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Clinical application of sentinel lymph node mapping in colon cancer: *in vivo vs. ex vivo* techniques

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Purpose: Clinical usefulness of sentinel lymph node (SLN) mapping in colorectal cancer remains controversial. The aim of this study is to evaluate the accuracy of the SLN mapping technique using serial sectioning, and to compare the results between *ex vivo* and *in vivo* techniques.
Methods: From February 2011 to October 2012, 34 colon cancer patients underwent SLN mapping during surgical resection. Eleven patients were analyzed with the *in vivo* method, and 23 patients with the *ex vivo* method. Patient characteristics and results of SLN mapping were evaluated.
Results: The SLN mapping was performed in 34 patients. Mean age was 67.3 years (range, 44–81 years). Primary tumors were located in the following sites: 13 in the right colon (38.2%) and 21 in the left colon (61.8%). SLN mapping was performed successfully in 88.2% of the patients. There was no significant difference in the identification rate between the two methods (90.9% *vs.* 87.0%, P = 1.000). Both the mapping methods showed a low sensitivity and high rate of skip metastasis.
Conclusion: This study showed that SLN evaluation using serial sectioning could not predict the nodal status with clinically acceptable accuracy despite the high detection rate.
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Key Words: Sentinel lymph node biopsy, Colon neoplasms

INTRODUCTION

Accurate pathologic staging is important for improving the long-term survival in patients with colorectal cancer because administration of adjuvant chemotherapy can improve the survival [1]. However, the benefit of adjuvant chemotherapy in patients with node-negative disease remains controversial [2,3], despite loco-regional recurrence and distant metastases in more than 30% of them. These data raise the question whether the current conventional nodal staging, which examines only one section of the lymph nodes by hematoxylin and eosin (H&E) staining, might be associated with a substantial rate of understaging.

It is recommended that a minimum of 12 lymph nodes be examined before the patient is considered free of lymph node metastases [4]. Actually, other reports recommended that more lymph nodes should be examined to ensure proper staging [5,6]. However, it is time-consuming and impractical to obtain more lymph nodes. Therefore, focusing on the lymph nodes that represent the nodal status is a more effective and reliable strategy.

The sentinel lymph node (SLN) is defined as the first lymph node to receive lymphatic drainage from a primary tumor [7]. Therefore, the SLN is most likely to contain metastases and to have the potential to improve lymph node staging accuracy by thorough examination of the SLN using serial sectioning and immunohistochemical (IHC) staining. However, prognostic impact of micrometastasis detected by the IHC technique

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remains undetermined [8-10].

Previous studies investigating the feasibility and reliability of the SLN mapping in colorectal cancer have reported SLN detection rates from 85% to 100% [11-13], regardless of whether an *in vivo* or *ex vivo* technique was performed. There are several reports comparing the *ex vivo* technique with the *in vivo* technique in colon cancer surgery [14,15]. However, there are few reports that studied the SLN using serial sectioning [16]. The purposes of this study are to evaluate the detection rate, accuracy, and false negative rate of SLN technique using serial sectioning and to compare the results between the *ex vivo* and the *in vivo* technique in colon cancer.

METHODS

Patients

From February 2011 to October 2012, 34 colon cancer patients underwent SLN mapping during surgical resection. Exclusion criteria were synchronous colon carcinoma, recurrent or metastatic colon cancer, previous chemotherapy or radiation therapy, and emergency operation. Surgery was performed either via an open or laparoscopic approach. We planned to perform *ex vivo* mapping in the beginning and change to *in vivo* mapping. Exceptionally, *ex vivo* method was selected in case of difficulty in administering a subserosal injection and a poor medical status during *in vivo* period.

Eleven patients were analyzed with the *in vivo* method, and 23 patients with the *ex vivo* method. Patient characteristics and results of SLN mapping were evaluated. All of the procedures will be performed by one colorectal surgeon. The study was approved by the Institutional Review Board of Ajou University Hospital..

SLN detection processing

In the *in vivo* technique, 1–2 mL of 1% methylene blue was injected into the subserosa in four quadrants around the tumor after detection of tumor location. Laparoscopically, the colonic segment containing the lesion was carefully moved to the abdominal wall using atraumatic graspers, and a 22-gauge spinal needle was inserted into the abdominal wall and into subserosal layer of the colon in the tangential plane. The blue-stained lymph node was detected and marked with a suture within the first 10 minutes after injection. After completion of the surgical procedure, the marked lymph node was dissected from the mesentery and sent to the surgical pathology department for evaluation. In the *ex vivo* technique, 1–2 mL of 1% methylene blue was injected into the subserosal layer in four quadrants around the tumor. The injection sites were gently massaged for 2 to 3 minutes. The mesentery was inspected for blue LNs by palpation and visual examination. The identified blue lymph nodes were dissected and sent to the surgical pathology department for evaluation. Blue lymph nodes, partially blue lymph nodes, and lymph nodes with connecting blue vessels were regarded as SLNs.

Pathologic examination

All of the SLNs were cut in serial sections, stained with H&E, and evaluated for tumor involvement. Sentinel nodes were serially sectioned at 0.5-mm intervals and assessed using H&E staining. All of the non-SLNs were bivalved and examined with routine H&E evaluation. The SLN mapping procedure was tested in 10 patients before the study. All SLN mapping procedure were performed by the same surgeon. A positive sentinel node was defined as a blue-stained node containing single cells or cell aggregates demonstrating morphologic features consistent with colon carcinoma apparent on evaluation of H&E.

Detection and accuracy rates of SLN mapping

The identification rate was defined as the number of mapping procedures finding at least one blue node out of the total number of mapping procedures performed. Sensitivity of SLN mapping was defined as the proportion of patients with positive nodes found by routine H&E examination to have positive SLNs. False-negative cases were defined as those where the SLNs were negative but the NSLNs were positive. Skip metastases were defined as metastases in non-SLNs with negative SLNs metastasis. Accuracy of SLN mapping and biopsy was defined as the proportion of patients with successful lymphatic mapping having SLN examination.

Statistics

Pearson chi-square or Fisher exact test was used to assess differences in the clinicopathological features. Continuous data were compared by Student t-test. All statistical tests were two-sided and performed using SPSS ver. 15.0.0 (SPSS Inc., Chicago, IL, USA). A P-value ≤ 0.05 was considered to indicate a significant difference.

RESULTS

Clinicopathological characteristics

The SLN mapping procedure was performed in 34 patients. Mean age was 67.3 years (range, 44–81 years). Of these 34 patients, two had T1 lesions, 4 were classified as T2, 24 were classified as T3, and 4 were as T4. Regional LN involvement was identified in 17 patients (50.0%), and 17 patients (50.0%) were node-negative. Five patients were stage 1, 12 were stage 2, and 17 were stage 3. Primary tumors were located in the following sites: 13 in the right colon (38.2%) and 21 in the left colon (61.8%). Mean tumor size was 5.1 cm for the *in vivo* technique, and 5.3 cm for the *ex vivo* technique (Table 1).

Sentinel lymph node mapping

SLN mapping was successful in 90.9 % of the patients with the *in vivo* technique and 87.0% of the patients with the *ex vivo* technique (P = 1.000). Mean number of total lymph nodes retrieved was significantly different between two techniques (13.4 ± 6.0 *vs.* 24.2 ± 13.4, P = 0.003). Mean number of SLNs retrieved was not significantly different between the two techniques (1.2 ± 0.6 *vs.* 1.2 ± 0.9, P = 0.907). There was no significant difference between the two techniques with respect to sensitivity, negative predictive value, and skip metastasis. For the *in vivo* technique, the sensitivity was 50.0% (3/6), negative predictive value was 70.0% (7/10), and rate of skip metastasis was 50.0% (3/6). For the *ex vivo* method, the sensitivity was 55.6% (5/9), negative predictive value was 80.0% (16/20), and rate of skip metastasis was 44.4% (4/9) (Table 2).

Table 1. Patient characteristics

Characteristic	*In vivo* (n = 11)	*Ex vivo* (n = 23)	P-value
Gender			0.705
Male	8 (72.7)	14 (60.9)	
Female	3 (27.3)	9 (39.1)	
Age (yr)	71.1 ± 4.7	65.5 ± 12.5	0.070
Tumor size (cm)	5.1 ± 2.5	5.3 ± 2.2	0.759
Tumor site			0.024
Right colon	1 (9.1)	12 (52.2)	
Left colon	10 (90.9)	11 (47.8)	
T stage			0.455
T1	1 (9.1)	1 (4.3)	
T2	0 (0)	4 (17.4)	
T3	8 (72.7)	16 (69.6)	
T4	2 (18.2)	2 (8.7)	
TNM stage			0.539
I	1 (9.1)	4 (17.4)	
II	3 (27.3)	9 (39.1)	
III	7 (63.6)	10 (43.5)	
Tumor grade			0.245
Well	0 (0)	3 (13.0)	
Moderately	11 (100)	18 (78.3)	

Values are presented as number (%) or mean ± standard deviation.

DISCUSSION

The nodal analysis in patients with colorectal cancer is a significant determining factor for further oncological treatment and for the prediction of survival. Patients with nodal disease should undergo adjuvant chemotherapy because of recurrence potential. The optimal number of lymph nodes required to accurately predict lymph node negativity has been a point of debate. The Working Party Report to the World Congress of Gastroenterology recommended that a minimum of 12 lymph nodes be examined before the patient is considered free of lymph node metastases [4]. Baxter et al. [17] demonstrated an overall poor compliance rate with the guideline of harvesting more than 12 nodes in colorectal cancers, with only 37% of cases meeting the guideline.

The number of nodes recovered from a surgical specimen is mainly related to the extent of the surgical resection and the completeness of the pathologic examination [18,19]. The extent of the dissection performed by surgeons directly affects the survival. Therefore, surgeons should strictly adhere to the guidelines for colon cancer surgery. For complete cure of cancer, radical *en bloc* removal of lymph nodes should be performed. This is the reason why SLN mapping cannot contribute to conservative treatment of colorectal cancer. A recent report suggested that sentinel node mapping may be used to detect the aberrant drainage of sentinel nodes in colon cancer, leading

Table 2. Sentinel lymph node mapping in patients with colon cancer

Variable	*In vivo* (n = 11)	*Ex vivo* (n = 23)	P-value
No. of harvested nodes	13.4 ± 6.0	24.2 ± 13.4	0.003
Right colon	14.0 ± 0.0	28.8 ± 11.8	
Left colon	13.3 ± 6.3	19.2 ± 13.8	
No. of nonsentinel nodes	12.2 ± 6.1	23.0 ± 13.8	0.003
Right colon	14.0 ± 0.0	27.8 ± 12.2	
Left colon	12.0 ± 6.4	17.8 ± 14.1	
No. of sentinel nodes	1.2 ± 0.6	1.2 ± 0.9	0.907
Sentinel lymph node mapping			1.000
Detection rate (patients with success)	10 (90.9)	20 (87.0)	
Failure rate (patients with failure)	1 (9.1)	3 (13.0)	
False negative (patients with skip metastases)	3/6 (50.0)	4/9 (44.4)	1.000
Sensitivity	3/6 (50.0)	5/9 (55.6)	1.000
Accuracy	7/10 (70.0)	16/20 (80.0)	0.657
Negative predictive value	4/7 (57.1)	11/15 (73.3)	

Values are presented as mean ± standard deviation or number (%).

to more extensive resection [20].

Pathologists should assess sufficient number of lymph nodes to detect tumor positive lymph nodes. Currently, the enlarged lymph nodes are removed manually, half-dissected, and examined to detect the tumor cell. To improve the nodal analysis, several methods have been introduced, such as SLN evaluation, IHC staining, and fat clearing technique. Fat clearing technique is time-consuming and difficult to perform in routine practice [21]. On the contrary, SLN mapping is a simple and inexpensive technique to improve the staging in a clinical setting. Furthermore, sentinel node mapping helps the pathologist to evaluate the nodal status using lesser nodes. Mainly the sentinel node mapping in colon cancer was used to assess the micrometastasis using serial sectioning and IHC staining. However, because its clinical significance remains unclear, we focused on the predictive ability of the SLN technique for nodal status.

Although the sentinel node mapping has a potential advantage to improve staging, there is controversy about the effectiveness of the sentinel node mapping in a clinical setting. Some papers reported that the sentinel node mapping does not improve staging accuracy in colon cancer because of its low sensitivity and high false-negative rate [22,23].

This study showed that the SLN mapping was successfully accomplished with an overall identification rate of 88.2%, with a detection rate of 90.9% for the *in vivo* technique and a detection rate of 87% for the *ex vivo* technique. Variable detection rates have been reported in the literature [11-13]. Our report suggested that there was no significant difference in the detection rate between the two procedures. Therefore, the *ex vivo* technique can be selected for SLN mapping although the *in vivo* technique is more physiological than the *ex vivo* technique.

The accuracy of the SLN mapping was 76.7% in our study. Previous studies reported accuracy between 78% and 100%. The reason that this study showed a little lower accuracy was that the number of the SLN s and the analysis technique used were different from those in previous studies. We removed one blue-stained node in most cases because we considered SLN as first node draining from a tumor. We analyzed the SLN by using the serial sectioning method because the clinical significance of lymph node micrometastases remains controversial [8-10].

Although this procedure was performed successfully with a good detection rate, it could not predict node positivity because the sensitivity was very low and the false negative rate was high in this study. This result is similar to those in some previous reports [13,24,25], although there are papers reporting contradictory results [11,12,26]. This can be explained by differences in inclusion criteria and analysis technique used. In our study, the high false negative rate may be due to high proportion of T3/T4 tumors. It is reported that skip metastases result from the obstruction of lymphatic channels due to bulky tumor or invasion and variations or connections in the lymphatic drainage pathway [22].

The number of SLNs was not different between the two groups. However, more number of lymph nodes were harvested after the *ex vivo* technique compared to the *in vivo* technique. This can be explained by the fact that the *ex vivo* mapping group included more cases of right colon cancers. The number of nodes recovered from a surgical specimen is related to the location of primary tumor [27]. Baxter et al. [17] reported that more nodes were examined in patients with right-sided colon cancer than in those with left-sided colon cancer. Chou et al. [28] reported that within the colon cohort, increased LN harvests were observed in tumors that were located in the ascending colon/hepatic flexure and transverse colon. This difference may be due to a longer resected specimen in right-sided colon cancer than in left-sided colon or rectal cancer. The limitations of this study are that it is not a randomized clinical trial with small sample size and inability to compare two methods with same injection technique. However, this study suggests that the SLN evaluation using serial sectioning in colon cancer is inappropriate in clinical practice.

In conclusion, this study showed that the *in vivo* technique is comparable to the *ex vivo* technique in detecting SLNs in colon cancer. Also, suggested that SLN evaluation using serial sectioning could not predict the nodal status with clinically acceptable accuracy despite a high detection rate. However, the clinical usefulness needs to be further investigated by performing large-scale trials in the future.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Cohen AM, Kelsen D, Saltz L, Minsky BD, Nelson H, Farouk R, et al. Adjuvant therapy for colorectal cancer. Curr Probl Surg 1997;34:601-76.
2. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106-30.
3. André T, Afchain P, Barrier A, Blanchard P, Larsen AK, Tournigand C, et al. Current status of adjuvant therapy for colon cancer. Gastrointest Cancer Res 2007;1:90-7.
4. Fielding LP, Arsenault PA, Chapuis PH, Dent O, Gathright B, Hardcastle JD, et al. Clinicopathological staging for colorectal cancer: an International Documentation System (IDS) and an International Comprehensive Anatomical Terminology (ICAT). J Gastroenterol Hepatol 1991;6:325-44.
5. Chen SL, Bilchik AJ. More extensive nodal dissection improves survival for stages I to III of colon cancer: a population-based study. Ann Surg 2006;244:602-10.
6. Choi HK, Law WL, Poon JT. The optimal number of lymph nodes examined in stage II colorectal cancer and its impact of on outcomes. BMC Cancer 2010;10:267.
7. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg 1992;127:392-9.
8. Yasuda K, Adachi Y, Shiraishi N, Yamaguchi K, Hirabayashi Y, Kitano S. Pattern of lymph node micrometastasis and prognosis of patients with colorectal cancer. Ann Surg Oncol 2001;8:300-4.
9. Liefers GJ, Cleton-Jansen AM, van de Velde CJ, Hermans J, van Krieken JH, Cornelisse CJ, et al. Micrometastases and survival in stage II colorectal cancer. N Engl J Med 1998;339:223-8.
10. Calaluce R, Miedema BW, Yesus YW. Micrometastasis in colorectal carcinoma: a review. J Surg Oncol 1998;67:194-202.
11. Bembenek A, Schneider U, Gretschel S, Fischer J, Schlag PM. Detection of lymph node micrometastases and isolated tumor cells in sentinel and nonsentinel lymph nodes of colon cancer patients. World J Surg 2005;29:1172-5.
12. Dahl K, Westlin J, Kraaz W, Winqvist O, Bergkvist L, Thorn M. Identification of sentinel nodes in patients with colon cancer. Eur J Surg Oncol 2005;31:381-5.
13. Bertagnolli M, Miedema B, Redston M, Dowell J, Niedzwiecki D, Fleshman J, et al. Sentinel node staging of resectable colon cancer: results of a multicenter study. Ann Surg 2004;240:624-8.
14. Tuech JJ, Pessaux P, Di Fiore F, Nitu V, Lefebure B, Colson A, et al. Sentinel node mapping in colon carcinoma: in-vivo versus ex-vivo approach. Eur J Surg Oncol 2006;32:158-61.
15. Park JS, Chang IT, Park SJ, Kim BG, Choi YS, Cha SJ, et al. Comparison of *ex vivo* and *in vivo* injection of blue dye in sentinel lymph node mapping for colorectal cancer. World J Surg 2009;33:539-46.
16. Vîlcea ID, Vasile I, Mirea CS, Meşina C, Enache SD, Tenovici M, et al. Sentinel lymph node study in colorectal cancer using serial sectioning and Hematoxylin-Eosin staining: importance and limitations. Rom J Morphol Embryol 2011;52(1 Suppl):379-83.
17. Baxter NN, Virnig DJ, Rothenberger DA, Morris AM, Jessurun J, Virnig BA. Lymph node evaluation in colorectal cancer patients: a population-based study. J Natl Cancer Inst 2005;97:219-25.
18. Bamboat ZM, Deperalta D, Dursun A, Berger DL, Bordeianou L. Factors affecting lymph node yield from patients undergoing colectomy for cancer. Int J Colorectal Dis 2011;26:1163-8.
19. Caplin S, Cerottini JP, Bosman FT, Constanda MT, Givel JC. For patients with Dukes' B (TNM Stage II) colorectal carcinoma, examination of six or fewer lymph nodes is related to poor prognosis. Cancer 1998;83:666-72.
20. Saha S, Johnston G, Korant A, Shaik M, Kanaan M, Johnston R, et al. Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation. Am J Surg 2013;205:302-5.
21. Wong JH, Johnson DS, Hemmings D, Hsu A, Imai T, Tominaga GT. Assessing the quality of colorectal cancer staging: documenting the process in improving the staging of node-negative colorectal cancer. Arch Surg 2005;140:881-6.
22. Cahill RA. What's wrong with sentinel node mapping in colon cancer? World J Gastroenterol 2007;13:6291-4.
23. Lim SJ, Feig BW, Wang H, Hunt KK, Rodriguez-Bigas MA, Skibber JM, et al. Sentinel lymph node evaluation does not improve staging accuracy in colon cancer. Ann Surg Oncol 2008;15:46-51.
24. Viehl CT, Hamel CT, Marti WR, Guller U, Eisner L, Stammberger U, et al. Identification of sentinel lymph nodes in colon cancer depends on the amount of dye injected relative to tumor size. World J Surg 2003;27:1285-90.
25. Feig BW, Curley S, Lucci A, Hunt KK, Vauthey JN, Mansfield PF, et al. A caution regarding lymphatic mapping in patients with colon cancer. Am J Surg 2001;182:707-12.
26. Paramo JC, Summerall J, Poppiti R, Mesko TW. Validation of sentinel node mapping in patients with colon cancer. Ann Surg Oncol 2002;9:550-4.
27. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 2005;353:2654-66.
28. Chou JF, Row D, Gonen M, Liu YH, Schrag D, Weiser MR. Clinical and pathologic factors that predict lymph node yield from surgical specimens in colorectal cancer: a population-based study. Cancer 2010;116:2560-70.