Prospective Multicenter Trial of Staging Adequacy in Colon Cancer

Preliminary Results

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Hypothesis: Lymph node evaluation is an important prognostic factor in colorectal cancer (CRC). A 25% recurrence rate in patients with node-negative CRC suggests that current staging practices are inadequate. Focused analysis of the sentinel node (SN) by multiple sectioning and immunohistochemistry improves staging accuracy.

Design: Prospective phase 2 multicenter trial.

Setting: Tertiary referral cancer centers.

Patients: Between March 2001 and June 2005, 132 patients were enrolled with clinical stage I and II CRC in a prospective multicenter trial (R01-CA90484).

Intervention: During a standard oncologic resection, lymphatic mapping was performed and the SN identified either by the surgeon or the pathologist. Hematoxylin-eosin staining was performed on all lymph nodes and immunohistochemistry, on lymph nodes negative by hematoxylin-eosin staining.

Main Outcome Measures: Micrometastases greater than 0.2 mm but less than 2 mm and isolated tumor cells less than 0.2 mm were defined according to the sixth edition of the American Joint Committee on Cancer Cancer Staging Manual.

Results: The 63 men and 69 women had a median age of 74 years. Sixty-eight patients (52%) underwent a right hemicolectomy; 3 (2.3%), a transverse colectomy; 9 (7%), a left colectomy; 15 (11%), a sigmoid colectomy; 34 (26%), a low anterior resection; 1 (1%), an abdominal perineal resection; and 2 (2%), a total colectomy. Of the 111 evaluable primary tumors, 19 (17%) were T1 lesions; 17 (15%), T2; 72 (65%), T3; and 3 (2.7%), T4 tumors. Thirty-three patients (30%) were classified as stage I; 46 (41%), stage II, and 32 (29%), stage III. The SN was identified by the surgeon in 127 patients (96%) and by the pathologist in 5 patients (4%). The median number of SNs and total lymph nodes examined were 3 and 14.5, respectively. The sensitivity of lymphatic mapping and SN analysis was 88.2% and the false-negative rate, 7.4% (6/81). Of the 6 false-negative results, 4 were attributed to lymphatic channels obliterated by tumor. Upstaging occurred in 28 patients (23.6%).

Conclusions: In a multicenter trial, ultrastaging of colon cancer is feasible and accurate. In stage II CRC, 24% of patients had nodal carcinoma cells not detected by conventional staging methods. Surgical technique (adequate lymph node retrieval) and focused pathological analysis may improve staging accuracy and the selection of patients for chemotherapy. The unnecessary toxicity and expense of chemotherapy may be avoided in those patients who are truly node negative.

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The purpose of this study was to evaluate the feasibility of this procedure in colon cancer in a multicenter trial, selecting experienced surgical and pathological teams that had performed LM in colorectal cancer at least 20 times.

**METHODS**

Between March 2001 and June 2003, 187 patients with clinical stage I and II colorectal cancer were accrued at 4 tertiary referral cancer centers (John Wayne Cancer Institute [Santa Monica, Calif], Century City Doctor’s Hospital [Century City, Calif], Michigan State University [Flint], and University of Colorado [Denver]). Patients were enrolled under Saint Johns Health Center/John Wayne Cancer Institute joint institutional review board–approved protocols in accordance with all institutional standard operating procedures.

Eligible patients had potentially curable colorectal cancer with no evidence of distant metastases confirmed via chest radiography and computed tomography of the abdomen and pelvis within 8 weeks of enrollment. All patients were at least 18 years of age with a performance status of less than or equal to 2 on the Eastern Cooperative Oncology Group/Zubrod scale. Patients requiring emergency surgery and those with a history of Crohn disease, chronic ulcerative colitis, familial polyposis, or prior malignancy (except for completely resected cervical or nonmelanoma skin cancers) within 3 years of enrollment were excluded. Patients found to have metastases intraoperatively or any other exclusionary criteria after enrollment were considered screen failures and removed from the study.

The data were reviewed independently by the data and safety monitoring board and institutional review board, and 132 patients were evaluated.

**SURGICAL TECHNIQUE**

A laparotomy or laparoscopy was performed and metastatic disease was excluded. The primary neoplasm was then mobilized without extensive dissection of lymphatic channels or blood vessels. Lymphatic mapping was performed by injecting 0.5 to 1 mL of isosulfan blue dye (Lymphazurin; BenVenue Laboratories Inc, Bedford, Ohio) circumferentially around the neoplasm using a tuberculin syringe (Figure 1). An afferent lymphatic channel was visualized within a minute after injection of the dye and was dissected to the first 1 to 3 blue-stained lymph nodes. Each of these SNs was marked with a silk suture (Figure 1).

The lymphatic channel containing the blue dye was then followed proximally to the site of the primary neoplasm to ensure that there were no SNs hidden in the mesenteric fat. On occasion, visualization of the lymphatic(s) and SNs required minor dissection of surrounding tissues. After all SNs were marked with a suture, an en bloc resection of the neoplasm and the regional lymph nodes was performed in the standard fashion.

If LM could not be undertaken before resection, it was performed after the tumor had been resected (ex vivo) and placed on a side table in the operating room. This was the preferred method for rectal cancers. If the surgeon could not identify the SN, the specimen was immediately sent to the pathology suite where a more aggressive dissection was performed and the SN identified by the pathologist.

**PATHOLOGICAL ANALYSIS**

Specimens were sectioned and processed within 4 hours of receipt in the laboratory to minimize postoperative movement of blue dye to nonsentinel nodes (NSNs). Standard processing of the tumor included reporting the tumor size and grade, T stage, and surgical margin status. Suture-tagged SNs were measured, described, and embedded in a separate cassette.

Figure 1. Lymphatic mapping of colon cancer demonstrating a blue-stained lymphatic channel and sentinel node.
Sentinel nodes were measured and sectioned at 2- to 3-mm intervals. If the SN was smaller than 5 mm, it was processed whole. Sentinel node tissue was frozen and prepared for cryosectioning. A face section was cut at a thickness of 5 µm for H&E staining. Twelve sections, representing approximately 72 µm of node, were preserved frozen for molecular analysis. The remaining nodal tissue was placed in 10% formalin and embedded in paraffin. Two additional 4-µm sections at approximately 200-µm intervals were taken for H&E and CK-IHC stains; CK-IHC staining was performed on H&E-negative nodes with a panspecific cocktail of antibodies for human CK, AE1/AE3 (DAKO, Carpinteria, Calif) and processed with an automated immunostainer (Ventana ES 320; Ventana Medical Systems, Tucson, Ariz). A CK-IHC stain was considered positive if it demonstrated strongly positive cell clusters or individual cells with anatomical and cytologic features of tumor cells.

Non-SNs were sectioned if larger than 5 mm, formalin fixed, and embedded in paraffin. Non-SNs were assessed by the same H&E and CK-IHC techniques.

Tumor deposits within lymph nodes were classified and staged according to the revised guidelines set by the American Joint Committee on Cancer and International Union Against Cancer.9,10 Pathology reports were individually reviewed and confirmed by the principal investigators. Metastases less than 2 mm and greater than 0.2 mm were considered micrometastases; isolated tumor cells had morphologic characteristics of tumor cells. Isolated tumor cells without cytologic features were not considered tumor cells. Of the 28 patients with micrometastases or isolated tumor cells in an SN, only 7 patients had isolated tumor cells or cell clusters that measured no greater than 0.2 mm and were usually detected by IHC.

STATISTICS

All data were reviewed and analyzed by the Biostatistical Unit at the John Wayne Cancer Institute. A χ² test was used to compare the proportions of positive to total SNs with positive to total NSNs. A P value of <.05 was considered statistically significant.

The percentage of upstaging, or improvement in the detection of positive SNs afforded by CK-IHC staining, was computed as the number of positive SNs detected by CK-IHC only (not by H&E staining) divided by the total number of SNs deemed negative by H&E staining.

RESULTS

The 63 men and 69 women had a median age of 74 years. Sixty-eight patients (52%) underwent a right hemicolec-
Patients were all negative by both H&E and CK-IHC staining. The sensitivity of LM-SN analysis (ie, the probability of detecting tumor in an SN when nodal metastasis is present) was 88.2% (45 patients with tumor-positive SNs divided by 51 patients with tumor-positive nodes). The false-negative rate (ie, the probability of not finding tumor in an SN when nodal metastasis is present) was 7.4% (6 patients with tumor-negative SNs but tumor-positive NSNs divided by 81 patients with tumor-negative nodes).

Of the 6 false-negative results, 4 were attributed to lymphatic channels obliterated by tumor (Figure 3), and 3 of these were in patients with rectal cancer. Thirty-three patients (30%) were classified as stage I; 46 (41%), as stage II; and 32 (29%), as stage III.

There was a much greater likelihood of tumor in SNs than NSNs. Of 496 SNs examined, 87 (18%) were positive (ratio, 0.175 [95% confidence interval, 0.143-0.212]); by comparison, of 1764 NSNs examined, 109 (6%) were positive (ratio, 0.062 [95% confidence interval, 0.051-0.074]). By binomial testing, the ratios were significantly different (P=.001).

Of the 17 patients with SN macrometastases, 14 had T3 lesions (82.3%), 1 patient had a T2 lesion (5.9%), and 2 patients had T4 lesions (11.8%). Of the 28 patients with micrometastases, 20 had T3 lesions (71%), 7 had T2 lesions (25%), and 1 had a T1 lesion (4%) (Figure 4).

**COMMENT**

One third of patients with node-negative colon cancers have a recurrence and die of the disease. A likely explanation for this is missed micrometastases or aberrant drainage pathways to lymph nodes beyond the field of resection. Retrospective analyses of recurrences in pa-
patients with H&E-negative nodes have identified more tumor deposits by multiple sectioning and IHC but the prognostic relevance is unclear. Prospective studies have been limited because the process of identifying micrometastases and isolated tumor cells in all draining lymph nodes is expensive and of unproven value.

Lymphatic mapping, as originally described in melanoma, allows the pathologist to focus on a limited number of lymph nodes, usually 1 or 2, that are representative of the lymph node basin. The SN paradigm is based on the premise that lymphatic drainage from a primary organ site occurs in an orderly and progressive fashion. In colon cancer, the lymphatic drainage proceeds from the submucosal lymphoid follicles through the bowel wall to the epicolic, paracolic, and finally the para-aortic nodes. The SLN is the first node to receive lymphatic drainage from a primary anatomical site and is therefore the most likely node to harbor a metastasis. In a previous feasibility study, we demonstrated that LM is applicable to colon cancer and improves staging accuracy. It has also been shown to assist with the identification of more lymph nodes, particularly nodes less than 5 mm. Despite this, the sensitivity and accuracy of LM in several studies in colorectal cancer have been inconsistent.

This may be explained by the lack of standardization, training, and interpretation of micrometastases. We therefore began a prospective trial at cancer centers that had successfully performed LM in colorectal cancer at least 20 times. Surgical technique as well as pathological analysis was standardized and closely monitored by the data and safety monitoring board. Our preliminary finding indicates a high rate of SN identification (96%) and a low rate of false-negative results (7.4%). These differ from a recently reported prospective multicenter colon SN trial in which 25 surgeons at 13 member institutions enrolled 72 patients. The SN was successfully identified in only 66% of patients and the false-negative rate was 54%. These results may be attributed to inadequate pathological evaluation or to surgical inexperience. In that study, 18 (72%) of 25 surgeons performed fewer than 5 LM procedures, and only 2 performed more than 10. Only half of the SNs in the study underwent multilevel sectioning and the majority of primary tumors (65%) were locally advanced (T3 and T4).

The original technical success rates of LM in melanoma and breast cancer were 82% and 60%, respectively. With well-orchestrated training programs and clinical trials, these rates have increased to 98% and are widely reproducible. The precise number of cases required in colon cancer to be proficient is unknown, but the learning curve appears less than breast cancer and melanoma.

Paramo and colleagues have demonstrated that the sensitivity of LM in colon cancer increased after 5 LM procedures were performed and then remained consistently high in a study of 7 surgeons. In a similar feasibility study in 100 patients, we demonstrated that all of the technical failures occurred in the first 50 patients undergoing LM and SLN analysis, with 3 of the 5 false-negative results in the first 30 operations. In the present trial, 3 of the 6 false-negative results occurred in rectal cancer in patients treated with neoadjuvant chemotherapy.

Lymphatic mapping has not only been shown to improve the detection of micrometastases but is also associated with an increase in lymph node retrieval, possibly because, with time, more smaller nodes stain blue. The median number of lymph nodes in this study was 14.5, significantly more than the number of lymph nodes retrieved at our institution prior to performing LM. Intuitively, the more nodes that are recovered the greater the likelihood of discovering an occult metastasis. In a study of 1816 patients with Dukes B colon cancer, Prandi et al noted lower overall survival when fewer lymph nodes were recovered in the colectomy specimen. They suggested that patients with Dukes B colon cancer with fewer than 7 nodes in the surgical specimen are potentially understaged and should be considered for adjuvant chemotherapy. However, because chemotherapy benefits only 30% of node-positive patients, its routine use would unnecessarily expose many patients to drug toxic effects.

Despite this, there is no consensus on the number of nodes necessary for adequate staging. Both the American Joint Committee on Cancer and the International Union Against Cancer recommend the examination of at least 12 lymph nodes per specimen. In a review of 108 patients with stage II colon cancer, Burdy et al found that the recovery of fewer than 14 uninvolved lymph nodes in the colectomy specimen was significantly associated with tumor recurrence. Joseph et al found that the prediction of nodal status with an 85% certainty required examination of more than 40 nodes for T1 and T2 lesions and at least 40 nodes for T3 lesions. While more than 40 nodes may be harvested from a properly resected specimen, thorough examination of these nodes would be costly and labor intensive.

The prognostic value of micrometastases is a subject of much debate. It is unclear whether IHC-detected cells within lymph nodes represent part of a metastatic cascade or hyperplastic mesothelial cells. Micrometastases have been reported in 10% to 76% of H&E-negative nodes by retrospective analysis. Using a multimarker RT-PCR assay, we upstaged 46% of cases identified as node negative by both H&E and IHC staining. Similarly, Liebers et al used RT-PCR for carcinoembryonic antigen to study the nodes of 26 patients with node-negative colorectal cancer, 54% of can-
cers were upstaged. More importantly, the 5-year survival rate was 91% for those who were node negative by RT-PCR compared with only 50% for those who were node positive by RT-PCR.

Ultrapragmatically, techniques are probably but remain costly and time-consuming for use on all nodes in a colectomy specimen. By concentrating the pathologist’s efforts on a few lymph nodes that are most likely to harbor a metastatic cancer, the potential to upstage cancer can be greatly increased without significantly increasing time and cost. Lymphatic mapping, by helping to identify high-risk lymph nodes, particularly those that may be small or in aberrant locations and difficult to see, would assist in the upstaging of colon cancer. Lymphatic mapping may allow an effective, focused pathological ultrastaging review of 1 or more SNs, without compromising standard pathological assessment of SNs.

Preliminary studies from this prospective phase 2 trial suggest that LM and SLN techniques are feasible and accurate in colon cancer. Focused pathological review identified micrometastases and/or isolated tumor cells in 24% of patients who otherwise may have been considered node negative. The full impact of the prognostic effect of micrometastases continues to be evaluated in this trial. The improved risk stratification afforded by standardization of both surgical and pathological techniques may improve the selection of patients for chemotherapy, thereby avoiding the unnecessary toxic effects and expense for those cured by surgery alone.

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REFERENCES


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Jan H. Wong, MD, Honolulu, Hawaii: In 1991, I, along with my coauthors Drs Cagle and Morton, published what has turned out to be, unfortunately, an infrequently cited manuscript describing an operative technique to identify the sentinel lymph node in a feline model (Wong JH, Cagle LA, Morton DL. Ann Surg 1991; 214:537–541). Approximately 1 year later and some 2 years after being presented at the annual meeting of the Society of Surgical Oncology, the technical details of intraoperative lymphatic mapping and sentinel node dissection was published. The difficulty in getting that report accepted for publication attests to the considerable skepticism that sentinel node staging was met with. His-tory, however, has confirmed and now repeatedly validated the simple yet eloquent hypothesis that solid tumors, if they metastasize to the regional lymphatics, will do so in an orderly fashion to a sentinel lymph node—at least in cutaneous melanoma and breast cancer.

Dr Bilchik and his colleagues report today on a multicenter trial evaluating the feasibility and accuracy of sentinel node stag-

ing in carcinoma of the colon and rectum. His results demonstrate that not only is sentinel node staging of the colon and rectum technically feasible (their success rate of identifying the sentinel node was 96%), but with the addition of 2-level sections and immunohistochemical staining to the routine analysis of the sentinel lymph nodes, there is a dramatic increase in the identification of tumor cells in the hematoxylin-eosin–negative nodes. Sentinel node staging in their hands has a sensitivity of 88% and false-negative rate of 6.9%, results that are comparable with those observed in cutaneous melanoma and breast cancer.

Although there is general uniformity in the sensitivity and false-negative rates reported in melanoma and breast cancer, the same cannot be said for carcinoma of the colon or rectum. We have reported similar results to those reported here today utilizing ex vivo lymphatic mapping techniques in carcinoma of the colon and rectum. However, other investigators have not been nearly as successful in reproducing Dr Bilchik’s outstanding results. My first question addresses these apparent discrepancies. Are these simply technical issues that can be overcome with experience or is this tumor model just not amenable to this approach?

If experience is a key and given the workload of the average general surgeon as compiled at the time of recertification, is sentinel node staging so precise that it can only be performed in experienced, high-volume settings? We have described the ex vivo technique to map the lymphatics of the colon and rectum in pathology (Wong JH, Johnson DS, Namiki T, Tauchi-Nishi P. Ann Surg Onc. 2004;11:772–777). Might this technique, in which all colorectal cancer specimens are examined and mapped by a single laboratory, perhaps shorten the ability to implement this approach to improved staging?

The majority of your upstaging (85% to be exact) was the result of the identification of isolated tumor cells. Is the upstaging that you observed, particularly of isolated tumor cells, simply the result of a more thorough examination of the sentinel lymph node or does this truly represent the initial site of metastases to the regional lymphatics?

Finally, as you discussed in your manuscript, the biologic and prognostic relevance of these immunohistochemical findings are controversial. It is apparent that if we look we will find. Do you have recommendations for what we should do when these small volumes of apparent disease are identified? Should these individuals be candidates for adjuvant systemic therapy? Is it feasible to design a clinical trial to address the prognostic relevance of these findings or should we focus on the burgeon-

ing technology of gene expression signatures and proteomic profiles?

Dr Bilchik: Thank you, Dr Wong. The pioneering investigations of sentinel node (SN) mapping conducted by you and Dr Morton have revolutionized the management of both melanoma and breast cancer.

In colon cancer, the accuracy of lymphatic mapping and focused analysis of the sentinel node depends on careful patient selection and on technical issues. For example, patients with large proximally tumors (T4) are poor candidates for sentinel node mapping because injection of the mapping agent around the tumor is difficult; also, a large tumor may obliterate lymphatic channels. Moreover, these patients probably will receive systemic chemotherapy regardless of the tumor status of the sentinel node. We would not expect the accuracy of lymphatic mapping to be as high in small studies that included patients with advanced tumors or in any study not based on standardized techniques and close communication between surgeon and pathologist.

Ex vivo lymphatic mapping may facilitate standardization because it can be performed in the pathology suite. Often the pathologist is able to identify small blue-stained lymph nodes
not identified intraoperatively. The only drawback of ex vivo lymphatic mapping is its inability to identify aberrant or unexpected lymphatic drainage that might change the operative approach. We favor the ex vivo approach when the presence of rectal cancer or a thickened mesentery would complicate in vivo identification of the sentinel node.

In our study, most upstaging of colon cancer was based on identification of tumor cell clusters; both immunohistochemical and molecular studies suggest that these clusters represent biologically active metastasis. Cells that stained positive by immunohistochemistry but had no morphologic characteristics of tumor cells were not considered a basis for upstaging. However, immunohistochemistry often detected small tumor cells initially missed by H&E but confirmed by rereview of H&E-stained sections.

Should a patient with micrometastases receive chemotherapy? Although most medical oncologists are inclined to say yes, the issue remains controversial. Retrospective studies have been limited by small numbers, inadequate lymph node retrieval, and lack of standardized methodology. We hope that results of our ongoing prospective multicenter trial will yield a more definitive answer. The design of a prospective trial based on gene expression is somewhat problematic given the need for adequate nodal sampling, standardized pathological and molecular analysis, and consensus on the selection of marker panels. At this time, the most important criterion for a successful trial of staging in patients with colon cancer is a carefully planned oncologic operation with adequate lymphadenectomy.

Baiba J. Grube, MD, Galveston, Tex: I do have one question regarding the whole concept of regional metastases as opposed to systemic disease, much like the Halstedian principle of regional spread in breast cancer as opposed to Fisher’s principle of disease being metastatic from the beginning. How will your trial answer the possibility of metastatic disease as an initial event rather than regional spread?

Dr Bilchik: The purpose of our trial is to evaluate the accuracy of sentinel node mapping and then to determine whether nodal micrometastasis impacts overall survival. The trial was not designed to address the possibility of metastatic disease without regional nodal involvement.

Tyler Hughes, MD, McPherson, Kan: One question is about lymphazurin in the breast. We are starting to see moving away from it because of allergic reactions, etc. So I wonder about potential morbidities of lymphazurin.

The other question I have, being an average general surgeon, if I were to use this technique and find an aberrant node, because in small towns everything turns out to be aberrant, what operation do I do at the point where I have a right colon lesion and I get a blue node in the sigmoid mesentery?

Dr Bilchik: In our experience, use of isosulfan blue dye to visualize lymphatic drainage to the sentinel node is safe and effective. There is essentially no morbidity and we have not encountered any anaphylactic reactions. The blue dye is inexpensive, and mapping adds only minutes to the operation. Fortunately, aberrant or unexpected drainage pathways are extremely unusual; in our experience, the tumor is usually a right-sided colon cancer that drains to a sentinel node located to the left of the middle colic artery. To change the operation from a right colectomy to an extended right hemicolectomy adds no morbidity and is undeniably gratifying when the SN tests positive for tumor. The possibility of aberrant drainage has major consequences for the laparoscopic surgeon, who otherwise might be tempted to skip sampling of the mesenteric nodes when the SN is at the root of the mesentery.

Anthony M. Vernava, MD, Rochester, NY: I would like to compliment you and your coauthors on attempting to deal with stage migration and would like to ask you a question regarding your sentinel lymph node study. Earlier studies which have evaluated patients for the presence of micrometastases have been successful in upstaging patients in whom micrometastases have been identified. However, those same studies did not demonstrate a clinical harm for those patients upstaged by micrometastases comparable to conventional stage III patients. For the most part, patients upstaged using micrometastases as an indicator of node-positive disease continued to behave biologically as having node-negative disease. How do your results using sentinel lymph node evaluation compare to those earlier studies using cytokeratin identify micrometastases? Does upstaging a patient using sentinel node technique confer useful clinical information beyond conventional operation and pathologic staging?

Dr Bilchik: We recently performed a meta-analysis of all retrospective studies evaluating micrometastases in lymph nodes of colon cancer patients. In most studies, immunohistochemistry and/or reverse transcriptase–polymerase chain reaction identified micrometastases in nodes that stained negative by H&E. Interestingly, identification of nodal micrometastasis by RT-PCR but not by immunohistochemistry was associated with a statistically significant difference in overall survival. However, the techniques were not standardized, the studies were retrospective, and most of the patients underwent inadequate nodal sampling. Databases on standardized, prospective assessment are necessary to resolve the clinical relevance of upstaging based on nodal micrometastasis.