Annals of Surgical Oncology, 9(10):944–953
DOI: 10.1245/ASO.2002.04.005

Educational Review

Significance of Micrometastases in Colorectal Cancer

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Key Words: Colon cancer—Micrometastases—Review—Sentinel node.

Colon cancer will be the cause of death for more than 50,000 people this year alone, and more than 133,200 new cases of colorectal cancer (CRC) will be diagnosed.[1] It is currently the third most common cancer in both women and men and is the third highest cause of cancer mortality.[1]

Like other solid tumors, CRC is staged pathologically on the basis of the extent of primary organ involvement and the metastatic spread to lymph nodes or to distant organs. With the tumor-node-metastasis classification system, patients with stage I and II disease have 90% and 75% 5-year survival rates, respectively. By definition, patients with stage I or II disease are node negative and lack evidence of extracolonic spread. The surgical resection offered to these patients is potentially curative, eradicating—at least in theory—all of the disease from their bodies. However, up to 30% of these patients develop metastatic disease and eventually die from colon cancer. Therefore, most likely there was extracolonic spread of disease at the time of surgery that was below the limits of detection with standard techniques.

The detection of extracolonic disease has practical, short-term implications: patients who have node-positive disease have been shown to have an overall reduction in disease-specific mortality of 30% with the addition of postoperative chemotherapy.[2] Hence, it is the standard of care to provide adjuvant therapy for patients with positive nodes. Missing positive nodes will understage patients and cause adjuvant therapy to be withheld from a group of patients that could derive benefit. By contrast, the administration of adjuvant chemotherapy to node-negative patients has not translated into clinical benefit.

It is possible that in some patients, extracolonic disease is too minute to be detected at the time of resection. More scrupulous examination of lymph nodes within a resected specimen could improve the detection of node-positive disease. This then begs an answer to the question of how the lymph nodes within the surgical specimen are examined: How many lymph nodes are adequate? By what method does one examine the lymph nodes? Ultrasensitive tests could perhaps identify metastatic disease beyond lymph nodes and into distant organs, the marrow, and even peripheral blood.

In this review, we discuss the various techniques of detection of small metastatic nodal deposits of disease, as well as the clinical implications of such findings. Clearly the advent of new technologies lowers the threshold of detection. It is our belief that at some point, we may cease to show clinical relevance for miniscule amounts of nodal disease. We must therefore relate technological advances to clinical utility.

SIGNIFICANCE OF MICROMETASTASES

By convention, the term *micrometastases* refers to deposits of single tumor cells or very small clusters of neoplastic cells. Historically, size criteria were used to determine the presence of micrometastases within a lymph node. Although some physicians formerly advocated defining micrometastases on the basis of the percentage of histological involvement in a cross-sectional area of a node,[3] the general consensus now is a focus of

Received April 6, 2002; accepted September 6, 2002.

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metastatic cells <2 mm in diameter.[4] This definition, however, is not based on clinical outcome and should therefore be considered somewhat arbitrary.[5] As newer technologies become cheaper, standardized, and more available, the onus rests on the clinician to determine the value of the detection of micrometastases. We must decide the level of micrometastatic nodal disease that warrants postoperative adjuvant therapy or altered primary therapy. The mere presence of neoplastic cells outside the site of the primary tumor may warrant systemic treatment. Alternatively, our examining eye may have become too sensitive, and such foci of disease may be meaningless to the patient's overall health and prognosis.

Just as there are no set pathologic criteria for the definition of a focus of metastatic disease, nor are there treatment implications of such, there is no clear comprehension of the role of such a cluster of cells in the oncological process. It has been hypothesized that tumor cells detected by techniques more sensitive than hematoxylin and eosin (H&E) staining may represent "shedding" from the primary tumor and not biologically active metastases.[6,7]

PATHOLOGIC EXAMINATION OF LYMPH NODES

Current Techniques

It is well established that the presence and number of lymph nodes involved with metastatic disease has an effect on survival.[8–12] Traditionally, the nodes from a resected specimen are identified by manual palpation, thereby subjecting the identification of these nodes to the pathologist's diligence and patience. Individual institutional practices differ greatly, but often the nodes will be bivalved and examined by H&E staining of serial sections. Approximately 55% of patients presenting with CRC will be determined to be node negative with this technique. Using this method of manual palpation to identify the lymph node, followed by light microscopy of a section of that node, skillful pathologists can detect deposits of metastatic disease as small as 100 μm in diameter, or approximately the size of 100 cancer cells clustered together.[13]

Ideal Number of Lymph Nodes

Several reports have documented that increasing the number of examined lymph nodes will increase the chance of detecting nodal positivity.[14–16] It is therefore logical to attempt to retrieve and examine all possible nodes from a specimen. In a report to the World Congress of Gastroenterology, Fielding et al.[8] recommended the examination of a minimum of 12 lymph nodes per colon cancer specimen before one could report accurately on the status of a patient's entire nodal basin. This recommendation subsequently was adopted by the American Joint Committee on Cancer and the TNM Committee of the International Union Against Cancer.[17] Conversely, Hernanz et al.[18] report that only six lymph nodes are required for accurate staging. Most clinicians believe that this figure is too low.

Additional studies have indicated that more than 12 lymph nodes must be examined. Goldstein et al.[14] and Goldstein[19] recommend 17 lymph nodes per resected specimen because in their experience, the percentage of node-positive patients plateaus with more than 17 nodes. Scott and Grace[15] recommend that 13 lymph nodes be examined. Jessup et al.[20] and Wong et al.[21] examined the percentage of node positivity as it varied with tumor size and found that with 14 lymph nodes examined, the rates of nodal disease reflected those estimated by the National Cancer Data Base Report.

The initiation of laparoscopic colectomy further stimulated the debate over the adequate number of examined lymph nodes needed to reflect accurately the status of the nodal basin. This was due to the preliminary observation that laparoscopic colectomy resulted in the retrieval of fewer lymph nodes compared with open colectomy. Hida et al.[22] propose adjusting the extent of lymph node dissection according to the tumor stage to improve the yield from laparoscopically resected specimens. Recently published data indicate that, in experienced hands, laparoscopic and open colectomy yield equivalent rates of nodal retrieval.[23]

Improved Yield From Surgical Specimens

It has been estimated that more than 70% of lymph nodes with metastatic disease are <.5 cm in diameter.[24,25] Such small nodes are very likely to be missed by simple manual palpation. The identification rate of lymph nodes within a given specimen can be significantly increased by using a technique called *fat clearance*.[16,24] Use of this process has been shown to upstage the disease,[24,26] but, at least thus far, no survival benefit has been noted from increased lymph node detection.[16]

Increased Pathologic Sampling

Serial sectioning from nodes within a pathologic specimen increases the detection of metastatic disease.[18,27] Closer examination of lymph nodes has been postulated to upstage up to 33% of all CRC patients.[28] It has been estimated that if a single lymph node is examined with one 5-μm-thick section, only .04% of the node will be examined, thereby potentially introducing significant

sampling error.[29] Examining multiple sections of the same node will reduce this error, although this could become too labor intensive. The literature from studies on axillary nodes from breast cancer patients notes increased numbers of higher-staged tumors with serial sectioning.[30] The CRC data are less extensive: van Wyk et al.[31] noted only a small number of patients who were upstaged on the basis of multiple sectioning of lymph nodes.

Immunohistochemistry of Lymph Nodes

Immunohistochemistry (IHC) represents the first of the pathology tools that surpasses the capabilities of H&E staining in the examination of a specimen for microscopic disease. For colon cancer detection, monoclonal antibodies against markers of epithelial histology are used. Early studies failed to show improved detection capabilities with IHC.[32] Davidson et al.[33] used IHC to identify nodal disease and retrospectively re-examined the H&E slides of the same nodes that were identified as IHC positive. There was evidence of metastatic disease in these slides on H&E, thereby indicating that the tumor cells were simply missed on routine H&E. In more recent studies, IHC routinely has increased the stage of patients with colon cancer.

The clinical utility of IHC in the evaluation of lymph nodes has been debated (Table 1). Isaka et al.[41] stained lymph nodes from 42 patients with Dukes' B (negative nodal specimens by H&E) rectal carcinoma with a monoclonal antibody, CAM5.2, which binds cytokeratins (CKs) 8 and 18. They found deposits of micrometastatic disease in 19 (2.9%) of 644 nodes from 9 different patients. Although the presence of metastatic disease was not correlated with the clinicopathology of the primary tumor, there were differences in recurrence rates, relapse-free survival, and 10-year survival between patients with and without micrometastases. In another study supporting the benefit of IHC, 568 lymph nodes from 50 patients with Dukes' B CRC were examined with IHC for CK and a specific tumor-associated glycoprotein, TAG-72. Six of the 14 IHC-positive patients died of CRC within 66 months, whereas only 1 of the 36 IHC-negative patients died of CRC ($P = .0009$).[35]

By contrast, a number of studies have failed to show increased patient survival despite pathologic upstaging on the basis of IHC[34,36,42] (Table 1). Andreola et al.[43] studied more than 100 cases of primary rectal carcinoma of the lower third of the rectum, all treated with resection and lymphadenectomy with coloanal anastomosis. The lymph nodes were identified by manual palpation, with an average of 42.3 lymph nodes per specimen, of which 6.5% were positive by H&E alone. The percentage of positive nodes increased to 40.4% with IHC with a pool of antibodies against CK. Each of the five patients who had recurrent disease had at least one definable oncological predisposition to metastases or recurrence: namely, positive surgical margins (distal or circumferential) or vascular invasion. The authors concluded that examining H&E-negative lymph nodes by immunostaining techniques was "time-consuming and unnecessary" provided that an ample number of lymph nodes had been examined.

Others also support the conclusion that nodal micrometastases detected by IHC do not predict relapse. Nakanishi et al.[42] compared patients with and without recurrence of CRC in a case-controlled manner. They

TABLE 1. *Upstaging detected by IHC performed on lymph nodes*

Study	n	MAb target	Positive IHC	Prognostic effect
Davidson[33]	47	CEA, EMA	NA	No
Cutait[34]	46	CEA, CK	12 (26%)	No
Greenson[35]	50	CK, TAG-72	14 (28%)	Yes
Jeffers[36]	77	CK	19 (25%)	No
Adell[37]	100	CK	39 (39%)	No
Broll[38]	49	CK, EP4	13 (26%)	No
Haboubi[24]	25	CK	15 (60%)	Yes
Oberg[39]	147	CK	47 (32%)	No
Clarke[40]	134	Mp53P[a]	35 (26%)	Yes
Isaka[41]	42	CK8, CK18	9 (21%)	Yes
Nakanishi[42]	44	CK, p5	30 (68%)	No
Tschmelitsch[29]	50	CK	38 (76%)	No
Andreola[43]	52	CK	21 (40%)	NA
Bilchik[44]	40	CK	4 (10%)	NA
Yasuda[45]	42	CK	32 (76%)	NA

MAb, monoclonal antibody; CEA, carcinoembryonic antigen; EMA, EP4, epithelial membrane antigen; CK, cytokeratin; NA, not available; IHC, immunohistochemistry.

[a] Mutant p53 protein.

found no difference in the frequency of micrometastatic disease between the two groups. Tschmelitsch et al.[29] found a higher rate of IHC-positive, H&E-negative patients among those who did not relapse than in those who did. In this study, specimens from 55 patients with stage II disease were examined. With 5 years of follow-up, 28 relapses occurred. All lymph nodes were re-examined with H&E staining initially, and in 4 of the 28 relapses, re-examination revealed nodal metastases, as compared with 1 of the 27 in the nonrelapsing group. Moreover, of the 24 patients who had disease relapse but negative lymph nodes by H&E on initial and subsequent re-examination, 16 had positive IHC staining for CK. This result is compared with 22 of 26 patients without relapsing disease whose nodes were negative for disease by H&E but tested positive for CK. These data support the claim that the detection capabilities with IHC are too great to be translated into clinical utility.

Yasuda et al.[45] have attempted to refine the conditions that qualify as clinically relevant micrometastatic disease on the basis of the location of the lymph node. They found that in Dukes' B CRC patients, tumor deposits detected by IHC for CK were prognostic of clinical failure only if more than four nodes had micrometastatic disease or if the nodes involved were within 5 to 10 cm of the primary tumor.

Intuitively, the combination of several methods of identification should improve diagnostic yield and pathologic accuracy. Haboubi et al.[24] showed better staging when xylene clearance and IHC were combined. In this study, there was a diminished 5-year survival for patients identified as having H&E node-negative disease but node-positive disease with IHC on nodes identified by fat clearance compared with patients who were node negative by both H&E and IHC.

Molecular Staging

Reverse transcription polymerase chain reaction (RT-PCR) is a method of amplifying a particular known sequence of RNA that encodes for a protein of interest by making the complementary DNA that encoded that RNA and then amplifying that segment of DNA. This allows detection of a single tumor cell within a population of 2 to 5 $\times$ 10^7 cells,[46] which is approximately 3 times the sensitivity of IHC.[47] This sensitivity increases by using subspecialized techniques such as immunobead-PCR, a technique in which immunomagnetic beads enrich the working medium for the epithelial cells.[48]

In CRC, the presence of RT-PCR–positive lymph nodes correlates with the stage of the primary tumor. Bernini et al.[49] performed RT-PCR on the gene that encodes for MUC2, a mucin protein: no patient with T1 disease had tumor-associated protein in the lymph nodes, whereas 17%, 40%, and 50% of patients with T2, T3, and T4 tumors, respectively, had nodal disease.

Bilchik et al.[44] used RT-PCR in colon cancer specimens to amplify a nucleic acid sequence encoding for β human chorionic gonadotropin, which is commonly overexpressed in gastrointestinal tumors; a hepatocyte growth factor (cMet) associated with CRC tumor progression; and a universal melanoma-associated antigen that is specific for neoplastic tissue (Table 2).[[50–53]] When RT-PCR was combined with IHC, micrometastatic disease was found in more than half of the patients who were node negative by H&E.[44] Mori et al.[54] examined the expression of carcinoembryonic antigen (CEA) in the lymph nodes of patients with gastric, breast, and rectal cancers. Of 23 nodes examined, 4 were positive by H&E, but 19 were positive by RT-PCR. Sanchez-Cespedes et al.[55] tested K-*ras* and p53 mutations and p16 promoter hypermethylation in RNA from perihepatic lymph nodes and liver metastases from CRC patients. Of 21 separate liver metastases, 76% had at least one of these three genetic alterations. The perihepatic lymph nodes of those patients with a mutation were then examined similarly: 28% were positive by H&E, and 45% were positive by PCR.

In contrast to investigating the presence of a particular protein in lymph nodes with PCR, some groups are using a variation of RT-PCR to detect mutations or genetic sequence repeats within the genome in lymph nodes of patients with CRC. This is a process known as *mutant*

TABLE 2. *Effects of RT-PCR on lymph nodes of CRC patients*

Study	n	PCR target	Positive (%)	Prognostic effect
Hayashi[50]	22	K-*ras*, p53 mutations	14 (64%)	NA
Hayashi[51]	120	K-*ras*, p53 mutations	37 (31%)	Yes (recurrence)
Liefers[52]	26	CEA	14 (54%)	Yes (survival)
Bernini[49]	43	*MUC2*	12 (28%)	NA
Bilchik[44]	40	β-hCG, cMET, uMAGE	12 (30%)	NA
Miyake[53]	11	CEA, CK	9 (82%)	NA

NA, not available; *MUC2*, mucin gene; RT-PCR, reverse transcription polymerase chain reaction; CRC, colorectal cancer; CEA, carcinoembryonic antigen; hCG, human chorionic gonadotropin; cMET, hepatocyte growth factor; uMAGE, universal melanoma-associated antigen; CK, cytokeratin.

allele specific amplification (MASA).[50] Hayashi et al.[50] examined 22 CRC specimens; 6 had K-*ras* and 9 had p53 mutations, and 1 tumor had both. The authors then examined the pericolonic lymph nodes of these patients with regular histopathologic techniques as well as MASA. One half (7 of 14) of the patients with mutations in the primary tumors had MASA-detected genetic alterations in their lymph nodes but had negative histopathology in these nodes.

Recently, there has been an increased effort to relate nodal status, as detected by molecular techniques, to clinical outcome. Hayashi et al.[51] examined patients with histologically negative lymph nodes: 37 patients had MASA-detected mutations of either K-*ras* or p53, and 34 did not. Twenty-seven of the 37 MASA-positive, but none of the MASA-negative, patients experienced a recurrence within 5 years.

Sanchez-Cespedes et al.[55] found that the median survival rate of patients with PCR-positive perihepatic lymph nodes was 165 days, compared with 1056 days for patients with PCR-negative nodes ($P = .0005$). However, this small set of patients was also demonstrated to have a significant difference in median survival when H&E-positive lymph nodes were compared with H&E-negative nodes.[55] The effect afforded by the results of RT-PCR alone, separate from H&E, is somewhat diluted and is difficult to determine in this and in other studies.

Liefers et al.[52] used RT-PCR for CEA to examine lymph nodes from 26 stage II CRC patients. The investigators found a higher 5-year survival rate among patients with RT-PCR–negative nodes as compared with patients with at least one RT-PCR–positive node (91% vs. 50%; $P = .02$). Miyake et al.[53] combined IHC and RT-PCR to examine 247 lymph nodes from 11 patients. Patients who were positive by both techniques had already been deemed positive for nodal disease by H&E. Retrospective analysis of the clinical data showed that 2 of their 11 patients had recurrent disease within 1 year after resection. In both of these cases, more than 70% of the resected lymph nodes tested positive for micrometastatic disease by RT-PCR. Moreover, the nodes that were positive were not anatomically confined to pericolonic tissues. The authors believe that the detection of distant (i.e., near the root of the inferior mesenteric artery) nodal micrometastases may relay valuable clinical data. Table 2 lists the upstaging effects seen with PCR performed on CRC nodes.

EXTRANODAL SPREAD

Bone Marrow

The original definitions of the term *micrometastases* included not only size criteria, but also histological architecture such that the cluster of cells could indeed become malignant. As stated by Tsavellas et al.,[47] "prerequisites for metastasis are tumor cell arrest, implantation, and proliferation with a surrounding stromal reaction." However, the term *micrometastases* now incorporates blood and bone marrow metastases, which by nature lack histological architecture. The literature from studies in patients with breast cancer has raised awareness of the potential significance of metastatic disease detected in the bone marrow. Staining for the presence of epithelial cells or epithelial cell markers in marrow may serve as a method of differentiating between normal mesenchymal cells and tumor deposits. This idea has caught attention in the literature, and now the presence of tumor cells detected in the bone marrow has been given a special tumor-node-metastasis classification notation: M1(i). A recent meta-analysis that included all types of carcinoma found a prevalence of bone marrow metastases of 35%.[56] Fourteen of the 20 studies reviewed showed a correlation between marrow metastases and relapse-free survival. But in only two studies was the presence of marrow metastases on multivariate analysis determined to be an independent predictor of survival.[56]

TABLE 3. *Summary of bone marrow micrometastases in colorectal cancer*

Study	n	Method	Marker	% Positive	Prognostic effect
Schlimok[57]	156	IHC	CK 18	27	Relapse
Lindemann[58]	88	IHC	CK 18	32	Relapse
Gerhard[46]	15[a]	RT-PCR	CEA	67	NA
Juhl[59]	58	IHC	CK	29	NA
O'Sullivan[60]	48	Flow cytometry	CK	23	Metastatic development
Soeth[61]	65	RT-PCR	CK 20	31	Survival
Weitz[62]	30	RT-PCR	CK 20	27	NA

IHC, immunohistochemistry; CK, cytokeratin; NA, not assessed; RT-PCR, reverse transcription polymerase chain reaction; CEA, carcinoembryonic antigen.

[a] Gastric, pancreatic, and colorectal cancers included.

The practical clinical application of the presence of marrow micrometastases remains in doubt (Table 3).[57–62] Lindemann et al.[58] reported a decrease in disease-free survival in CRC patients with positive bone marrow aspirates. Moreover, a Cox regression analysis showed that the presence of bone marrow metastases was an independent predictor of relapse. Schlimok et al.[57] found a higher disease relapse rate in patients whose marrow tested positive for IHC against CK 18. Using immunomagnetic assays, Flatmark et al.[63] found micrometastatic disease in the marrow of 17% of all patients undergoing CRC surgery. There was a trend in their data toward higher detection of micrometastatic disease in the marrow with advanced clinical stages.

Peripheral Blood Examination

In parallel to the examination of bone marrow, attention has been focused on the detection of cancer micrometastases from peripheral blood samples. Molecularly detected evidence of tumor in the peripheral blood of patients with prostate cancer and melanoma has been associated with poorer survival,[64,65] but as yet no consistent results have been identified. A single colon cancer cell can be detected with RT-PCR in a milliliter of peripheral blood.[66] Some suggest that the mere presence of colon cancer cells in the peripheral blood portends a worse survival than in patients without such cells. In colon cancer patients, Hardingham et al.[48] found that PCR positivity significantly correlated with disease-free survival. Others doubt the reported deleterious effects of tumor cells in the peripheral blood because of the stability of the DNA molecule.[67] In addition, some argue that circulating tumor cells routinely are cleared by the body's own defense mechanisms.[68] To support the benignity of circulating tumor cells, Fidler et al.[69] showed in an animal model that <.1% of circulating tumor cells develop into metastases.

Not all agree that circulating tumor cells lack prognostic clinical value. Using a monoclonal antibody and immunobeads to select epithelial cells and then quantitatively measuring the messenger RNA (mRNA) produced from complementary DNA genes encoding CK, Denis et al.[70] found circulating colon cancer cells in 20% of patients with stage A or B CRC and 77% of patients with stage C or D. Patel et al.[68] examined the peripheral blood of 116 patients with known CRC in the preoperative period. Eighty-one (70%) of 116 patients had positive RT-PCR for CEA or CK 20 in peripheral blood. Furthermore, the number of RT-PCR–positive patients decreased significantly 24 hours after surgery, but on subgroup analysis, only the patients with Dukes' A or B cancer had statistically significant decreases in RT-PCR positivity.

Several technical challenges have been raised in the detection of peripheral-blood CRC cells. First, these cells possess a tendency to cluster, thereby increasing the potential for false-negative results due to the inhomogeneity of circulating blood.[71] Others argue that surgical manipulation of the tumor launches tumor cells into the bloodstream. Although not uniformly accepted,[68] this theory is supported by the detection of higher mRNA concentrations after surgery.[72–75]

Portal Circulation

A corollary to the examination of peripheral blood for the presence of tumor cells is the examination of portal blood, because the liver is the primary extranodal site of metastases in colon cancer. Sadahiro et al.[76] measured mRNA for CEA in the portal venous system and the peripheral venous system of patients with CRC. Although this was not statistically different from the percentage of patients with positive CEA in peripheral blood, 51% of patients had positive CEA in portal blood. However, there was a 91% concordance rate between peripheral and portal blood, leading the investigators to conclude that there was little utility in sampling portal blood. Taniguchi et al.[77] report a decreased 2-year disease-free survival in CRC patients who had CEA mRNA detected in the portal circulation compared with those patients who did not, although this difference was also seen with samples of peripheral blood.

SENTINEL LYMPH NODE

Overview

The impetus to examine the existence and clinical significance of micrometastatic disease is fueled by advances in technology, particularly in the fields of immunology and molecular biology; an era of cost containment, wherein it would be impractical and unfeasible to meticulously examine thin sections of every lymph node within a given specimen; and, last, the advent of technology that allows pathologists to "hedge their bets" with focusing their efforts on a single node or a few nodes.

The concept of a sentinel lymph node (SLN) was first introduced in the field of otolaryngology as early as the 1950s.[78] More recently, the concept has been applied to melanoma[79] and breast cancer.[80] This is based on the notion that, first, there is an orderly progression of lymphatic drainage from a given site and, second, that the SLN is the initial node to receive lymphatic drainage from an anatomical location. The SLN should be the first node to contain cancer cells if the cancer has spread

along the lymphatics. For colon cancer, as first reported in 1935, the presumed sequence of drainage is first through the bowel wall, because these are mucosal tumors, followed by pericolic lymph nodes and subsequently para-aortic lymph nodes.[81] However, skip metastases have been reported to occur,[82] which could render the SLN falsely negative.

There is a key distinction between the use of the SLN in breast cancer and melanoma and its use in CRC. The status of the SLN in the former two cancers may limit or dictate the extent of further surgery. The SLN in CRC does not limit the planned operative resection but rather is a potential means of allowing a focused pathologic examination. In some instances, mapping the lymphatic drainage from a particular site within the gastrointestinal tract actually expands the planned margins of resection. In one published series of 130 patients, 3 patients underwent extended right hemicolectomies instead of the planned standard right hemicolectomy because the SLN drained to the left side of the middle colic vessels, as detected during surgery.[83] When such a procedure is performed, it is therefore imperative to minimize the mobilization of the colon before injection of radiocolloid or dye so as not to disrupt the lymphatics.[84]

Technique of SLN Identification

There are two distinct methods of identifying the SLN, regardless of the organ being studied (e.g., colon, breast, skin, and so on): lymphoscintigraphy and visible dye. Even in fields where the use of SLN is more established, there is no academic consensus as yet of the best technique. With regard to the use of SLN in CRC, there are two additional variables besides the material used: the timing of the lymphatic mapping in relation to surgical dissection and the histological injection site. Certainly the location of the primary tumor (low rectal, and so on) and operative technique (open or laparoscopic) can affect the ease with which the SLN mapping is performed.

Furthermore, some surgeons advocate injecting radionucleotide or dye while the specimen is still in vivo, whereas others propose injecting dye on the back table of the operating suite once the specimen has been resected. Wood et al.[85] propose that the in vivo technique allows greater confidence in the identification of the lymphatics and use the ex vivo technique only when the in vivo technique fails. Others have demonstrated good ability to detect SLNs with an ex vivo technique.[86]

There also is discordance among various groups as far as the injection site per se: subserosal, peritumoral, or submucosal. One requires endoscopic instrumentation of the colon, whereas the others may sacrifice accuracy of direct localization of the lesion in question. The nuances of the technique of SLN injection are beyond the scope of this article.

Results From SLN Biopsy

Table 4 [[88–91]] lists several studies that have examined the utility of SLN biopsy. As with any procedure, and especially germane to SLN mapping, there is a learning curve. Joosten et al.[87] reported an inability to ascertain meaningful clinical data based on SLN mapping, given a 30% failure rate of identifying a single node with lymphoscintigraphy and a 60% false-negative rate of the status of the SLN. On retrospective review, it was noted that most of the failures were due to an incorrect injection technique. Very likely, as with trials of SLN in breast cancer and melanoma, the level of internal surgeon-specific quality control must be high and constantly monitored.

When the procedure is successfully performed, Wood et al.[85] found that analysis of SLN upstaged 20% of their patients. Others report a sensitivity of 100%, a sensitivity

TABLE 4. *The use of SLN in CRC*

Study	n	Success, n (%)[a]	Pathologic technique of SLN examination	False-negative, n (%)[b]	Positive SLN, n (%)[b]	SLN as the only positive node, n (%)[b]
Joosten[87]	50	35 (70%)	H&E; IHC	12 (24%)	NA	NA
Saha[83]	131	130 (99%)	Multiple sections for H&E; IHC	4 (3%)	47 (36%)	25 (19%)
Saha[88]	86	85 (99%)	Multiple sections for H&E; IHC	3 (3%)	29 (34%)	15 (18%)
Bilchik[84]	40	40 (100%)	H&E; IHC; RT-PCR	0 (0%)	26 (65%)	7 (18%)
Merrie[89]	26	23 (88%)	H&E; RT-PCR	5 (45%)	NA	NA
Wong[86]	26	24 (92%)	H&E; IHC	1 (4%)	15 (58%)	4 (15%)
Wood[85]	75	72 (96%)	Multiple sections for H&E; IHC	5 (20%)	50 (67%)	17 (23%)
Bendavid[90]	20	18 (90%)	Multiple sections for H&E; IHC	1 (5%)	NA	5 (25%)
Patten[91]	43	41 (95%)	H&E; IHC	7 (16%)	NA	2 (5%)

SLN, sentinel lymph node; CRC, colorectal cancer; H&E, hematoxylin and eosin; IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction; NA, not available.

[a] Success was defined as the ability of the investigator to find at least one sentinel node.

[b] Numbers reflect the number of patients, not nodes. Percentages were calculated as the proportion of total patient number.

of 92%, and a negative predictive value of 95%.[83] Merrie et al.[89] found a poor correlation between the SLN status and the status of the total nodal basin, yielding a sensitivity of 55% and a false-negative rate of 45%. They attributed these dismal findings to the occurrence of skip metastases and concluded that there is little clinical value in the use of SLN in CRC. Nevertheless, two recent studies also demonstrated low sensitivity and a high false-negative rate for sentinel node detection of nodal metastases.[89,92] Therefore, the value of the SLN procedure for CRC remains in doubt.

SLN in Laparoscopic Colectomy

The potential benefit of SLN mapping extends to the resurging use of laparoscopy for colon resections. The technique was formerly abandoned because of reported cases of port site metastases. As noted by Wood et al.,[93] the number of lymph nodes obtained from the resected specimen from laparoscopic procedures is less than that from open cases. However, as surgeons become more experienced with laparoscopic resections, this notion has been challenged recently. Nevertheless, SLN biopsy would perhaps correct the possibility of understaging because of undersampling. Wood et al.[93] performed SLN biopsy successfully in nine patients who underwent laparoscopic colectomy, with a 100% success rate.

CONCLUSION

The combined effort of pathologists, molecular biologists, and surgeons has allowed a very focused examination on select lymph nodes within a specimen. As such, clinicians are afforded the pathologic diagnosis with a smaller and smaller extent of disease. At present, the detection threshold has exceeded the clinical knowledge: that is to say, the mere presence of disease in minute amounts has not yet been studied extensively enough to identify prognostic information from such or to alter the current therapy offered to patients. Continued efforts to define "clinically relevant micrometastases" should persist.

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