Annals of Surgical Oncology, **8**(2):150–157
Published by Lippincott Williams & Wilkins

Validation of Lymphatic Mapping in Colorectal Cancer: In Vivo, Ex Vivo, and Laparoscopic Techniques

Thomas F. Wood, MD, Sukamal Saha, MD, Donald L. Morton, MD, George J. Tsioulias, MD, DMSc, Decio Rangel, MD, William Hutchinson Jr., MD, Leland J. Foshag, MD, Anton J. Bilchik, MD, PhD

Background: The use of lymphatic mapping (LM) is being investigated to improve the staging of colorectal cancer (CRC) and thereby identify patients who might benefit from adjuvant chemotherapy. This study evaluated in vivo, laparoscopic, and ex vivo approaches for the ultrastaging of CRC.

Methods: Seventy-five CRC patients were enrolled in a study of LM with peritumoral injection of isosulfan blue dye. LM was undertaken during open colon resection (OCR) in 64 patients, during laparoscopic colon resection (LCR) in 9 patients, and after specimen removal (ex vivo) in 2 patients. Ex vivo LM was also undertaken in 6 patients after unsuccessful in vivo LM. All nodes were examined by hematoxylin and eosin (H&E) staining; in addition, sentinel lymph nodes (SNs) were multisectioned and examined by immunohistochemical staining with cytokeratin (CK-IHC).

Results: At least one SN was identified in 72 patients (96%). In vivo LM identified SNs in 56 of 64 (88%) patients undergoing OCR and in 9 of 9 (100%) patients undergoing LCR. Ex vivo LM was undertaken as the initial mapping procedure in 2 cases of intraperitoneal colon cancer and after in vivo LM had failed in 6 cases of extraperitoneal rectal carcinoma; an SN was identified in 7 of the 8 cases. Focused examination of the SN correctly predicted nodal status in 53 of 56 OCR cases, 9 of 9 LCR cases, and 6 of 7 ex vivo cases. Multiple sections and CK-IHC identified occult micrometastases in 13 patients (17%), representing 10 OCR, 1 LCR, and 2 ex vivo cases.

Conclusions: LM of drainage from a primary CRC can be accurately performed in vivo during OCR or LCR. Ex vivo LM can be applied when in vivo techniques are unsuccessful and may be useful for rectal tumors. During LCR, colonoscopic injection can be used to mark the primary tumor and define the lymphatic drainage so that adequate resection margins are obtained. These LM techniques improve staging accuracy in CRC.

Key Words: Colorectal carcinoma—Sentinel node—Lymphatic mapping—Staging—Laparoscopic colon resection—Ex vivo.

Colorectal cancer (CRC) is the second leading cause of deaths from cancer in the United States. The 5-year survival rate is high (90%) after treatment of American Joint Committee on Cancer (AJCC) stage I CRC but decreases substantially as the disease progresses to stage II (75%) and stage III (50%).[1] Hence, the presence of lymph node metastasis is one of the most important prognostic factors.

Approximately one third of patients initially diagnosed with AJCC stage I or II CRC develop systemic disease despite "negative" lymph nodes. This implies that these patients have occult disease not detected by current techniques. Previous studies have demonstrated that lymph node micrometastases documented by ultrastaging correlate with poorer survival.[2,3] Because the average CRC resection specimen contains 15 or more lymph nodes, the use of ultrastaging techniques on each lymph node would be labor and cost intensive. Therefore, a means of focusing an examination on the lymph

Received March 17, 2000; accepted July 27, 2000.

From the John Wayne Cancer Institute (TFW, SS, DLM, GJT, DR, WH, LJF, AJB), Santa Monica, California and the Department of Surgery (SS), McLaren Regional Medical Center, Michigan State University, Flint, Michigan.

Presented at the 53rd Annual Meeting of the Society of Surgical Oncology, New Orleans, Louisiana, March 16-19, 2000.

Address correspondence to: Anton J. Bilchik, MD, PhD, John Wayne Cancer Institute, 2200 Santa Monica Boulevard, Santa Monica, CA 90404; Fax: 310-449-5261; Email: bilchika@jwci.org

nodes most likely to contain metastases would be advantageous.

Morton and colleagues[4] popularized the sentinel lymph node (SN) concept in melanoma. Giuliano and co-workers[5] later applied the concept in breast cancer. Because the first ("sentinel") lymph nodes to receive the lymphatic drainage from the primary tumor are the most likely to contain metastasis, examination of these SNs could be used to determine which patients should not be subjected to the morbidity associated with complete lymphadenectomy. More recently, Bilchik et al.[6] and Saha et al.[7] have applied the lymphatic mapping (LM) technique to identify SNs in patients with CRC. However, unlike in melanoma and breast cancer, LM in CRC is not used to limit the extent of lymph node dissection, but rather to improve staging by a focused ultrastaging examination of the SNs.

In our early experience using LM in CRC (unpublished data, 1999), we successfully mapped the SN in more than 90% of cases, and most SNs were identified during open colon resection (OCR). One limitation of the technique, however, involved failures of LM for rectal tumors below the peritoneal reflection. Unpublished data from investigators at the University of Hawaii (JH Wong, May, 1999) indicate that LM of the drainage from a CRC can be performed ex vivo, i.e., after the tumor has been removed. We hypothesized that ex vivo LM might be useful when in vivo LM failed to identify an SN, especially in patients with rectal tumors, and that the approach could be applied during laparoscopic colon resection (LCR). This study expanded our initial experience with in vivo LM, and it evaluated the potential of ex vivo and laparoscopic LM to improve staging of CRC.

METHODS

Between August 1996 and February 2000, 75 patients with clinically localized CRC were enrolled in a study of LM undertaken in vivo during OCR or laparoscopic colon resection (LCR), or ex vivo after the tumor had been resected. Informed consent was obtained prior to surgery.

In Vivo Techniques

Laparotomy and Routine Abdominal Exploration

The tumor was localized. After resectability had been determined and before mobilization of the colon, .5–1 cc of isosulfan blue dye (Lymphazurin, Ben Venue Laboratories, Inc., Bedford, OH) was carefully injected subserosally around the periphery of the tumor using a tuberculin syringe (Fig. 1). The dye traveled from the injection site along the lymphatics to the SN(s) typically within 30–60 seconds. Occasionally gentle dissection of the mesentery was performed to trace the lymphatic path to the SN. Each blue-stained node was marked with sutures, and the colectomy performed in the standard

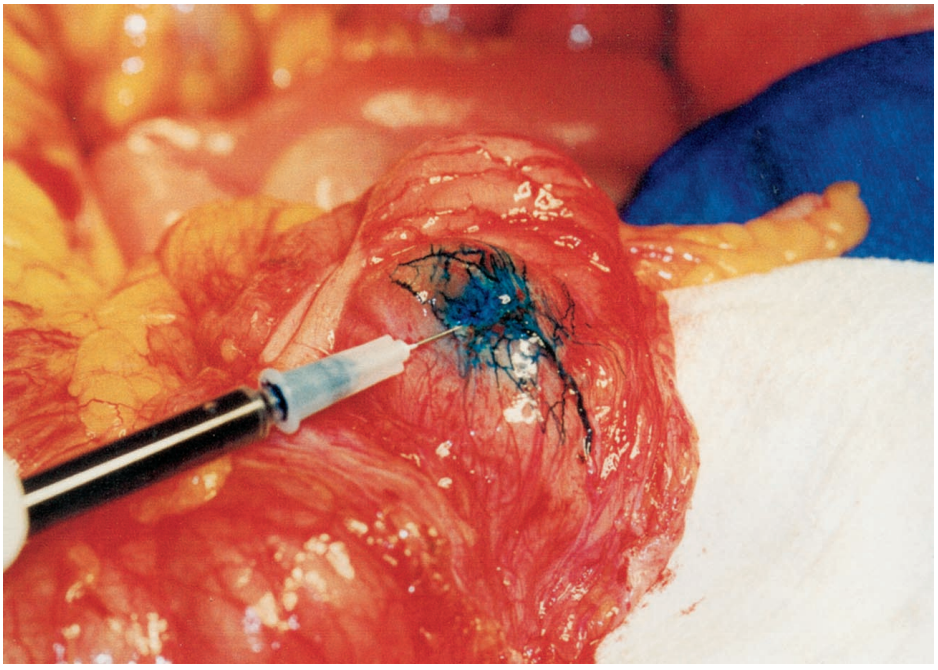

FIG. 1. A tuberculin syringe is used to inject isosulfan blue dye subserosally around the periphery of the tumor. The blue dye immediately flows in the lymphatic channels toward the SN(s).

fashion, including all blue nodes in the specimen. The specimen was submitted for pathologic review.

Laparoscopy/Colonoscopy

Patients were placed on the operating table in the low lithotomy position. The abdomen was insufflated. Laparoscopic abdominal exploration was performed to confirm suitability for LCR, and operative ports placed in appropriate locations for the specific procedure planned. The bowel was run using endobabcocks. The appropriate segment of bowel was mobilized by sharp dissection of the peritoneal attachments. The bowel was clamped distal to the tumor and colonoscopy performed. The tumor or polypectomy site was visualized and transilluminated (Fig. 2A) and LM was performed. The site was injected submucosally with .5–1 cc of blue dye via colonoscope. The site of the primary tumor injection was visualized by the laparoscope (Fig. 2B). The appropriate segment of colon and mesentery was exposed. Under the magnification of laparoscopy, the blue dye was followed from the primary tumor along the lymphatics to the SN(s). Each SN was marked with a clip or suture. A minilaparotomy was then performed and any additional SNs marked with sutures. The bowel resection and anastomosis were performed extracorporeally. All blue nodes and lymphatics were included in the mesenteric resection (Fig. 3). The specimen was submitted for pathologic review.

Ex Vivo Technique

The ex vivo technique was used either as a primary LM procedure, or secondarily for failed in vivo attempts at LM. After routine completion of the colectomy, the specimen was immediately taken to a side table. Isosulfan blue dye in a volume of 1–2 cc was then carefully injected subserosally around the tumor using a tuberculin syringe. The dye was visualized as it traveled from the primary site along the lymphatic channels to the SN(s) within the mesentery (Fig. 4). Each SN was marked with a suture. The specimen was submitted for pathologic review.

Histopathology Protocol

Pathologic review entailed routine microscopic analysis of the tumor, margins and all lymph nodes via hematoxylin and eosin (H&E) staining. In addition, each marked SN was examined by a focused technique originally developed for the examination of SNs draining primary breast carcinomas.[8] The pathologist bisected or sectioned each SN into slices no thicker than 2–3 mm. Paraffin sections, each approximately 4 μm thick, were cut at two levels separated by 200 μm. One section from each level was stained with H&E and another with CK-IHC. A false-negative SN was defined as an SN that contained no tumor cells when one or more nonsentinel nodes in the specimen were tumor-positive.

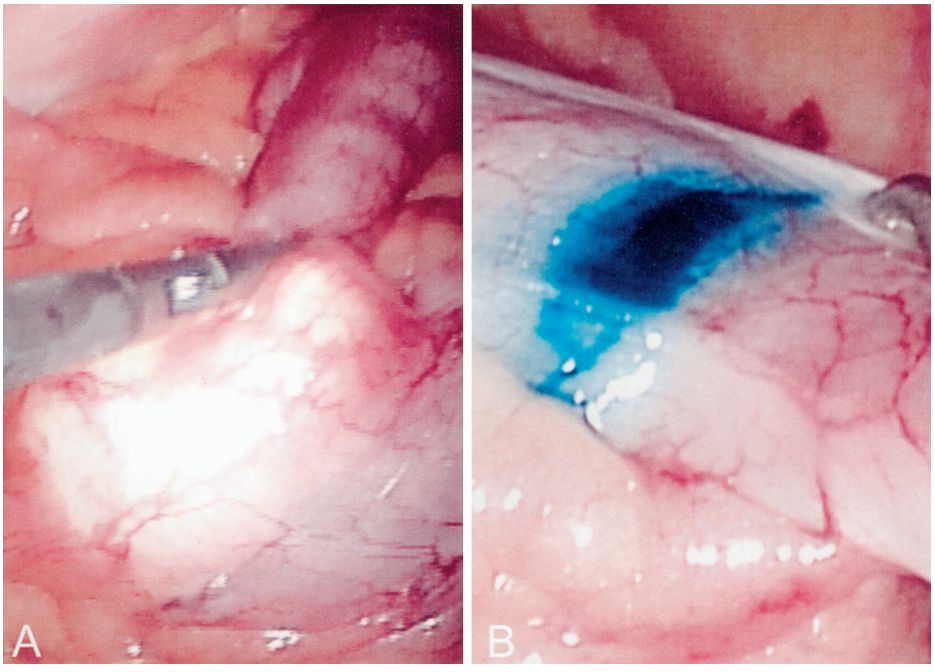

FIG. 2. (A) Colonoscopic transillumination of the tumor/polypectomy site during laparoscopy. (B) Isosulfan blue tattooing of the tumor site after colonoscopic injection during laparoscopy. Note the lymphatic channel running inferior to the blue tattoo.

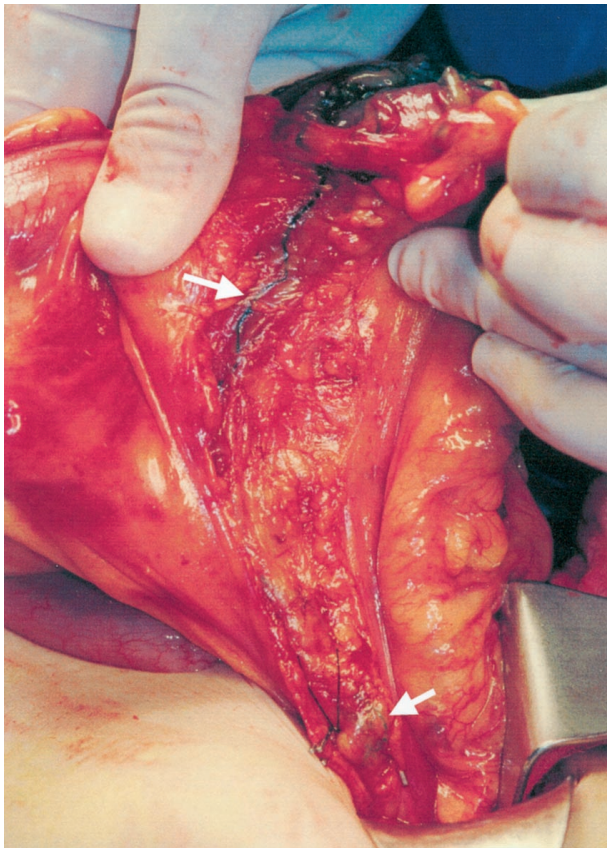

FIG. 3. LM undertaken during LCR of a T1 cecal carcinoma reveals blue-stained lymphatic channels running from the primary tumor site to the SN deep at the base of the mesentery. Minilaparotomy has been performed and the specimen delivered extracorporeally. Arrows indicate the lymphatic channel superiorly and inferiorly as it enters the SN (marked with a suture and clips).

Immunohistochemical Staining

Paraffin sections for CK-IHC were placed on charged slides (Superfrost Plus M6416-plus, Baxter Diagnostics Inc, McGaw Park, IL). A standardized procedure used an automated immunostainer (Ventana ES, Ventana Medical Systems, Tucson, AZ) with enzyme digestion (Protease 1) of tissue sections for 8 minutes and AE-1/AE-3 CK antibody (Dako Corporation, Carpinteria, CA) staining (1:200 dilution) for 32 minutes. Diaminobenzidene was the chromogen. IHC stains were interpreted according to strict criteria that required strong immunoreactivity combined with microanatomic and cytologic features compatible with CRC.

RESULTS

The 75 study patients represented a male:female ratio of 30:45 and had an average age of 68 years (range, 28–97 years). Primary tumors were in the right colon (n = 24), sigmoid colon (n = 18), rectum (n = 14), cecum (n = 10), left colon (n = 5), and transverse colon (n = 4). LM identified at least one SN in 72 (96%) patients. The average number of SNs identified was 2 (range, 1–4), and the average number of nodes in each CRC specimen was 15 (range, 2–28). In 7 cases, LM demonstrated primary lymphatic drainage to SNs outside the margins of conventionally planned resections. In each of these cases the operative procedure was altered (Table 1). For example, during four of the LCRs, an SN was mapped deep at the base of the mesentery, and the resection was extended to encompass this area. In two other right colon carcinomas, LM demonstrated SNs to the left of the middle colic vessels, and extended right hemicolectomies were performed to include the SNs in the mesentery of the transverse colon.

Overall, focused examination of the SNs correctly reflected the tumor status of the nodal basin in 68/72 (94%) cases (Table 2). Of the 35 patients with nodal metastases, 30 had positive SNs; thus the sensitivity of the SN as an overall indicator of nodal status was 88%. Thirteen (17%) of the 75 patients had occult nodal micrometastases identified only during a focused examination of the SN (Table 3); five tumors were upstaged by examining multiple sections of an SN, and the eight remaining tumors were upstaged by the results of CK-IHC. Another tumor was upstaged when micrometastasis was identified by routine H&E staining of an aberrantly positioned SN. The patient, who had a right colon cancer, underwent extended right colectomy (see above) after LM identified an SN in the mesentery of the transverse colon, left of the middle colic vessels. This SN was the only positive node of 18 in the resection specimen. The SN was the only positive node in 17 of the 75 cases; in 11 of these cases, the positive SN was demonstrated only after the focused pathologic examination (Table 3).

Tumor (T) stage correlated with increasing probability of node positivity ($P = .0001$, Wilcoxon sign rank test) (Table 4). Although only 1 of 14 (7%) T1 tumors and 7 of 22 (32%) T2 tumors had positive nodes, 22 of 33 (67%) T3 and 5 of 6 (83%) T4 tumors were associated with nodal metastasis. Similarly, advancing T-stage correlated significantly with SN positivity ($P = .004$, Wilcoxon sign rank test).

In Vivo Techniques

In 64 patients, LM was undertaken during OCR (Table 2). In 56 of these cases (88%), at least one SN was identified (range, 1–4). Of the 8 unsuccessful cases, 7 were rectal tumors below the peritoneal reflection and 1 was a right colon tumor. The tumor status of the SN was an accurate indicator of nodal status in 53 of the 56

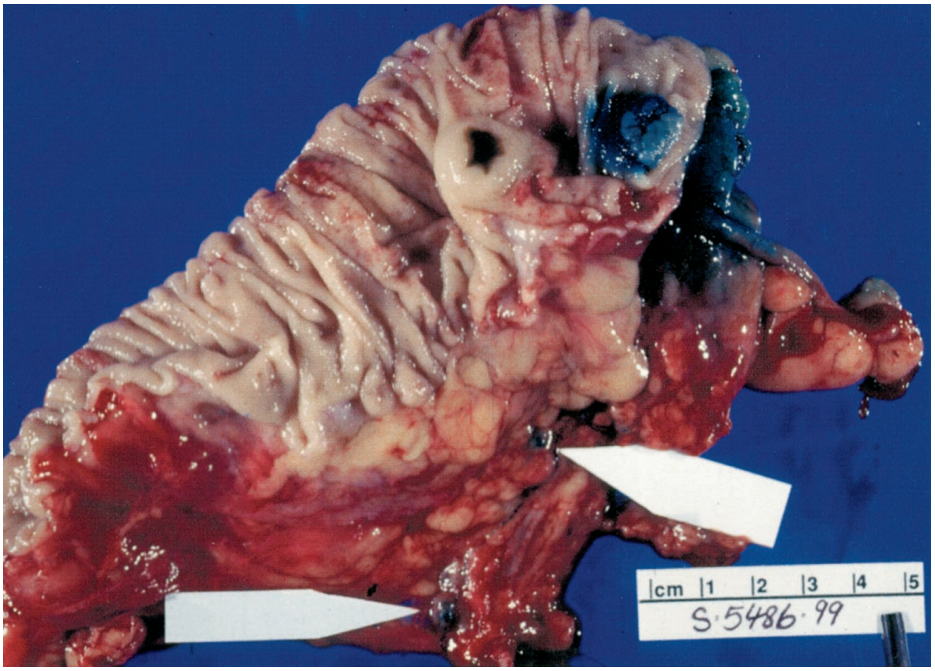

FIG. 4. Ex vivo LM identified SNs (arrows) in the mesentery. Injection of isosulfan blue dye was undertaken at a side table after the specimen had been removed from the body.

patients (95%), and focused examination of this node upstaged CRC in 10 patients.

In nine patients, LM was undertaken during LCR (Table 2). In all of these cases, at least one SN was identified (range, 1–3). In four cases, LM identified an SN deep at the base of the mesentery, requiring a more extensive resection. The tumor status of the SN correctly reflected the status of the entire specimen in all cases. In the only node-positive case of the nine laparoscopic cases, micrometastasis was found using the focused examination of a 2.8-cm moderately differentiated T3 sigmoid adenocarcinoma. In this case, the CK-IHC positive SN was the only positive lymph node of seven recovered in the specimen.

Ex Vivo Technique

Ex vivo LM was attempted in eight cases and was successful in seven cases (88%) (Table 2). Six of the seven cases were low rectal tumors whose drainage could not be mapped during OCR LM. Ex vivo LM identified at least one SN (range, 1–3) in five of the six cases.

SNs identified during ex vivo LM accurately reflected the nodal status in six of seven patients (86%) (Table 2). Interestingly, one of these patients had a low rectal carcinoma associated with SN micrometastases identified by CK-IHC. This SN was the only positive lymph node of the 16 harvested from the specimen. In two cases, the tumor was upstaged after identification of

TABLE 1. *Unexpected lymphatic drainage in 7 patients undergoing LM for CRC*

		Type of operation	
Location of tumor	Location of SN	Planned	Performed
Cecum (2 cases)	Deep at base of mesentery	Laparoscopic right hemicolectomy	Laparoscopic right hemicolectomy including extended deep mesenteric resection
Proximal right colon	Left of the middle colic vessels	Right hemicolectomy	Extended right hemicolectomy
Mid-right colon	Left of the middle colic vessels	Right hemicolectomy	Extended right hemicolectomy
Mid-right colon	Deep at base of mesentery	Laparoscopic right hemicolectomy	Laparoscopic right hemicolectomy including deep mesenteric resection
Mid-right colon	Deep at base of mesentery	Right hemicolectomy	Right hemicolectomy including deep mesenteric resection
Left colon	Deep at base of mesentery	Laparoscopic left hemicolectomy	Laparoscopic left hemicolectomy including deep mesenteric resection

TABLE 2. *Technical success rates of in vivo and ex vivo LM techniques*

Approach	n	Mapping successful (%)	Avg. no. of nodes (range)		Accuracy* (%)
			Total	Sentinel	
In vivo					
Open	64	56 (88)	15 (3–28)	2 (1–4)	53/56 (95)
Laparoscopic	9	9 (100)	12 (2–20)	2 (1–3)	9/9 (100)
Ex vivo	8	7 (88)	16 (8–24)	2 (1–3)	6/7 (86)
Overall	81**	72/75 patients (96)	15 (2–28)	2 (1–4)	68/72 (94)

* Correspondence between the SN status (metastasis positive or negative) and the regional lymph node basin status as a whole.

** Overall, 81 LM procedures were performed in the 75 patients; 6 patients in whom in vivo mapping failed to map a SN were attempted via the ex vivo approach.

occult micrometastasis in an SN identified during ex vivo LM.

DISCUSSION

Published reports offer no consensus on the prognostic significance of nodal micrometastases in CRC. In a study of 46 patients initially reported as node-negative, re-examination using CK- and carcinoembryonic antigen (CEA)-IHC demonstrated evidence of micrometastases in 12 patients (26%). When compared with patients without evidence of nodal disease, however, there was no significant difference in 5-year survival.[9] Similarly, Jeffers and associates[10] detected CK-IHC micrometastases in 25% of 77 patients whose CRCs were initially staged as Duke's B. Again, the presence of micrometastases had no significant effect on survival; however, random use of microsectioning for nodal specimens may have missed micrometastases. In fact, survival for true node-negative Duke's B (stage II) disease could be better than now appreciated.

More recent studies have reported inferior survival in CRC patients with nodal micrometastases. Greenson and colleagues[2] demonstrated an adverse effect on survival for micrometastatic disease missed by routine H&E staining but identified by CK-IHC. Similarly, Hayashi et al.[11] demonstrated decreased survival in patients with p53 or K-ras mutations in colonic lymph nodes. In a study of Duke's B CRC patients, Liefers and co-workers[3] reported a 5-year survival rate of 50% for patients whose nodes expressed CEA, versus 91% for those whose nodes did not express CEA.

Multiple sectioning and IHC are too time-consuming and expensive for examination of all nodes in a CRC specimen; however, these ultrastaging techniques can be cost-effectively focused on the one to four nodes identified during LM. Unlike in melanoma[4,12] and breast[5,7,13] cancer, in which LM is used to avoid unnecessary radical lymphadenectomies, in CRC the LM technique is used to improve staging.[6,7] The most straightforward approach to identifying the SNs draining a CRC is LM undertaken during OCR. In our study, OCR LM was successful in all but nine patients, six of whom had rectal tumors below the peritoneal reflection. Subsequent ex vivo LM in the six rectal specimens was successful in five cases.

It is unclear how blue dye is pushed through the lymphatics in the ex vivo specimen. Certainly the injection generates hydrostatic pressure, but there also may be a contribution from the pumping action of the myoendothelium of the lymphatics. Alternatively, surgical disruption may disrupt the neural pathways that regulate constriction of the lymphatics, thereby facilitating lymphatic flow. In any case, our initial findings indicated that ex vivo LM could successfully localize SN(s) de novo or after failure of in vivo LM. To confirm the accuracy of the ex vivo technique, we remapped the SNs in 10 operative specimens from patients who had undergone successful in vivo LM. In all cases, ex vivo injection of the dye intensified the staining of nodes identified during in vivo LM and did not stain any new nodes. Although

TABLE 3. *Node positive and SN only positive cases*

Approach	n	Node positive cases total/by ultrastaging	SN only positive node total/by ultrastaging
In vivo*			
Open	58	29/10	14/8
Laparoscopic	9	1/1	1/1
Ex vivo	8	5/2	2/2
Overall	75	35/13	17/11

* In vivo mapping was performed as the only lymphatic mapping procedure in these 58 patients.

TABLE 4. *Relationship of tumor (T) stage to nodal status*

Tumor stage	n	Node-negative	Node-positive	SN positive	SN only positive node	Upstaged to node positive by SN status
T1	14	13	1	1	1	1
T2	22	15	7	7	6	5
T3	33	11	22	20	9	7
T4	6	1	5	22	1	0

Increasing T-stage correlates significantly with overall nodal ($P = .0001$) and SN positivity ($P = .004$), by the Wilcoxon sign rank test.

we performed ex vivo LM on a back table in the operating room, it could also be undertaken in the pathology department. The ex vivo technique may therefore be a practical means for the pathologist to focus his or her analysis on SNs in CRC.

The appropriateness of LCR for malignancy is currently being studied in a randomized multicenter trial supported by the National Cancer Institute.[14] To date, multiple American[15–19] and international[20–24] trials have demonstrated the safety and efficacy of LCR. In these studies, an average of 8 to 16 lymph nodes were harvested during LCR, which is about the same or slightly fewer than the number of nodes harvested during OCR. Because fewer nodes may be harvested at LCR, some question the oncologic soundness of the technique. In our experience, the average number of lymph nodes removed during LCR was 12, compared with 15 removed during OCR. It is unknown whether these larger resections have an oncological impact. Because we have largely limited LCR to small, early-stage tumors, only one of the nine patients had lymph node-positive disease (one CK-IHC positive SN draining a T3 lesion). However, if the ongoing multicenter trial proves LCR to be appropriate for more advanced cases, LM may become even more useful to outline all SNs, and their lymphatics for inclusion into the resection margins. Another advantage of LM during LCR is that colonoscopic injection tattoos the tumor with blue dye, precisely localizing the lesion. This was helpful because most of our patients had early-stage tumors and had undergone recent colonoscopic biopsies/polypectomies removing most if not all of the tumor. Therefore, these lesions were not usually visible or "palpable" laparoscopically.

Our successful application of LM for CRC[6,7,25] is in part based on careful ongoing review of our failures to map at least one SN. In the present study, we were unable to identify an SN in three cases, two of which were low rectal tumors (operations performed before we began the ex vivo technique). In one of these cases, a locally advanced T4 tumor had grossly positive nodes (13 of 15 nodes positive on pathology). In such cases with grossly positive nodes, tumor replacement of the lymph node may inhibit the ability of the dye to traverse the lymphatics. This has been observed in melanoma and breast cancer. We do not recommend LM in such cases because analysis of SN status is unlikely to provide useful staging information.

Overall, extraperitoneal rectal tumors have proven to be quite problematic. Unlike intraperitoneal colon tumors, these tumors are not readily accessible and visible for injection upon exploration of the abdomen. Whether the dye injection was performed via sigmoidoscope with a spinal needle before mobilization of the rectum, or via the routine in vivo technique performed after rectal mobilization, visualization of the blue dye traveling in the lymphatic channels was more difficult and hence these were the most technically difficult cases in our series.

We have also analyzed cases in which the SN failed to reflect the tumor status of all nodes in the CRC specimen. In the present study, four cases had false-negative SNs. Each of these four failures occurred in locally advanced (T3 or T4) tumors: two right colon and two rectal tumors. It is not clear whether these failures reflected skip metastases. However, it is interesting to note that in three of the four false-negative cases, RT-PCR ultrastaging was positive for evidence of micrometastasis (unpublished data, 2000).

In conclusion, LM of the SNs draining primary CRC can be performed accurately by in vivo or ex vivo techniques. Focused examination of SNs demonstrates micrometastases missed by conventional techniques, upstaging almost one fifth of CRCs. Although the significance of nodal micrometastases found by ultrastaging techniques is currently unknown, a multicenter American College of Surgeons Oncology Group study (ACOSOG Z0170) is being designed to confirm the accuracy of LM and determine the prognostic significance of micrometastases in CRC. The ex vivo technique can be used successfully de novo or if in vivo LM is unsuccessful. LM at the time of LCR tattoos the primary tumor and outlines its primary lymphatic drainage.

Acknowledgments: Supported in part by grant T32 CA 09689 from the National Cancer Institute and by funding from the Rogovin-Davidow Foundation, Los Angeles. The authors

would like to thank Ms. Gwen Berry for her invaluable editorial support.

REFERENCES

1. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. *CA Cancer J Clin* 2000;50:7–33.
2. Greenson JK, Isenhart CE, Rice R, Mojzisik C, Houchens D, Martin EW, Jr. Identification of occult micrometastases in pericolic lymph nodes of Duke's B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. Correlation with long-term survival. *Cancer* 1994;73:563–69.
3. Liefers GJ, Cleton-Jansen AM, van de Velde CJ, et al. Micrometastases and survival in stage II colorectal cancer. *N Engl J Med* 1998;339:223–8.
4. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg* 1992;127:392–9.
5. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220:391–8.
6. Bilchik AJ, Giuliano A, Essner R, et al. Universal application of intraoperative lymphatic mapping and sentinel lymphadenectomy in solid neoplasms. *Cancer J Sci Am* 1998;4:351–8.
7. Saha S, Wiese D, Badin J, et al. Technical details of sentinel lymph node mapping in colorectal cancer and its impact on staging. *Ann Surg Oncol* 2000;7:120–4.
8. Turner RR, Ollila DW, Stern S, Giuliano AE. Optimal histopathologic examination of the sentinel lymph node for breast carcinoma staging. *Am J Surg Pathol* 1999;23:263–7.
9. Cutait R, Alves VA, Lopes LC, et al. Restaging of colorectal cancer based on the identification of lymph node micrometastases through immunoperoxidase staining of CEA and cytokeratins. *Dis Colon Rectum* 1991;34:917–20.
10. Jeffers MD, O'Dowd GM, Mulcahy H, Stagg M, O'Donoghue DP, Toner M. The prognostic significance of immunohistochemically detected lymph node micrometastases in colorectal carcinoma. *J Pathol* 1994;172:183–7.
11. Hayashi N, Ito I, Yanagisawa A, et al. Genetic diagnosis of lymph-node metastasis in colorectal cancer. *Lancet* 1995;345: 1257–9.
12. Morton DL, Thompson JF, Essner R, et al. Validation of the accuracy of intraoperative lymphatic mapping and sentinel lymphadenectomy for early-stage melanoma: a multicenter trial. Multicenter Selective Lymphadenectomy Trial Group. *Ann Surg* 1999; 230:453–63.
13. Giuliano AE, Dale PS, Turner RR, Morton DL, Evans SW, Krasne DL. Improved axillary staging of breast cancer with sentinel lymphadenectomy. *Ann Surg* 1995;222:394–9.
14. Stocchi L, Nelson H. Laparoscopic colectomy for colon cancer: trial update. *J Surg Oncol* 1998;68:255–67.
15. Franklin ME Jr, Rosenthal D, Abrego-Medina D, et al. Prospective comparison of open vs. laparoscopic colon surgery for carcinoma. Five-year results. *Dis Colon Rectum* (Suppl) 1996;39:S35–S46.
16. Lord SA, Larach SW, Ferrara A, Williamson PR, Lago CP, Lube MW. Laparoscopic resections for colorectal carcinoma. A three-year experience. *Dis Colon Rectum* 1996;39:148–54.
17. Vukasin P, Ortega AE, Greene FL, et al. Wound recurrence following laparoscopic colon cancer resection. Results of the American Society of Colon and Rectal Surgeons Laparoscopic Registry. *Dis Colon Rectum* (Suppl) 1996;39:S20–S23.
18. Bouvet M, Mansfield PF, Skibber JM, et al. Clinical, pathologic, and economic parameters of laparoscopic colon resection for cancer. *Am J Surg* 1998;176:554–8.
19. Khalili TM, Fleshner PR, Hiatt JR, et al. Colorectal cancer: comparison of laparoscopic with open approaches. *Dis Colon Rectum* 1998;41:832–8.
20. Huscher C, Silecchia G, Croce E, et al. Laparoscopic colorectal resection. A multicenter Italian study. *Surg Endosc* 1996;10: 875–9.
21. Leung KL, Yiu RY, Lai PB, Lee JF, Thung KH, Lau WY. Laparoscopic-assisted resection of colorectal carcinoma: five-year audit. *Dis Colon Rectum* 1999;42:327–2.
22. Melotti G, Tamborrino E, Lazzaretti MG, Bonilauri S, Mecheri F, Piccoli M. Laparoscopic surgery for colorectal cancer. *Semin Surg Oncol* 1999;16:332–6.
23. Poulin EC, Mamazza J, Schlachta CM, Gregoire R, Roy N. Laparoscopic resection does not adversely affect early survival curves in patients undergoing surgery for colorectal adenocarcinoma. *Ann Surg* 1999;229:487–92.
24. Santoro E, Carlini M, Carboni F, Feroce A. Colorectal carcinoma: laparoscopic versus traditional open surgery. A clinical trial. *Hepatogastroenterology* 1999;46:900–4.
25. Saha S, Wiese D, Beutler T, et al. Accuracy of diagnosis of micrometastases in colorectal cancer by sentinel lymph node mapping technique. *Proc Am Soc Clin Oncol* (abstract) 1999; 18:236a.