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Sentinel Lymph Node Mapping in Colon Cancer: Current Status

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Background: The primary role of sentinel lymph node (SLN) mapping in colon cancer is to increase the accuracy of nodal staging by identifying those lymph nodes with the greatest potential for harbouring metastatic disease. Ultrastaging techniques aim to identify the otherwise undetected metastases. Until now, no consensus exists as to the most optimal procedure in patients with colon cancer.

Methods: A systematic literature search on the value of different SLN mapping techniques in patients with colon cancer was performed using the electronic search engine PubMed. Prospective studies published before 1 December 2005 were included and further articles were selected by cross-referencing. The results of different techniques using either blue dye or radiocolloid, were investigated.

Results: The literature search yielded 17 relevant articles. SLN mapping using blue dye was described in 15 studies. Two studies reported the results of SLN mapping using a combination of blue dye and radiocolloid. The reported results on identification rate varied between 71 and 100%. Accuracy rates were between 78 and 100%, sensitivity rates between 25 and 100% and true upstaging rates between 0 and 26%. The results were not affected by the addition of radiocolloid to blue dye.

Conclusions: Sentinel lymph node mapping in patients with colon cancer remains an experimental procedure with varying results. Further evaluation may lead to a standardized technique that offers the potential for significant upstaging of stage II patients. This may have important implications as to tailor adjuvant chemotherapeutic regimens in these patients.

Key Words: Sentinel lymph node mapping—Colon cancer—Micrometastasis.

Over the last few years, colon cancer has proven to be an increasing health problem. In The Netherlands, the incidence increased from 5,205 in 1998[1,2] to 9,700 in 2004. Nodal status remains the most important prognostic indicator of recurrence and survival.[3] In patients with lymph node-positive disease, the 5-year survival rate decreases by 20–30%.[4] The presence of lymph node metastases is the primary determinant of adjuvant chemotherapy. This results in decreased recurrence and mortality rates by 40 and 33%, respectively, compared with untreated controls.[5] These benefits of adjuvant chemotherapy could not be demonstrated in patients with lymph node-negative disease (stage II).[6,7] Therefore, chemotherapy is not considered as the standard of care in stage II patients.[6,7] Unfortunately, 20–30% of node-negative patients will eventually die

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from local tumour relapse or overwhelming metastatic disease.[4] Inaccuracy of the current staging method is a possible explanation for this phenomenon. In current nodal staging, only one or two sections of lymph nodes are selected for histopathological evaluation,[8] resulting in a substantial risk of missing metastases in other parts of the lymph node. Additionally, microscopic error, due to a failure of the conventional histopathological analysis to detect micrometastases may contribute to understaging.[9] Another explanation may be that not all lymph nodes are harvested from the specimen and small lymph nodes with metastatic disease remain undetected. Finally, aberrant lymphatic drainage, defined as lymphatic drainage, identified outside the usual resection margins, may be problematic for adequate staging.[10] To improve the accuracy of staging in patients with colon cancer, it would be necessary to perform a detailed analysis of all lymph nodes recovered from the specimen. The routine use of ultrastaging techniques such as multisectioning, combined with immunohistochemistry (IHC) on all lymph nodes would however be impractical, time consuming, labour intensive and expensive. Sentinel lymph node (SLN) mapping aims to resolve these problems by selecting only a few lymph nodes for detailed histopathological analysis. The use of this procedure makes it possible to select patients, normally considered node-negative, who potentially might benefit from adjuvant chemotherapy. Since 1997, several studies have been undertaken to assess the feasibility of SLN mapping in colon cancer.[11] The accuracy of nodal staging with the use of SLN mapping relies on both surgical and pathological techniques. SLN mapping makes it possible to identify those lymph nodes, most likely to be harbouring metastatic disease. [12,13] Also, SLN mapping may help to identify any unusual pattern of lymphatic drainage from the primary tumour site which could lead to an extended regional lymphadenectomy.

As no standardization exists concerning the most optimal technique for SLN mapping, we performed a literature search, to assess the current status regarding feasibility and accuracy of different SLN mapping techniques in colon cancer. We especially focused on the results, using blue dye and/or radiocolloid as different tracers.

METHODS

Search Strategy

A systematic literature search was performed, using the electronic search engine PubMed to identify potentially relevant English-language references on the value of different SLN mapping techniques in patients with colon cancer. The following keywords were used: 'sentinel node', 'colon cancer', 'colorectal cancer' and 'colloid'. Potentially relevant articles were selected by reviewing the titles and abstracts. Prospective studies assessing SLN mapping in patients with colon cancer, published before the first of December 2005 and of which the complete English text could be acquired, were included. Levels of evidence were determined, guided by the ranking of evidence as mentioned in Table 1.[14] Further articles were selected by cross-referencing from initially retrieved papers. The results of different mapping techniques using either blue dye or radiocolloid during in vivo or ex vivo procedures were investigated. Rectal cancer was excluded from this search, because of its different pattern of spread and recurrence, its more difficult anatomical access and its different operative treatment.[15] Moreover, adjuvant chemotherapy is not standard treatment for lymph node-positive rectal cancer patients. Also, the frequently applied preoperative radiotherapy in rectal cancer might disrupt the lymphatic architecture making SLN mapping less accurate.[12]

TABLE 1. *Ranking of evidence*[14]

Level	Definition
1a	Systematic review of randomized controlled trials (RCTs) with consistent results
1b	RCT of good quality
2a	Systematic review of observational or case-control studies with consistent results
2b	RCT of less quality or observational or case-control study
2c	Outcomes research (descriptive study)
3	Patient series, observational or case-control study of poor quality
4	The experts' opinion or generally accepted practice

Definitions

Most studies have evaluated the feasibility of SLN mapping by determining the identification rate, the accuracy of the SLN in predicting the nodal status of the regional lymphatic basin, false-negative rates and upstaging percentages. The identification rate is defined as the number of patients with one or more SLNs identified. The accuracy of the SLN reflects the agreement between the nodal status of the SLN and the nodal status of the regional lymphatic basin. Negative SLNs are called false-negative nodes if one or more of the other lymph nodes in the regional

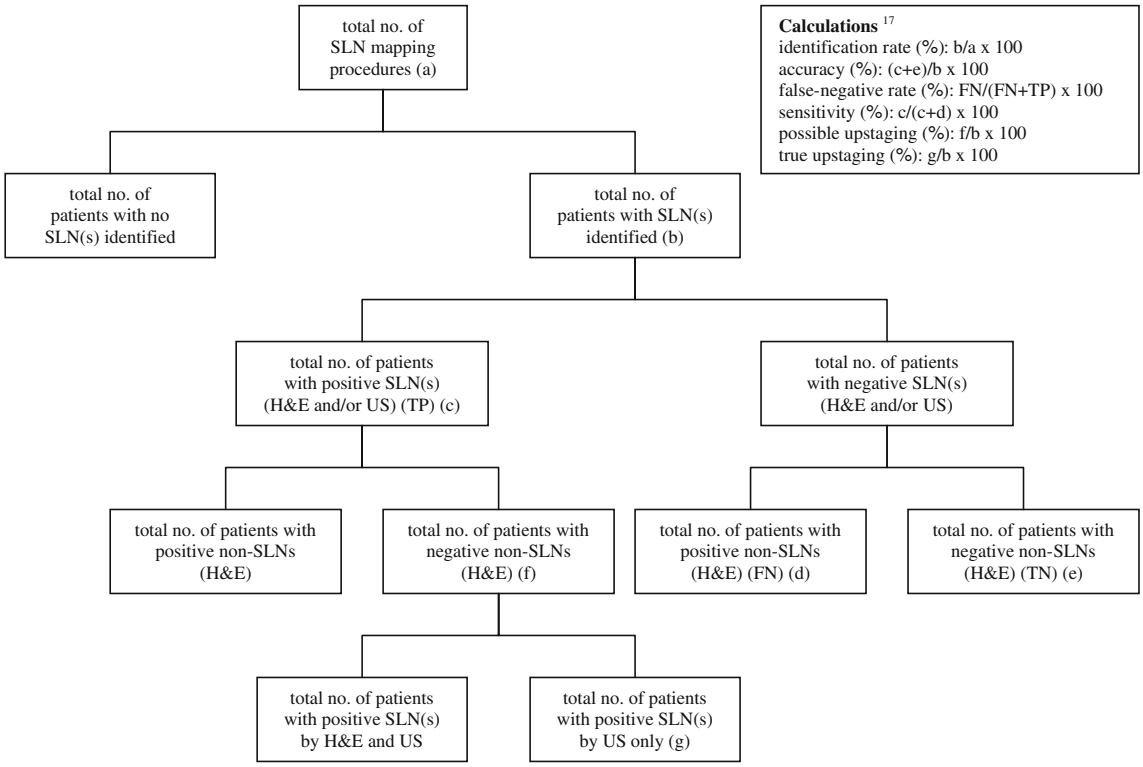

FIG. 1. Flowchart. *SLN* sentinel lymph node, *US* ultrastaging (multisectioning, immunohistochemistry and/or reverse transcription-polymerase chain reaction), *FN* false-negative group, *TN* true-negative group, *TP* true-positive group. Adapted from Viehl et al.[16]

lymphatic basin are tumour-positive. Upstaging may be subdivided into possible and true upstaging. Patients with SLNs as the only site of lymph node metastases are possibly upstaged, as with conventional histopathological examination, these lymph nodes might have been missed. A detailed examination of SLNs by ultrastaging techniques may reveal metastases that are regularly missed with routine hematoxylin and eosin (H&E)-analysis, resulting in true upstaging.[16] To compare the results of the different published studies accurately, we applied the criteria of the aforementioned definitions and (re)calculated the results on the basis of a standard flowchart when possible (Fig. 1).[17]

RESULTS

The literature search identified 17 studies, 15 describing SLN mapping using blue dye [16,18–31] and two describing SLN mapping using a combination of blue dye and radiocolloid.[9,32] For one study that used both blue dye and radiocolloid in the first few cases, but, since the same SLNs were identified with both tracers, subsequently only used blue dye in the remaining cases, the reported results were ascribed to blue dye only.[29] All of the retrieved publications concerned prospective patient series and were therefore ranked as level 3 studies (Table 1). Remaining studies regarding SLN mapping in colorectal cancer failed to provide the needed information for a subset analysis solely of patients with colon cancer and were excluded.

Pathological Analysis

In conventional nodal staging, the total number of resected lymph nodes correlates with staging accuracy and has a significant impact on survival.[33] The optimal number of lymph nodes that should be assessed for accurate nodal staging appears to be 7–14.[34] However, the number of lymph nodes retrieved from the resection specimen differs widely between centers for colorectal surgery, reflecting a surgical variability in the extent of the operation.[35] Inaccurate staging can occur, when an insufficient number of lymph nodes are evaluated. Because of this limitation of current nodal staging, efforts have been made to improve the gross identification of lymph nodes in resected specimens. Fat clearance techniques have

been applied to the pericolic and mesenterial fat, facilitating the retrieval of small lymph nodes.[36–38] Although more lymph nodes are retrieved, this technique is expensive, time consuming and in combination with SLN mapping, a partial disappearance of blue dye is reported.[38,39] These techniques are therefore, not widely used. Furthermore, different studies have demonstrated that multisectioning, IHC and reverse transcription-polymerase chain reaction (RT-PCR) are able to identify (micro)metastases, that would not have been detected with conventional histopathological analysis.[40–43] For example, multisectioning detects an additional 6–8% of metastatic disease in comparison to the evaluation of only one or two lymph node sections.[4,44] Generally, examination of four representative levels of each lymph node is recommended.[12] After identification of the SLN(s), microscopic examination using conventional H&E-staining was performed first in several of the retrieved studies.[9,18–32] Furthermore, multisectioning, H&E- and/or immunohistochemical staining were performed on SLNs in most of these studies.[9,16,18–28,31] Immunohistochemical staining was carried out using antibodies against cytokeratin (CK) and in one study against carcinoembryonic antigen (CEA).[23] The use of RT-PCR was only described in one study.[32] The remaining lymph nodes in the specimen (non-SLNs) usually only underwent conventional H&E-examination, but in four studies these lymph nodes were examined in the same way as the SLNs.[16,27,28,32] In all studies, the remainder of the surgical specimen was processed in the standard manner for colon cancer specimens.

SLN Mapping Using Blue Dye

Most studies used an open (in vivo) technique.[16,18–25,27–31] This was generally performed according to the recommendations described in detail by Saha et al.[4] During laparotomy, the affected colon segment is minimally mobilized after which 0.5–5.0 ml of isosulfan blue dye (Lymphazurin) or patent blue dye (Patent Blue V) is injected into the subserosa, in four quadrants or circumferentially around the tumour. Subsequently, blue stained lymphatic channels and lymph nodes are visualized in vivo. After marking or excision of the identified SLNs, the procedure is completed by a standard colectomy and lymphadenectomy. In one study, the ex vivo technique was used.[26] After resection of the colon, the specimen is incised longitudinally on the antimesenteric border. Injection of blue dye is performed in four quadrants around the tumour. The injection site is then gently massaged to encourage flow of dye. Blue stained lymphatic channels and lymph nodes are identified in the mesentery and harvested separately.[26,45–48] SLN mapping was performed laparoscopically in three studies.[21,24,25] Besides the laparoscopic approach and intraoperative colonoscopy for submucosal injection of blue dye, the technique was comparable to the open procedure. The time between injection of blue dye and identification of SLNs varied between studies, but in general, this period lasted several minutes. In most studies, the first four blue-stained lymph nodes were considered as SLNs, but this also differed between studies.

In ten studies, the identification rate was between 90 and 100% (Table 2).[18,21–27,29,31] The other five studies reported an identification rate of 71, 79, 82, 85 and 87%, respectively.[16,19,20,28,30] In three publications using the laparoscopic method, the identification rate amounted 100%.[21,24,25] An intra-operative identification rate of 50% was found in one study, owing principally to the fat in the mesocolon. However, during subsequent pathological analysis, the identification rate increased to 90%.[22]

The percentages in which an aberrant lymphatic drainage was reported varied between 0 and 36%.[18–22,24–27,29,31] The highest percentages (27, 28 and 36%) were again registered using laparoscopy.[21,24,25] If an aberrant lymphatic drainage pattern was identified, lymphadenectomy was extended to include all SLNs. Aberrant lymph nodes were mostly situated deep at the base of the mesentery.[21,24]

The reported accuracy varied between 78 and 100%.[16,19–21,23–27,29,30] In four studies we were not able to calculate the accuracy using the reported data.[18,22,28,31] In nine studies the false-negative rate varied between 0 and 10%.[19–21,23–26,28,31] Five other papers mentioned false-negative rates of 17, 24, 38, 50 and 54%, respectively.[16,18,27,29,30] In several studies, we could not recalculate the reported false-negative rate[18,19,24,25] and in one study no false-negative rate was mentioned.[22]

Possible upstaging percentages were between 3 and 20%.[16,18–21,24–27,30] The percentage of true upstaging varied between 0 and 26%.[16,18–28,31] Several studies did not show sufficient data to calculate the percentage possible upstaging.[22,23,28,29,31]

SLN Mapping Using a Combination of Blue Dye and Radiocolloid

The literature reveals conflicting results regarding the feasibility and accuracy of SLN mapping using blue dye, leading to the question whether the addition

TABLE 2. *Lymphatic mapping technique in colon cancer using blue dye*

Study (first author)	Ranking (level)	No. of patients	Mapping technique	Identification rate (%)	Accuracy (%)	False-negative rate (%)	Sensitivity (%)	Upstaging (%)		Aberrant lymphatic drainage (%)	Total no. of LNs [mean (range)]	No. of SLNs [mean (range)]	Added operating time (min)
								Possible	True				
Feig[18]	3	48	Open	98	IC	38[c]	62[c]	8.5[e]	10[c]	0	13 (4–46)	2.6 (0–7)	NM
Paramo[20]	3	35	Open	71	100	0	100	17[c]	11[c]	0	10	2 (1–4)	NM
Paramo[a19]	3	55	Open	82	98	3[c]	97[c]	20[e]	11[d]	2	12	1.9 (1–4)	NM
Viehl[16]	3	31	Open	87	78	50	50	7.4	0	NM	21[f] (5–40)	2[f] (1–8)	NM
Wood[21]	3	11	Lap	100	100	0	100	9[e]	9[e]	36	13 (2–21)	1.8 (1–3)	15–20
Bendavid[22]	3	20	Open	90	IC	IC	IC	IC	25[c]	11	NM	3.9	NM
Waters[23]	3	22	Open	91	100	0	100	IC	5	NM	11.6	NM	5
Tsioulias[24]	3	14	Lap	100	93	7[d]	93[d]	14[e]	14	28	13.5 (2–21)	1.7 (1–3)	15–20
Bilchik[b25]	3	30	Lap	100	93	7[c]	93[c]	13[e]	14[c]	27	14 (2–21)	1.8 (1–3)	15–20
Braat[26]	3	35	Open and ex vivo	94	97	9	91	14[c]	3	0	9 (1–23)	1.7 (0–4)	5
Bertagnolli[27]	3	72	Open	92	80	54	46	3[e]	1.5[e]	0	17.3	2.1	NM
Dahl[29]	3	30	Open	100	93	17	83[e]	NM	NM	13	17.4 (4–35)	2.2[g] (0–6)	NM
Bembenek[28]	3	55	Open	85	IC	4[e]	96[e]	IC	26[e]	NM	26[f] (10–59)	2[f]	NM
Read[30]	3	38	Open	79	97[c]	24[c]	25[c]	3[c]	IC	NM	14[f] (7–45)	2[f] (1–3)	NM
Saha[31]	3	336	Open	99	IC	4	IC	IC	13	4	15.2	2.1	NM

LN lymph node, *SLN* sentinel lymph node, *Lap* laparoscopic, *IC* impossible to calculate, *NM* not mentioned.
[a] Continuation of an earlier performed pilot-study.[20]
[b] Continuation of an earlier performed study.[24]
[c] Discrepancy between reported value and calculated value (reported values are mentioned in this table).
[d] Impossible to recalculate reported value.
[e] Calculated value.
[f] Median.
[g] Detected intraoperatively.

of radiocolloid could improve the results of SLN mapping in colon cancer. One of the reported studies used a mixture of 40 MBq of 99mTc colloidal antimony sulfide with 2 ml Patent Blue Dye V.[32] The other study performed the procedure using 0.5 millicuries of technetium-labeled sulfur colloid, followed by 3–5 ml of isosulfan blue dye.[9] An identical open technique was used for the administration of both tracers. Once the tumour was identified by exploratory laparotomy, blue dye and radiocolloid were injected subserosally in multiple injections around the tumour. Whenever exposure of the tumour was necessary, careful mobilization of the colon was undertaken prior to injection. For the identification of SLNs, different techniques were used. The first published study performed a lymphoscintigraphy of the specimen ex vivo.[32] Afterwards, mesenteric lymph nodes were dissected from the specimen and their position and colour were mapped on an anatomic diagram. Comparison of the lymphoscintigram and the anatomic diagram made it possible to determine the relationship between radioactive and blue coloured lymph nodes.[32] Of all blue-stained nodes, only first echelon nodes were considered SLNs.[32] In the most recently published study, SLNs were identified and marked in vivo several minutes after injection of both tracers, using both a hand-held gamma probe and visualization of blue dye.[9] After resection, tagged lymph nodes were excised and the level of radioactivity and presence or absence of blue staining was recorded. Highly radioactive and/or blue-stained lymph nodes were defined as SLNs. No maximum number of SLNs was determined.

The study that used the ex vivo technique reported an identification rate of 88%, together with a sensitivity rate of 55% and a false-negative rate of 45% (Table 3).[32] Only 51% of blue nodes proved radioactive. In contrast, 81% of radioactive nodes were found to be blue.[32] Using the in vivo technique, an identification rate of 98% was found.[9,32] With the use of ultrastaging techniques, a sensitivity rate of 83% and a false-negative rate of 17% were reported.[9] A lack of data made it impossible to recalculate these reported values. A true upstaging rate of 19% was found after performing IHC-analysis on the SLNs.[9] Finally, ten additional SLNs (5%) were identified by the use of radiocolloid. However, only one additional positive SLN was revealed that would not have been found by blue dye alone.[9]

DISCUSSION

The primary role of SLN mapping in colon cancer is to increase the accuracy of staging by identifying

TABLE 3. *Lymphatic mapping technique in colon cancer using radiocolloid/blue dye combination*

Study (first author)	Ranking (level)	No. of patients	Mapping technique	Identification rate (%)	Accuracy (%)	False-negative rate (%)	Sensitivity (%)	Upstaging (%)		Aberrant lymphatic drainage (%)	Total no. of LNs [mean (range)]	No. of SLNs [mean (range)]	Added operating time (min)
								Possible	True				
Merrie[32]	3	25	Ex vivo	88	IC	45[a]	55[a]	IC	IC	NM	17[b](4–52)	3[b] (0–8)	NM
Patten[9]	3	57	Open	98	IC	17[a]	83[a]	IC	19[c]	0	NM	3.5 (0–11)	NM

LN lymph node, *SLN* sentinel lymph node, *IC* impossible to calculate, *NM* not mentioned.
[a] Impossible to recalculate reported value.
[b] Median.
[c] Discrepancy between reported value and calculated value (reported values are mentioned in this table).

and analyzing those nodes with the greatest potential for harbouring metastatic disease.[49] The necessity for improved staging is reflected by the fact that 20–30% of patients with stage II colon cancer will eventually die from a local tumour relapse or distant metastases.[4] It is reasonable to assume that a considerable percentage of these patients represent a subset of patients with occult nodal metastases not detected by conventional histopathological analysis. However, to expose all stage II patients to adjuvant chemotherapy, would result in unnecessary toxicity and high costs, in a considerable number of patients.[50,51] Clearly, accurate staging of patients with colon cancer is important not only for prognostic purposes but also to identify those patients who can truly benefit from adjuvant chemotherapy.

The type of tracer is a crucial aspect in SLN mapping. Possible advantages of the use of radiocolloid in comparison with blue dye are the slower diffusion of radiocolloid through the lymphatic channels, no need for direct visualization of blue-stained lymphatic channels and the use of scintigraphic imaging for improving the identification of SLNs and aberrant lymphatic drainage.[52] One of the difficulties of SLN mapping in patients with colon cancer lies in the fact that the lymphatic drainage pattern of the colon is variable, possibly leading to the presence of SLNs in unpredictable locations. Preoperative lymphoscintigraphy notes the distribution and the number of SLNs and helps to identify SLNs in unexpected areas distant from the primary tumour (Fig. 2).[52,53] The rate of tracer movement through the lymphatic channels is closely related to the particle size of the tracer.[52] Because of its smaller particle size, blue dye travels through the lymphatic channels relatively quickly and will rapidly pass on to second echelon lymph nodes.[32,49,52,53] These lymph nodes subsequently may be incorrectly defined as SLNs. The radiocolloid particles travel through the lymphatic channels at a much slower rate and therefore will detect fewer nodes. Furthermore,

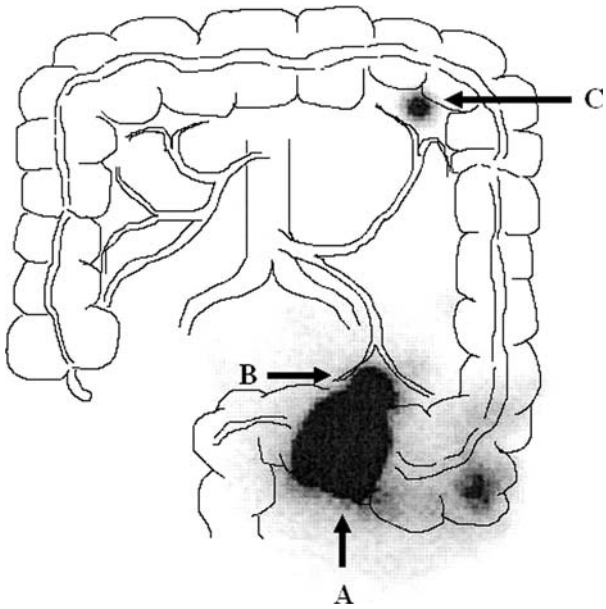

FIG. 2. Overlay of an anatomic diagram on a lymphoscintigram, demonstrating aberrant lymphatic drainage from the sigmoid colon. **a** Hot spot of submucosally injected radiocolloid in the sigmoid colon, **b** hot spot of a paracolic lymph node, **c** hot spot of an aberrant lymph node in the transverse mesocolon.

incorporation of radiocolloid particles by phagocytosis in first echelon lymph nodes also depends on particle size, making the choice of particle size a crucial factor in the detection of SLNs.[54] Radiocolloid-detected nodes are more likely true SLNs and therefore more likely to contain metastatic disease.[49,55]

The addition of radiocolloid to blue dye in SLN mapping for both patients with breast cancer and patients with melanoma has been shown to increase the identification rate and accuracy of the procedure.[9,49] In the retrieved articles, the addition of radiocolloid does not seem to improve the results of SLN mapping for colon cancer.[9,32] The use of different techniques for the identification of SLNs could

be responsible for the reported differing results. Large prospective trials using a combination of blue dye and radiocolloid, such as used in SLN mapping for breast cancer and melanoma, may reveal higher success rates of this technique in colon cancer.[49] Kitagawa et al.[56] reported the only study to date that investigated the feasibility of SLN mapping using preoperative endoscopic injection of only technetium-labeled tin colloid in patients with sigmoid colon and rectal cancer. Since only limited results are available regarding the feasibility and accuracy of radiocolloid-guided SLN mapping in colon cancer, more studies should be performed in which the value of radiocolloid as a tracer is investigated. In our institute, we recently started a study in which the use of radiocolloid as a single tracer will be analyzed during laparoscopic colon resections.

Unsuccessful SLN mapping may be caused by technical errors such as intraluminal injection, incomplete circumferential injection around the tumour or application of the procedure in large tumours that have a disturbed lymphatic drainage. Tumour manipulation with disruption of lymphatic drainage can also influence the results. Obstruction of lymph flow as a result of complete replacement of lymph nodes by tumour burden and previous colon surgery that may alter the lymphatic flow patterns by disrupting lymphatic channels are other factors that may influence the results of SLN mapping. Therefore, SLN mapping seems to be especially feasible for colon tumours without extensive nodal tumour burden and obstruction of lymphatic flow.[39] Other responsible factors for disappointing and varying results could be a discrepancy between the pattern of lymphatic drainage of the injection site and the primary neoplasm and a learning curve.[18–20,26,27,39,57] Direct visualization of lymphatic channels can contribute to a high identification rate,[10] as reflected by the laparoscopic studies.[21,24,25]

The in vivo and ex vivo mapping techniques show comparable results.[26,32] An advantage of the ex vivo technique is its possible application when the open technique has been unsuccessful.[45,58] Moreover, this technique can be applied outside the operating room. On the other hand, possibilities for the identification of aberrant lymphatic drainage patterns are lacking. Possible disadvantages, which could negatively influence the accuracy of this technique are the disruption of lymphatic channels during surgery and the need for artificial massage of the injection site. Clearly, this technique requires further evaluation.

Regarding the reported accuracy rates between 93 and 100%, sensitivity rates between 90 and 100% and true upstaging rates between 5 and 14% in most of the published papers, it can be concluded that SLN mapping should be considered as a mandatory step towards optimal staging in colon cancer.[19–21,23–26] Nonetheless, the prognostic significance of IHC- and/or RT-PCR-detected micrometastases remains unclear (Table 4).[8,59–69] Only 3 of the 12 reported studies found that the presence of nodal micrometastases correlates with a significantly worse survival.[62,63,65] Three studies reported that the detection of micrometastases is related to a higher risk for recurrent disease.[61,62,69] These diverging results are partially explained by a lack of uniform techniques, different study designs and patient populations, different numbers of lymph nodes evaluated, and paucity of prospective data (Table 4). Several studies included both colon and rectal cancer patients,[59–64,66–69] and one study also included stage III patients.[60] The discrepancy between studies is emphasized by the opposite conclusions drawn by the three studies ranked as best evidence (level 2b).[8,61,69] All three studies compare the presence of micrometastases between recurrent and non-recurrent cases. However, despite comparable study designs, they strongly differ in their results.

Compared to RT-PCR, IHC is relatively inexpensive, fast and widely available. RT-PCR, on the other hand, is costly and not sufficiently specific for malignancy, but highly sensitive and less subject to sampling error.[34] Consequently, using different ultrastaging techniques, various definitions of micrometastases are employed and different rates of SLN positivity are reported, subsequently affecting survival rates among authors.[40,42] Furthermore, ultrastaging techniques enable the identification of isolated tumour cells, undetermined whether these cells are cancer cells, hyperplastic epithelial cells or benign marker-positive mesothelial cells (Fig. 3).[41] The lack of a correlation between isolated tumour cells and survival confirms that these cells might not represent true metastases. Lymph nodes with isolated tumour cells are therefore considered negative in modern staging systems.[70] Alltogether, clear definitions are needed to stratify submicroscopic nodal tumour deposit that form a solid basis for future studies. It is recommended that the new guidelines for the classification of micrometastases and isolated tumour cells from the International Union Against Cancer are used uniformly (Table 5).[70]

Furthermore, a standardized SLN mapping technique may also be useful in determining the prognostic significance of nodal micrometastases.[55] Long-term follow-up of these patients is important to

TABLE 4. *Prognostic relevance of nodal micrometastases*

Study (first author)	Ranking (level)	Study design	No. of Patients	Total no. of LNs per patient [mean (range)]	Histo-pathological technique	Follow-up (months)	Antibody or marker	Patients with micro-metastases (%)	Results (micrometastases versus no micrometastases)
Yasuda[61]	2b	RS	42	18[a] (3–94)	IHC	60	anti-CAM5.2	76[b]	Rate of micrometastases in recurrence versus non-recurrence group 92 versus 70%
Tschmelitsch[8]	2b	RS	55	16.4[a](2–47)	IHC	60	anti-AE1:AE3	76	Overall greater rate of micro-metastases in non-recurrence group
Sasaki[69]	2b	RS	19	18.8[b]	IHC	73–114[c]	anti-CAM5.2	NM	Significant greater rate of positive lymph nodes in recurrence versus non-recurrence group (38%[b] vs. 13%[b])[d]
Liefers[62]	3	PS	26	7.4 (2–16)	RT-PCR	60	CEA-mRNA	19	Survival rate 50 versus 91%[e,f], recurrence rate 58 versus 8%[g]
Greenson[63]	3	RS	50	11.3	IHC	60.3	anti-AE1:AE3 and anti-CC49	76[b]	Survival rate 57.2 versus 97.2%[e]
Bukholm[65]	3	RS	156	4[a] (1–23)	IHC	NM	anti-CAM5.2	38	Reduced relative survival[h,j]
Choi[59]	3	RS	93	15[a] (6–53)	IHC	66[a]	anti-MNF116	31	Cancer-related death 17.2 versus 14.1%[k]
Broll[60]	3	RS	49	NM	IHC	84[a]	anti-AE1:AE3 and anti-BerEP4	26.5	No significant difference in recurrence and survival
Oberg[68]	3	RS	147	4[a] (1–15)	IHC	30–114	anti-CAM5.2	32	No significant survival difference
Cutait[66]	3	RS	46	13.1[b]	IHC	64–135	anti-CEA and anti-AE1:AE3	26[b]	No significant survival difference[l]
Adell[64]	3	RS	100	4[a] (1–18)	IHC	49[m]	anti-cytokeratin	39	No significant survival difference[n]
Jeffers[67]	3	PS	77	7 (1–37)	IHC	81	anti-AE1:AE3	25	No survival difference[o]

LN lymph node, *RS* retrospective study, *PS* prospective study, *IHC* immunohistochemistry, *RT-PCR* reverse transcription-polymerase chain reaction, *NM* not mentioned, *CEA* carcinoembryonic antigen.
[a] Median.
[b] Calculated value.
[c] For non-recurrent cases (all recurrent cases developed within 5 years after initial surgery).
[d] ($P < 0.006$).
[e] Adjusted for only cancer deaths.
[f] ($P = 0.02$).
[g] ($P = 0.02$).
[h] ($P = 0.019$).
[j] Univariate analysis.
[k] ($P = 0.65$).
[l] ($P = 0.472$).
[m] Mean.
[n] ($P = 0.89$)
[o] ($P > 0.1$)

determine whether upstaging of these patients based on micrometastases and isolated tumour cells is appropriate.

CONCLUSIONS

Currently, SLN mapping for patients with colon cancer remains an experimental procedure with varying results reflecting the lack of a standardized technique and an univocal definition of which stained lymph node(s) should be considered as SLNs. Therefore, interpretation of identification rates is difficult while it remains unknown whether all identified SLNs can be considered true SLNs. Further prospective trials may lead to a standardized technique resulting in a more accurate identification of SLNs, which, in combination with a focused histopathological examination of these nodes, offers the potential for significant upstaging of patients. The

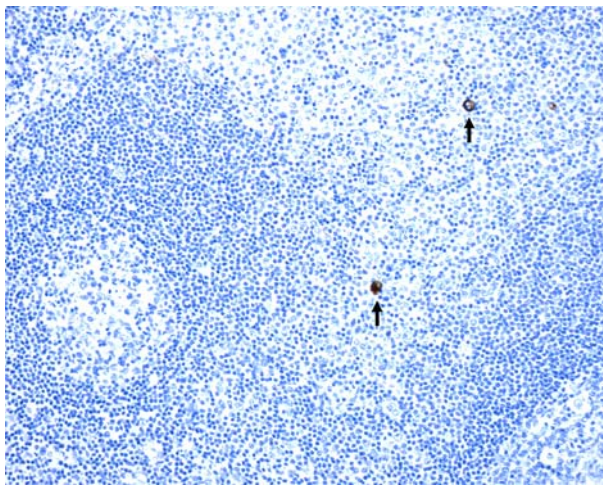

FIG. 3. Serial section (5 μm, CAM5.2 immunostaining, 100×) of SLN with two isolated tumour cells in the paracortical area (*arrows*).

TABLE 5. *Classification of micrometastases and isolated tumour cells (ITCs) (International Union Against Cancer)*[70]

	Definition
pN0	No lymph node metastasis histologically, no examination for isolated tumour cells
pN0(i−)	No lymph node metastasis histologically, negative immunohistochemical findings for isolated tumour cells
pN0(i+)	No lymph node metastasis histologically, positive immunohistochemical findings for isolated tumour cells
pN0(mol−)	No lymph node metastasis histologically, negative molecular findings for isolated tumour cells
pN0(mol+)	No lymph node metastasis histologically, positive molecular findings for isolated tumour cells
pN1(mi)	Histologically proven micrometastasis

true value of SLN mapping for improving nodal staging in colon cancer can only be demonstrated by an improved survival of patients with accurately staged stage II colon cancer.

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