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REVIEW ARTICLE

Lymph node micrometastasis in gastrointestinal tract cancer—a clinical aspect

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Abstract Lymph node micrometastasis (LNM) can now be detected thanks to the development of various biological methods such as immunohistochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR). Although several reports have examined LNM in various carcinomas, including gastrointestinal (GI) cancer, the clinical significance of LNM remains controversial. Clinically, the presence of LNM is particularly important in patients without nodal metastasis on routine histological examination (pN0), because patients with pN0 but with LNM already in fact have metastatic potential. However, at present, several technical obstacles are impeding the detection of LNM using methods such as IHC or RT-PCR. Accurate evaluation should be carried out using the same antibody or primer and the same technique in a large number of patients. The clinical importance of the difference between LNM and isolated tumor cells ($\leq$0.2 mm in diameter) will also be gradually clarified. It is important that the results of basic studies on LNM are prospectively introduced into the clinical field. Rapid diagnosis of LNM using IHC and RT-PCR during surgery would be clinically useful. Currently, minimally invasive treatments such as endoscopic submucosal dissection and laparoscopic surgery with individualized lymphadenectomy are increasingly being performed. Accurate diagnosis of LNM would clarify issues of curability and safety when performing such treatments. In the near future, individualized lymphadenectomy will develop based on the establishment of rapid, accurate diagnosis of LNM.

Keywords Lymph node metastasis · Micrometastasis · Esophageal cancer · Gastric cancer · Colorectal cancer

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Introduction

One of the characteristics of malignant tumor is the ability to metastasize. If a tumor has high malignant potential, metastasis is often seen in wide areas. Thus, lymph node metastasis is one of the most important prognostic factors in various carcinomas, including gastrointestinal (GI) cancer. Even if complete lymph node dissection is performed in patients with early cancer, recurrent disease is sometimes encountered. Usually, histological examination for lymph node metastasis is performed using representative sections from the removed nodes. However, lymph node micrometastasis (LNM) may be identified in multiple sections of lymph nodes despite not being detected by routine histological examination using hematoxylin and eosin (HE) staining. Even in early gastric cancer, we found lymph node metastasis in 10.5 % of patients when additional sections of nodes were examined [1]. However, such procedures are labor-intensive and not cost-effective in active clinical practice.

The development of sensitive immunohistochemical techniques and reverse transcription-polymerase chain reaction (RT-PCR) has led to the detection of LNM that could not be found on routine histological examination. According to previous reports, cytokeratin (CK) AE1/AE3 and CAM5.2 monoclonal antibodies are often used for immunohistochemistry (IHC). Each technique has

specific advantages and disadvantages. Since IHC is relatively simple, the techniques are available in many institutions. However, problems arise in determining how many sections are sufficient for detection of LNM, the high cost of antibody, and false-positive results. On the other hand, RT-PCR offers an objective method for estimating LNM. Epithelial markers are usually available for detecting LNM, because epithelial components are not normally present in the lymph node. Although this approach offers high sensitivity, false-positive results are sometimes seen because of the presence of pseudogenes. Several epithelial markers can be used to recognize LNM in lymph nodes, but one of the key problems is determining what kind of marker is suitable for each carcinoma. Usually, CK, carcinoembryonic antigen (CEA) and squamous cell carcinoma-related antigen (SCC) are used for the detection of LNM.

This review focuses on the clinical significance of LNM detected by IHC and RT-PCR methods in carcinomas of the GI tract such as esophageal, gastric and colorectal cancer. Several reports have investigated LNM in specific lymph nodes such as recurrent nerve lymph nodes in esophageal cancer, para-aortic lymph nodes in gastric cancer, and lateral lymph nodes in colorectal cancer. Excluding those papers, we here review only reports in which LNM was examined in all dissected lymph nodes in GI cancer.

Definition of lymph node micrometastasis

Historically, several terms for tiny metastatic foci have been used, including occult metastasis, harbored metastasis, tumor microinvolvement and tumor deposit. Micrometastasis is currently defined according to the criteria of the tumor–node–metastasis (TNM) classification established by the International Union Against Cancer (UICC) in 2002, and is completely differentiated from isolated tumor cells (ITC) by size [2]. ITC represent either single tumor cells or small clusters of cells measuring $\leq$0.2 mm in greatest dimension and are commonly identified by IHC, but can be confirmed by routine HE staining. Moreover, ITC basically do not demonstrate evidence of metastatic activity, such as proliferation or stromal reaction, or penetration of vascular or lymphatic sinus walls. Patients with ITC in lymph nodes are staged as pN0 (i+). On the other hand, micrometastasis refers to tumor cell clusters measuring >0.2 mm but $\leq$2.0 mm in greatest dimension. Patients with micrometastasis in lymph nodes are staged as pN1 (mi). Furthermore, patients with node positivity as diagnosed by non-morphological findings using RT-PCR are staged as pN0 (mol+).

Lymph node micrometastasis in esophageal cancer

Several reports have investigated LNM detected by IHC in esophageal cancer (Table 1) [3–14]. The numbers of patients were relatively small, with all but two reports involving less than 100 patients. Two reports focused on T1 tumors, but the remaining reports covered advanced esophageal cancer. In Eastern countries, squamous cell carcinoma was a major histological type, while both squamous cell carcinoma and adenocarcinoma were included in Western countries. CK antibody (AE1/AE3) was commonly used for IHC. Single sections were used in 5 reports, and multiple sections in 7 reports. The definition of LNM varied. Seven authors defined LNM as identification of tumor cells in patients classified as pN0 according to routine HE staining. The remaining authors defined LNM by tumor size. The incidence of LNM ranged from 8.1 to 55.5 %. Since the diagnosis of LNM was based on morphology, this discrepancy might be due to the estimation of each author. Shiozaki et al. [11] conducted a multi-institutional study and the results of LNM were compared between institutional researchers and pathologists. Among 164 patients with pN0, 51 patients were diagnosed as micrometastasis-positive by institutional evaluation, but the pathologists identified only 25 patients as having micrometastasis-positive lymph nodes. Institutional positivity for micrometastasis was negated by these pathologists for the following reasons: (1) lack of nuclei in CK-positive cells; (2) location of stained cells outside the lymph node structure; or (3) stained cells appearing morphologically different from cancer cells or epithelial cells. If the evaluation of LNM detected by IHC differs between each institution, the results from different reports will naturally also be different. Common criteria for identifying LNM using IHC are thus necessary. Regarding the prognostic impact, 7 of 13 authors reported that the presence of LNM was related to poor prognosis. In particular, the two reports that included more than 100 cases both found significant differences in prognosis between the presence and absence of LNM [7, 11].

The relationship between LNM detected by RT-PCR and clinical significance was investigated in five studies (Table 2) [15–19]. Numbers of patients and numbers of examined nodes were not high. All reports included both early and advanced carcinoma. Two reports included only squamous cell carcinoma, two reports covered both squamous cell and adenocarcinoma and one report examined only adenocarcinoma. The primers for RT-PCR varied, including CEA, CK19, TACSTD-1, MUC1 and SCC. Double markers were used in two reports. The incidence of LNM ranged from 8.7 to 36.7 %, and all authors found a significant difference in prognosis between positive and negative LNM, with the single exception of a study that did

Table 1 Immunohistochemical studies in patients with histologically node-negative esophageal cancer diagnosed by hematoxylin–eosin staining

Years	Study	No. of patients	Average no. of LNs	Depth of invasion	Histological type	Method	Antibody	Sections for IHC	Definition of micrometastasis	No. of patients with micrometastases (%)	5-year survival (positive vs. negative)	*P*	Prognostic significance
1998	Natsugoe et al. [3]	41	–	T1–T3	SCC	IHC	CK (AE1/AE3)	Single	<0.5 mm	13 (31.7)	–	<0.05	Yes
1999	Glickman et al. [4]	78	7.4	–	SCC, AC	IHC	CK (AE1/AE3)	Multiple	≤2 mm	20 (25.6)	–	–	No
2000	Matsumoto et al. [5]	59	46.0	T1–T3	SCC	IHC	CK (AE1/AE3)	Single	pN0 by HE staining	39 (55.5)	44.6 vs. 91.0 %	0.002	Yes
2001	Sato et al. [6]	50	36.8	T1–T4	SCC	IHC	CK (AE1/AE3)	Single	pN0 by HE staining	20 (40.0)	78.0 vs. 75.0 %	0.91	No
2002	Komukai et al. [7]	104	74.7	T1–T3	SCC	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	47 (45.2)	34.0 vs. 72.0 %	<0.01	Yes
2002	Nakamura et al. [8]	53	47.4	T1–T3	SCC	IHC	CK (AE1/AE3)	Single	pN0 by HE staining	14 (26.4)	–	0.16	No
2002	Doki et al. [9]	41	52.9	T3–T4	SCC	IHC	CK (AE1/AE3)	Single	pN0 by HE staining	11 (26.8)	28.0 vs. 79.0 %	0.0188	Yes
2003	Tanabe et al. [10]	46	–	T1	SCC	IHC	CK (AE1/AE3)	Multiple	≤5 cells	12 (26.1)	–	–	No
2007	Shiozaki et al. [11]	167	–	T1–T3	SCC	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	25 (15.0)	20.0 vs. 70 % (cluster)	0.0462	Yes
2009	Koenig et al. [12]	33	–	T1–T3	SCC, AC	IHC	CK (AE1/AE3)	Multiple	≤10 cells	3 (27.3)	30.0 vs. 76.0 %	0.009	Yes
2009	Zingg et al. [13]	86	14.0	T1–T3	SCC, AC	IHC	CK (Lu-5)	Multiple	≥0.2, ≤2 mm	7 (8.1)	35.7 vs. 61.1 %	n.s.	No
2012	Prenzel et al. [14]	48	28.0	T1	SCC, AC	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	7 (14.6)	57.0 vs. 79.0 %	0.002	Yes

Table 2 RT-PCR studies in patients with histologically node-negative esophageal cancer diagnosed by hematoxylin–eosin staining

Years	Study	No. of patients	Total no. of LNs	Depth of invasion	Histological type	Method	Markers	No. of patients with micrometastases (%)	5-year survival (positive vs. negative)	*P*	Prognostic significance
2001	Godfrey et al. [15]	30	387	T1–T3	SCC, AC	RT-PCR	CEA	11 (36.7)	–	<0.0001	Yes
2005	Xi et al. [16]	34	314	Tis–T3	AC	RT-PCR	CK19, TACSTD-1	5 (14.7)	–	0.0023	Yes
2007	Li et al. [17]	93	426	T1–T3	SCC	RT-PCR	MUC1	32 (34.4)	18.8 vs. 47.6 %	0.004	Yes
2011	Sun et al. [18]	82	501	T1–T3	SCC	RT-PCR	MUC1	23 (28.1)	21.7 vs. 62.7 %	0.0001	Yes
2013	Hagihara et al. [19]	46	–	T1–T2	SCC, AC	RT-PCR	CEA, SCC	4 (8.7)	–	–	–

not refer to prognosis. The RT-PCR method is more sensitive than IHC for detecting LNM because of the greater quantity of sample. However, several problems remain for RT-PCR examination. Since these epithelial markers are not specific for cancer, how many markers are necessary? What primers are suitable? If esophageal cancer-specific markers become available, the results of RT-PCR examinations will become more reliable.

Lymph node micrometastasis in gastric cancer

We collected 16 reports in which LNM was investigated by IHC for gastric cancer (Table 3) [20–35]. The definition of LNM varied. A few studies examined the incidence of ITC and micrometastasis classified on the basis of the TNM classification criteria for gastric cancer [30, 31, 34, 36]. LNM is basically defined as the presence of a single or small clusters of gastric tumor cells identified by IHC in lymph nodes classified as pN0 from HE staining. Table 3 summarizes studies reported since 1996 on LNM determined by IHC in patients with pN0 gastric cancer. Numbers of patients and average number of lymph nodes examined ranged from 34 to 308, and from 9.0 to 41.9, respectively. Seven reports included only early gastric cancer, while the others included both early and advanced cancer. All researchers used CK antibody to detect LNM, and several kinds of CKs such as CAM5.2, AE1/AE3 and MNF116 were used. The percentage of patients with LNM ranged from 10.0 to 36.0 %. Even in the 7 reports limited to early cancer, the incidence of LNM was found in the range of 10.0 to 31.8 %. This suggests that LNM has frequently already occurred in T1 tumor even if lymph node metastasis is not identified on routine histological examination. Prognosis was described in 14 of the 16 reports. Regarding the relationship between presence and absence of LNM and prognosis, nine authors found a significant correlation. The authors who did not find a correlation between LNM and prognosis indicated that standard gastrectomy with D2 lymphadenectomy was an appropriate treatment for gastric cancer, even in the presence of LNM determined by IHC [24]. In contrast, in a study of 160 gastric cancer patients with pT1N0 tumors, Cao et al. [34] recently reported LNM as one of the most important prognostic factors in multivariate survival analysis. When Yonemura et al. [30] focused on the clinical significance of ITC (single tumor cells or small clusters of cells measuring $\leq$0.2 mm by TNM classification), patients with ITC showed a significantly poorer prognosis than those without ITC. Furthermore, they examined immunohistochemically the proliferative activity of ITC using Ki-67 (MIB-1) and demonstrated positive MIB-1 labeling in 12 of 25 ITC (48.0 %) with a single tumor cell and in 49 of 52 ITC (94.2 %) with clusters. Similarly, when we assessed the proliferative activity of ITC and micrometastasis by double-staining IHC analysis with CY and Ki-67 mAb, Ki-67 positivity rates for LNM and ITC were 92 and 29 %, respectively [36]. These two studies suggest that, at the very least, micrometastatic tumor cells in lymph nodes display proliferative activity. Residual ITC when complete lymph node dissection is not performed might thus represent a high risk factor for tumor recurrence.

Some researchers have tried to examine LNM using RT-PCR (Table 4) [37–41]. According to these studies, simplex or multiplex RT-PCR assay using target molecular markers is performed for the detection of LNM in gastric cancer. The number of patients was relatively small, ranging from 10 to 80, and the markers used varied, including CEA, CK, Mage3, MUC2 and TFF1. The incidence of LNM detected by RT-PCR was over 20 %. We compared the incidence of LNM between IHC and RT-PCR assay in 1,862 lymph nodes obtained from 80 patients

Table 3 Immunohistochemical studies in patients with histologically node-negative gastric cancer diagnosed by hematoxylin–eosin staining

Years	Study	No. of patients	Average no. of LNs	Depth of invasion	Method	Antibody	No. of sections for IHC	Definition of micrometastasis	No. of patients with micrometastases (%)	5-year survival (positive vs. negative)	*P*	Prognostic significance
1996	Maehara et al. [20]	34	12.4	T1	IHC	CK (CAM5.2)	–	pN0 by HE staining	8 (23.5)	–	<0.05	Yes
2000	Cai et al. [21]	69	25.0	T1b	IHC	CK (CAM5.2)	Single	pN0 by HE staining	17 (24.6)	82.0 vs. 100.0 %	<0.01	Yes
2000	Harrison et al. [22]	25	9.0	T1–T4	IHC	CK (CAM5.2)	–	pN0 by HE staining	9 (36.0)	35.0 vs. 66.0 %	0.048	Yes
2001	Nakajo et al. [23]	67	26.3	T1–T3	IHC	CK (AE1/AE3)	Single	pN0 by HE staining	10 (14.9)	–	<0.05	Yes
2001	Fukagawa et al. [24]	107	41.9	T2–T3	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	38 (35.5)	94.0 vs. 89.0 %	0.86	No
2001	Morgagni et al. [25]	139	10.7	T1	IHC	CK (MNF 116)	Multiple	pN0 by HE staining	24 (17.3)	87.0 vs. 88.0 %	0.6564	No
2002	Choi et al. [26]	88	25.8	T1b	IHC	CK (35βH11)	Single	pN0 by HE staining	28 (31.8)	92.9 vs. 95.0 %	0.6836	No
2002	Yasuda et al. [27]	64	31.9	T2–T4a	IHC	CK (CAM5.2)	Multiple	pN0 by HE staining	20 (31.3)	66.0 vs. 95.0 %	<0.01	Yes
2003	Morgagni et al. [28]	300	18.0	T1	IHC	CK (MNF 116)	Multiple	pN0 by HE staining	30 (10.0)	94.0 vs. 89.0 %	0.7797	No
2006	Miyake et al. [29]	120	29.1	T1	IHC	CK (AE1/AE3)	Multiple	≤0.2 mm	27 (22.5)	–	–	–
2007	Yonemura et al. [30]	308	39.0	T1–T4	IHC	CK (AE1/AE3)	–	≤0.2 mm	37 (12.0)	–	0.014	Yes
2008	Kim et al. [31]	184	27.1	T1–T4a	IHC	CK (AE1/AE3)	–	pN0 by HE staining	31 (16.8)	58.5 vs. 91.8 %	<0.001	Yes
2008	Ishii et al. [32]	35	29.4	T1b–T2	IHC	CK (O.N.352)	Multiple	pN0 by HE staining	4 (11.0)	–	–	–
2009	Kim et al. [33]	90	39.2	T1	IHC	CK (AE1/AE3)	–	≤2 mm	9 (10.0)	100 vs. 100 % (DSS)	–	No
2011	Cao et al. [34]	160	10.4	T1	IHC	CK (AE1/AE3)	–	pN0 by HE staining	34 (21.3)	55.9 vs. 92.9 %	<0.001	Yes
2011	Wang et al. [35]	191	22.0	T1–T3	IHC	CK (AE1/AE3)	Multiple	>0.2 and ≤2 mm	54 (28.3)	27.8 vs. 87.1 %	<0.001	Yes

Table 4 RT-PCR studies in patients with histologically node-negative gastric cancer diagnosed by hematoxylin–eosin staining

Years	Study	No. of patients	No. of total LNs	Depth of invasion	Method	Markers	No. of patients with micrometastases (%)
2001	Okada et al. [37]	24	335	Tl–T4a	RT-PCR	CEA, CK20, MAGE3	10 (41.7)
2002	Matsumoto et al. [38]	50	312	T1–T4	RT-PCR	CEA	14 (28.0)
2005	Arigami et al. [39]	80	1,862	T1–T3	RT-PCR	CEA	25 (31.3)
2006	Sonoda et al. [40]	33	310	Tl	RT-PCR	MUC2, TFF1	11 (33.3)
2007	Wu et al. [41]	10	–	–	RT-PCR	CK20	2 (20.0)

with pN0 gastric cancer [39]. LNM was identified in 9 of 80 patients (11.3 %) and in 34 of 1,862 nodes (1.8 %) by IHC, whereas RT-PCR assay demonstrated LNM in 25 patients (31.3 %) and 66 nodes (3.5 %). Of those 66 nodes, 33 were detected only by RT-PCR. The detection rate of LNM was generally higher by RT-PCR than by IHC due to the high sensitivity of RT-PCR. These reports did not examine the relationship between LNM and prognosis, so further investigation will be necessary in the future.

Lymph node micrometastasis in colorectal cancer

Table 5 summarizes findings for LNM determined by RT-PCR in patients with colorectal cancer [42–55]. According to 14 reports, the number of patients and average number of lymph nodes ranged from 30 to 395 and from 5.3 to 21.3, respectively. Almost all reports dealt with relatively early-stage cancer, such as stage II or Dukes A–B. CK antibody was commonly used for detection of LNM, as for esophageal and gastric cancer. LNM was examined using multiple sections in many reports. LNM was defined as newly found metastasis in patients showing pN0 status on routine HE staining in 9 of 16 reports. In the others, LNM was defined according to the size of metastasis. The incidence of LNM ranged from 5.1 to 70.9 % and the detection rate was >30 % in half of the reports (7/14). Detection rates were >30 % for 33.3 % (4/12) of reports on esophageal cancer and 25.0 % (4/16) of reports on gastric cancer. The incidence of LNM was thus higher in colorectal cancer than in esophageal and gastric cancer. In terms of prognostic impact, a significant correlation was found in only 3 of 13 reports (23.1 %). Positive rates for a prognostic impact of LNM were high in both esophageal and gastric cancer, at 58.3 % (7/12) and 64.3 % (9/14), respectively, compared with colorectal cancer. Rahbari et al. [56] conducted a systematic review with meta-analyses of studies that evaluated the prognostic significance of molecular tumor-cell detection in regional lymph nodes. Meta-analysis revealed that molecular tumor-cell detection in regional lymph nodes was associated with poor overall survival, disease-specific survival, and disease-free survival. Subgroup analyses showed the prognostic significance of molecular tumor-cell detection independent of the applied detection method, molecular target, or number of retrieved lymph nodes. They concluded that molecular detection of occult disease in regional lymph nodes is associated with an increased risk of disease recurrence and poor survival in patients with node-negative colorectal cancer. In node-negative patients, LNM is thought to represent a crucial prognostic factor, since it indicates metastatic potential.

Four studies have examined LNM detected by RT-PCR in colorectal cancer (Table 6) [44, 57–59]. The numbers of patients and numbers of examined nodes were relatively small. Like esophageal and gastric cancer, CEA and/or CK were used as markers. The detection rate of LNM was high, at >50 % in three of the four reports. In esophageal and gastric cancer, no reports showed detection rates over 50 %. As with IHC, a high positive rate of LNM with RT-PCR was seen for colorectal cancer. The difference may be due to organ specificity. Interestingly, all authors found a significant correlation between LNM and prognosis. In comparison, a significant association was found in only 23 % of studies using IHC, differing markedly from the RT-PCR method. As the meta-analysis by Rahbari et al. [56] included results from both IHC and RT-PCR, LNM might be a prognostic factor in colorectal cancer. Comparing prognostic significance of LNM between IHC and RT-PCR in the same cases thus seems warranted.

Clinical utility and future perspectives for lymph node micrometastasis

The presence of LNM means that the process of metastasis from the primary tumor has already started. According to the results of this review, a high incidence of LNM ≥10 % is present in patients with pN0 GI cancer. Whether all tiny tumor cells implant and grow in lymph nodes remains unclear, but the potential presence of LNM should be kept in mind. In our study, LNM already showed proliferative activity even in ITC [36]. If LNM is present in patients diagnosed as pN0, we think that such patients should be

Table 5 Immunohistochemical studies in patients with histologically node-negative colorectal cancer diagnosed by hematoxylin–eosin staining

Year	Study	No. of patients	Average no. of LNs	Tumor stage	Method	Antibody	No. of sections for IHC	Definition of micrometastasis	No. of patients with micrometastases (%)	5-year survival (positive vs. negative)	*P*	Prognostic significance
2001	Yasuda et al. [42]	30	21.3	Dukes B	IHC	CK (CAM5.2)	Multiple	pN0 by HE staining	21 (70.0)	–	–	–
2002	Noura et al. [43]	55	12.0	T1–T3	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	27 (49.1)	–	0.817	No
2002	Noura et al. [44]	64	5.5	Stage II	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	35 (54.7)	90.8 vs. 85.1 %	n.s.	No
2003	Palma et al. [45]	38	10.3	Dukes B	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	6 (15.8)	–	0.804	No
2003	Bukholm et al. [46]	156	5.5	Stage II	IHC	CK (CAM5.2)	Multiple	≤0.2 mm	59 (37.8)	–	0.029	Yes
2005	Perez et al. [47]	56	9.6	Stage II (post-CRT)	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	4 (7.1)	–	n.s.	No
2006	García-Sáenz et al. [48]	105	6.3	Stage II	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	26 (24.8)	–	0.759	No
2006	Messerini et al. [49]	395	20.9	Stage IIA	IHC	CK (CK20; clone K 20.8)	Multiple	>0.2 mm and < 2 mm	39 (9.9)	64.1 vs. 78.1 %	0.046	No
2008	Davies et al. [50]	105	5.3	Dukes A–B	IHC	CK (AE1/AE3, MNF 116)	–	pN0 by HE staining	49 (46.7)	–	0.54	No
2008	Bosch Roig et al. [51]	39	9.8	Stage II	IHC	CK (AE1/AE3)	Multiple	>0.2 and <2 mm	2 (5.1)	–	<0.0001	Yes
2008	Park et al. [52]	160	17.8	Stage I–II	IHC	CK (CK20; clone K 20.8)	Multiple	pN0 by HE staining	8 (5.0)	91.7 vs. 93.1 %	0.59	No
2010	Uribarrena-Amezaga et al. [53]	85	10.8	Dukes A-B	IHC	CK (AE1/AE3)	–	pN0 by HE staining	31 (36.5)	–	0.2916	No
2011	Oh et al. [54]	124	19.2	Stage II	IHC	CK (AE1/AE3)	Single	<2 mm	33 (26.6)	96.3 vs. 97.6 %	0.75	No
2011	Faerden et al. [55]	126	–	Stage I–II	IHC	CK (CAM5.2)	Multiple	≤2 mm	39 (31.0)	75.0 vs. 93.0 %	0.012	Yes

Table 6 RT-PCR studies in patients with histologically node-negative colorectal cancer diagnosed by hematoxylin–eosin staining

Years	Study	No. of patients	No. of total LNs	Tumor stage	Method	Markers	No. of patients with micrometastases (%)	5-year survival (positive vs. negative)	*P*	Prognostic significance
1998	Futamura et al. [57]	13	202	Stage I-III	RT-PCR	CEA, CK20	13 (100)	–	–	–
1998	Liefers et al. [58]	26	192	Stage II	RT-PCR	CEA	14 (53.8)	50.0 vs. 91.0 %	0.02	Yes
2002	Noura et al. [44]	64	350	Stage II	RT-PCR	CEA	19 (29.7)	78.2 vs. 95.3 %	0.015	Yes
2002	Rosenberg et al. [59]	85	25 (median)	Stage I–II	RT-PCR	CK20	44 (51.8)	70.6 vs. 95.9 %	0.001	Yes

categorized as pN1. Examination of LNM is thus useful for accurate staging, particularly in pN0 patients. Since prognosis differs significantly between patients with and without LNM according to several reports, adjuvant therapy seems to be necessary for patients with LNM. Prospective randomized controlled studies should be conducted to examine the effectiveness of adjuvant therapies in patients with LNM.

Recently, rapid examination using IHC and RT-PCR has been developed to detect LNM even during surgery. Particularly when performing less-invasive surgeries, intraoperative diagnosis of lymph node metastasis, including LNM, is essential. For example, we applied intraoperative diagnosis of LNM to esophageal cancer surgery in which supraclavicular lymphadenectomy was omitted if negative results were obtained for LNM at the recurrent nerve and cervical paraesophageal nodes [60]. In recent years, sentinel node navigation surgery (SNNS) has been clinically introduced for breast cancer and malignant melanoma [61, 62]. SNNS has also been trialed for GI cancer. We investigated LNM in all dissected lymph nodes, including the sentinel node (SN), as SN mapping using IHC and RT-PCR, yielding good results in patients with esophageal and gastric cancer classified as clinical T1 and N0 [63, 64]. We thus think that SNNS is applicable to clinical T1 and N0 patients based on intraoperative identification of LNM. In fact, if intraoperative histological and molecular examinations demonstrate no metastasis in any SNs identified from cT1 and cN0 patients, treatment using thoracoscopic and laparoscopic approaches with SN dissection may be feasible. On the other hand, standard surgery with standard lymph node dissection is currently necessary in patients with SN metastasis verified by intraoperative diagnostic tools. Furthermore, in the future, endoscopic submucosal dissection (ESD) with thoracoscopic and laparoscopic SN dissection might serve as an ultimate organ-preserving surgery to avoid lymph node recurrence in selected patients with extended indications for ESD. SNNS will add to the development of minimally invasive surgeries with individualized lymphadenectomy and good postoperative quality of life.

In conclusion, LNM needs to be recognized as the first step on the path to lymphatic metastasis. Minimally invasive surgery can be safely performed in clinical situations with accurate diagnosis of LNM. New treatment strategies applying the diagnosis of LNM are to be expected for each type of cancer.

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Conflict of interest The authors declare that they have no conflict of interest.

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