Clinical Aspect: Esophageal Cancer

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Abstract

Lymph node micrometastasis (LNM), including isolated tumor cells (ITC), has recently been the focus of study for the development of a biological method to detect lymph node metastasis in various malignant neoplasms. The applicability of immunohistochemistry (IHC) and reverse transcription-polymerase chain reactions (RT-PCR) to the detection of LNM in esophageal cancer has already been reported. However, the clinical significance of LNM currently remains unclear in patients with esophageal cancer. The presence of LNM is clinically important in patients without nodal metastasis in a routine histological examination (pN0) because patients with pN0, but also with LNM already exhibit metastatic potential. Accurate evaluations need to be performed using the same antibody or primer as well as the same technique in a large number of patients. A rapid diagnosis of LNM using IHC and RT-PCR during surgery will be clinically useful. Minimally invasive treatments such as endoscopic submucosal dissection and laparoscopic surgery with individualized lymphadenectomy are now being increasingly performed in consideration of postsurgical quality of life (QOL). However, it is important to maintain the balance between QOL and curability when selecting surgical treatments for patients with esophageal cancer. We reviewed the clinical significance of LNM as an important strategic target in patients with esophageal cancer.

Keywords

Lymph node micrometastasis · Esophageal cancer · Minimally invasive surgery

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9.1 Introduction

One of the characteristics of a malignant tumor is its ability to metastasize. If a tumor exhibits high malignant potential, metastasis is often detected in a wide range of areas. The prognosis of esophageal cancer is poor. It frequently metastasizes to any of a number of lymph nodes, including the cervical, mediastinal, and abdominal lymph nodes. Lymph node metastasis is one of the most important prognostic factors in patients with esophageal cancer [1, 2]. Even if complete lymph node dissection is performed in patients with early cancer, recurrent disease is sometimes encountered. Therefore, in Japan, radical lymph node dissection, such as extended three-field lymphadenectomy, is performed on patients with esophageal cancer. However, this type of surgical procedure in patients with esophageal cancer is associated with a higher incidence of postoperative complications and hospital mortality than surgical treatments for patients with other gastrointestinal tract cancers [3–5]. If it is possible to perform minimally invasive surgery to treat esophageal cancer, the mortality rate after surgery and postsurgical quality of life (QOL) may be improved. There are currently several therapeutic strategies in the clinical management of patients with early esophageal cancer. Regarding surgical treatments, minimally invasive surgery, such as endoscopic mucosal resection (EMR), endoscopic submucosal dissection (ESD), and blunt dissection, are selected and performed based on the stages and preoperative conditions of patients [6, 7]. In surgical procedures, the clinical efficacy of sentinel node navigation surgery (SNNS) has been investigated in patients preoperatively free of lymph node metastasis (cN0), and many investigators have reported that SNNS is applicable to patients who are preoperatively diagnosed with cT1 and cN0 esophageal cancer [8–10]. Since patients who undergo esophagectomy with standard lymphadenectomy have a promising prognosis, oncological curability needs to be secured in patients receiving less-invasive treatments, such as ESD and SNNS; disease recurrence following less-invasive treatments is undesirable. Accordingly, the precise assessment of the intraoperative lymph node status is extremely important in the strategic process when performing less-invasive treatments.

A histological examination for lymph node metastasis is typically 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 a routine histological examination using hematoxylin and eosin (HE) staining. Even in early gastric cancer, lymph node metastasis was detected in 10.5% of patients when additional sections of nodes were examined [11]. However, these procedures are labor-intensive and not cost-effective in active clinical practice. To date, several investigators have demonstrated the clinical impact of LNM identified by immunohistochemistry (IHC) [12–14]. Furthermore, real-time reverse transcription-polymerase chain reactions (RT-PCR) have been reported to detect LNM better than IHC [15–17]. However, few studies have focused on SN mapping based on LNM assessed by RT-PCR in patients with esophageal cancer.

This review will focus on the clinical significance of LNM as an important therapeutic target in esophageal cancer, including recent advances.

9.2 Definition of LNM

Historically, several terms for very small metastatic foci have been used, including occult metastasis, harbored metastasis, tumor microinvolvement, and tumor deposits. Micrometastasis (MM) is currently defined according to the criteria of the tumor–node–metastasis (TNM) classification established by the International Union Against Cancer (UICC) in 2002 [18] and is completely differentiated from isolated tumor cells (ITC) by size [19]. ITC represent either single tumor cells or small clusters of cells measuring <0.2 mm at their greatest dimension and are commonly identified by IHC, but may also be confirmed by routine HE staining. Moreover, ITC do not basically demonstrate evidence of metastatic activity, such as proliferation or a stromal reaction, or the penetration of vascular or lymphatic sinus walls. Patients with ITC in the lymph nodes are staged as pN0 (i+). On the other hand, MM refers to tumor cell clusters measuring >0.2 mm, but <2.0 mm at the greatest dimension. Patients with MM in the lymph nodes are staged as pN1 (mi). Furthermore, patients with node positivity diagnosed by non-morphological findings using RT-PCR are staged as pN0 (mol+).

9.3 Detection of MM

Many researchers have reported several procedures for the detection of LNM in patients with esophageal cancer. The development of sensitive IHC techniques and RT-PCR has led to the detection of LNM that cannot be found in routine histological examinations. IHC as well as conventional HE staining has been clinically utilized as a standard tool for detecting LNM in esophageal cancer. Furthermore, due to advances in molecular biological techniques, RT-PCR is now available for the detection of LNM. Epithelial markers are commonly used to identify LNM in IHC. Cytokeratin (CK) is representative of epithelial markers. According to previous studies, CK AE1/AE3 and CAM5.2 monoclonal antibodies are often used for IHC [9, 13, 14, 20–28]. Each technique has specific advantages and disadvantages. Since IHC is relatively simple and has the capacity to morphologically identify a single tumor cell or small clusters of tumor cells in lymph nodes, it is a technique this is available in many institutions. Matsumoto et al. [29] established a rapid IHC procedure with the ability to diagnose LNM within 30 min, and this procedure has recently been applied to the detection of LNM during surgery for upper gastrointestinal tract cancer, including esophageal cancer. However, difficulties are associated with selecting a sufficient number of sections for the detection of LNM. Noura et al. [30], in a study on 98 patients with colorectal cancer, demonstrated that the diagnosis of LNM by immunostaining requires staining of at least five slices and therefore is expensive, and generates false-negatives.

On the other hand, RT-PCR offers an objective method for estimating LNM. In RT-PCR assays, several epithelial markers may be used to detect LNM in lymph nodes; however, one of the key issues is selecting what kind of marker is suitable for each carcinoma. CK, carcinoembryonic antigen (CEA), and squamous cell

carcinoma-related antigen (SCC) are typically used for the detection of LNM in esophageal cancer. CEA, CK, and Mucin 1 (MUC 1) are used as target markers of LNM [9, 31–34]. CEA is an epithelial-specific antigen that is expressed in most cancers as well as in normal gastrointestinal tissues [35]. The MUC1 gene is one of the specific markers of epithelial tissues that does not appear in normal lymph nodes [36, 37]. Epithelial markers are generally available for the detection of LNM because epithelial components are not normally present in the lymph nodes. Although this approach offers high sensitivity for detecting low numbers of occult cancer cells in lymph nodes, false-positive results are sometimes obtained as a result of contamination and the presence of pseudogenes. Moreover, false-negatives may be obtained due to the heterogeneous expression of a target marker. Therefore, a detailed assessment using a multiplex RT-PCR assay is currently recommended in order to decrease the rate of false-negative results [38].

In order for RT-PCR assays to be applied as an intraoperative diagnostic tool for the detection of LNM, they need to enable rapid analyses during surgery and retain high sensitivity and specificity. Yanagita et al. reported the clinical availability of another RT-PCR assay named the SmartCycler system as an intraoperative diagnostic tool for detecting LNM in patients with gastric cancer [39]. The reverse transcription of cDNA from target mRNA and the amplification of cDNA are automatically performed by one step in this system. Moreover, the SmartCycler system using a prototype kit may assess the expression of CEA and CK 19 mRNAs and complete the detection of lymph node metastasis within approximately 40 min. According to their study on 47 overt metastatic lymph nodes from 8 patients with advanced gastric cancer and 22 benign lymph nodes from patients without malignant tumors, the sensitivity of the multiplex assay using double markers was 100%. Since the further development of RT-PCR assays will continue in the future, this molecular system may be a promising tool for the intraoperative detection of LNM when performing minimally invasive surgery with personalized lymphadenectomy on patients with esophageal cancer.

9.4 Incidence of MM in Esophageal Cancer

Several studies have investigated LNM detected by IHC in esophageal cancer (Table 9.1) [9, 13, 14, 20–28, 40, 41]. Marked differences were noted in the number of patients and dissected lymph nodes, the depth of tumor invasion, antibodies used for IHC, and the number of node sections assessed by IHC. LNM is basically defined as the presence of a single or small clusters of esophageal tumor cells identified by IHC in pN0 lymph nodes assessed by HE staining [9, 14, 20, 21, 23, 25–27, 40]. The incidence of LNM ranged between 8.1 and 55.5% in all studies. Since the diagnosis of LNM was based on morphology, this discrepancy may be due to the estimations performed by each author. Shiozaki et al. [23] 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 MM-positive by institutional evaluations, whereas pathologists only

Table 9.1 Immunohistochemical studies in patients with esophageal cancer

Prognostic significance	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	No	Yes	No	No
P	<0.05	–	0.002	0.91	<0.01	0.16	0.0188	–	0.0462	0.009	n.s.	0.002	–	–
5-year survival (positive vs. negative)	–	–	44.6 vs. 91.0%	78.0 vs. 75.0%	34.0 vs.72.0%	–	28.0 vs. 79.0%	–	20.0 vs. 70%	30.0 vs. 76.0%	35.7 vs. 61.1%	57.0 vs. 79.0%	–	–
No. of patients with micrometastasis (%)	13 (31.7)	20 (25.6)	39 (55.5)	20 (40.0)	47 (45.2)	14 (26.4)	11 (26.8)	12 (26.1)	25 (15.0)	3 (27.3)	7 (8.1)	7 (14.6)	8 (14.0)	7 (20.5)
Definition of micrometastasis	<0.5 mm	<2 mm	pN0 by HE staining	pN0 by HE staining	pN0 by HE staining	pN0 by HE staining	pN0 by HE staining	<5 cells	pN0 by HE staining	<10 cells	>0.2, <2 mm	pN0 by HE staining	pN0 by HE staining	pN0 by HE staining
Sections for IHC	Single	Multiple	Single	Single	Multiple	Single	Single	Multiple	Multiple	Multiple	Multiple	Multiple	Single	Multiple
Antibody	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)	CK (Lu-5)	CK (AE1/AE3)	CK (AE1/AE3)	CK (AE1/AE3)
Method	IHC	IHC	IHC	IHC	IHC	IHC	IHC	IHC	IHC	IHC	IHC	IHC	IHC	IHC
Histological type	SCC	SCC, AC	SCC	SCC	SCC	SCC	SCC	SCC	SCC	SCC, AC	SCC, AC	SCC, AC	SCC, AC	SCC, AC
Depth of invasion	T1–3		T1–3	T1–4	T1–3	T1–3	T1–4	T1	T1–3	T1–3	T1–3	T1	T1–2	T1–4
Average no. of LNs	–	7.4	46.0	36.8	74.7	47.4	52.9	–	–	–	14.0	28.0	22.8	30.3
No. of patients	41	78	59	50	104	53	41	46	167	33	86	48	46	34
Study	Natsugoe et al. [13]	Glickan et al. [27]	Matsumoto et al. [14]	Sato et al. [26]	Komukai et al. [24]	Nakamura et al. [39]	Doki et al. [25]	Tanabe et al. [23]	Shiozaki et al. [22]	Koenig et al. [21]	Zingg et al. [40]	Prenzel et al. [20]	Hagihara et al. [9]	Kinjo et al. [19]
Years	1998	1999	2000	2001	2002	2002	2002	2003	2007	2009	2009	2012	2013	2014

identified 25 patients with MM-positive lymph nodes. Institutional positivity for MM 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 with morphologically different appearances from cancer cells or epithelial cells. If the evaluation of LNM detected by IHC differs between each institution, the results from different studies will also naturally be different. Therefore, common criteria for identifying LNM using IHC are necessary. Even patients with mucosal and submucosal tumors have 10% or more LNM in pN0 esophageal cancer [21, 24]. Tanabe et al. [24], in a study on 46 node-negative patients with pT1 tumors, such as mucosal and submucosal tumors, reported a high incidence (26.1%) of LNM by IHC using a CK AE1/AE3 antibody. Furthermore, patients with deeper tumor invasion showed a slightly higher incidence of LNM than those with pT1 tumors in pN0 esophageal cancer. Matsumoto et al. [14] showed that LNM was identified by IHC in 1 (4.3%) out of 23 node-negative patients with pT1 tumors, but in 32 (88.9%) out of 36 node-negative patients with pT2 or pT3 tumors. Similarly, Sato et al. [27] detected LNM by IHC in 13 (54.1%) out of 24 node-negative patients with pT2-pT4a tumors.

Table 9.2 summarizes studies on LNM assessed by RT-PCR in patients with pN0 esophageal cancer. According to these studies, simplex or multiplex RT-PCR assays using target molecular markers were performed for the detection of LNM in patients with esophageal cancer [9, 31–34]. Hagihara et al. compared the incidence of LNM between IHC and RT-PCR assays in 1284 lymph nodes obtained from 50 patients with pN0 esophageal cancer [9]. Lymph nodes were cut into two blocks at the plane of the largest dimension. Half of each lymph node was then used in an RT-PCR analysis of CEA and SCC mRNA and sections of the remaining halves were stained for IHC using CK AE1/AE3 mAb. LNM was identified in 4 out of 50 patients (8.0%) and in 19 out of 1284 nodes (1.5%) by IHC, whereas RT-PCR assays detected

Table 9.2 RT-PCR studies in patients with esophageal cancer

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

LNM in 7 patients (14.0%) and 25 nodes (1.9%) [9]. Only 3 out of the 25 LNM were detected by RT-PCR [9]. On the other hand, only one LNM was detected by IHC alone [9]. These findings indicate that an RT-PCR assay is the most sensitive tool for detecting LNM in patients with esophageal cancer.

9.5 Clinical Significance of MM

A large number of studies have investigated the clinical impact of LNM in various malignant tumors, such as breast cancer, non-small cell lung cancer, gastric cancer, colorectal cancer, pancreatic cancer, and biliary cancer [42–49]. Although many investigators have also demonstrated the clinical significance of LNM in patients with esophageal cancer, it currently remains controversial [9, 13, 14, 20–28, 40, 41].

Shiozaki et al. [23], in a study on 164 esophageal cancer patients with pT1-3N0 tumors, reported that 51 out of 164 patients with pN0 were diagnosed as LNM-positive by institutional evaluations, and LNM based on an institutional diagnosis did not have a significant impact on survival. Based on diagnoses made by pathologists, LNM, including IHC-positive single cells and clusters, did not have a clinical impact on survival, whereas metastasis with clusters of IHC-positive cells only had a significant clinical impact on prognosis, with 5-year overall survival rates of 20% and 70%, respectively. They indicated a need to correlate MM-IHC-positive cells with the morphological aspects of stained cells and that only LNs with clusters of stained cells are prognostically significant in esophageal carcinoma. They suggested that patients with clusters of positive cells showed worse prognoses if they had pathologically positive lymph nodes, which suggests that cluster-type-positive cells in lymph nodes are a biological feature of malignant potential in esophageal carcinoma. Zingg et al. [41], in a study on 86 esophageal cancer patients (32 with squamous cell carcinoma and 54 with adenocarcinoma), reported that there was no significant difference in the frequency of LNM between adenocarcinoma and squamous cell carcinoma (11.3% vs. 3.1%, p = n.s.). In this study, the definition of LNM was as follows: intra-nodal tumor cell infiltrates measuring between 0.2 and 2 mm were classified as MM, while those measuring less than 0.2 mm were classified as ITC according to the proposition of Hermanek et al. [50]. Cytokeratin-positive material devoid of any evidence of vital nuclei was classified as “avital cytokeratin-positive material” (ACPM). They demonstrated that IHC-negative patients with squamous cell carcinoma showed significantly better overall survival ($p < 0.02$) and disease-free intervals ($p < 0.01$). No significant differences were observed in adenocarcinoma. They identified differences in biological behavior and outcomes, indicating that it is inappropriate to treat adenocarcinoma and squamous cell carcinoma as one entity. Kinjo et al. [20], in a study on 77 esophageal cancer patients with pT1-pT4 tumors, classified each esophageal tumor into 1 of 3 categories in accordance with the sixth edition of the Tumor–Node–Metastasis (TNM) Classification of Malignant Tumors, a cancer staging system developed by the International Union Against Cancer (UICC), based on the relationship between the initial tumor status and applicability of upfront R0 resection for esophageal cancer. In terms of tumor

categories, IHC-positive LNM was present in 12 (30%), 11 (52.4%), and 11 (68.8%) of 40, 21, and 16 Category 1, 2, and 3 patients, respectively. A significant difference in the frequency of IHC-positive LNM was observed among these three patient groups ($p = 0.019$). They also reported that 5-year survival rates in patients with or without LNM were 42.5% and 61.8%, respectively. However, the survival rates were not significantly different according to the presence or absence of micrometastasis by immunostaining, although the 5-year survival rate of 27 cases positive for both lymph node metastasis by HE staining and micrometastasis by immunostaining was 30.6%, significantly different from 65.1% in the remaining 50 cases. In addition, they identified simultaneous HE-positive lymph node metastasis and IHC-positive LNM and pT as independent prognostic predictors that correlated with survival. Matsumoto et al. [14] examined clinicopathological factors in 59 patients with T1-4 tumors without lymph node metastasis. They reported that the rate of recurrent disease was significantly higher in patients with than in those without LNM (94.1% vs. 40.5% respectively). LNM was immunohistochemically detected in all patients with lymph node recurrence. The 5-year survival rate was significantly lower in patients with than in those without LNM (91.0% vs. 44.6%, respectively). They demonstrated that the frequency of micrometastases increases in T2 and 3 tumors. It should be noted that such tumors are associated with micrometastasis, especially, lymph node recurrence. However, since 17 patients with LNM did not develop recurrence, these patients benefited from lymph node dissection. They concluded that the presence of LNM positively correlated with disease recurrence and poor outcomes. Extended lymphadenectomy and postoperative adjuvant therapy may be indicated for patients with esophageal SCC. Koenig et al. [22], in a study on 33 esophageal cancer patients (18 with squamous cell carcinoma and 15 with adenocarcinoma) with pT1-3N0 tumors, reported that 9 patients were diagnosed with IHC-positive LNM, and 5-year overall survival probability was 76% in patients without LNM, but was 30% in patients with LNM ($P = 0.009$, the Log-rank test). Further analyses revealed that 5-year overall survival probability in patients with nodal microinvolvement was similar to that of pN1 patients (the Log-rank test; $P = 0.875$). They also identified the LNM ratio as the most powerful predictive variable for overall survival in patients with esophageal carcinoma irrespective of the histological tumor type in a multivariate analysis. They concluded not only that the global presence or absence of nodal microinvolvement may serve as a tool for differentiating high-risk from low-risk patients, but also that the IHC ratio of affected lymph nodes to the total number of lymph nodes appears to enable improved risk stratification for esophageal cancer patients.

Hagihara et al. [9] focused on SNs in a study on 57 esophageal cancer patients with cT1-2N0 tumors. They reported that conventional HE staining detected histological lymph node metastasis in 7 out of 57 patients (12.3%). Lymph node metastasis, including MM, was identified in 11 patients (19.3%) by IHC. In the remaining 46 node-free patients assessed by HE staining and IHC, MM was identified in 4 patients (7.0%) by RT-PCR. They suggested the applicability of RT-PCR to the detection of a very small number of tumor cells within lymph nodes. Li et al. [31], in a study on 93 esophageal squamous cell carcinoma patients with pT1-3N0

tumors, detected MUC1 mRNA in 32 patients, which accounted for 34.4% of all 93 patients. Tumor relapse developed during the follow-up in 61 (65.6%) out of the 93 patients, 26 of whom had LNM while 35 did not. Patients with LNM had a significantly shorter disease-free interval than those without LNM (26 vs. 32 months). The 5-year survival rate of patients with LNM was significantly lower than that of those without LNM (18.8 vs. 47.6%). They also indicated that the T status and LNM were independent prognostic factors. They concluded that TNM staging needs to involve LNM, and improved staging may be expected with further information on LNM, whereby a subgroup of patients who may benefit greatly from adjuvant therapy may be identified.

Xi et al. [33], in a study on 34 esophageal adenocarcinoma patients with pTis-3N0 tumors, detected CK19/TACSTD-1 mRNA in the nodes of 5 patients. Quantitative RT-PCR (QRT-PCR)-positive patients had significantly worse disease-free survival than QRT-PCR-negative patients ($P = 0.0023$). The ongoing clinical trial of chemotherapy by the Eastern Cooperative Oncology Group demonstrated that chemotherapy is effective for patients with trace amounts of residual lesions, such as micrometastasis. Furthermore, a major benefit of more accurate staging may be the ability to identify low-risk, truly node-negative patients, thereby avoiding the potential morbidity of unnecessary chemotherapy for these patients. The RT-PCR method is more sensitive than IHC for detecting LNM because of the greater quantity of the sample available. However, several issues are still associated with RT-PCR examinations. Since these epithelial markers are not specific to cancer, the number of markers needed remains unclear. Furthermore, suitable primers have not yet been identified. If esophageal cancer-specific markers become available, the results of RT-PCR examinations will become more reliable.

9.6 Future Possibilities for MM

The existence of LNM indicates that metastasis from the primary tumor has already begun. According to the findings of this review, a high incidence of LNM > 10% exists in patients with pN0 esophageal cancer. It currently remains unclear whether all small tumor cells graft and grow in lymph nodes; however, the potential existence of LNM in patients with pN0 needs to be considered. In our study, LNM already exhibited proliferative activity, even in ITC [51]. If LNM exists in patients diagnosed as pN0, these patients need to be considered as pN1. Therefore, examinations of LNM are favorable for correct staging, particularly in pN0 patients. The detection of LNM, and subsequently improved patient staging, may have significant consequences for the treatment of esophageal cancer. Since prognoses differ significantly between patients with and without LNM according to several studies, adjuvant therapy appears to be necessary for patients with LNM. Due to the lack of systemic adjuvant therapy for esophageal cancer, any correct staging system currently lacks clinical significance for decision-making in individual patients and is only of prognostic importance. This may change as soon as advances are achieved in the field of new adjuvant chemotherapeutic and targeted therapy regimens.

Prospective randomized controlled studies need to be conducted in order to examine the effectiveness of adjuvant therapies in patients with LNM.

Surgical approaches and the extent of lymph node dissection may also be selected based on the lymph node status, with some surgeons advocating extensive lymph node dissection in node-positive patients. Furthermore, there is strong evidence that patients with extensive lymph node metastasis have a poor prognosis. Therefore, curative surgery may be difficult for such patients. These findings indicate that the revision of the TNM staging system is necessary. The examination of LNM in patients with pN0 assessed by routine HE staining may facilitate screening and validation of whether they are truly node-negative patients. The former subgroup may benefit from more extensive nodal dissection, whereas the latter group may achieve curative resection with less aggressive surgical resection.

An accurate intraoperative diagnosis of the lymph node status, including LNM, by molecular methods is necessary when performing minimally invasive surgery with individualized lymphadenectomy. For example, the supraclavicular lymph nodes are not dissected in patients negative for micrometastasis in the recurrent neural and cervical paraesophageal lymph nodes [52]. Currently, SNNS is performed for breast cancer and malignant melanoma [53, 54]. We investigated LNM in all dissected lymph nodes, including the SN, because SN mapping using IHC and RT- PCR yields good results in patients with esophageal and gastric cancer classified as clinical T1 and N0 [8, 55]. It is reasonable to apply less-invasive procedures than surgical treatments when intraoperative histological and molecular diagnoses reveal that SNs in cT1N0 patients are negative for metastasis. On the other hand, standard surgery with standard lymph node dissection is currently recommended for patients with SN metastasis verified by intraoperative diagnostic tools. Furthermore, ESD with thoracoscopic and laparoscopic SN dissection may serve as the ultimate esophageal-preserving surgery in the future to avoid lymph node recurrence in selected patients with extended indications for ESD. Thus, if SNNS based on the LNM status is clinically developed as a surgical treatment for patients with esophageal cancer, minimally invasive surgery with individualized lymphadenectomy may be safely performed in the near future and achieve good results for the balance between postsurgical QOL and curability. Future studies on the biological behavior of MM tumor cells will greatly contribute to the development of further treatments for patients with esophageal cancer.

In conclusion, LNM needs to be recognized as the first and important step in the path to lymphatic metastasis. Minimally invasive surgery may be safely performed in clinical situations with a correct diagnosis of LNM. New treatment strategies that apply the diagnosis of LNM are expected for esophageal cancer.

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