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Original Article

Sentinel lymph node dissection in colorectal cancers: A single-center, prospective study

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Abstract

Aim: In this study, we aimed to investigate the feasibility of ex vivosentinel lymph node (SLN) mapping and to evaluate efficacy of this technique on staging in patients with colorectal cancer.

Material and Methods: : This single-center, prospective study included a total of 35 patients (25 males, 10 females; mean age: 55 years; range, 35 to 85 years) who were diagnosed with Stage 2 colorectal cancer between May 2015 and August 2017. All patients underwent curative surgery and SLN dissection.

Results: Tumor was located in rectum in 17, in sigmoid colon in six, in right colon in seven, and in left colon in five patients. Total abdominal colectomy was performed in six, left hemicolectomy in three, right hemicolectomy in six, low anterior resection in 14, anterior resection in two, and abdominoperineal resection in four patients. Of 17 patients with clinical Stage 2 rectal cancer, 15 underwent neoadjuvant chemoradiotherapy (CRT). All patients were histopathologically diagnosed with an adenocarcinoma. Median total number of SLNs dissected was 13 with 16.9 per patient. In two patients receiving neoadjuvant CRT due to rectal cancer, a pathological complete response was achieved. The failure rate of SLNs detection was statistically significantly higher for rectal tumors than the other tumors (p=0.041) and in the patients in whom ≤7 lymph nodes dissected (p=0.023).

Conclusion: Our study results suggest that SLN mapping is a useful technique with high success ratesas well as further immunohistochemical examination of the SLNs doesn't cause stage migration. However, the success rate is lower in rectal tumors than the other tumors and in the patients with ≤7 lymph nodes dissected.

Keywords: Colorectal cancer; sentinel; lymph node

INTRODUCTION

Colorectal cancers are the fourth most common cancer and the second leading cause of cancer-related death after lung cancer (1). With the introduction of innovative concepts in the therapeutic field in recent years, its mortality has been gradually decreased (1). Currently, lymph node status is one of the major prognostic factors in colorectal cancer and determines the need for adjuvant chemotherapy, as well. Although surgery is often considered curative in node-negative disease, recurrence occurs in 20 to 30% of these patients (2). In Stage 2 colon cancer, undiagnosed lymphatic metastases and downstaging may be a possible cause of recurrence (3).

Sentinel lymph nodes (SLNs) are the first lymph nodes which receive lymphatic drainage from the primary tumor (4). In vivo or ex vivo SLN dissection is used in definitive staging of colon cancers (4). Theoretically, SLNs in all specimens should be ideally examined by immunohistochemical (IHC) testing or by further tests; however, it is not practical due to its high cost and time-

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consuming nature with a considerable loss of work productivity. To increase the accuracy of staging, ex vivo SLNs mapping can be performed using methylene blue dye.

In the present study, we aimed to investigate the feasibility of ex vivo SLNs mapping and to evaluate efficacy of this technique on staging in patients with colorectal cancer.

MATERIAL and METHODS

This single-center, prospective study was conducted at out center between May 2015 and August 2017. Patients who were diagnosed with Stage 1-2 colorectal cancer according to the preoperative Tumor, Node, Metastasis (TNM) staging were screened (5). Those with clinical and pathological Stage 3-4 colorectal cancer were excluded from the study.Data including demographic characteristics of the patients, histopathological characteristics, stage, and location of the tumor, the number and region of SLNs dissected, and IHC examination results were recorded.
A written informed consent was obtained from each patient. The study protocol was approved by the Ethics Committee of Cukurova University, Faculty of Medicine, Non-Interventional Clinical Trials with the date&number of 2.10.2015/46. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Surgical technique

Curative surgery was performed in all patients.In the ex vivo technique, 5 cc methylene blue dye (Blumet,DEFARMA Ilaç San.Tic. A.S. Istanbul/Turkey)was injected into the subserosal layer of four quadrants around the tumor (Figure 1).

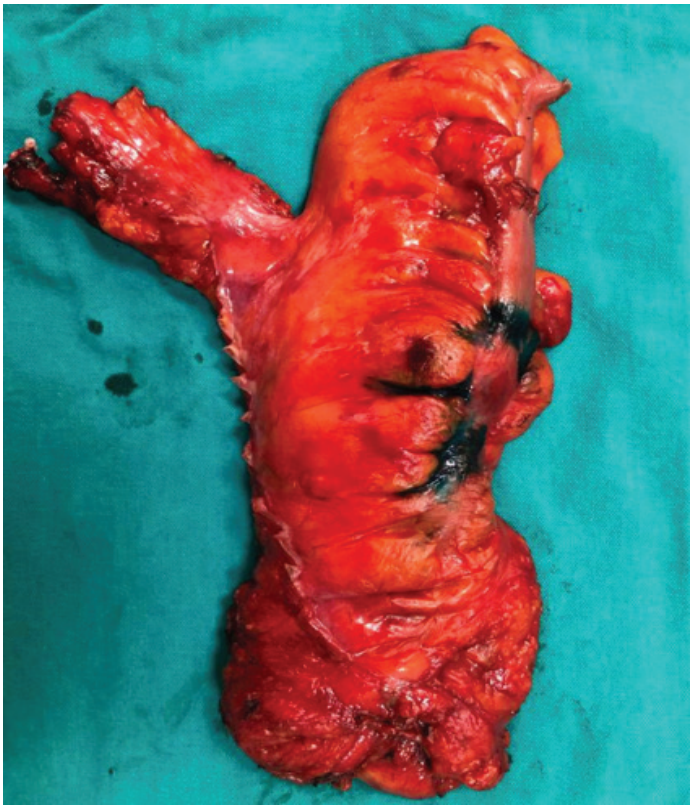

Figure 1. Methylene blue dye was injected into the subserosal layer of four quadrants around the tumor

The injection sites were gently massaged for 4 to 6 min (Figure 2). With the collaboration of a pathologist, the peritumoral region (1 cm away from the tumor; Level 1), adjacent mesenteric fat (area between the SLN 1 cm away from the tumor and mesenteric root; Level 2), and mesenteric root (3 cm distance from the mesenteric vessels ends; Level 3) were identified by palpation and visual examination. Blue lymph nodes were, then, identified on gross examination and SLN dissection was performed (Figure 3). Blue SLNs were recorded according to the location of the dissection as Level 1, Level 2, and Level 3 and were placed in embedding cassettes. The SLNs which were not identified as non-malignant through routine hematoxylin and eosin (H&E) staining were examined IHC using pancytokeratin (CK-PAN) stain.

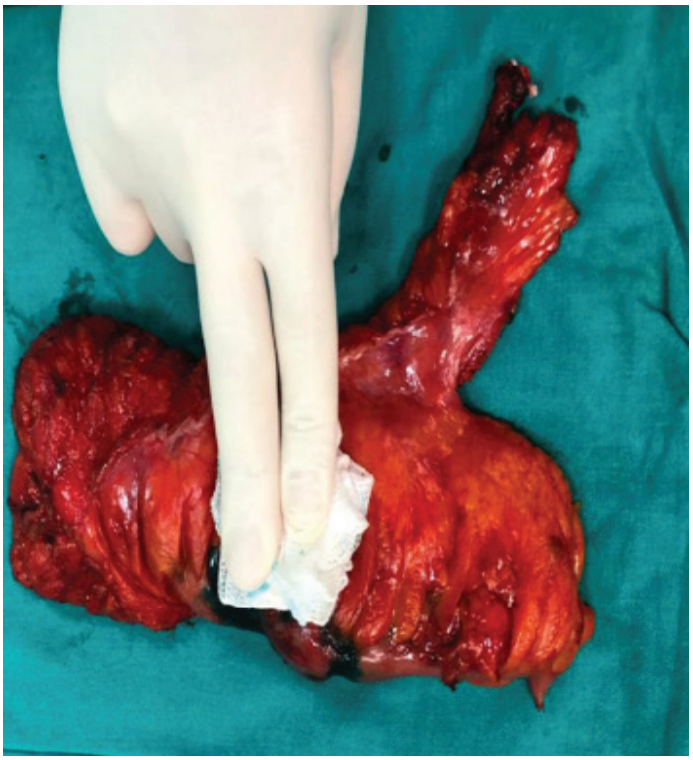

Figure 2. The injection sites were gently massaged for 4 to 6 min

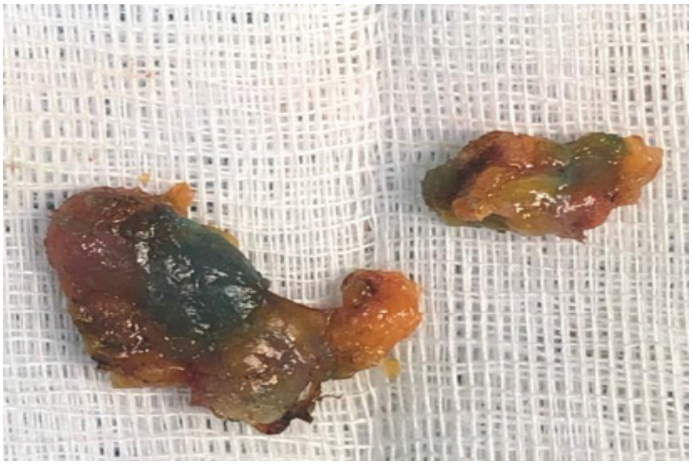

Figure 3. Blue sentinel lymph nodes

The fresh colorectal specimens were sampled, fixed in formaldehyde for a night.The formalin-fixed, paraffin-embedded (FFPE) tissues were stained with H&E. The pathological examination of both microscopic and macroscopic sections was based on the College of American Pathologists (CAP) protocols for colorectal specimens. The SLNs were stained with cytokeratin by immunohistochemistry in addition to H&E slides. The micro-invasiveness of metastasis of the tumor and isolated tumor cells which could be misdiagnosed as negative in H&E sections only were evaluated.

Statistical Analysis

Statistical analysis was performed using the Statistics Package for the Social Sciences (SPSS) version 22 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in median (min-max) values or number and frequency. The distribution of the data was analyzed using normality tests. When the data were not normally distributed, the Mann-Whitney U test was used for the comparison of two groups. The chi-square and Fisher exact tests were used for the comparison of categorical variables. Model discrimination was measured using the receiver operating characteristic (ROC) curve and expressed in the area under the curve (AUC). The AUC requires binary outcomes (presence or absence of the event). An AUC of 0.5 represents no discriminating ability, while a value of 1.0 represents perfect accuracy. Univariate analysis was also performed to analyze possible factors affecting SLN mapping. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 35 patients who were diagnosed with Stage 2 colorectal cancer based on routine H&E staining were included in the study.Of the patients, 25 were males and 10 were females with a mean age of 55 (range, 35 to 85) years.

Tumor was located in rectum in 17 (48.5%), in sigmoid colon in six (17%), in right colon in seven (20%), and in left colon in five patients (14%). Total abdominal colectomy was performed in six (17%), left hemicolectomy in three (8.5%), right hemicolectomy in six (17%), low anterior resection in 14 (40%), anterior resection in two (5.7%), and abdominoperineal resection in four patients (11%). Of 17 patients with clinical Stage 2 rectal cancer, 15 (88.2%) underwent neoadjuvant chemoradiotherapy (CRT). Twelve of them received CRT due to ≥T3, while three received CRT to preserve the sphincter. All patients were histopathologically diagnosed with an adenocarcinoma.

In all patients, negative margins were achieved postoperatively. The median number of SLNs dissected was 2 (range, 0 to 8). A total of 81 SLNs were identified in 35 patients with 2.31 per patient. In 17 patients with rectal cancer, the median number of SLNs dissected was 1 (range, 0 to 5) with 1.52 per patient. In four patients (11.4%), no SLN was identified. The overall rate of SLNs was 88.6% (n=31). The median total number of SLNs dissected was 13 (range,0 to 67) with 16.9 per patient. In two patients receiving neoadjuvant CRT due to rectal cancer, a pathological complete response was achieved. In one of these patients (2.7%), no SLN was dissected, while one SLN was identified in the other patient. Demographic and clinicopathological characteristics of the patients are presented in Table 1.

Table 1. Demographic and clinicopathological characteristics of patients

Variable	n (%)
Age, median (range)	55(35-85)
Sex	
Male	25 (71.4)
Female	10 (29.6)
Tumor localization	
Rectum	17 (48.5)
Sigmoid colon	6 (17.1)
Left colon	5 (14.2)
Right colon	7 (20)
Type of surgery	
Low anterior resection	16 (45.7)
Anterior resection	2 (5.7)
Abdominoperineal resection	4 (11.4)
Total abdominal colectomy	6 (17.1)
Right hemicolectomy	6 (17.1)
Left hemicolectomy	3 (8.5)
NeoAdjuvant CRT (for rectal tumors, n=17)	15 (42.8)
≥T3	12 (34.2)
Sphincter-sparing	3 (8.5)
Pathological complete response after CRT	2/15 (13.3)
SLN mapping	
Successful	31 (88.6)
Failed	4 (11.4)
Number of SLN harvested, median	
Range of all patients (n=35)	2 (0-8)
Range of rectal cancer patients (n=17)	1 (0-5)
Per patient for all (n=35)	2.31
Per patients for rectal cancers (n=17)	1.51
Number of lymph nodes harvested, median	
Total	13 (0-67)
Lymph nodes per patient	16.9
Upstaging after IHC (isolated tumor cells or micrometastases)	0 (0%) n=35

Data are given in number (%), unless stated otherwise. CRT, chemoradiotherapy; SLN, sentinel lymph node; IHC, immunohistochemistry.

Table 2. Demographic and clinicopathological characteristics of patients

Variable	Odds ratio	p value
Age, years		
<55	0.00	0.998
≥55		
Sex		
Male	0.00	0.992
Female		
Tumor localization		
Rectum	0.12	**0.041**
Other		
Staging (TNM)		
0		
1		
2A	0.48	0.553
2B		
2C		
T stage		
0		
1	0.42	
2		0.390
3		
4		
Lymphovascular invasion	0.44	0.524
Perineural invasion	0.47	0.489
Neoadjuvant CRT		
Yes	0.39	
No		0.325
Histopathological subtype		
Mucinous	0.00	
Non-mucinous		0.990
Peritumoral lymphocytic response		
Yes	0.00	0.990
No		
Total number of lymph nodes harvested		
≤7	0.02	**0.023**
>7		
Grade		
High-grade	0.5	0.596
Low-grade		

CRT, chemoradiotherapy; SLN, sentinel lymph node; TNM, Tumor, Node, Metastasis.

Using the CK-PAN antibody, there were no isolated tumoral cells or micrometastasis which led to stage migration. According to the tumor location, the failure rate of SLNs detection was statisticallysignificantly higher for rectal tumors than the other tumors (p=0.041). However, there was no significant difference in the failure rate ofSLNs between those receivingneoadjuvant CRT and those not (p>0.05). According to the SLN regions, the median number of SLNs in Level 1 was statistically significantly higher than Level 2 and Level 3 (p<0.001). According to the SLNs finding success or failure rates, however, there was no significant difference in terms of the distribution of lymphovascular invasion, perineural invasion, tumor nodule, T stage, and N stage (p>0.05). On the other hand, failurerate of SLNs was statistically significantly higher in the patients in whom ≤7 lymph nodes dissected (p=0.023) (Table 2 and Figure 4).

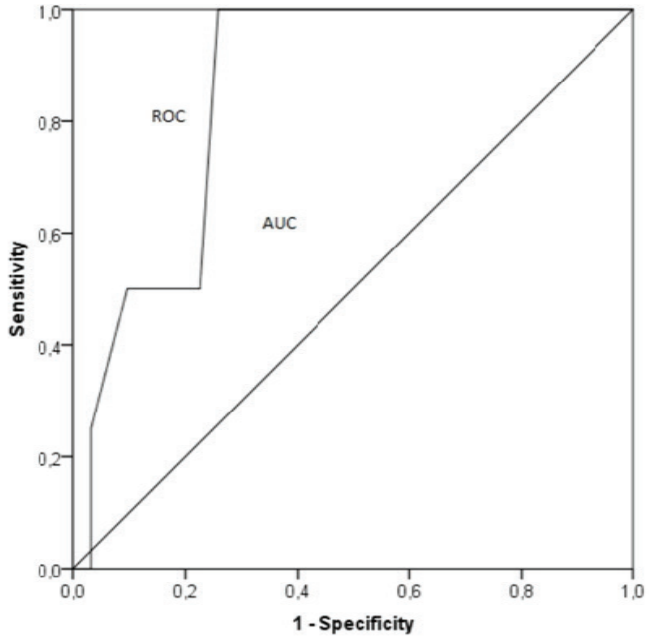

Figure 4. Failurerate of SLNs was statistically significantly higher in the patients in whom ≤7 lymph nodes dissected. The schemaof ROC curve and AUC

DISCUSSION

Accurate staging of colorectal cancers is of utmost importance not only to determine prognosis, but also to identify the need for chemotherapy and follow-up interval. Currently, adjuvant chemotherapy is a must for patients with Stage 3 colorectal cancer. About one-third of these patients benefit from adjuvant chemotherapy (6,7). Therefore, it is essential to identify Stage 3 patients who are downstaged as Stage 2 in clinical practice. One of the main goals of SLN mapping is to identify these patients who are likely to benefit from adjuvant chemotherapy.

According to American Joint Committee on Cancer (AJCC), minimum 12 lymph nodes are required for accurate staging of colorectal cancer, although this figure may vary from 9 to 18 in clinical setting (5). In our study, the median total number of lymph nodes dissected was 13 (range, 0 to

67) with a ratio of 16.9 per patient. In a systemic review of SLN mapping procedure in colorectal cancer, van der Zaag et al. (7) reported that the mean number of harvested total lymph nodes was 16.7 (range, 7.5 to 30.0) and that the overall mean number of SLNs identified was 2.8 (range, 1.0 to 7.1) across the studies. Higher number of lymph nodes in our study than those reported in the international guidelines may be attributed to our meticulous technique during surgery and pathological examination.

In the literature, the success rate of SLN identification has been reported ranging from 58 to 100%; however, the majority of studies have shown a success rate of 82 to 92% (8-11). In our study, the success rate was found to be 88.6% in 31 patients, consistent with the literature.

Currently, SLN mapping can be performed in the in vivo or ex vivo setting using patent blue, isosulfan blue, methylene blue dye, or Tc99 (8). Taken together, the rate of upstaging in further examinations varies from 0 to 46% (8). However, the majority of studies have reported a rate of upstaging between 7.4 and 23% (12-15). Inconsistent with previous studies, in our study, the IHC examination revealed no isolated tumoral cell or micrometastasis which led to stage migration. On the other hand, in a study including 58 patients, Finan et al. (16) found an upstaging rate of 0% and suggested that SLN technique used did not affect the disease stage. In our study, small sample size may have yielded misinterpretation of the results. In addition, a relatively high number of rectal tumors (48.5%) and high number of rectal cancer patients receiving neoadjuvant CRT (42.8%) may have precluded upstaging. Of note, our SLN technique is similar to those used in previous studies (12-16). Therefore, we believe that materials used during SLN identification and pathological examination did not affect the study results. Also, the SLN technique was performed by the same operators in our study, which abolishes the impact of different operators on the results.

It has been demonstrated that neoadjuvant CRT reduces the lymph node dissection rate (17). The number of lymph nodes to be harvested is 7, but not 12 for these patients which is associated with reduced SLN identification rates. In the literature, the success rate of SLN identification is relatively low in patients receiving neoadjuvant CRT. In a study, Finanet al. (16) reported that 88% patients received neoadjuvant therapy and the SLN detection rate was 85% and the accuracy of SLN mapping was 71% with an average SLN harvest of 2.2 nodes per patient. This finding is consistent with our results showing a patient ratio receiving neoadjuvant CRT of 88.2% and 82% accuracy rate with 1.52 nodes per patient.

As the number of lymph nodes in colorectal specimen increases, it is theoretically expected to obtain a higher number of SLNs and to achieve a high level of success for SLN mapping. Consistently, the failure rate of SLNs was statistically significantly higher in the patients in whom ≤7 lymph nodes dissected in our study. This finding is one of the major findings of the present study and there are no relevant data available in the literature.

The SLN is defined as the first draining lymph node which is mostly likely to spread from a primary tumor. Although this spread is known as a skip metastasis, the SLN is theoretically located peritumorally. In their study, Mogoanta et al. (9) reported that SLNs were mostly located in the immediate proximity of the tumor. Similarly, the SLNs were frequently located in the proximity of the tumor in our study. However, there is no study regarding the location of SLNs in colorectal cancer in the literature.

The clinical relevance of upstaging based on SLNs is uncertain in colorectal cancer. In a randomized-controlled study, Nissan et al. (14) investigated whether targeted nodal assessment and ultrastaging improved disease-free survival (DFS) in colorectal cancer. They reported that a 15% absolute difference in the five-year DFS between the study groups with node-negative patients was clinically meaningful. According to the National Comprehensive Cancer Network (NCCN), the use of SLN and detection of cancer cells by IHC alone should be investigational and the results should be used with caution in clinical management decisions (5). According to the AJCC Cancer Staging Manual and Handbook, micrometastases are defined as clusters of 10 to 20 tumor cells of clumps of tumor ≥0.2 mm in diameter and these micrometastases should be considered standard positive nodes (5). In a systemic review, van der Zaag (8) concluded that SLN mapping could be used to refine staging, but not to tailor treatment. In a meta-analysis, Cleothoak et al. (19) found micrometastases to be poor prognostic factors. In a multicenter, prospective study, Protic et al. (20) detection of isolated tumor cells yielded 10% decrease in survival in only T3 and T4 tumors. In another prospective study, Pallares et al. (21) reported a higher local recurrence rate in N0 patients with SLN positivity. Unlike the aforementioned studies, in the present study, we examined the feasibility and results of the SLN mapping, rather than the clinical relevance and survival rates. Consistent with our objective, Bembenek et al. (22) concluded that SLN biopsy appeared more promising in patients with limited disease than those with advanced disease and that SLN biopsy was not ready for routine clinical use in rectal cancer.

Pathological point of view showed that intensive sentinel lymph node sampling and histopathologşcal evaluation are strictly related to catch isolated tumor cells. Micrometastasis or macrometastasis are usually not a problematic issue and these diagnoses have high interobserver agreement. Immunohistochemical staining of sentinel lymph nodes doesn't improve diagnosis of isolated tumor cells.

Nonetheless, there are some limitations to this study. This is a prospective study with the small number of cases. There are some lacking of balanced distribution of the tumor location consequently treatment algoritm.

CONCLUSION

In conclusion, the clinical relevance of SLN mapping is still controversial in colorectal cancer. Based on our study, it seems to be a useful technique with high success rates.

However, the success rate was lower in rectal tumors than the other tumors and in the patients with ≤7 SLNs dissected. Of note, the SLNs were primarily located in the mesenteric region adjacent to the tumor. Unlike previous studies, SLN mapping was not found to be associated with stage migration in this patient population. We believe that the present study is valuable as it shows the feasibility and high success of the SLN mapping. However, further prospective, randomized-controlled studies are needed to evaluate its use in routine clinical practice.

Competing interests: TThe authors declare that they have no competing interest.

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Ethical approval: The study was approved by the Ethics Committee of Cukurova University, Faculty of Medicine, Non-Interventional Clinical Trials (date&number: 2.10.2015/46).

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