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Factors affecting retrieval of 12 or more lymph nodes in pT1 colorectal cancers

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Abstract

Objective: The aim of this study was to identify clinicopathological factors that affect the number of lymph nodes (LNs) (12 or more) retrieved from patients with colorectal cancer (CRC), particularly those with pathologic T1 (pT1) disease.

Methods: From 429 CRC patients, 75 pT1 cancers were identified and digitally scanned. Binary logistic regression analysis was performed to identify the clinicopathological factors affecting the number of LNs retrieved from all 429 patients and from the subset of patients with pT1 CRC.

Results: For the 429 patients, the mean number of harvested LNs per specimen was 20 (median, 19). The number of retrieved LNs was independently associated with maximum tumor diameter $>$ 2.3 cm and right-sided tumor location. The mean number of LNs retrieved from the 75 patients with pT1 CRC was 14 (median, 15); retrieval of 12 or more LNs from this group was independently associated with maximum tumor diameter $>$ 14.1 mm.

Conclusion: The number of LNs retrieved from patients with CRC was associated with maximum tumor diameter and right-sided tumor location. For patients with pT1 CRC, maximum tumor diameter was independently associated with the harvesting of 12 or more LNs.

Keywords

Colorectal cancer, pT1, lymph node, harvest, retrieval, adenocarcinoma

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Introduction

Metastasis of colorectal cancer (CRC) to lymph nodes (LN) is a major step in cancer progression; therefore, it has a marked effect on prognosis and therapeutic stratification.[1] LN involvement is a critical factor in prognostic classification according to the American Joint Conference on Cancer and Union Internationale Contre le Cancer (AJCC/UICC) TNM system. Metastasis to LNs is associated with an adverse clinical outcome and indicates the requirement for post-operative adjuvant chemotherapy. By contrast, a lack of LN involvement is associated with a better clinical outcome.[2] These facts underscore the importance of accurate LN assessment.

Accurate assessment of LN involvement depends upon the retrieval of a sufficient number of LNs via appropriate surgical resection.[3] The number of LNs harvested is an independent prognostic factor for clinical outcome. A low LN yield increases the risk of inaccurate assessment,[4] whereas a greater number of harvested LNs is associated with a more favorable outcome, particularly for patients with stage II CRC.[3,5,6] It should be noted, however, that conflicting data suggest that the outcome for CRC patients is independent of the number of LNs acquired.[7]

AJCC/UICC guidelines recommend histopathological evaluation of at least 12 LNs to prevent the degree of LN involvement from being underestimated.[8] Successful harvesting of at least 12 LNs represents both a prognostic marker and an indicator of the quality of surgical resection.[9] In cases where fewer than 12 LNs are harvested, adjuvant chemotherapy is recommended, regardless of nodal status.[10] However, it is unclear whether retrieval of more than 12 nodes improves staging accuracy and prognosis.[3,5]

The number of retrieved LNs may be influenced by several parameters, including surgical radicality, pathological work-up, and patient- and tumor-specific factors; however, data are frequently inhomogeneous.[3,5,11,12] Furthermore, the number of LNs retrieved from patients with pathologic T1 or T2 status is consistently low.[7,13–15] However, few studies have examined factors affecting the number of LNs retrieved from CRC patients with low pathologic T-classification. Therefore, we analyzed patient- and tumor-specific factors affecting the number of LNs retrieved from patients with CRC, focusing on pathologic T1 (pT1) stage, and evaluated the prognostic impact of retrieving more than 12 LNs.

Methods

Patient cohort

Data from patients with a histologic diagnosis of adenocarcinoma who underwent surgical resection for CRC between 2014 and 2016 at Chungbuk National University Hospital (Cheongju, Korea) were retrospectively evaluated. Patients who received neoadjuvant chemotherapy or radiotherapy were excluded. In all patients, D2 or D3 lymph node dissection was performed, according to the Japanese classification system. High-risk stage II patients and stage III patients received adjuvant chemotherapy of fluoropyrimidine and oxaliplatin as specified in the Korean clinical practice guidelines for colon and rectal cancer. Stage I patients did not receive adjuvant therapy. Patients with stage IV disease were treated with various combinations of chemotherapeutic agents. Informed consent was not required for this retrospective study. The study protocol was reviewed and approved by the Institutional Review Board of Chungbuk National University Hospital (approval no. 2018-02-001).

Clinicopathological data

Original histopathology slides were evaluated independently by two gastrointestinal

pathologists (S.M.S. and H.C.L.). Over the 3 years, all specimens were examined first by one pathologist (S.M.S.) and then reviewed by the other (H.C.L.). If the number of LNs retrieved by one pathologist did not meet the limit of 12, additional LNs were harvested from the resected tissue by another pathologist. The following clinicopathological data were collected from patient medical records: gender, age, tumor stage, tumor location, length of resected specimen, maximum tumor diameter, differentiation grade, lymphovascular invasion, and mismatch repair (MMR) gene status. Tumor stage was assessed according to the 8th edition of the AJCC/UICC TNM classification.[16] Histological tumor type and tumor grade were analyzed according to WHO guidelines.[17] Tumors located from the splenic flexure to the rectum were defined as left-sided cancers, while tumors located from the transverse colon to the caecum were defined as right-sided cancers. MMR status was analyzed by polymerase chain reaction using five Bethesda guideline panel loci (BAT25, BAT26, D2S123, D5S346, and D17S250).

Histopathological analysis

Maximum tumor diameter was measured macroscopically for all patients. For the cases of pT1 cancer, digital scanning at ×200 magnification was performed on the slide that included the widest and deepest area of invasion (Pannoramic SCAN, 3DHISTECH, Budapest, Hungary).[18] Computer-based morphometry was performed using a digital slide viewer (CaseViewer v2.1, 3DHISTECH). The following quantitative factors were analyzed:[18] (1) maximum tumor diameter (defined as the maximum size of the neoplastic lesion, i.e., both adenoma and carcinoma components) (Figure 1); (2) maximum carcinoma diameter (defined as the width of submucosal invasion) (Figure 1); (3) maximum vertical depth of carcinoma from the luminal surface (Figure 2); and (4) maximum vertical depth of carcinoma from the muscularis mucosae (Figure 2).

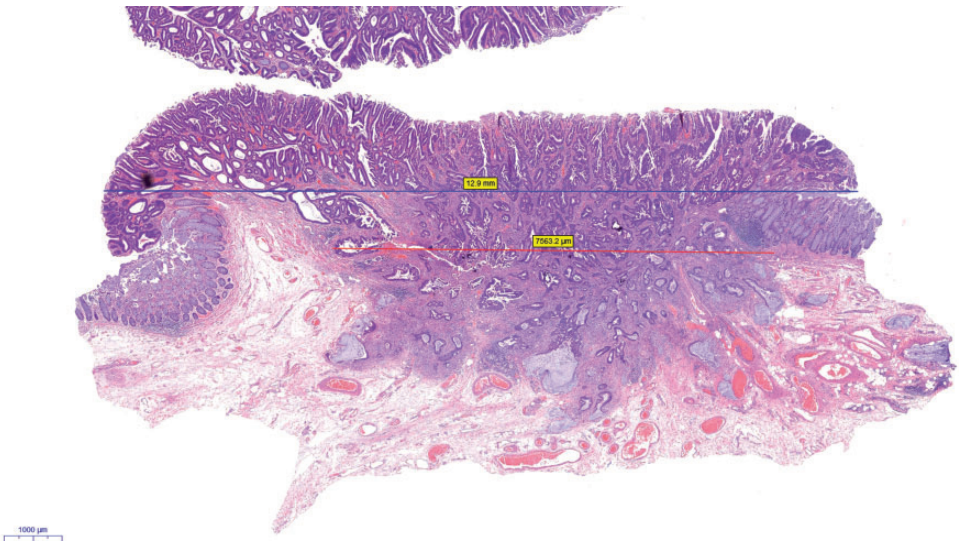

Figure 1. Measurement of tumor/carcinoma diameter in a patient with pT1 cancer. The figure shows that the diameter of the total tumor is 12.9 mm (blue line) and that the diameter of the carcinoma is 7.6 mm (red line).

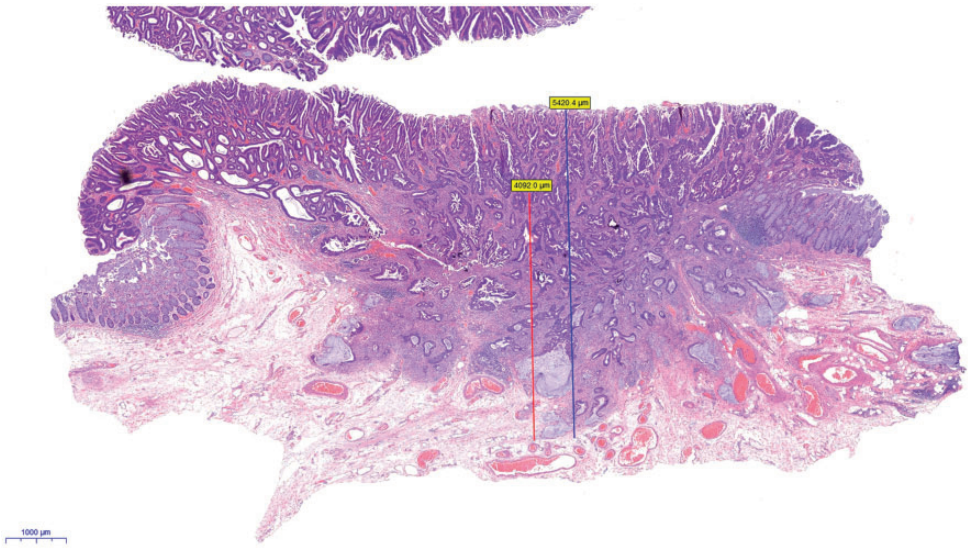

Figure 2. Example of the depth of invasion from the luminal surface and muscularis mucosae. The depth from the luminal surface of the tumor is 5.4 mm (blue line), and that from the muscularis mucosae is 4.1 mm (red line).

Statistical analyses

The χ^2 and Fisher's exact tests were used to examine the association between clinicopathologic factors and the number of retrieved LNs. A receiver operating characteristic (ROC) curve was generated to determine cutoff values for quantitative factors identified as significant. Binary logistic regression analysis was subsequently used to identify variables affecting the number of retrieved LNs. A Student's t-test or χ^2 test was used for comparisons among study groups. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. The Cox proportional hazard model was used for univariate and multivariate survival analyses. Multivariate analysis included variables identified as predictive by univariate analysis. Progression-free survival (PFS) was calculated from the date of the diagnostic biopsy until confirmed disease progression or death. All statistical analyses were performed using the SPSS statistical package, version 18.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$ (two sided).

Results

Factors associated with retrieval of ≥12 LNs from patients with CRC

The study cohort comprised 429 patients with CRC, and the patient characteristics are summarized in Table 1. There were 264 men (62%) and 165 women (38%), with a median age of 70 years (range, 31–92 years). The mean number of retrieved LNs was 20 (95% confidence interval (CI), 19–21), and the median number was 19 (range, 0–58); 384 patients (90%) had ≥12 lymph nodes sampled. Using 12 retrieved LNs as a guide (as per AJCC/UICC guidelines), the number of retrieved LNs was classified into two groups: <12 LNs retrieved and ≥12 LNs retrieved. ROC curves for specimen length and maximum tumor diameter were generated and

Table 1. Relationship between clinicopathologic parameters and number of lymph nodes retrieved.

Characteristics	Total (n = 429)	LN < 12 (n = 45)	LN ≥ 12 (n = 384)	*p*-value
Sex, n (%)				0.018
Male	264 (62)	35/45 (78)	229/384 (60)	
Female	165 (38)	10/45 (22)	155/384 (40)	
Age, years				0.902
Median (range)	70 (31–92)	70 (44–80)	70 (31–92)	
<70, n (%)	206 (48)	22/45 (49)	184/384 (48)	
≥70, n (%)	223 (52)	23/45 (51)	200/384 (52)	
T-classification, n (%)				<0.001
T1–2	125 (29)	33/45 (73)	92/384 (24)	
T3–4	304 (71)	12/45 (27)	292/384 (76)	
N-classification, n (%)				<0.001
N0	249 (58)	38/45 (84)	211/384 (55)	
N1–2	180 (42)	7/45 (16)	173/384 (45)	
AJCC/UICC stage (8th edn)				<0.001
Stage I	109 (25)	28/45 (62)	81/384 (21)	
Stage II	129 (30)	10/45 (22)	119/384 (31)	
Stage III	150 (35)	7/45 (16)	143/384 (37)	
Stage IV	41 (10)	0/45 (0)	41/384 (11)	
Specimen length, cm				<0.001
Median (min, max)	18.0 (4.5, 145.0)	14.5 (5.5, 64.0)	18.0 (4.5, 145.0)	
≤15.5, n (%)	161 (38)	29/45 (64)	132/384 (34)	
>15.5, n (%)	268 (62)	16/45 (36)	252/384 (66)	
Maximum diameter of tumor, cm				<0.001
Median (min, max)	4.0 (0.4, 14.0)	1.5 (0.5, 10)	4.0 (0.4, 14)	
≤2.3, n (%)	112 (26)	35/45 (78)	77/384 (20)	
>2.3, n (%)	317 (74)	10/45 (22)	307/384 (80)	
Tumor location, n (%)				0.004
Right	116 (27)	4/45 (9)	112/384 (29)	
Left	313 (73)	41/45 (91)	272/384 (71)	
Tumor differentiation, n (%)				0.095
Well or moderate	377 (88)	43/45 (96)	334/384 (87)	
Poor	52 (12)	2/45 (4)	50/384 (13)	
Lymphovascular invasion, n (%)				0.865
Negative	378 (88)	40/45 (89)	338/384 (88)	
Positive	51 (12)	5/45 (11)	46/384 (12)	
MMR gene status				0.513
MSS	204 (82)	13/17 (76)	191/231 (83)	
MSI	44 (18)	4/17 (24)	40/231 (17)	

LN, lymph node; MMR, mismatch repair; MSS, microsatellite stability; MSI, microsatellite instability.
p values for categorical data were obtained using the χ^2 and Fisher's exact tests.

used to identify optimal cutoff values for predicting the retrieval of ≥ 12 LNs. The cutoff values for specimen length and maximum tumor diameter were 15.5 cm and 2.3 cm, respectively. Retrieval of ≥ 12 LNs was associated significantly with female gender ($p = 0.018$), T-classification ($p < 0.001$), N-classification ($p < 0.001$), AJCC/UICC stage ($p < 0.001$), specimen length > 15.5 cm ($p < 0.001$), maximum tumor diameter

>2.3 cm ($p<0.001$), and right-sided tumor location ($p=0.004$). No association was identified with age, tumor differentiation, lymphovascular invasion, and MMR gene status, which was analyzed in 248 of the 429 participants (Table 1).

Table 2 shows the results of multivariate logistic regression analysis of variables statistically associated (in univariate analysis) with harvesting of ≥ 12 LNs. Maximum tumor diameter >2.3 cm ($p<0.001$) and right-sided tumor location ($p=0.026$) were associated independently with retrieval of the recommended ≥ 12 LNs. The mean number of harvested LNs was higher in cases with a maximum tumor diameter >2.3 cm than in cases with a maximum diameter ≤ 2.3 cm (21.9 *vs.* 14.6, respectively; $p<0.001$) (Figure 3a). Tumor location had a significant effect on the mean number of harvested LNs (right side, 21.7 *vs.* left side, 19.4; $p=0.012$) (Figure 3b).

Factors associated with harvesting of ≥ 12 LNs from patients with pathologic T1 colorectal cancer

Seventy-eight patients had pT1 stage disease. Of these, 75 were included in the study (microscopy slides from three patients

Table 2. Multivariate logistic regression analysis of factors predicting retrieval of ≥ 12 lymph nodes from patients with CRC.

Variable	Category	OR	95% CI	*p*-value
Sex	Female	1.805	0.794–4.105	0.159
T-classification	3, 4	1.631	0.625–4.256	0.318
N-classification	1, 2	2.157	0.795–5.854	0.131
Specimen length	>15.5 cm	1.656	0.755–3.632	0.209
Maximum diameter of tumor	>2.3 cm	8.115	3.167–20.796	<0.001
Tumor location	Right	3.814	1.169–12.438	0.026
Tumor differentiation	Poor	0.834	0.154–4.509	0.833
Lymphovascular invasion	Present	0.998	0.310–3.214	0.997

OR, odds ratio; CRC, colorectal cancer; CI, confidence interval.

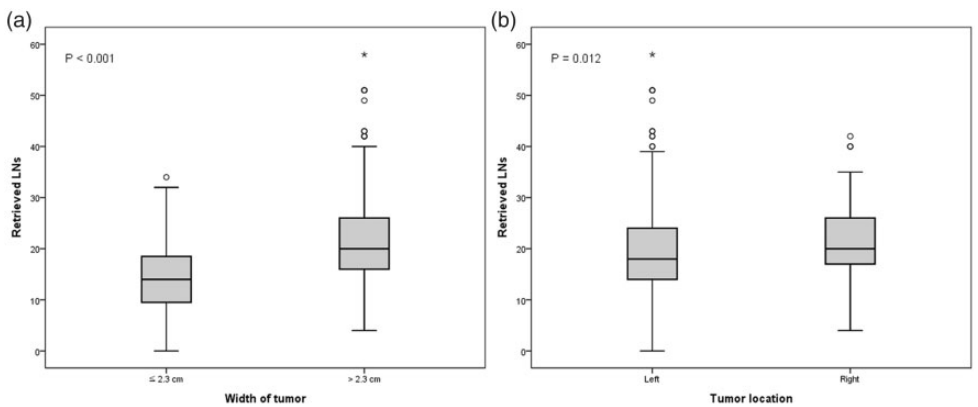

Figure 3. (a) Mean number of harvested LNs from cases with a maximum tumor diameter of >2.3 cm was greater than that in groups with a maximum tumor diameter ≤ 2.3 cm ($p<0.001$). (b) A significant difference was observed in the mean number of LNs harvested from tumors at different locations ($p=0.012$). LN, lymph node.

were damaged and were therefore unsuitable for digital scanning). The clinicopathological factors for these 75 patients are summarized in Table 3. The mean number of retrieved LNs was 14 (95% CI, 13–16), and the median number was 15 (range, 3–32). LN metastasis was identified in 5 (7%) of the 75 cases. ROC curves were generated using data for specimen length, tumor diameter, carcinoma diameter, depth of invasion from the surface, and depth of invasion from the muscularis mucosae. This information was used to identify optimal cutoff values for predicting the retrieval of $\geq$12 LNs from patients with pT1 CRC. The cutoff values determined were as follows: specimen length, 15.0 cm; tumor diameter, 14.1 mm; carcinoma diameter, 10.4 mm; depth of invasion from the surface, 3237 μm; and depth of invasion from the muscularis mucosae, 2551 μm. Cases where $\geq$12 LNs were harvested had a significantly greater specimen length ($p = 0.03$), greater tumor diameter ($p = 0.025$), and lesser depth of invasion from the muscularis mucosae ($p = 0.017$) and were more likely to have a right-sided tumor ($p = 0.006$) when compared with cases from whom $<$ 12 LNs were harvested. No association with sex, age, N-classification, maximum diameter of carcinoma, depth of invasion from the surface, tumor differentiation, lymphovascular invasion, and MMR gene status was identified (Table 3).

Multivariate logistic regression analysis of variables showing statistical significance in univariate analysis identified maximum tumor diameter $>$ 14.1 mm ($p = 0.04$) as the only significant predictor of the retrieval of $\geq$12 LNs (Table 4). The mean number of harvested LNs was higher in cases with a maximum tumor diameter of $>$ 14.1 mm than in cases with a maximum diameter $\leq$14.1 mm (16.1 *vs.* 13.4, respectively; $p = 0.044$) (Figure 4).

Prognostic factors related to PFS in patients with more or less than 12 LNs dissected

Analysis of 399 out of 429 (93%) patients with available follow-up data revealed progressive disease in 78 (20%) patients after a median follow-up of 44.1 months (range, 3.5–72.5). The median PFS was 42.3 months (95% CI, 38.1–41.7 months). By the end of follow-up, 34 (9%) patients had died from cancer.

Table 5 presents the results of univariate and multivariate survival analyses for 399 patients; data include age, sex, number of LNs retrieved, T-classification, N-classification, tumor location, tumor differentiation, and lymphovascular invasion. Disease progression occurred in one of 44 (2%) patients with $<$ 12 retrieved LNs compared with 77 of 355 (22%) patients with $\geq$12 retrieved LNs ($p = 0.021$). Cox proportional hazards regression analysis revealed that T-classification and N-classification retained prognostic significance ($p = 0.004$ and $p < 0.001$, respectively), and that the retrieval of $\geq$12 LNs had no independent prognostic impact ($p = 0.271$).

Among patients with stage II disease (n = 123), disease progression occurred in one of nine (11%) patients with $\leq$12 retrieved LNs and in nine of 114 (8%) patients with $>$ 12 retrieved LNs ($p = 0.728$). In patients with stage I disease (n = 105), disease progression occurred in only one of 78 (1%) patients with $>$ 12 retrieved LNs ($p = 0.662$).

Discussion

This study examined factors affecting the retrieval of at least 12 LNs from patients with CRC defined according to AJCC guidelines. The number of harvested LNs depends on four major factors: the surgeon, the pathologist, the patient, and the tumor.[3,5,11,19] However, the number of LNs required for accurate staging is

Table 3. Relationship between clinicopathologic parameters and number of lymph nodes retrieved from patients with pT1 CRC.

Characteristics	Total (n = 75)	LN < 12 (n = 24)	LN ≥ 12 (n = 51)	*p*-value
Sex, n (%)				0.343
Male	54 (72)	19/24 (79)	35/51 (69)	
Female	21 (28)	5/24 (12)	16/51 (31)	
Age, years				
Median (range)	65 (39–80)	64 (44–80)	65 (39–80)	0.812
<65, n (%)	36 (48)	12/24 (50)	24/51 (47)	
≥65, n (%)	39 (52)	12/24 (50)	27/51 (53)	
N-classification, n (%)				0.319
N0	70 (93)	21/24 (88)	49/51 (96)	
N1–2	5 (7)	3/24 (13)	2/51 (4)	
Specimen length, cm				0.030
Median (min, max)	14.0 (4.5, 64.0)	13.0 (5.5, 64.0)	15.0 (4.5, 35.0)	
≤15.0, n (%)	46 (61)	19/24 (79)	27/51 (53)	
>15.0, n (%)	29 (39)	5/24 (21)	24/51 (47)	
Maximum diameter of tumor, mm				0.025
Median (min, max)	11.8 (3.1, 39.5)	9.9 (4.6, 32.3)	12.2 (3.1, 39.5)	
≤14.1, n (%)	49 (65)	20/24 (83)	29/51 (57)	
>14.1, n (%)	26 (35)	4/24 (17)	22/51 (43)	
Maximum diameter of carcinoma, mm				0.093
Median (min, max)	6.0 (0.6, 24.5)	6.1 (2.4, 10.5)	6.0 (0.6, 24.5)	
≤10.4, n (%)	64 (85)	23/24 (96)	41/51 (80)	
>10.4, n (%)	11 (15)	1/24 (4)	10/51 (20)	
Maximum depth of invasion of carcinoma from surface, μm				0.084
Median (min, max)	3922 (770, 9898)	3969 (1805, 9898)	3798 (770, 9557)	
≤3237, n (%)	26 (35)	5/24 (21)	21/51 (41)	
>3237, n (%)	49 (65)	19/24 (79)	30/51 (59)	
Maximum depth of invasion of carcinoma from muscularis mucosae, μm				0.017
Median (min, max)	2464 (155, 7878)	2940 (747, 7878)	2167 (155, 7585)	
≤2551, n (%)	40 (53)	8/24 (33)	32/51 (63)	
>2551, n (%)	35 (47)	16/24 (67)	19/51 (37)	
Tumor location, n (%)				0.006
Right	57 (76)	23/24 (96)	34/51 (67)	
Left	18 (24)	1/24 (4)	17/51 (33)	
Tumor differentiation, n (%)				0.952
Well	34 (45)	11/24 (46)	23/51 (45)	
Moderate	41 (55)	13/24 (54)	28/51 (55)	
Lymphovascular invasion, n (%)				0.717
Negative	65 (87)	20/24 (83)	45/51 (88)	
Positive	10 (13)	4/24 (17)	6/51 (12)	
MMR status				1.000
MSS	20 (83)	5 (83)	15 (83)	
MSI	4 (17)	1 (17)	3 (17)	

LN, lymph node; CRC, colorectal cancer; MMR, mismatch repair; MSS, microsatellite stability; MSI, microsatellite instability.

p values for categorical data were obtained using the χ^2 and Fisher's exact tests.

Table 4. Multivariate logistic regression analysis of factors predicting retrieval of ≥12 lymph nodes from patients with pT1 CRC.

Variable	Category	OR	95% CI	*p*-value
Specimen length	>15 cm	0.942	0.211–4.230	0.941
Maximum diameter of tumor	>14.1 mm	5.136	1.077–24.492	0.040
Maximum diameter of carcinoma	≤10.4 mm	3.483	0.278–43.607	0.333
Maximum depth of invasion of carcinoma from surface	≤3237 μm	0.499	0.084–2.957	0.444
Maximum depth of invasion of carcinoma from muscularis mucosae	≤2551 μm	0.295	0.058–1.491	0.140
Tumor location	Right	9.311	0.893–97.082	0.062

OR, odds ratio; CRC, colorectal cancer; CI, confidence interval.

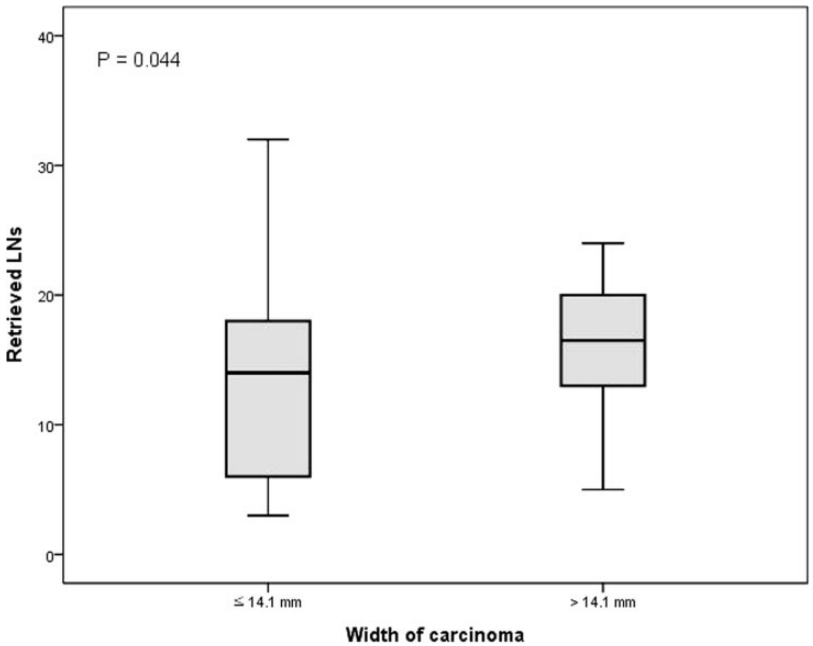

Figure 4. The mean number of LNs harvested from patients with pT1 CRC with a maximum tumor diameter > 14.1 mm was higher than that in patients with a maximum tumor diameter ≤14.1 mm ($p = 0.044$). LN, lymph node.

controversial. In 1990, the Working Party Report to the World Congress of Gastroenterology in Sydney recommended the retrieval of a minimum of 12 LNs.[20] In 2001, the AJCC recommended the assessment of at least 12 LNs for accurate staging.[8] Recently, Ng et al.[21] proposed a formula based on age, tumor site, and tumor dimensions that could be used to calculate the minimum number of LNs required to accurately stage patients with CRC.

Here, we identified maximum tumor diameter as an independent factor that

Table 5. Univariate and multivariate analyses for progression-free survival in 399 patients.

		Univariate analysis			Multivariate analysis		
Variable	Category	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	≥65 years	0.993	0.637–1.548	0.974			
Sex	Female	1.130	0.719–1.777	0.595			
LN retrieval	LN ≥ 12	10.295	1.431–74.040	0.021	3.090	0.414–23.074	0.271
T-classification	T3–4	13.442	4.238–42.635	<0.001	5.930	1.779–19.771	0.004
N-classification	N1–2	6.650	3.880–11.396	<0.001	3.870	2.189–6.842	<0.001
Tumor location	Right	0.689	0.398–1.194	0.185			
Tumor differentiation	Poor	2.328	1.359–3.987	0.002	1.215	0.692–2.134	0.499
Lymphovascular invasion	Present	2.451	1.446–4.154	0.001	1.410	0.801–2.479	0.233

LN, lymph node; HR, hazard ratio; CI, confidence interval.

influenced retrieval of ≥12 LNs; tumor size is an established predictor of LN yield.[7,14,22–26] It could be argued that larger tumors are associated with LN enlargement due to necrosis caused by an inadequate blood supply, which then induces reactive changes in regional nodes.[27] Larger tumors are thought to induce stronger antigenic immune responses in the draining LNs; this causes the reactive enlargement of regional LNs, which ultimately facilitates their detection by the pathologist during gross examination of the specimen.[26]

The results presented herein also show that right-sided tumor location is an independent factor that influences retrieval of ≥12 LNs ($p = 0.027$). Other studies also report a higher number of LNs retrieved from right-sided cancers.[6,7,14,22,23,28–32] Differences in embryological development or a large amount of mesenteric fat obtained from larger surgical specimens during right *versus* left colectomy may account for this finding.[3,33] In addition, right-sided tumors are often accompanied by microsatellite instability, which is characterized by the presence of tumor-infiltrating lymphocytes.[23] However, although we examined MMR gene status in 248 out of 429 patients, we found no association between microsatellite instability and LN retrieval.

We found no evidence that other previously described factors were related to LN yield. Many studies have identified patient age,[6,7,11,22,24,26,28,30,32,34] T-classification,[7,11,14,28,35] and N-classification[11,14,24,35] as being significantly associated with LN count; however, our data do not support these findings. In addition, multivariate analysis did not identify the length of the resected colon or tumor differentiation grade as being associated with the number of retrieved LNs.

LN retrieval from patients with early stage CRC is affected by maximal tumor length, location of the tumor, and invasion depth; neither the surgeon nor the pathologist has a significant effect on the number of retrieved LNs.[36] Here, we found that 19 out of the 75 patients with pT1 CRC showed histologic evidence of adenocarcinoma coexistent with adenoma. We therefore measured the total diameter of the tumor (the sum of the adenoma and carcinoma components) and the diameter of the carcinoma component alone as separate variables using digital pathology (similar to the method used by Toh et al.[18]). Additionally, we measured the depth of invasion from the tumor surface and from the muscularis

mucosae. Univariate analysis revealed that the following factors were associated with the retrieval of ≥12 LNs: length of the resected specimen ($p = 0.03$), maximum tumor diameter ($p = 0.025$), depth of invasion from the muscularis mucosae ($p = 0.017$), and tumor location ($p = 0.006$) (Table 3). Univariate analysis identified depth of invasion from the muscularis mucosae as being inversely related to the number of retrieved LNs. However, multivariate logistic regression analysis identified only maximum tumor diameter as a significant independent predictor of LN retrieval ($p = 0.04$) (Table 4). Our data suggest that it is the total size of the neoplastic lesion (including both adenoma and carcinoma components) rather than the size of the carcinoma that determines LN yield in patients with pT1 CRC. Given that larger tumors are likely to generate more intense immune responses, thereby increasing the size of regional LNs,[26] it could be assumed that immune system activation starts before lesions become invasive.

LN retrieval can be time-consuming and difficult when there is excess adipose tissue in the resected specimen. Therefore, the examining pathologist has a significant effect on the number of LNs harvested from a resected specimen.[35] However, other studies report that techniques used to increase LN yield are highly effective, although they are not associated with upstaging of cancer specimens.[37] Here, we were careful to control the influence of the pathologist on LN harvesting. The surgical specimens in our cohort were examined first by one pathologist and then reviewed by another to minimize the effect of the pathologist on an inadequate LN yield. A median of 19 LNs were retrieved from each of the 429 patients in the cohort. McDonald et al.[5] reported that the median number of LNs harvested ranged from 6 to 21. Taken together with our data, this suggests that the LN retrieval technique used by the pathologists was not a limiting factor in the present study.

There was no significant association between the number of LNs harvested and PFS. Nevertheless, many studies have reported a directly proportional relationship between the number of LNs removed and survival, particularly in patients with stage II disease.[6,11,25,35,38] By contrast, Wong et al.[39] reported an analysis of Surveillance Epidemiology and End Results data (1995–2005) from 30,625 non-metastatic colon cancer patients and concluded that the number of LNs examined following colon resection was not associated with survival. In addition, Moro-Valdezate et al.[7] showed that the recovery of ≥12 LNs made no significant difference to overall and disease-free 5-year survival. Nevertheless, the mechanisms underlying these associations are uncertain and remain in dispute.

The present study had some limitations. First, since the follow-up period was relatively short, the number of patients who experienced recurrence or died from CRC was small. Second, although operative techniques were standardized, and all patients underwent radical curative resection, the effect of different surgeons or surgical procedures was not accounted for. Finally, although we excluded patients with rectal cancer who received neoadjuvant chemotherapy or radiotherapy to generate a more homogeneous cohort, the study cohort was still heterogeneous since it included both colon and rectal cancer cases.

Conclusion

Maximum tumor diameter and right-sided tumor location are factors that affect the number of LNs retrieved from patients with CRC. In particular, with the help of digital microscopy, we identified maximum diameter of tumor as being independently associated with retrieval of ≥12 LNs from patients with pT1 CRC. Retrieval of ≥12

LNs had no effect on the survival of patients with CRC.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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