant ($p < 0.001$),^{*} the difference between the RhC and LhC groups were similar ($p = 0.94$).

The LNR was the highest in the LhC group, therefore the LNR/cm was higher than in the other groups and, a statistically significant difference was found between the groups in terms of LNR and LNR/cm. Comparison among different types of surgical resection according to lymph node dissection is listed in Table II.

The length of the resected specimen divided into 4 groups; Group1: 0-10cm, Group2: 11-30cm, Group3: 31-50cm, Group4 :> 50cm; and they were similar in terms of age and gender (p : 0.656 and 0.979). The length of the specimen resected in emergency operations was mostly over 30 cm; and Group 4 patients mostly underwent emergency operations. Group 1, Group 2 and Group 4 consisted of left colon tumors, while Group 3 consisted mostly right colon tumors. In all three groups, the majority of the patients were with T3 tumors. LhC ($p < 0.001$) for Group 1 and Group 2, RhC ($p < 0.001$) for Group 3 and StC ($p < 0.001$) for Group 4 were preferred resection type.

The length of the resected specimen had a direct proportion with the closest surgical margin distance to the tumor ($p < 0.001$).

As the length of the resected specimen increased, the number of patients with ≥ 12 lymph node dissection and the number of lymph nodes dissected increased ($p < 0.001$); however, the LNh/cm ratio decreased. Although there was not statistically difference between Group 1 and Group 2 in terms of LNh/cm, but there was a difference between Group 1 and Group 2 with Group 3 and Group 4 ($p < 0.001$).

The rate of inadequate lymph node dissection decreased as the length of the resected specimen increased ($p < 0.001$) in patients with metastatic LN. The highest LNR was found in Group 2 (p : 0.435) and the LNR/cm ratio decreased as the specimen length increased. Although there was not statistically significant difference between Group 1 and Group 2, the difference between Group 1 and Group 2 with Group 3 and Group 4 was significant for LNR/cm ($p < 0.001$): adjusted using the Bonferroni correction).

The comparison of different specimen lengths, according to the number of lymph node dissected, is listed in Table III. The length of the colon for adequate LN dissection was found be 25.5cm (sensitivity: 51.1%, specificity: 75%) for right colon tumors, and 22.75cm (sensitivity: 38.7%, specificity: 80.8%) for left colon tumors.

DISCUSSION

Curative resection of colon tumors and accurate identification of positive lymph nodes are important in terms of staging the disease, postoperative management, prognosis, and survival rates have an inverse proportion with the number of dissected metastatic lymph nodes.¹⁴ The number of harvested lymph nodes can vary depending on patient-related factors (age, gender, tumor biology), surgeon-induced factors (surgeon's experience, type of surgery) and pathologist-derived factors (experience of the pathologist, specific dissection techniques). While patient-induced factors are not alterable, surgeon and pathologist originated factors are manageable.^{15,16} It has been reported that adequate lymph node dissection (LNh ≥ 12) in colon cancer is still at the level of 70-80%.^{17,18} In this study, this rate was determined as 81.4% (524/644), comparable with the literature.

Radical techniques, such as mesocolic excision and high ligation of vascular structures, have been developed in colorectal cancer surgery for adequate LN dissection. In addition, the idea of 'longer colon resection, more dissected LN' seemed to be valid by many surgeons and some studies have shown that there is a direct proportion between the length of the resected specimen and the number of lymph nodes dissected.^{8,19} However, although the average length of the specimen resected varies according to the location of the tumor and the type of surgery performed, the localisation of the tumor also affects the number of lymph nodes dissected.²⁰

There are many studies reported that the number of lymph nodes dissected in tumors located in the left colon and rectum is less than right or transvers colon located tumors.^{7,21} Studies have reported that the surgeon avoids a high anterior resection especially in left colon tumors located in the distal sigmoid and rectosigmoid, resulting in the resection of the shorter colon segment and therefore less lymph node dissection.²² In addition, the fact that the vascular anatomy allows the resection of a longer colon segment and wider mesentery in right colon tumors is seen as another factor in removing more lymph nodes in tumors located in the right colon compared to tumors located in the left colon.²³ Microsatellite instability (MSI), which is an important pathway in tumor biology and detected in 20-25% of right colon tumors should be considered as another important factor, because MSI has been found to be associated with a high level of locoregional lymph nodes metastasis.²⁴ In abdominoperineal resection and low anterior resection operations performed for rectal tumors, although the resected

segment is long, less lymph node dissection than left colon tumor operations has been reported to be associated with preoperative chemoradiotherapy.²⁵

The number of harvested lymph nodes was analysed, according to the resection type; and it was found that the length of the resected specimen and the number of lymph nodes harvested were higher in RhC than in LhC, similar with the literature. It is concluded that the longer right colon mesentery and the presence of dense lymph nodes in the ileocecal region cause this result. Besides, segmental resections of the colon (sigmoidectomy, segmental resection for splenic flexure tumor) can be associated with short specimen length.

The surgical margin was shorter in the LhC group where the number of rectosigmoid tumors was high compared to the other two groups in our study. We conclude that this situation is caused by the surgeon's avoidance of mesorectal dissection and an inferior anastomosis. However, it is noticed that this situation not only leads to a short surgical margin, but also to shorter specimen length and an increase in the number of inadequate lymph node dissection. Comparison of resection types showed that highest LNR was in the LhC group. Although the number of metastatic LN patients among the groups was relatively similar, the high LNR in these patients seemed to be an expected result due to the high number of patients with inadequate lymph node dissection in the LhC group. There are many studies indicating the prognostic importance of the LNR ratio, especially in stage 1-3 colorectal cancer patients. However, this view leads to the conclusion that prognostic evaluation would be more accurate in patient groups with ≥ 12 lymph node dissection according to the LNR result.

Comparison of resected colon lengths showed that there was a direct proportion between length of the specimen and number of harvested LN, similar with the literature. It is found that the LNR is similar in different specimen lengths. However, considering that the definition of adequate lymph node dissection is aimed to capture the possible metastatic lymph node, it is seen that the number of LN + patients detected increases up to 50 cm specimen length, but this rate decreases when the specimen length is over 50 cm. In this case, although specimen length and LNh have a linear relationship, it led to the conclusion that "more is not necessarily better" as stated by Amri *et al.*¹⁰.

There are a few studies indicating the minimum length of the colon specimen that needs to be resected for an adequate LN dissection. Stracci *et al.* reported that a colon resection of at least 20 cm is required for an adequate LN dissection regardless of the location of the tumor.⁹ Gravante *et al.* stated that the specimen should be at least 36 cm in abdominoperineal resection and 42 cm in Hartmann's resection for an adequate LN dissection, especially for rectum and rectosigmoid tumors.⁷ The length of resected colon segment

should be 25.5 cm in the RhC and 22.75 cm in the LhC, in order to harvest 12 LN with a low sensitivity and $>75\%$ specificity.

The limitations of the study are that retrospective design and operations were performed by different surgeons that would affect the surgery type.

CONCLUSION

The length of the resected colon segment in colon tumors is associated with the number of harvested LN mathematically, but clinically anatomical location of the tumor, vascular and lymphatic anatomy are the major factors for the number of LN harvested, so colon tumor surgery should be done by oncological principles.

ETHICAL APPROVAL:

The study was approved by the local Ethics Committee of Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey (27.11.2017 / 43-24).

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MTB, IY: Material preparation, data collection and analysis. The first draft of the manuscript was written and all authors commented on previous versions of the manuscript; all authors read and approved the final manuscript; and contributed to the study conception and design.

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