
Colon Sentinel Lymph Node Mapping: Practical Surgical Applications

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The mainstay of treatment for nonmetastatic colorectal cancer is complete surgical resection of the tumor-bearing colon, with en bloc regional lymphadenectomy. The surgical technique for en bloc lymphadenectomy is based on the blood supply to the segment of colon affected by the malignancy. Considerable surgeon-to-surgeon variability exists in the extent of operation, both with respect to the amount of colon resected and the degree to which the node-bearing mesentery is removed. There can be inconsistency between patients with respect to the number of surgically resected and pathologically evaluated lymph nodes. When this occurs, inaccurate staging can also occur.

Metastatic spread to the draining lymph nodes is one of the most important prognostic factors for patients with colorectal cancer.¹⁻³ Patients with node-negative disease experience 5-year survival rates of 75%, and those with node-positive disease experience 5-year survival rates of 30% to 60%.² En bloc lymphadenectomy has been shown to be both diagnostic and therapeutic for colorectal carcinoma. The number of nodes resected has a significant impact on survival in node-negative and node-positive patients.² This survival benefit is likely a reflection of completeness of resection and staging accuracy.

Nodal status is not only a primary factor in determining stage but also for determining the need for adjuvant chemotherapy. Randomized trials have demonstrated a 40% reduction in recurrence and 33% improvement in

survival with the use of adjuvant 5-FU-based chemotherapy.⁴ Statistically significant survival benefits have not been found with use of adjuvant chemotherapy in patients with node-negative disease, even though a third of node-negative patients will recur and die from the disease within 5 years of diagnosis.^{5,6} Identification of a high-risk subset of node-negative patients can justify use of adjuvant chemotherapy in selected patients.

Although distant hematogenous disease spread can occur in the absence of lymphatic involvement, this bypassing of regional nodes is an uncommon phenomenon. Disease recurrence and tumor-related mortality in early-stage colorectal cancer after potentially curative operations can reflect either incomplete surgical resection or pathologic understaging. Conventional pathologic techniques for colorectal specimen processing and evaluation have limitations that make identification of low-volume nodal metastasis challenging. Failure to identify micrometastatic disease results in false-negative pathologic staging. This understaging can contribute to unfavorable oncological outcomes of supposed node-negative patients who otherwise are not routinely treated with adjuvant chemotherapy.

Accuracy of pathologic staging requires diligence and depends significantly on the quantity of nodes evaluated, as the number of lymph nodes examined correlates directly with the proportion of patients correctly staged as node-positive.⁷⁻⁹ Number of lymph nodes resected and evaluated influences not only the accuracy of nodal staging in colon cancer but also cancer outcomes. For example, survival for patients with node-negative T2 or T3 colorectal cancers with six or fewer pathologically staged nodes is significantly worse than that for comparable patients having more than six nodes evaluated.¹⁰ One lymph node measuring 5 mm in greatest dimension can be sectioned into 1,000 5- μ m slices; but standard histopathologic processing evaluates < 1% of the entire node. Microscopic examination of one or two sections of each node identified limits pathologic assessment to a small portion of overall nodal tissue volume, in turn

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Abbreviations and Acronyms

CK-20	= cytokeratin-20
H&E	= hematoxylin and eosin
IHC	= immunohistochemistry
RT-PCR	= reverse transcriptase-polymerase chain reaction
SLN	= sentinel lymph node

contributing to false-negative cancer staging, particularly for isolated tumor cells (single tumor cells or small cell clusters ≤ 0.2 mm) and micrometastatic (0.2 to 2.0 mm) tumor cell deposits.

Standard gross pathologic dissection of the resected mesentery might not identify nodes critical to accurate staging. A significant proportion (up to 78%) of lymphatic tumor metastases are found in nodes < 5 mm in size, which can be overlooked in a large mesenteric specimen.¹¹⁻¹⁴ Pathologic nodal sampling and evaluation can be inconsistent and vary from hospital to hospital. The optimal minimum number that should be examined to accurately stage the regional nodal basin appears to be 12 to 14 lymph nodes.¹⁵⁻¹⁷ Insufficient nodal resection or pathologic evaluation, lymph node sampling error, and limitations of conventional hematoxylin and eosin-stained evaluation are three limitations of standard pathologic staging of colorectal carcinoma.¹⁸

Various techniques have been developed to improve the accuracy of regional nodal staging in colorectal cancer. These techniques include careful specimen stretching, pinning and fixation, mesenteric fat clearing, multiple microscopic step sections, and ultrastaging methods, including immunohistochemistry (IHC) and reverse transcriptase-polymerase chain reaction (RT-PCR).^{7,12,19,20} Comprehensive evaluation of the entire nodal sample using such specialized histopathologic methods would be unwieldy and impractical in terms of time, human resource requirement, and cost. Focused pathologic examination and ultrastaging of a limited number of lymph nodes draining a tumor-bearing segment of bowel that is at highest likelihood of harboring tumor metastases would be practical and can improve staging accuracy.

SENTINEL LYMPH NODE PARADIGM

History of sentinel node

One of the first descriptions of the term *sentinel node* appeared in 1960, when Gould and colleagues²¹ referred to a normal-appearing node at the junction of the ante-

rior and posterior facial vein containing parotid cancer on frozen section, forming the basis for radical neck dissection in conjunction with planned parotidectomy. Early contrast-enhanced lymphangiographic studies of breast anatomy conducted by Kett and colleagues²² in 1970 defined the first draining node to receive contrast medium as the "*sorgius node*." In 1977, Cabañas²³ meticulously detailed the lymphatic drainage patterns in patients with penile carcinoma using lymphangiography, directed lymphadenectomy, and histopathologic staging. He identified the sentinel node as the first site of regional nodal metastasis and suggested that it was predictive of the remaining "inguinofemoroiliac" nodal basin; this served as the groundwork for his recommended surgical therapy (selective lymphadenectomy) on the pathologic basis of the sentinel node.

Ground-breaking studies of lymphatic mapping and sentinel lymph node (SLN) biopsy applied to melanoma by Morton and colleagues at the John Wayne Cancer Institute led to one of the most significant developments in clinical oncology in the late 20th century.²⁴⁻²⁷ In a landmark study reported initially in 1989, Morton and colleagues²⁷ injected blue dye around clinical stage I melanoma to allow intraoperative identification of the node first to receive drainage from the primary tumor and the one most likely to harbor lymphatic metastases when present, the SLN. They recommended selective lymphadenectomy for patients with positive sentinel nodes, sparing those with negative sentinel nodes unnecessary operative morbidity, as the status of the SLN was predictive of the entire remaining regional nodal basin. Likelihood of a positive nonsentinel node when the SLN was negative was $< 1\%$ (2 of 3,079 nodes).

Theory of lymphatic drainage

The fundamental basis of SLN biopsy is that there exists an orderly, sequential, and predictable dissemination of solid tumor cells through regional lymphatics and that the first draining node effectively traps malignant cells. Numerous studies in the decade after Morton and colleagues²⁷ seminal report have validated SLN mapping and biopsy using radiolabeled colloids or blue dye, or both, for melanoma and breast cancer.²⁸⁻³⁸ The success in identifying the sentinel node and accuracy in its ability to predict overall regional nodal status led to general acceptance and widespread use of the technique for these two common cancers for the purpose of guiding focused pathologic ultrastaging without conceding diagnostic

accuracy and selecting patients most likely to benefit from lymphadenectomy.

SLN mapping and biopsy has been validated for a number of epithelial cancers as a method to improve accuracy of cancer staging and a way to select patients who can benefit from regional lymphadenectomy and spare patients with negative sentinel nodes the morbidity of that operation. Significant morbidity can be associated with lymphadenectomy performed as part of treatment for melanoma and breast cancer. Complications after colon cancer resection, on the other hand, are generally unrelated to the extent of lymph node dissection. Minimizing the extent of lymphadenectomy, as is done with melanoma and breast cancer, is of little use and risks inaccurately staging the patient.

The existing literature on the subject of lymphatic mapping for colorectal cancer emphasizes the value of focused pathologic assessment and highly sensitive ultra-staging of sentinel node(s), given that a significantly greater proportion of patients is upstaged (~20% to 30%) with this technique than with standard pathologic evaluation alone. Sentinel nodal staging has been shown to be feasible, both in terms of time and cost effectiveness. This paradigm provides a valuable avenue of inquiry into the biology of lymphatic spread of cancer. Therapeutic implications associated with micrometastases identification remain to be defined.

How sentinel node mapping could benefit patients with colon cancer

Regional lymphadenectomy with en bloc primary tumor resection remains the gold standard for surgical treatment of colorectal cancer. For the majority of patients, SLN mapping for colorectal cancer does not contribute additional information that will alter the extent of operation. Aberrant lymph node drainage has been shown to occur in 8% to 30% of patients and in these instances lymphatic mapping should alter the extent and site of nodal dissection. In the majority of patients, the importance of SLN mapping will rest in this technique's ability to more accurately identify the node most likely to harbor metastasis and allow focused evaluation of that node. Focused evaluation can allow a more accurate identification of node-positive patients. This will result in more precise prognostic information and will identify a group of patients who can likely benefit from adjuvant chemotherapy.

That the sentinel node is not uniformly peritumoral

in location and that lymphatic drainage can be unpredictable at times underscore the importance of lymphatic mapping to precisely locate the sentinel node(s). Various techniques have been described for SLN mapping for colorectal carcinoma. The lack of a standardized approach to lymphatic mapping for colorectal carcinoma, notwithstanding published findings, support the sentinel node concept for cancer of the colon and rectum in an effort to overcome the limitations of conventional pathologic staging.

TECHNICAL ASPECTS OF SLN MAPPING

Blue dye

The potential benefit of sentinel node mapping for colorectal cancer begins with the accurate identification of the tumor-draining lymph nodes. Isosulfan blue is the dye of choice for lymphatic mapping in colorectal cancer. It is inexpensive, easy to use, and its uptake into lymphatic channels and draining nodes is brisk (within 5 to 10 minutes). Based on its relatively small particle size, the distribution of dye through lymphatic channels is more rapid than that of technetium sulfur colloid.

In vivo sentinel node mapping

The majority have used in vivo, subserosal, circumferential, and peritumoral injection (25- to 30-gauge needle) of isosulfan blue dye (without opening the bowel). Mesenteric lymphatic disruption is minimized during mobilization of the tumor-bearing colon before lymphatic mapping. Peritumoral injection of the dye into the subserosal space should be circumferential to optimize SLN(s) identification. Care must be taken to avoid spillage of dye onto the adjacent mesentery, as doing so can obscure sentinel nodes in close proximity to the tumor. Using a tuberculin syringe, small-caliber needle, and applying negative pressure to the syringe when the needle is withdrawn from the submucosal or subserosal space between injections best achieve this end. Volume of dye injected in published reports has varied considerably, from 0.25 to 5.0 mL. Care must be taken to avoid intraluminal injection of dye, as it can contribute to blue staining of nonsentinel nodes or lack of adequate dye distribution to properly demonstrate the sentinel node(s).

The blue dye is distributed rapidly through afferent lymphatic channels visible within the colonic mesentery; the window of opportunity from time of injection to sentinel identification is narrow. This window depends on how one defines the SLN(s). Although some

authors define sentinel nodes as the first one to four blue nodes with the most direct drainage from the tumor to appear within 5 to 10 minutes of dye injection, others have regarded all blue-staining nodes as sentinel nodes.³⁹⁻⁴² Consequently, there is a large variation in examined sentinel nodes (0 to 21) (Table 1) in the literature. This may directly impact the false negative rate due to sampling error. Blue-staining afferent lymphatics and sentinel nodes can be better visualized if the mesentery is transilluminated. If the first sentinel node is replaced by tumor, the node will not stain blue; blue staining lymphatic channels can be visualized, leading up to the tumor-replaced node and an adjacent blue-staining node. Both nodes are regarded as sentinel node(s) in this case.⁴³ During the *in vivo* technique for sentinel lymphatic mapping, blue nodes are not dissected out after identification; rather, the nodes are tagged with suture immediately after they are found for later identification by the pathologist. Suture marking of the sentinel nodes is critical for the purpose of pathologic processing, as initially blue-staining true sentinel nodes can fade as dye distributes further after the initial 10 minutes of dye administration. This applies, of course, to sentinel nodes defined only as the first 1 to 4 nodes staining blue within the first 10 minutes of injection. Resection of the colon-bearing tumor and supporting mesentery is undertaken after marking. Mesenteric dissection and SLN harvest should occur before formalin fixation of the specimen to avoid washout of the dye.⁴⁴

The specimen is submitted fresh to pathology for SLN dissection and processing with serial step microscopic sectioning (20- to 40- μ m intervals), hematoxylin and eosin (H&E) staining, and IHC with or without RT-PCR testing. IHC is cost effective and relatively straightforward. The highly sensitive technique of RT-PCR, on the other hand, is more costly, might not be sufficiently specific for malignancy, and requires specialized pathologic handling and processing; it is not available or feasible for general use at the present time. RT-PCR is less subject to sampling error than IHC, as it can be used to evaluate the entire node. The biologic relevance of RT-PCR-detected nodal disease remains to be defined.

Ex vivo sentinel node mapping

The principal argument in favor of the *in vivo* mapping technique is the occurrence (approximately 10%) of aberrant lymphatic drainage that warrants a more extended

nodal resection. Proponents of the *ex vivo* SLN mapping technique have cited several limitations of the *in vivo* blue dye mapping approach: possible tumor cell shedding and increase in local recurrence, obscuring of pericolic nodes, and adverse dye reactions ranging from urticaria to anaphylactic shock, artifactual interference with pulse oximetry monitoring, along with increase in operating time and unfeasibility in rectal cancer. The issue of metastatic tumor cell seeding during mechanical manipulation to increase lymphatic flow and reduction in local control is unproven and adverse dye reactions are exceedingly rare.

The *ex vivo* technique can be achieved by either subserosal (colon specimen left intact) or submucosal (colon specimen opened along antimesenteric aspect) peritumoral injection of isosulfan blue dye using a tuberculin syringe. Published techniques generally use 0.25 mL (submucosal injection around the exposed primary tumor through an antimesenteric incision) to 2.0 mL (subserosal injection around the intact primary tumor and colon) of blue dye for the purpose of lymphatic mapping.^{42,45-47} The specimen is massaged for 5 minutes after injection of the dye to facilitate lymphatic distribution of the dye. The specimen is delivered in a fresh state to the pathologist within 30 minutes of lymphatic mapping. Advocates of the *ex vivo* technique have emphasized that it can be applied to all anatomic colorectal sites precisely, without risk of adverse event, tumor shedding, fascial plane disruption, or increase in operative time; and *ex vivo* lymphatic mapping lends itself to improved technical quality control by standardized, uniform specimen processing and assessment.⁴⁸ That the technique of sentinel node mapping for colorectal cancer can increase tumor shedding is a concern obviated by *ex vivo* peritumoral dye administration. Investigators at the John Wayne Cancer Institute have demonstrated comparable success and accuracy with open or laparoscopic *in vivo* and *ex vivo* lymphatic mapping in colorectal cancer and have shown that the *ex vivo* technique can be implemented effectively after attempts at *in vivo* mapping have failed.^{49,50} An important finding in their experience was the lack of additional blue staining nodes with *ex vivo* blue-dye injection after successful *in vivo* localization of sentinel nodes.

Radioisotope for lymphatic mapping

The use of radioisotope in conjunction with blue dye has been used in breast cancer and melanoma to increase

sensitivity and accuracy of the SLN technique. Relatively little attention has been given to radioisotope/ γ -probe-guided mapping for colorectal sentinel nodes. Few authors have used radioisotope-directed lymphatic sentinel nodal mapping with or without blue dye.^{41,51} Increased nodal radioactivity twice that of baseline defines sentinel nodes sought after by γ -probe-directed mapping after administration of technetium sulfur colloid.⁵² Lymphoscintigraphy is time consuming and costly, impractical, and requires a coordinated multispecialty approach that has not been shown to definitively improve either the success rate or accuracy of SLN mapping for colorectal cancer. In one study, only half of blue nodes demonstrated uptake of radiocolloid and agreement between blue staining and hot nodes was 76%.⁵¹ Tumor proximity to epiploic sentinel nodes can limit the ability to find some sentinel node(s) with radioisotope alone. Another study concluded that radioisotope-guided lymphatic mapping provided little incremental yield, as it identified a single additional sentinel node (9%) not evident by *in vivo* blue dye mapping.⁴¹ A more recent study compared isosulfan blue dye with technetium sulfur colloid for lymphatic mapping in colorectal cancer and found predictably higher mapping success (100% versus 89%), though comparable accuracy (93% versus 92%), with the blue dye mapping technique.⁵² Isosulfan blue detected a significantly higher number of sentinel nodes (152 versus 100 of 156 SLNs). The relatively larger particle size of technetium sulfur colloid accounts for slower lymphatic distribution of tracer and fewer nodes found to concentrate radiotracer than isosulfan blue dye. Blue staining non-SLNs can occasionally be included inadvertently as part of the harvested sentinel nodes, depending on timing of harvest because of small particle size and rapidity of lymphatic uptake of blue dye; this is much less likely to occur with radioisotope.⁴⁴

Saha and colleagues⁵² acknowledge the inflated accuracy of radioisotope-guided mapping as the hot nodes identified were already blue, but argue that technetium-identified nodes are “likely primary nodes in the natural sequential pathway of lymphatic flow away from the tumor and are therefore more likely to contain metastatic disease.” The higher percentage of histologically positive sentinel nodes in the combined modality (blue dye + technetium) group (19.5% versus 10.7%) forms the basis of this argument and the authors’ recommendation for both blue dye and radioisotope/ γ -probe-

guided mapping for sentinel nodes in colorectal cancer.^{52,53} This combined approach has not been validated in large, multicenter trials and the blue dye-only technique has met with greater clinical approval based on its efficacy, safety and simplicity, time, and cost effectiveness.

Laparoscopic sentinel node mapping

Lymphatic mapping for colon cancer can be achieved successfully during laparoscopic colectomy as well. Two techniques have been applied to this end, one using intraoperative endoscopic submucosal peritumoral injection of blue dye (0.5 to 1.0 mL) or the more expedient preoperative endoscopic tattooing of the tumor followed by percutaneous subserosal peritumoral dye injection with a spinal needle.⁵⁴ Preoperative colonoscopic tumor marking has apparent advantages for intraoperative laparoscopic tumor localization. The alternative approaches would be direct manual subserosal injection using a laparoscopic hand assist port or *ex vivo* lymphatic mapping. Wood and coworkers⁵⁴ demonstrated in a pilot study of 11 patients undergoing colectomy that sentinel node mapping can be achieved laparoscopically with equal success and accuracy as can be achieved using open technique.

Feasibility of sentinel lymphatic mapping at time of laparoscopic colectomy has been supported by other published reports.^{42,50,55} The technique of SLN mapping for colorectal cancer is straightforward and technically feasible and can be done quickly and inexpensively. Consistently high accuracy, sensitivity, and disease upstaging rates associated with lymphatic mapping in high-volume centers reflects a coordinated effort with careful attention to rigorous technical detail with comprehensive specimen handling and processing procedures, and institutional commitment to an interdisciplinary approach to optimizing the accuracy of staging for colorectal cancer. This improvement in staging accuracy will enhance consistency and comparability of outcomes of patients enrolled in clinical trials.

Learning curve

Intraoperative SLN mapping is clear cut, accurate, and cost effective, adding little to overall operative time. Material requirements are few and the blue dye is inexpensive. Unlike that of breast cancer and melanoma, the learning curve is relatively flat for colorectal cancer SLN mapping. Successful sentinel node identification remains consistently high after the first five cases.⁵⁶

Paramo and colleagues⁵⁶ plotted the “average learning curve” according to the number of consecutive operations incorporating lymphatic mapping (in vivo isosulfan blue dye) performed by seven surgeons and the sentinel node detection rate for colon cancer. The curve flattened or stabilized after the first five lymphatic mapping procedures with sentinel node identification success rates > 98% thereafter.

Large series have demonstrated that technical experience correlates with successful lymphatic mapping. Bilchik and colleagues⁴² reported a success rate of 97% with SLN mapping in 100 patients with colorectal cancer, all of the technical failures having occurred in the first 50 patients. Three of the 5 false-negative patients in that series were identified during the first 30 operations.

One recent multicenter trial demonstrated the importance of training and standardization of lymphatic mapping before large-scale cooperative group clinical trials.⁵⁷ Twenty-five surgeons participated in the CALGB 80001 trial, which evaluated the feasibility of sentinel node mapping, serial step sectioning, and staging with H&E alone among 72 patients with colon carcinoma enrolled at 13 member institutions. The sentinel node was identified successfully in only 66% of patients, where 18 of 25 (72%) surgeons performed fewer than five lymphatic mapping procedures, what others have shown to be at the cusp of the learning curve. Only 2 surgeons performed 10 or more SLN mappings. Surgical volume did not correlate significantly with successful sentinel nodal localization, leading the authors to conclude, “surgeons performing more SN procedures do not have greater success in SN localization.” This appears to be at variance with other published reports demonstrating lymphatic mapping success, accuracy, and sensitivity in over 95% of patients when conducted at institutions with considerable technical experience.^{17,47,52,58}

RESULTS FROM PUBLISHED REPORTS (1999–2004)

Success rate and upstaging

A review of the published results of colorectal SLN mapping with isosulfan blue dye alone (Table 1) demonstrates generally high success rates (studies with 30 to 99 cases, 58% to 100%) and even greater success in identifying at least 1 sentinel node in centers with extensive experience with the technique (studies with 100+ cases, 97% to 100%). Status of the SLN accurately reflects the status of the remaining nodal basin with 92% to 96%

accuracy and with a low risk of “skip metastasis” (negative SLN/positive non-SLN, 4% to 8%) in such experienced centers (Table 1). SLN mapping has been associated with an increase in the nodal yield over historical controls, a finding that can contribute to lower overall rates of understaging, and improved cancer outcomes.^{2,18}

One recently published series compared patients undergoing lymphatic mapping with those undergoing resection with conventional pathologic evaluation and found significant differences in mean number of nodes examined (14 versus 10 lymph nodes) and incidence of nodal isolated tumor cells and micrometastases (29.4% versus 1.9% isolated tumor cells and micrometastases) favoring lymphatic mapping and focused pathologic assessment of the sentinel node(s).¹⁷ This study confirms earlier findings that conventional histopathologic assessment of one or two H&E-stained sections of randomly selected nodes from colorectal cancer specimens overlooks a significant proportion of nodal tumor cell deposits that can be consistently and accurately identified with focused sentinel nodal ultrastaging (Table 1).

The techniques implemented to ultrastage colorectal cancer SLNs are highly variable and include multilevel step sectioning with H&E staining alone or in combination with cytokeratin, CEA IHC, or RT-PCR. If one considers patients with tumor-negative nodes by H&E examination found to have micrometastatic disease by focused examination of the SLN(s), the proportion of patients upstaged by centers specializing in the technique is approximately 20% (Table 1); but the overall rate reported is inconsistent, 7% to 45%. Reported upstaging rate denominators vary in an attempt to portray the proportion of patients with negative nodes by routine H&E pathologic examination found to have micrometastatic disease by focused examination of the sentinel node(s) among those in the study population. The rate most commonly reported uses the entire patient population studied; but the rate consistently applied in Table 1 reflects the difference between the patient population and the sum of all node-positive patients, excluding those upstaged by sentinel node ultrastaging. To summarize, the sentinel node is the only positive node in 35% of node-positive patients, lymphatic mapping upstages approximately 20% of node-negative patients, and the false-negative rate varies from 5% to 18% (according to variations in definition) in the most experienced hands.

Although most regard occult sentinel nodal metasta-

Table 1. Published Reports of Sentinel Lymph Node Mapping for Colorectal Cancer (1999–2004)

Year	Lead author	n	Technique	SNB success	Mean No. of LNs, range	Node(+) patients*	Mean No. of SLNs, range	Accuracy of SNB†	Sensitivity of SNB†	SNB upstaging [§]	False(-) SNB	Aberrant LN drainage [¶]
1999	Cserni ⁷⁸	25	In vivo	96	16	52	4	79	62	14	21	—
			Patent blue (2.0 mL) H&E of 2–3 sections	24/25	2–34	13/25	0–12	19/24	8/13	2/14	5/24 38	
1999	Joosten ⁶²	50	In vivo	70		52		66	38	8	33	—
			Patent blue (1.0–2.0 mL) CK-p and CK-19 IHC	35/50	2–37	26/50	1–16	23/35	10/26	2/26	12/36 62	
2000	Wiese ⁵⁹	83	In vivo	99	16	41	2	96	91	—	4	2
			Isosulfan blue (1.0–2.0) AE1/AE3 CK and CEA IHC	82/83	2–39	34/83	1–4	79/82	31/34		3/82 9	2/82
2000	Saha ³⁹	86	In vivo	99	16	37	2	96	91	22	4	2
			Isosulfan blue (1.0–1.5 mL) AE1 CK IHC	85/86		32/86	1–4	82/85	29/32	15/69	3/85 9	2/85
2000	Waters ⁷⁹	22	In vivo	91	12	27	—	100	100	6	0	—
			Isosulfan blue (1.0 mL) CEA and CK IHC	20/22	—	6/22	—	20/20	6/6	1/17	0/20 0	
2001	Saha ⁵⁸	203	In vivo	98	—	40		96	90	18	4	—
			Isosulfan blue (0.5–2.0 mL) AE1/AE3 CK IHC	198/203		81/203	1–4	190/198	73/81	27/149	8/198 10	
2001	Merrie ⁵¹	26	In vivo	88	—	42	3	—	55	21	45	17
			^{99m} Tc colloidal antimony mixed with patent Blue Dye V (2.0 mL) CK 20 RT-PCR	23/26	4–52	11/26	0–8	—	—	4/19	—	4/23
2001	Esser ¹⁸	31	In vivo	58	15	10	—	94	67	7	6	—
			Isosulfan blue (1.0–2.0 mL) H&E of 1–2 sections	18/31	12–16	3/31	0–5	17/18	2/3	2/30	1/18 33	
2001	Paramo ⁵⁶	35	In vivo	75	10	29	—	100	100	14	0	0
			Isosulfan blue (1.0 mL) CK CAM 5.2 IHC	25/35		10/35	1–4	25/25	10/10	4/29	0/25 0	0/25
2001	Wood ⁵⁴	11	In vivo, laparoscopic	100	13	9	2	100	100	9	0	36
			Isosulfan blue (0.5–1.0 mL) or India ink AE1/AE3 CK IHC	11/11	2–20	1/11	1–3	11/11	1/1	1/11	0/11 0	4/11

(continued)

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Year	Lead Author	n	Technique	SNB success	Mean No. of LNs, range	Node(+) patients*	Mean No. of SLNs, range	Accuracy of SNB [†]	Sensitivity of SNB [†]	SNB upstaging [§]	False(-) SNB	Aberrant LN drainage ^{¶v}
2001	Wood ⁴⁹	75	In vivo plus ex vivo	96	15	47	2	94	88	25	6	10
			Open plus laparoscopic Isosulfan blue (0.5–2.0 mL) AE1/AE3 CK IHC	72/75	2–28	35/75	1–4	68/72	30/35	13/53	4/72 12	7/72
2001	Feig ⁴⁰	48	In vivo	98	13	33	3	79	38	11	21	0
			Isosulfan blue (3.0–5.0 mL) AE1/AE3 CK IHC	47/48	4–46	16/48	0–7	37/47	6/16	4/36	10/47 62	0/47
2001	Wong ⁴⁵	26	Ex vivo	92	18	62	3	96	94	29	4	—
			Isosulfan blue (0.25 mL) AE1/AE3, CAM 5.2, 35bH11 IHC	24/26	8–36	16/26	0–6	23/24	—	4/14	1/24 6	—
2001	Bilchik ⁴⁶	40	In vivo	100	15	35	2	100	100	13	0	8
			Isosulfan blue (0.5–1.0 mL) MAK-6 Ab cocktail CK IHC β -HCG, c-Met, uMAGE RT-PCR	40/40	2–28	14/40	1–3	40/40	14/14	4/30	0/40 0	3/40
2002	Bilchik ⁵⁵	30	In vivo	100	14	20	2	93	—	14	7	29
			Isosulfan blue (0.5–1.0 mL) or India ink, AE1/AE3 CK IHC	30/30	2–21	6/30	1–3	28/30	—	4/28	2/30 —	8/30
2002	Bilchik ⁴²	100	In vivo and ex vivo	97	15	44	2	95	—	24	5	8
			Isosulfan blue (1.0–2.0 mL) AE1/AE3 CK IHC β -HCG, c-Met, uMAGE RT-PCR	97/100	2–28	44/100	1–4	92/97	—	18/74	5/97 —	8/97
2002	Cox ⁸⁰	17	In vivo and ex vivo	100	18	41	6	100	100	29	0	—
			Isosulfan blue (1.0 mL) AE1/AE3 CK IHC	17/17	4–33	7/17	2–11	17/17	7/7	4/14	0/17 0	—
2002	Tsioulis ⁵⁰	14	In vivo	100	14	21	2	93	67	15	7	28
			Isosulfan blue (0.5–1.0 mL) AE1/AE3 CK IHC	14/14	2–21	3/14	1–3	13/14	2/3	2/13	1/14 33	4/14

(continued)

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2002	Bendavid ⁸¹	20	In vivo Isosulfan blue (1.0 mL) AE1/AE3 CK IHC	90 18/20	—	65 13/20	4	94 17/18	—	42 5/12	6 1/18 —	—
2002	Fitzgerald ⁸²	26	Ex vivo Isosulfan blue (1.0 mL) Anti-CK MoAb Cam 5.2	88 23/26	15	19 5/26	3	91 21/23	60 3/5	9 2/23	9 2/23 40	—
2002	Paramo ⁵⁶	55	In vivo Isosulfan blue (1.0 mL) CAM 5.2, 1:2 CK IHC	82 45/55	12	27 15/55	— 1-4	98 44/45	93 14/15	13 6/46	2 1/45 7	2 1/45
2003	Turner ⁶⁰	51	In vivo Isosulfan blue (0.5-1.0 mL) AE1/AE3 CK, p53, E-cadherin, calretinin IHC	100 51/51	11 1-42	61 31/51	3 1-13	92 47/51	87 27/31	29 8/28	8 4/51 13	—
2003	Viehl ⁶¹	31	In vivo Isosulfan blue (1.0-5.0 mL) Pancytokeratin CK22/Lu5	87 27/31	21 5-40	48 15/31	2 1-8	78 21/27	50 6/12	11 2/18	22 6/27 50	—
2003	Bilchik ¹⁷	120	In vivo and ex vivo Isosulfan blue (0.5-1.0 mL) 99mTc sulfur colloid (0.5 mCi) AE1/AE3 CK IHC	96 115/120	14	53 63/120	2	96 110/115	92 58/63	31 26/83	4 5/115 8	—
2004	Saha ⁵²	57	In vivo Isosulfan blue (1.0-2.0 mL) 99mTc sulfur colloid unfiltered (0.5-1.0 mCi) CK IHC	100 57/57	12	33 19/57	3 1-4	95 54/57	84 16/19	5 2/40	5 3/57 16	—
2004	Wong ⁴⁷	124	Ex vivo Isosulfan blue (0.5 mL) AE1/AE3, CAM 5.2, 35bH11 IHC	97 120/124	30 5-111	41 51/124	4 0-21	92 61/66	54 27/51	19 13/66	8 5/66 [#] 46	—
2004	Patten ⁴¹	57	In vivo Isosulfan blue (3.0-5.0 mL) 99mTc sulfur colloid unfiltered (0.5 mCi) AE1/AE3 CK IHC	98 56/57	14	45 27/57	4 0-11	89 50/56	83	20 5/25	11 6/56 17	0 0/56

(continued)

Table 1. (continued)

Year	Lead author	n	Technique	SNB success	Mean No. of LNs, range	Node (+) patients*	Mean No. of SLNs, range	Accuracy of SNB†	Sensitivity of SNB‡	SNB upstaging§	False(-) SNB	Aberrant LN drainage¶
2004	Saha ⁴³	209	In vivo and ex vivo Isosulfan blue/fluorescein Technetium sulfur colloid	100 209/209	14	41 85/209	2 1-4	96 201/209	92 78/85	13 19/143	4 8/209	—
2004	Bertagnoli ⁵⁷	72	In vivo Isosulfan blue (1.0 mL) Multilevel sectioning, H&E	92 66/72	17	33 24/72	2	80 53/66	46 11/24	0 0	20 13/66	0 0/66

Values are percents and n/total, except where indicated.

*Node(+), proportion of study patients with metastatic disease identified in any node by H&E, IHC, or RT-PCR, or a combination of methods.

†Accuracy of SNB, proportion of patients with successful lymphatic mapping having sentinel lymph node examination correctly reflect the tumor status of the nodal basin.

‡Sensitivity of SNB, proportion of patients with positive nodes by routine H&E examination found to have positive sentinel lymph nodes.

§SNB upstaging, proportion of patients with negative nodes by routine H&E examination found to have micrometastatic disease by focused examination of the sentinel lymph node (denominator = patient population – all node-positive patients – upstaged patients).

¶False(-) SNB, proportion of patients with successful lymphatic mapping having tumor-positive nonsentinel lymph node(s), but sentinel lymph node(s) without apparent tumor cells. This does not reflect false-negative rate, ie, (FN/FN + TP) × 100%, which is listed in the same column in the row immediately below. The false-negative rate and sensitivity (hit rate) of sentinel node mapping add up to 100% (ie, false-negative rate = 1 – sensitivity).

‡Aberrant LN drainage, proportion of patients with successful lymphatic mapping having primary tumor lymphatic drainage to the sentinel lymph nodes outside the margins of conventional colon resection.

¶All patients with histologically negative nodes by H&E underwent nodal step sectioning and pancytokeratin immunostaining of all nonsentinel as well as sentinel lymph nodes.
CK, cytokeratin; H&E, hematoxylin and eosin; IHC, immunohistochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction; SLN, sentinel lymph node; SNB, sentinel node biopsy; 99mTc, technetium 99m.

ses identified with ultrastaging techniques (generally multiple step sectioning and H&E ± IHC as true positive cases, others contend that these are negative cases, as the biology of micrometastasis remains undefined, and basing clinical decisions on such information is inappropriate at this time.^{40,41} To date, there has been no prospective randomized trial that has defined the role of SLN mapping and ultrastaging in colorectal carcinoma—or established a biologic significance of nodal micrometastasis.

Wiese and colleagues⁵⁹ also addressed the question of how many nodal sections are required to optimize pathologic staging given the practical limitations of time and resources. All patients in that study with positive sentinel nodes were staged correctly with four nodal sections and a single cytokeratin immunostain. Multilevel step sectioning of all resected nodes in patients with negative sentinel nodes would upstage < 1% of nodes and < 5% of patients. Accurate detection of sentinel nodal metastasis can be achieved with four representative sections of the node(s) and one immunostain in patients with colorectal cancer. This has been validated in another study with considerable institutional experience with lymphatic mapping.⁶⁰ More than 90% of patients with nodal metastases from colorectal carcinoma are staged correctly by focused pathologic evaluation of the SLN(s) (Table 1). The sentinel node(s) is the only site of nodal disease in 35% of all node-positive patients with colorectal cancer undergoing lymphatic mapping.⁴⁴

Although the sentinel node hypothesis has been validated for breast cancer and melanoma, until recently the assumption has been made that improved nodal staging directly reflected mapping of the node(s) most likely to harbor metastasis rather than a more comprehensive pathologic study of the node per se. Wong and colleagues⁴⁷ validated the sentinel node hypothesis by conducting multilevel step sectioning and pathologic analysis with H&E staining and cytokeratin IHC of sentinel nodes identified ex vivo and nonsentinel nodes from patients node negative by conventional pathologic staging.⁴⁷ There was a significantly higher conversion rate (upstaging) from H&E to IHC positivity among sentinel nodes (2 of 66 to 13 of 66 patients or 19.5%) than among nonsentinel nodes (3 of 66 to 5 of 66 patients or 7.5%, $p = 0.04$). Metastases were identified in 13 of 278 sentinel and 5 of 1,829 nonsentinel nodes. These findings support the hypothesis that blue-stained nodes are

indeed sentinel nodes and significantly more likely to harbor colorectal cancer metastases.

False-negative rate

A false-negative sentinel node is one with no apparent tumor cells in the presence of nonsentinel nodal metastases. Presentation of the false-negative sentinel node data varies in the literature (Table 1). These divergent false-negative rates can be attributed to a variety of factors, such as patient selection; disease extent; technique used for injection of dye or radiotracer, or both; number of sentinel nodes examined; method of nodal sectioning; staining and labeling; definition of "false-negative rate"; and interpretation of H&E-negative/IHC-positive patients.

The varying definition of SLN positivity is a major reason for the large discrepancy in reported false-negative rates. Some authors regard sentinel nodes positive only by presence of micrometastases or cytokeratin-positive staining, and other investigators do not. Studies relying only on multilevel sections of the sentinel node and H&E staining, defining nodal micrometastasis as sentinel node negative, have reported false-negative rates as high as 58%.⁵⁷ When similar definitions are applied to other large published series, and micrometastatic cases are classified as node negative, the false-negative rate expectedly increases three- to fourfold, from 4% to 5% to 12% to 18%, suggesting that conventional pathologic nodal staging is inadequate, even with the addition of multilevel step sectioning, to detect isolated tumor cells, cell clusters, and micrometastases.^{17,47,52,58} Wong and co-workers⁴⁷ reported significant improvement in sensitivity associated with ex vivo lymphatic mapping when pathologic assessment of the sentinel node changed from analysis of a single H&E-stained nodal section to cytokeratin IHC of the sentinel node (58.3% to 93.7%). Additional multilevel step sectioning in conjunction with IHC improved detection of micrometastases.

A few authors have emphasized the importance of reporting the false-negative rate as defined by false-negative cases divided by the sum of false-negative and true positive cases $[(FN/FN+TP) \times 100\%$ or 1-sensitivity].^{40,59} In this case, the false-negative rate and sensitivity (hit rate) of sentinel node mapping should add up to 100%. Contrary to the majority of published series, some researchers have not regarded H&E-negative/IHC-positive cases as true positive ones, on the premise that clinical decisions about adjuvant chemotherapy are not and should not be influenced by micro-

metastases, given that the prognostic significance of such low-volume disease remains indeterminate.^{40,41,57} Lower false-negative rates are reported in published reports that consider micrometastases true positive cases (Table 1). Table 1 shows both the proportion of false-negative cases (tumor-positive non-SLN[s], but SLN[s] without apparent tumor cells) among those mapped successfully (denominator is number of SLN success) and the false-negative rate (1-sensitivity or $[FN/FN+TP] \times 100\%$) to allow consistent comparisons across studies.

Additionally, a number of technical factors have been identified to contribute to false-negative sentinel node results, including volume and location of blue-dye injection, primary tumor site (colon versus rectum) and extent, nodal disease burden, and preoperative radiation for rectal cancer. Clearly, SLN mapping is not indicated in the setting of distant metastatic disease. Technical factors, such as position on the learning curve and site of injection, also account for false-negative sentinel node staging. Earlier colon resection can disrupt regional lymphatic channels, thereby contributing to subsequent inaccurate lymphatic mapping. A large series of sentinel node mapping for colorectal cancer identified 3 of 5 false-negative cases during the first 30 operations.⁴² Incomplete peritumoral circumferential injection of dye and injection of dye directly into the tumor or inadvertently into the bowel lumen will predictably fail to identify the sentinel node(s). Larger tumors can require increased volume of dye to achieve complete circumferential peritumoral dye distribution. Generally, 0.25 to 2.0 mL of isosulfan blue dye administered subserosally or submucosally around the tumor is used for colorectal lymphatic mapping. Several investigators have used what some might consider excessive dye volumes (3.0 to 5.0 mL) for the purpose of lymphatic mapping.^{40,41} Viehl and colleagues,⁶¹ recognizing the high false-negative lymphatic mapping rates with bulky primary (T3/T4) tumors, analyzed the volume of blue dye administered as a function of tumor volume, and found significantly better sentinel node mapping success with higher dye volumes (up to 5.0 mL) for larger tumors.⁶¹ For that reason, consideration of tumor size and extent is important in determining amount of dye to be used for lymphatic mapping of colorectal cancers, as dye volume is a significant predictor of successful sentinel node identification. The minimum amount of isosulfan blue dye to be injected around the tumor using a subserosal approach (as opposed to ex vivo submucosal injection)

recommended by some surgeons is 0.5 mL per centimeter tumor diameter (ie, 2.5 mL around a 5-cm tumor).⁶¹

Extent of disease contributes to false-negative sentinel node staging as well. Tumor-replaced lymph nodes, those with extranodal disease extension, and nodes directly invaded by tumor are unlikely to stain blue after peritumoral, subserosal injection of blue dye because of lymphatic channel obstruction by tumor.^{49,56,62} Sentinel lymphatic mapping is applied to T4 colon tumors in the absence of distant metastasis and in the setting of clinical trials with the understanding that lymphatic mapping is more challenging in the face of increased tumor burden and that greater volumes of dye will need to be administered. The infrequent occurrence of multiple or synchronous primary cancers will make lymphatic mapping challenging. SLN mapping and biopsy cannot be justified in the presence of clinically overt nodal or distant metastases.

Location of disease impacts success of SLN mapping, as tumors located in the rectum, particularly inaccessible cancers located extraperitoneally or those treated with preoperative radiation, are associated with high false-negative results.^{49,63} In vivo mapping of the sentinel node for rectal cancer is impractical, given the requirement for disrupting the mesorectum to identify the blue node. Surgeons have been reluctant to do so to ensure appropriate pathologic assessment of radial margins of resection. As such, rectal cancers have represented only 13% of studies evaluating lymphatic mapping for colorectal cancer.⁴⁴

One way to approach sentinel mapping for mid or low rectal cancers is by way of rigid proctoscopy-guided submucosal peritumoral injection of blue dye using a spinal needle, followed by mesorectal excision and ex vivo identification of sentinel lymph node(s) with the pathologist after inking of resection margins. This approach addresses concerns raised over disrupting lymphatic vessels during mesorectal excision that precedes in vivo peritumoral dye injection. Others have advocated precise ex vivo peritumoral instillation of dye into the submucosal plane as a feasible solution for lymphatic mapping of rectal cancer.⁴⁸ The relatively high false-negative rate associated with lymphatic mapping of rectal cancer has quelled enthusiasm for application of this technique to tumors originating in the rectum. Nonetheless, a cooperative approach between surgeon and pathologist dedicated to and experienced with SLN mapping for colorectal cancer improves patient selection

and helps to limit false-negative results. This is particularly important considering that the median maximum dimension of the largest SLN per patient is about half a centimeter in size.⁶⁰ This underscores not only the importance of a coordinated team approach to sentinel lymphatic mapping but also the value of careful patient selection.

Among SLN-negative patients ($n = 51$), Wiese and colleagues⁵⁹ identified disease in only 1% (8 of 802) of additional non-SLNs. This was confirmed later in a multicenter trial that evaluated 2,546 nonsentinel nodes among 203 sentinel node-negative patients finding metastasis in only 13 (0.5%) nonsentinel nodes.⁵⁸ Saha and colleagues⁴³ recently reported the distribution of nodal metastases in over 3,000 nodes evaluated, finding significantly higher frequency of metastases in sentinel than nonsentinel nodes (118/470 [25.1%] versus 189/2,541 [7.4%]). Sentinel nodes were the exclusive site of metastases in 10% of cases and the exclusive site of metastases in only 0.4% of nonsentinel nodes. These findings are similar to those reported from other disease processes, such as breast cancer and melanoma, and should allay concerns that sentinel node biopsy overlooks potential lymphatic spread to nonsentinel nodes. Based on the data here, if nodal metastases are not apparent in sentinel node(s) after serial step sectioning, IHC, and RT-PCR, it is improbable that disease exists in nonsentinel nodes. Even so, sentinel nodal mapping for colorectal cancer should in no way limit attempts to remove surgically en bloc with tumor-bearing colon or rectum—and evaluate pathologically a substantial volume of nodes, 12 to 14 at a minimum.

Aberrant lymphatic drainage

Intent of SLN mapping for colorectal carcinoma is improved pathologic staging, not refinement of the extent of operation. In the majority of patients, SLNs are identified in proximity to the primary colon cancer. In a small proportion of patients, blue nodes can be identified beyond the intended extent of lymphadenectomy, thereby extending the limits of resection. Skip metastases were rarely encountered when using conventional pathologic staging. SLN ultrastaging has identified an increased proportion of patients with aberrant lymphatic drainage, on average 4% (range 0% to 10%) in most large studies (Table 1). This implies that lymphatic spread of colon cancer from submucosal lymphatic channels, through epicolic, paracolic, and intermediate

nodes en route to paraaortic nodes, might not be uniformly orderly and predictable. For patients undergoing total mesorectal excision as part of surgical treatment of mid to low rectal cancer lymphatic drainage pathways are not apparent before en bloc lymphadenectomy unless one uses preoperative *in vivo* lymphatic mapping by proctoscopy-guided submucosal peritumoral injection of blue dye.

Reports of “aberrant lymphatic drainage” often state that the lymphatic mapping identified blue node(s) outside the limits of initially planned resection, but do not specify the exact location of the aberrant node(s).^{39,59,62} Many of the aberrant node(s) are identified at the base of the mesentery; these are nodes included routinely by some but not other surgeons during resection of colon carcinoma. The definition of aberrant lymphatic drainage in some situations depends on the individual surgeon and what he considers the usual limits of resection. There remains variability among surgeons in terms of extent of lymphadenectomy for any given colon cancer, much like the inconsistency in number of nodes retrieved by pathologists. It remains to be determined what the likelihood is that an aberrant sentinel node represents the only positive manifestation of lymphatic disease spread among the two to four sentinel nodes identified typically. It is unclear whether skip metastases represent unique tumor biology or variable lymphatic anatomy. Additional study is warranted to determine if aberrant lymph node metastases have an impact on tumor staging, response to treatment, or cancer-specific outcomes.

PROGNOSTIC IMPLICATIONS OF MICROMETASTASES

There is a paucity of prospective data on the biology of colorectal nodal micrometastasis. Tumor cell clusters unapparent by conventional H&E staining are evident in about 20% of sentinel nodes staged with IHC and over 40% evaluated with RT-PCR (Table 1).⁴⁶ Recognizing inherent limitations of single-marker RT-PCR assays for ultrastaging, Bilchik and colleagues^{42,46} conducted a multicenter phase II trial of molecular staging of colon cancer using three tumor mRNA markers as part of a semiquantitative assay (β -HCG, *c-Met*, and *uMAGE*, markers absent in nonmalignant tissue) in addition to cytokeratin IHC of sentinel nodes. Sentinel nodes with two or three mRNA markers were considered tumor positive. IHC upstaged 4 of 40 (10%) H&E-

negative sentinel nodes and RT-PCR upstaged 12 of 26 (46%) H&E-negative/IHC-negative sentinel nodes. Combined upstaging of IHC and multimarker RT-PCR was 53% (16 of 30 histopathologically negative sentinel nodes).

Biology of submicroscopic nodal metastasis and the prognostic significance of micrometastasis is a question of great controversy that remains unanswered. The controversy will likely endure until outcomes appraisals of patients with and without nodal tumor deposits are based on strict definitions that stratify according to disease burden—isolated tumor cells versus micrometastasis, IHC positive versus RT-PCR positive, and single versus multiple mRNA marker-positive assays. Most authorities would regard the uncommon finding of a single cytokeratin-positive “tumor cell” identified in lymph nodes of otherwise node-negative patients as “negative.” It is undetermined at present if these so-called tumor cells are truly malignant cells and not apoptotic or degenerating cells or mesothelial cells. Lack of specificity of this rare finding, single cytokeratin-positive cell, is thought to represent cytokeratin-positive reactive, hyperplastic mesothelial cells, cytokeratin staining normal, or dysplastic epithelial cells.^{17,60}

A common taxonomy is of particular importance as isolated tumor cells (pN0i+), unlike micrometastatic disease (pN1[mi]), do not typically demonstrate lymphatic sinus, vascular invasion, or signs of metastatic activity (cellular proliferation, stromal reaction, and so forth) and are considered N0 in modern staging systems. The new TNM classification defines precisely terminology relevant to extent of nodal disease burden—micrometastasis: “metastasis ≤ 0.2 cm”; isolated tumor cells: “single tumor cells or small clusters of cells ≤ 0.2 mm in greatest dimension that are usually detected by immunohistochemistry or molecular methods, but can be verified with H&E stains.”⁶⁴ Revised American Joint Committee on Cancer and the International Union Against Cancer guidelines for classification of sentinel nodes are listed in Table 2 and should form the basis of defining factors in prospective studies of sentinel lymphatic mapping.^{64,65}

Perhaps the most significant predictor of outcomes for colorectal cancer is lymph node status.¹⁻³ A considerable number (~20% to 30%) of node-negative patients who have undergone seemingly curative resection of colorectal cancer recur and die of disease; interestingly, this number resembles closely the sentinel node upstag-

Table 2. Sentinel Lymph Node Classification According to Ultrastaging Results

Descriptor	Definition
pN0 (i-)(sn)	No SN metastasis histologically, negative morphologic findings by IHC
pN0 (i+)(sn)	No SN metastasis histologically, positive morphologic findings by IHC
pN0 (mol-)(sn)	No SN metastasis histologically, negative molecular findings by RT-PCR
pN0 (mol+)(sn)	No SN metastasis histologically, positive morphologic findings by RT-PCR

IHC, immunohistochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction; SN, sentinel node.

ing rate in several studies (Table 1). Some patients originally staged as “node negative” are identified with greater pathologic scrutiny to have occult nodal metastases. It is unclear currently if those node-negative patients who recur and those harboring micrometastatic disease are one and the same; but several preliminary studies suggest that nodal micrometastases in patients otherwise staged as node negative (Dukes’s B or American Joint Committee on Cancer stage II) can adversely affect outcomes.⁶⁶⁻⁶⁸ One preliminary study evaluated two peritumoral nodes with RT-PCR for cytokeratin-20 (CK-20) in conjunction with CK-20 IHC in node-negative patients according to conventional staging.⁶⁹ Expression of CK-20 mRNA was found to be an independent predictor of tumor-specific mortality (5-year overall survival, 96% versus 71%, RT-PCR negative versus positive), and addition of CK-20 IHC significantly reduced the false-positive rate and improved the prognostic ability of RT-PCR.

Other studies have not demonstrated a significant effect on outcomes of micrometastases reflecting either actual tumor biology, study population size, false-positive ultrastaging in studies using common epithelial antigens, or sampling error—overlooked small tumor cell clusters by random nodal microsectioning.⁷⁰⁻⁷⁴ The finding that number of involved nodes (assessed with conventional pathologic methods) rather than nodal tumor volume impacts survival in colorectal cancer is an important finding, suggesting a similar prognosis for micrometastatic and macrometastatic disease when the number of nodes involved is the same.⁷⁵ Prognostic similarity between micro- and macrometastatic nodal disease suggests that sentinel nodal ultrastaging can identify biologically important and prognostically relevant tumor deposits. Replication of this finding in sentinel nodes subjected to multilevel sectioning, H&E staining,

and IHC with adequate longterm followup would provide valuable insight into the biology of colorectal cancer nodal micrometastases.

The surgical community’s decision to adopt or reject SLN mapping will not rest solely on settling of the micrometastasis controversy, for to do so would neglect a common problem in the community: insufficient number of nodes evaluated and attendant understaging. SLN mapping directs pathologists to the nodes most likely to contain metastasis and most representative of the entire nodal basin with the aim of optimizing staging accuracy. In fact, H&E staining of one or two sections of the sentinel node, without adjunctive IHC, RT-PCR, or consideration of micrometastasis, initially considered to have adequate staging accuracy, has been shown to have significant limitations in terms of identifying isolated tumor cell clusters and micrometastases.^{17,18} Before SLN mapping can be adopted as part of colorectal cancer treatment, the prognostic significance of IHC- or RT-PCR-detected tumor micrometastases will have to be determined.

There has been great reluctance to include results of sentinel node ultrastaging in pathology reports. To do so creates a formidable clinical predicament when tumor boards debate the relative risks and uncertain benefits of chemotherapy for patients with node-positive colorectal cancer on the basis of micrometastases alone. This problem is magnified when patients respond to sentinel nodal ultrastaging information in pathology reports with specific ardent requests for adjuvant chemotherapy and oncologists are in turn hard-pressed to justify treatment in the absence of supportive prospective trial data.

Even though some authors have recommended strong consideration of adjuvant chemotherapy for stages I and II colorectal cancer upstaged by SLN ultrastaging, this is not advisable outside of a clinical trial, as the benefit of such treatment remains unproven.⁷⁶ Three fundamental questions remain to be addressed definitively for sentinel mapping in colorectal carcinoma:

1. Does SLN mapping significantly upstage or increase staging accuracy?
2. Do patients with H&E-negative nodes but nodal micrometastases have significantly worse oncologic outcomes than those without micrometastases?
3. Does treatment of nodal micrometastasis with adjuvant chemotherapy translate into meaningful survival benefit?

The United States Military Cancer Institute has

launched multicenter trials to address two of these clinically relevant and important questions. One randomized trial compares conventional pathologic nodal assessment to standard pathology and sentinel node mapping and ultrastaging with serial step sectioning and IHC, hypothesizing that lymphatic mapping and SLN ultrastaging can more accurately stage colon cancer and identify micrometastasis undetected by conventional histopathologic techniques. The second large prospective observational trial evaluates the biologic relevance of nodal micrometastasis, the main objective being the prognostic significance of molecular staging of colon carcinoma on the basis of sentinel node mapping and ultrastaging. The question of whether adjuvant systemic therapy prolongs the survival of patients with disease identified with sentinel node ultrastaging methods (pN0[i+][sn], pN0[mol+][sn], or pN1[mi]) remains to be answered by large-scale cooperative cancer group randomized trials. Potential advantages of applying the SLN paradigm to colorectal cancer are evident not only in the ability to detect nodal metastasis earlier in the natural history of the disease and to stage disease more accurately, but also in the ability to define with greater fidelity, homogeneous clinical trial study groups to make cross-trial comparisons possible and trial data interpretation more meaningful.⁷⁷ Until the three fundamental questions are addressed definitively, sentinel lymphatic mapping for colorectal cancer will likely remain investigational.⁷⁸⁻⁸²

REFERENCES

1. Cancer facts and figures 2004. American Cancer Society Inc; 2004.
2. Le Voyer TE, Sigurdson ER, Hanlon AL, et al. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. *J Clin Oncol* 2003;21:2912-2919.
3. American Joint Committee on Cancer. Colon and rectum. In: Greene FL, Page DL, Fleming ID, et al, eds. *AJCC cancer staging manual*. 6th ed. New York: Springer Publishers; 2002:113-119.
4. Moertel CG, Fleming TR, Macdonald JS, et al. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. *Ann Intern Med* 1995; 122:321-326.
5. Cohen AM, Kelsen D, Saltz L, et al. Adjuvant therapy for colorectal cancer. *Curr Probl Surg* 1997;34:601-676.
6. Cohen AM, Tremitterra S, Candela F, et al. Prognosis of node-positive cancer. *Cancer* 1991;67:1859-1861.
7. Crucitti F, Doglietto GB, Bellantone R, et al. Accurate specimen preparation and examination is mandatory to detect lymph nodes and avoid understaging in colorectal cancer. *J Surg Oncol* 1992;51:153-157.
8. Goldstein NS, Weldon S, Coffey M, et al. Lymph node recovery from colorectal resection specimens removed for adenocarcinoma: trends over time and a recommendation for a minimum number of lymph nodes to be recovered. *Am J Clin Pathol* 1996;106:209-216.
9. Joseph NE, Sigurdson ER, Hanlon AL, et al. Accuracy of determining nodal negativity in colorectal cancer on the basis of the number of nodes retrieved on resection. *Ann Surg Oncol* 2003; 10:213-218.
10. Caplin S, Cerottini JP, Bosman FT. For patients with Dukes' B (TNM Stage II) colorectal carcinoma, examination of six or fewer nodes is related to poor prognosis. *Cancer* 1998;83:666-672.
11. Herrera-Ornelas L, Justiniano J, Castillo N, et al. Metastases in small lymph nodes from colon cancer. *Arch Surg* 1987;122: 1253-1256.
12. Haboubi NY, Clark P, Kaftan SM, Schofield PF. The importance of combining xylene clearance and immunohistochemistry in the accurate staging of colorectal carcinoma. *J R Soc Med* 1992; 85:386-388.
13. Rodriguez-Bigas MA, Maamoun S, Weber TK, et al. Clinical significance of colorectal cancer: metastases in lymph nodes < 5 mm in size. *Ann Surg Oncol* 1996;3:124-130.
14. Ratto C, Sofio L, Ippoliti M, et al. Accurate lymph-node detection in colorectal specimens resected for cancer is of prognostic importance. *Dis Colon Rectum* 1999;142:143-157.
15. Wong JH, Severino R, Honnebler MB, et al. Number of nodes examined and staging accuracy in colorectal carcinoma. *J Clin Oncol* 1999;17:2896-2900.
16. Tepper JE, O'Connell MJ, Niedzwiecki D, et al. Impact of number of nodes retrieved on outcome in patients with rectal cancer. *J Clin Oncol* 2001;19:157-163.
17. Bilchik AJ, Nora DT, Sobin LH, et al. Effect of lymphatic mapping on the new tumor-node-metastasis classification for colorectal cancer. *J Clin Oncol* 2003;21:668-672.
18. Esser S, Reilly WT, Riley LB, et al. The role of sentinel lymph node mapping in staging of colon and rectal cancer. *Dis Colon Rectum* 2001;44:850-854.
19. Scott KW, Grace RH. Detection of lymph node metastases in colorectal carcinoma before and after fat clearance. *Br J Surg* 1989;76:1165-1167.
20. Scott KW, Grace RH, Gibbons P. Five-year follow-up study of the fat clearance technique in colorectal carcinoma. *Dis Colon Rectum* 1994;37:126-128.
21. Gould EA, Winship T, Philbin PH, Kerr HH. Observations on a "sentinel node" in cancer of the parotid. *Cancer* 1960;13:77-78.
22. Kett K, Varga G, Lukacs L. Direct lymphography of the breast. *Lymphology* 1970;1970:13-12.
23. Cabañas RM. An approach for the treatment of penile carcinoma. *Cancer* 1977;39:456-466.
24. Robinson DS, Sample WF, Fee HJ, et al. Regional lymphatic drainage in primary malignant melanoma of the trunk determined by colloidal gold scanning. *Surg Forum* 1977;28:147-148.
25. Fee HJ, Robinson DS, Sample WF, et al. The determination of lymph shed by colloidal gold scanning in patients with malignant melanoma: a preliminary study. *Surgery* 1978;84:626-632.
26. Morton DL, Wen DR, Cochran AJ. Pathophysiology of regional lymph node metastases in early melanoma studied by intraop-

- erative mapping of the cutaneous lymphatics [abstract]. Second International Conference on Melanoma 1989:131.
27. Morton DL, Wen DR, Wong JH, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg* 1992;127:392–399.
 28. Krag DN, Weaver DL, Alex JC, et al. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. *Surg Oncol* 1993;2:335–340.
 29. Giuliano AE, Kirgan DM, Guenther JM, et al. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg* 1994;220:391–401.
 30. Turner RR, Ollila DW, Krasne DL, et al. Histologic validation of the sentinel lymph node hypothesis for breast carcinoma. *Ann Surg* 1997;226:271–278.
 31. Veronesi U, Paganelli G, Galimberti V, et al. Sentinel node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. *Lancet* 1997;349:1864–1867.
 32. Hill AD, Tran KN, Akhurst T et al. Lessons learned from 500 cases of lymphatic mapping for breast cancer. *Ann Surg* 1999;229:528–535.
 33. Giuliano AE, Haigh PI, Brennan MB, et al. Prospective observational study of sentinel lymphadenectomy without further axillary dissection in patients with sentinel node-negative breast cancer. *J Clin Oncol* 2000;18:2553–2559.
 34. Thompson JF, McCarthy WH, Bosch CM, et al. Sentinel lymph node status as an indicator of the presence of metastatic melanoma in regional lymph nodes. *Melanoma Res* 1995;5:255–260.
 35. Krag DN, Meijer SJ, Weaver DL, et al. Minimal-access surgery for staging of malignant melanoma. *Arch Surg* 1995;130:654–660.
 36. Albertini JJ, Cruse CW, Rapaport D, et al. Intraoperative radio-lympho-scintigraphy improves sentinel lymph node identification for patients with melanoma. *Ann Surg* 1996;223:217–224.
 37. Gershenwald JE, Colome MI, Lee JE, et al. Patterns of recurrence following a negative sentinel lymph node biopsy in 243 patients with stage I or II melanoma. *J Clin Oncol* 1998;16:2253–2260.
 38. Gershenwald JE, Thompson W, Mansfield PF, et al. Multi-institutional melanoma lymphatic mapping experience: the prognostic value of sentinel lymph node status in 612 stage I or II melanoma patients. *J Clin Oncol* 1999;17:976–983.
 39. 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;7:120–124.
 40. Feig BW, Curley S, Lucci A, et al. A caution regarding lymphatic mapping in patients with colon cancer. *Am J Surg* 2001;182:707–712.
 41. Patten LC, Berger DH, Rodriguez-Bigas M, et al. A prospective evaluation of radiocolloid and immunohistochemical staining in colon carcinoma lymphatic mapping. *Cancer* 2004;100:2104–2109.
 42. Bilchik AJ, Nora D, Tollenaar RAEM, et al. Ultrastaging of early colon cancer using lymphatic mapping and molecular analysis. *Eur J Cancer* 2002;38:977–985.
 43. Saha S, Dan AG, Beutler T, et al. Sentinel lymph node mapping technique in colon cancer. *Semin Oncol* 2004;31:374–381.
 44. Mulrow J, Winter DC, Keane JCO, O'Connell PR. Sentinel lymph node mapping in colorectal cancer. *Br J Surg* 2003;90:659–667.
 45. Wong JH, Steinman S, Calderia C, et al. Ex vivo sentinel node mapping in carcinoma of the colon and rectum. *Ann Surg* 2001;233:515–521.
 46. Bilchik AJ, Saha S, Wiese D, et al. Molecular staging of early colon cancer on the basis of sentinel node analysis: a multicenter phase II trial. *J Clin Oncol* 2001;19:1128–1136.
 47. Wong JH, Johnson DS, Namiki T, Tauchi-Nishi P. Validation of ex vivo lymphatic mapping in hematoxylin-eosin node-negative carcinoma of the colon and rectum. *Ann Surg Oncol* 2004;11:772–777.
 48. Johnson DS, Wong JH. The impact on nodal staging of lymphatic mapping in carcinoma of the colon and rectum. *Semin Oncol* 2004;31:403–408.
 49. Wood TF, Saha S, Morton DL, et al. Validation of lymphatic mapping in colorectal cancer: in vivo, ex vivo, and laparoscopic techniques. *Ann Surg Oncol* 2001;8:150–157.
 50. Tsoulfas GJ, Wood TF, Spirt M, et al. A novel lymphatic mapping technique to improve localization and staging of early colon cancer during laparoscopic colectomy. *Am Surg* 2002;68:561–566.
 51. Merrie AE, van Rij AM, Phillips LV, et al. Diagnostic use of the sentinel node in colon cancer. *Dis Colon Rectum* 2001;44:410–417.
 52. Saha S, Dan AG, Berman B, et al. Lymphazurin 1% versus 99mTc sulfur colloid for lymphatic mapping in colorectal tumors: a comparative analysis. *Ann Surg Oncol* 2004;11:21–26.
 53. Trocha S, Nora D, Saha S, et al. Combination probe and dye-directed lymphatic mapping detects micrometastases in early colorectal cancer. *J Gastrointest Surg* 2003;7:340–346.
 54. Wood TF, Spirt M, Rangel D, et al. Lymphatic mapping improves staging during laparoscopic colectomy for cancer. *Surg Endosc* 2001;15:715–719.
 55. Bilchik AJ, Trocha SD. Lymphatic mapping and sentinel node analysis to optimize laparoscopic resection and staging of colorectal cancer: an update. *Cancer Control* 2003;10:219–223.
 56. Paramo JC, Summerall J, Poppiti R, Mesko TW. Validation of sentinel node mapping in patients with colon cancer. *Ann Surg Oncol* 2002;9:550–554.
 57. Bertagnolli M, Miedema B, Redston M, et al. Sentinel node staging of resectable colon cancer: results of a multicenter study. *Ann Surg* 2004;240:624–630.
 58. Saha S, Bilchik A, Wiese D, et al. Ultrastaging of colorectal cancer by sentinel lymph node mapping technique—a multicenter trial. *Ann Surg Oncol* 2001;8:94S–98S.
 59. Wiese DA, Saha S, Badin J, et al. Pathologic evaluation of sentinel lymph nodes in colorectal carcinoma. *Arch Pathol Lab Med* 2000;124:1759–1763.
 60. Turner RR, Nora DT, Trocha SD, Bilchik AJ. Colorectal carcinoma nodal staging. Frequency and nature of cytokeratin-positive cells in sentinel and non-sentinel lymph nodes. *Arch Pathol Lab Med* 2003;127:673–679.
 61. Viehl CT, Hamel CT, Marti WR, 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;27:1285–1290.
 62. Joosten JJA, Strobbe LJA, Wauters CAP, et al. Intraoperative lymphatic mapping and the sentinel node concept in colorectal carcinoma. *Br J Surg* 1999;86:482–486.
 63. Tsioulfas GJ, Wood TF, Morton DL, Bilchik AJ. Lymphatic mapping and focused analysis of sentinel lymph nodes upstage gastrointestinal lymph nodes. *Arch Surg* 2000;135:926–932.
 64. Sobin LH, Wittekind C, eds. TNM classification of malignant tumors. 6th ed. New York: Wiley; 2002.

65. Hermanek P, Hutter RV, Sobin LH, et al. International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. *Cancer* 1999;86:2668–2673.
66. Greenson JK, Isenhardt CE, Rice R, et al. 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;73:563–569.
67. Hayashi N, Ito I, Yanagisawa A, et al. Genetic diagnosis of lymph-node metastasis in colorectal cancer. *Lancet* 1995;345:1257–1259.
68. Liefers GJ, Cleton-Jansen AM, van de Velde CJ, et al. Micrometastases and survival in stage II colorectal cancer. *N Engl J Med* 1998;339:223–228.
69. Rosenberg R, Hoos A, Mueller J, et al. Prognostic significance of cytokeratin-20 reverse transcriptase polymerase chain reaction in lymph nodes of node-negative colorectal cancer patients. *J Clin Oncol* 2002;20:1049–1055.
70. Jeffers MD, O'Dowd GM, Mulcahy H, et al. The prognostic significance of immunohistochemically detected lymph node micrometastases in colorectal carcinoma. *J Pathol* 1994;172:183–187.
71. Adell G, Boeryd B, Franlund B, et al. Occurrence and prognostic importance of micrometastases in regional lymph nodes in Duke's B colorectal carcinoma: an immunohistochemical study. *Eur J Surg* 1996;162:637–642.
72. Oberg A, Stenling R, Tavelin B, Lindmark G. Are lymph node micrometastases of any clinical significance in Duke's Stages A and B colorectal cancer? *Dis Colon Rectum* 1998;41:1244–1249.
73. Broll R, Schauer V, Schimmelpenning H, et al. Prognostic relevance of occult tumor cells in lymph nodes of colorectal carcinomas: an immunohistochemical study. *Dis Colon Rectum* 1997;40:1465–1471.
74. Choi HJ, Choi YY, Hong SH. Incidence and prognostic implications of isolated tumor cells in lymph nodes from patients with Duke's B colorectal carcinoma. *Dis Colon Rectum* 2002;45:750–755.
75. Wong JH, Steinemann S, Tom P, et al. Volume of lymphatic metastases does not independently influence prognosis in colorectal cancer. *J Clin Oncol* 2002;20:1506–1511.
76. Bilchik AJ, Nora DT. Lymphatic mapping of nodal micrometastasis in colon cancer: putting the cart before the horse? *Ann Surg Oncol* 2002;9:529–531.
77. Balch CM, Lange JR. Lymphatic mapping and sentinel node lymphadenectomy for cancer: an overview. *Ann Surg Oncol* 2001;8:1S–4S.
78. Cserni G, Vajda K, Tarjan M, et al. Nodal staging of colorectal carcinomas from quantitative and qualitative aspects. Can lymphatic mapping help? *Pathol Oncol Res* 1999;5:291–296.
79. Waters GS, Geisinger KR, Garske DD, et al. Sentinel lymph node mapping for carcinoma of the colon: a pilot study. *Am Surg* 2000;66:943–945.
80. Cox ED, Kellicut D, Adair C, et al. Sentinel lymph node evaluation is technically feasible and may improve staging in colorectal cancer. *Curr Surg* 2002;50:301–306.
81. Bendavid Y, Latulippe JF, Younan RJ, et al. Phase I study on sentinel lymph node mapping in colon cancer: a preliminary report. *J Surg Oncol* 2002;79:81–84.
82. Fitzgerald TL, Khalifa MA, Al Zahrani M, et al. Ex vivo sentinel lymph node biopsy in colorectal cancer: a feasibility study. *J Surg Oncol* 2002;80:27–32.