**Name of journal:** **World Journal of Gastroenterology**

**ESPS Manuscript No: 6562**

**Columns: TOPIC HIGHLIGHTS**

WJG 20th Anniversary Special Issues (8): Gastric cancer

**Clinical significance of molecular diagnosis for gastric cancer lymph node micrometastasis**

Sonoda H *et al.* Molecular diagnosis for GC LNM

Hiromichi Sonoda, Tohru Tani

**Hiromichi Sonoda, Tohru Tani,** Department of Surgery, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan

**Author contributions:** Sonoda H and Tani T wrote the paper.

**Correspondence to: Hiromichi Sonoda MD, PhD,** Department of Surgery, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga 520-2192, Japan.hirosono@belle.shiga-med.ac.jp

**Telephone:** +81-77-5482238  **Fax:** +81-77-5482240

**Received:** October 23, 2014 **Revised:** March 14, 2014

**Accepted:** June 26, 2014

**Published online:**

**Abstract**

Advances in molecular diagnostic tools have allowed the identification of lymph node micrometastasis (LNM), including isolated tumor cells, in cancer patients. While immunohistochemistry and reverse transcription-polymerase chain reaction have been used to identify LNM in patients with gastric cancer, the clinical significance of this finding remains unclear. Recently, minimally invasive treatments, such as endoscopic submucosal dissection and laparoscopic surgery, are widely performed to help improve postsurgical quality of life (QOL). However, it is important to maintain the balance between QOL and curability when making treatments decision for patients with gastric cancer. If minimally invasive surgery based on accurate intraoperative LNM diagnosis was established, it could be performed safely. Therefore, we reviewed the clinical significance of LNM detected by molecular techniques as an important target for treatment decision making with gastric cancer patients.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Gastric cancer; Lymph node micrometastasis; Molecular technique; Sentinel lymph node; Minimally invasive surgery

**Core tip:** Advances in molecular diagnostic tools have allowed the identification of lymph node micrometastasis in cancer patients. Minimally invasive treatments, such as endoscopic submucosal dissection and laparoscopic surgery, are widely performed to help improve postsurgical quality of life (QOL). However, it is important to maintain the balance between QOL and curability.

Sonoda H, Tani T. Clinical significance of molecular diagnosis for gastric cancer lymph node micrometastasis. *World J Gastroenterol* 2014; In press

**Introduction**

Regional lymph node metastasis is the most important prognostic factor for patients with gastric cancer. Therefore, radical gastrectomy with D2 lymph node dissection is recognized as the standard surgical treatment for early gastric cancer (EGC). Patients with EGC have good prognosis, with a 5-year survival rate for mucosal and submucosal gastric cancer of 95%-100% and 85%-95%, respectively[1-3]. Nevertheless, some patients without lymph node metastasis who received radical gastrectomy based on conventional histological hematoxylin-eosin (HE) staining occur disease recurrence[4-6]. Such reserches have concluded that lymph node micrometastasis (LNM) might be a key causative factor for gastric cancer recurrence. However, it is difficult to preoperatively diagnose lymph node metastasis, including micrometastasis, using imaging examinations (*e.g.*, ultrasonography, computed tomography, and positron emission tomography) in patients with gastric cancer[7].

To increase the quality of life (QOL) of patients with EGC, minimally invasive treatments, such as endoscopic submucosal dissection (ESD), laparoscopic surgery and a reduced form of a lymphadenectomy are preferred treatment options. Thus, it is more important to select those patients in which lymph node metastatic disease is not present. Recently, the clinical efficacy of sentinel lymph node navigation surgery (SLNNS) has been investigated in patients without lymph node metastasis preoperatively (cN0), and many reserchers have reported that SLNNS is applicable in patients who are preoperatively diagnosed with cT1 and cN0 gastric cancer[8-11]. Accordingly, the precise intraoperative assessment whether lymph node metastasis, including micrometastasis, is present or not is extremely important for treatment planning purposes when utilizing less invasive treatments. This review will focus on the clinical significance of molecular diagnosis for LNM as an important therapeutic target in gastric cancer and will discuss the recent progress in this field.

**Literature search**

A Pubmed search using the terms gastric cancer, micrometastasiswas performed for article published from 1946 to 2013. The literature search was limited to English language only. From these searched articles, we selected and reviewed clinical studies about LnM of gastric cancer. Moreover, we described future perspective based on the molecular techniques for detection of the LnM.

**DEFINITION OF LYMPH NODE MICROMETASTASIS**

Micrometastasis was defined as tumor cell clusters measuring from 0.2 mm to 2.0 mm in their greatest dimension, and classified as pN1 (mi) by the criteria of the tumor-node-metastasis (TNM) classification established by the International Union Against Cancer (UICC)[12]. On the other hand, isolated tumor cells (ITC) are defined as single tumor cells or small clusters of cells measuring ≤ 0.2 mm in their greatest dimension, and classified as pN0 (i+). Furthermore, patients with lymph node metastasis detected only by using reverse transcription-polymerase chain reaction (RT-PCR) are classified as pN0 (mol+).

**DETECTION OF MICROMETASTASIS**

Several procedures have been used for the detection of LNM in gastric cancer patients. First, Isozaki *et al*[13] reported that the detection rate of lymph node metastases of gastric cancer by serial sectioning was significantly higher than that of routine histological diagnosis. Immunohistochemistry (IHC) has also been used to detect LNMs. Epithelial marker, such as cytokeratin (CK), is commonly used to identify LNM in IHC. According to the previous studies, CK AE1/AE3 and CAM5.2 monoclonal antibodies (mAb) were often used to detect LNM of gastric cancer. The greatest advantage of IHC is that we can morphologically recognize cells in lymph nodes. However, there are some problems to apply IHC for the detection of LNM, such as the serial numbers of lymph node section required. Noura *et al*[14] demonstrated that the diagnosis of LNM in colorectal cancer patients should be assessed by IHC for at least five sections. IHC can be time consuming, but Matsumoto *et al*[15] established a rapid IHC procedure that could diagnose LNM within 30 min. They have been recently applied this rapid IHC technique for the detection of LNM during surgery in the upper gastrointestinal tract cancer. However, this method has not been widely accepted.

Due to progress in molecular biological techniques, RT-PCR can now be used for the detection of LNM. CK and carcinoembryonic antigen (CEA) are usually used as target markers for the detection of LNM[16-19]. CEA is a 200 kDa glycoprotein that is not expressed in normal gastric mucosa but expressed in most gastric cancer cells[20]. Although the RT-PCR assay has high sensitivity for the detection of small numbers of occult cancer cells in lymph nodes, several reserchers have reported that there are some problems to apply the RT-PCR assay for the detection of LNM[21,22]. First, false-positives may be produced by RT-PCR because of the contamination or the presence of pseudogene[21]. Second, there is a possibility of false-negatives because of the heterogeneous expression of target markers[22]. We previously reported that the MUC2 RT-PCR assay was a sensitive and specific method to detect LNM in gastric cancer patients[23]. Furthermore, detailed assessment by duplex[24] or multiplex[25] RT-PCR assay has been recommended in order to decrease the rate of false-negative results. Moreover, the use of RT-PCR assay as an intraoperative diagnostic tool for the detection of LNM requires rapid analysis during operation in a highly sensitive and specific manner. Horibe *et al*[26] developed a rapid method to detect LNM with the use of a reverse transcription loop mediated isothermal amplification (RT-LAMP) reaction. This technique requires less than 1 hour to obtain the final results. Recently, Sysmex Corp. (Kobe, Japan) developed a one-step nucleic acid amplification (OSNA) assay, which is an automated system that uses the RT-LAMP method for gene amplification. In this system, the cytokeratin 19 (CK19) mRNA, cancer specific molecular marker, is directly and rapidly amplified from the homogenized lymph nodes. Results are available within 30 min for one lymph node because the mRNA purification process that is usually performed in RT-PCR methods is not required in this assay. We can also analyze four lymph nodes simultaneously. Kumagai *et al*[27] reported that the OSNA assay could detect LNM as accurately as the histological examination of blocks sectioned at 2-mm intervals in the patients with gastric cancer. Therefore, they concluded that the OSNA assay is a useful tool for the intraoperative diagnosis of LNM in gastric cancer patients. Moreover, Yaguchi *et al*[28] described the use of the OSNA assay with SLNNS in patients with gastric cancer. There is some doubt as to whether the OSNA assay using a single marker is sufficient for detection of LNM in gastric cancer. However, advances in the RT-PCR assay are likely, and will likely enhance the clinical utility of this molecular system for intraoperative detection of LNM when we intend to perform minimally invasive surgery, such as SLNNS, in patients with gastric cancer.

**INCIDENCE OF MICROMETASTASIS**

Since 1996, there have been many studies regarding LNM detected by IHC in pN0 gastric cancer patients (Table 1). When comparing studies, there are remarkable differences in the number of patients, dissected lymph nodes, the depth of tumor invasion, and the number of node sections assessed by IHC. However, the incidence of LNM ranged from 10% to 36 % in all studies[1,4,29-42]. It is noteworthy that the incidence of LNM in pN0 gastric cancer was 10% or more even in the patients with mucosal and submucosal cancer[1,4,32,33,36-39].

Some studies have investigated LNM as determined by RT-PCR in pN0 gastric cancer patients[16-19,24]. In our study[23], we detected 49 of 286 histologically node negative lymph nodes (17.1%) by RT-PCR analysis of MUC2 mRNA. Of these 49 LNM, only six were detected by IHC using AE1/AE3 mAb. Similarly, Arigami *et al*[18] and Kubota *et al*[43] also reported that the incidence of LNM detected by RT-PCR was higher than that of LNM detected by IHC. These results suggest that the RT-PCR assay is now the most sensitive method for detection of LNM in gastric cancer patients.

**CLINICAL SIGNIFICANCE OF MICROMETASTASIS**

Many studies have reported the clinical impact of LNM in various cancers. Particularly, sentinel lymph node micrometastases were reported to be associated with adverse outcomes in patients with malignant melanoma[44] and breast cancer[45]. However, the clinical significance of LNM in patients with gastric cancer remains controversial.

Some studies have investigated the clinical impact of LNM in gastric cancer using IHC. Yasuda *et al*[29] studied 64 patients with pT2-3N0 gastric cancer and reported that the 5-year overall survival rates in patients with or without LNM were 66% and 95% (*P* < 0.01), respectively. Cao *et al*[32] and Yonemura *et al*[35] also reported that patients with LNM had significantly worse outcomes than those without LNM in pN0 gastric cancer. In contrast, Morgagni *et al*[37] studied 300 patients with pT1N0 gastric cancer and reported that there were no significant differences in the 10-year overall survival rates regardless of the presense of LNM. Fukagawa *et al*[40] studied 107 gastric cancer patients with pT2N0 or pT3N0 tumors at the Japanese National Cancer Center and also reported that there were no significant difference in the 5-year survival rates and 10-year survival rates in patients with or without LNM. On the other hand, no investigations have studied the relationship between the incidence of LNM detected by RT-PCR and patient outcomes (Table 2).

To verify these results from the viewpoint of tumor biology, Yonemura *et al*[35] immunostained sections of lymph nodes diagnosed as pN0 by H-E staining using Ki-67 mAb (MIB-1). This IHC analysis demonstrated positive MIB-1 labeling in 12 of 25(48.0%) with single isolated cancer cells and in 49 of 52(94.2%) with clusters of cancer cells. Similarly, Yanagita *et al*[46] also assessed the proliferative activity of ITC and micrometastasis using IHC analysis with Ki-67 mAb. According to this study, the Ki-67 positivity rates for macrometastasis, micrometastasis and ITC were 96%, 92% and 29%, respectively. These two studies suggest that LNM could have proliferative activity.

 Although it remains clinically difficult to draw definitive conclusions regarding this issue, the clinical outcome was not affected by the presence of LNM who underwent curative gastrectomy with D2 lymph node dissection in Japan.

**FUTURE PERSPECTIVE**

Gastrectomy with regional lymph nodes dissection has been widely accepted as the standard treatment even for the EGC. However, such extensive surgery is associated with long-term reduction of patients' QOL. The patients with EGC could have received curative endoscopic treatment by using ESD even with the submucosal invasion. A large number of studies have been published so as to the good long-term outcome of EGC treated by ESD. In the cases of the contraindication of ESD, surgery is usually required. Recently, minimally invasive surgery, such as SLNNS, has been widely performed. An accurate and rapid intraoperative diagnosis of lymph node metastasis, including LNM, is essential when performing SLNNS. Kumagai *et al*[27] reported that the OSNA assay could detect LNM as accurately as that detected by histological examination of blocks sectioned at 2-mm intervals in the patients with gastric cancer. Moreover, such results are available within 30 min. From these points of view, the OSNA assay is considered to be the most accurate and rapid method for the intraoperative detection of LNM in patients with gastric cancer. Therefore, if intraoperative molecular examinations, such as the OSNA assay, demonstrate no metastasis in all SLN, then patients could be received laparoscopic partial or segmental gastrectomy with SLN dissection. However, Shimizu *et al*[47] reported that seven (6.8%) of 103 patients had LNM in non-sentinel lymph nodes (all within the same lymphatic basin), according to RT-PCR performed with multiple markers (CK19, CK20, and CEA). The lymphatic basin is regarded as the most important lymphatic area in which lymph node metastasis may develops. Kinami *et al*[48] reported that the gastric lymphatic basins were divided in the following five directions along the main arteries around the stomach: left gastric artery area (l-GA), right gastric artery area (r-GA), right gastroepiploic artery area (r-GEA), left gastroepiploic artery area (l-GEA), and posterior gastric artery (p-GA). They also reported that nodal metastasis generally occurred in the SLNs or the lymphatic basin, and rarely extended outside the lymphatic basin. Therefore, those investigators concluded that if the all SLNs are negative for cancer metastasis by intraoperative diagnosis, patients could be received limited gastrectomy, such as partial or segmental gastrectomy with SLN basin dissection. On the other hand, in the case of positive for SLN metastasis confirmed by intraoperative diagnosis, standard gastrectomy with regional lymphadenectomy is considered necessary. Recently, Bok *et al*[49] reported that combined ESD and sentinel lymph nodes surgery (ESN) is a feasible minimally invasive procedure for cT1 (< 3 cm) N0 EGC. We believe that, in the near future, such advanced technology allows the patients with EGC to be treated with the procedures that balance between curability and QOL. Future studies will be needed to achieve such objectives.

**REFERENCES**

1 **Cai J**, Ikeguchi M, Maeta M, Kaibara N. Micrometastasis in lymph nodes and microinvasion of the muscularis propria in primary lesions of submucosal gastric cancer. *Surgery* 2000; **127**: 32-39 [PMID: 10660756 DOI: 10.1067/msy.2000.100881]

2 **Kim DY**, Joo JK, Ryu SY, Kim YJ, Kim SK. Factors related to lymph node metastasis and surgical strategy used to treat early gastric carcinoma. *World J Gastroenterol* 2004; **10**: 737-740 [PMID: 14991950]

3 **Maehara Y**, Orita H, Okuyama T, Moriguchi S, Tsujitani S, Korenaga D, Sugimachi K. Predictors of lymph node metastasis in early gastric cancer. *Br J Surg* 1992; **79**: 245-247 [PMID: 1555092]

4 **Maehara Y**, Oshiro T, Endo K, Baba H, Oda S, Ichiyoshi Y, Kohnoe S, Sugimachi K. Clinical significance of occult micrometastasis lymph nodes from patients with early gastric cancer who died of recurrence. *Surgery* 1996; **119**: 397-402 [PMID: 8644003]

5 **Siewert JR**, Kestlmeier R, Busch R, Böttcher K, Roder JD, Müller J, Fellbaum C, Höfler H. Benefits of D2 lymph node dissection for patients with gastric cancer and pN0 and pN1 lymph node metastases. *Br J Surg* 1996; **83**: 1144-1147 [PMID: 8869330]

6 **Ishida K**, Katsuyama T, Sugiyama A, Kawasaki S. Immunohistochemical evaluation of lymph node micrometastases from gastric carcinomas. *Cancer* 1997; **79**: 1069-1076 [PMID: 9070482]

7 **Sim SH**, Kim YJ, Oh DY, Lee SH, Kim DW, Kang WJ, Im SA, Kim TY, Kim WH, Heo DS, Bang YJ. The role of PET/CT in detection of gastric cancer recurrence. *BMC Cancer* 2009; **9**: 73 [PMID: 19250554 DOI: 10.1186/1471-2407-9-73]

8 **Kitagawa Y**, Fujii H, Mukai M, Kubota T, Otani Y, Kitajima M. Radio-guided sentinel node detection for gastric cancer. *Br J Surg* 2002; **89**: 604-608 [PMID: 11972551 DOI: 10.1046/j.1365-2168.2002.02065.x]

9 **Arigami T**, Natsugoe S, Uenosono Y, Mataki Y, Ehi K, Higashi H, Arima H, Yanagida S, Ishigami S, Hokita S, Aikou T. Evaluation of sentinel node concept in gastric cancer based on lymph node micrometastasis determined by reverse transcription-polymerase chain reaction. *Ann Surg* 2006; **243**: 341-347 [PMID: 16495698 DOI: 10.1097/01.sla.0000201453.65534.f1]

10 **Tajima Y**, Yamazaki K, Masuda Y, Kato M, Yasuda D, Aoki T, Kato T, Murakami M, Miwa M, Kusano M. Sentinel node mapping guided by indocyanine green fluorescence imaging in gastric cancer. *Ann Surg* 2009; **249**: 58-62 [PMID: 19106676 DOI: 10.1097/SLA.0b013e3181927267]

11 **Aikou T**, Higashi H, Natsugoe S, Hokita S, Baba M, Tako S. Can sentinel node navigation surgery reduce the extent of lymph node dissection in gastric cancer? *Ann Surg Oncol* 2001; **8**: 90S-93S [PMID: 11599911]

12 **Sobin LH**, Wittekind C. International Union against Cancer: TNM: classification of malignant tumours, 6th edn. New York: Wiley-Liss; 2002

13 **Isozaki H**, Okajima K, Fujii K. Histological evaluation of lymph node metastasis on serial sectioning in gastric cancer with radical lymphadenectomy. *Hepatogastroenterology* 1997; **44**: 1133-1136 [PMID: 9261612]

14 **Noura S**, Yamamoto H, Miyake Y, Kim Bn, Takayama O, Seshimo I, Ikenaga M, Ikeda M, Sekimoto M, Matsuura N, Monden M. Immunohistochemical assessment of localization and frequency of micrometastases in lymph nodes of colorectal cancer. *Clin Cancer Res* 2002; **8**: 759-767 [PMID: 11895906]

15 **Matsumoto M**, Natsugoe S, Ishigami S, Uenosono Y, Takao S, Aikou T. Rapid immunohistochemical detection of lymph node micrometastasis during operation for upper gastrointestinal carcinoma. *Br J Surg* 2003; **90**: 563-566 [PMID: 12734863 DOI: 10.1002/bjs.4083]

16 **Okada Y**, Fujiwara Y, Yamamoto H, Sugita Y, Yasuda T, Doki Y, Tamura S, Yano M, Shiozaki H, Matsuura N, Monden M. Genetic detection of lymph node micrometastases in patients with gastric carcinoma by multiple-marker reverse transcriptase-polymerase chain reaction assay. *Cancer* 2001; **92**: 2056-2064 [PMID: 11596020]

17 **Matsumoto M**, Natsugoe S, Ishigami S, Nakashima S, Nakajo A, Miyazono F, Hokita S, Takao S, Eizuru Y, Aikou T. Lymph node micrometastasis and lymphatic mapping determined by reverse transcriptase-polymerase chain reaction in pN0 gastric carcinoma. *Surgery* 2002; **131**: 630-635 [PMID: 12075175]

18 **Arigami T**, Natsugoe S, Uenosono Y, Arima H, Mataki Y, Ehi K, Yanagida S, Ishigami S, Hokita S, Aikou T. Lymphatic invasion using D2-40 monoclonal antibody and its relationship to lymph node micrometastasis in pN0 gastric cancer. *Br J Cancer* 2005; **93**: 688-693 [PMID: 16136051 DOI: 10.1038/sj.bjc.6602739]

19 **Wu ZY**, Li JH, Zhan WH, He YL, Wan J. Effect of lymph node micrometastases on prognosis of gastric carcinoma. *World J Gastroenterol* 2007; **13**: 4122-4125 [PMID: 17696234]

20 **Gerhard M**, Juhl H, Kalthoff H, Schreiber HW, Wagener C, Neumaier M. Specific detection of carcinoembryonic antigen-expressing tumor cells in bone marrow aspirates by polymerase chain reaction. *J Clin Oncol* 1994; **12**: 725-729 [PMID: 7512130]

21 **Ruud P**, Fodstad O, Hovig E. Identification of a novel cytokeratin 19 pseudogene that may interfere with reverse transcriptase-polymerase chain reaction assays used to detect micrometastatic tumor cells. *Int J Cancer* 1999; **80**: 119-125 [PMID: 9935241]

22 **Kuo CT**, Hoon DS, Takeuchi H, Turner R, Wang HJ, Morton DL, Taback B. Prediction of disease outcome in melanoma patients by molecular analysis of paraffin-embedded sentinel lymph nodes. *J Clin Oncol* 2003; **21**: 3566-3572 [PMID: 12913098 DOI: 10.1200/JCO.2003.01.063]

23 **Sonoda H**, Yamamoto K, Kushima R, Okabe H, Tani T. Detection of lymph node micrometastasis in gastric cancer by MUC2 RT-PCR: usefulness in pT1 cases. *J Surg Oncol* 2004; **88**: 63-70 [PMID: 15499573 DOI: 10.1002/jso.20143]

24 **Sonoda H**, Yamamoto K, Kushima R, Yamamoto H, Naitoh H, Okabe H, Tani T. Detection of lymph node micrometastasis in pN0 early gastric cancer: efficacy of duplex RT-PCR with MUC2 and TFF1 in mucosal cancer. *Oncol Rep* 2006; **16**: 411-416 [PMID: 16820924]

25 **Mori K**, Aoyagi K, Ueda T, Danjoh I, Tsubosa Y, Yanagihara K, Matsuno Y, Sasako M, Sakamoto H, Mafune Ki, Kaminishi M, Yoshida T, Terada M, Sasaki H. Highly specific marker genes for detecting minimal gastric cancer cells in cytology negative peritoneal washings. *Biochem Biophys Res Commun* 2004; **313**: 931-937 [PMID: 14706632]

26 **Horibe D**, Ochiai T, Shimada H, Tomonaga T, Nomura F, Gun M, Tanizawa T, Hayashi H. Rapid detection of metastasis of gastric cancer using reverse transcription loop-mediated isothermal amplification. *Int J Cancer* 2007; **120**: 1063-1069 [PMID: 17139607 DOI: 10.1002/ijc.22397]

27 **Kumagai K**, Yamamoto N, Miyashiro I, Tomita Y, Katai H, Kushima R, Tsuda H, Kitagawa Y, Takeuchi H, Mukai M, Mano M, Mochizuki H, Kato Y, Matsuura N, Sano T. Multicenter study evaluating the clinical performance of the OSNA assay for the molecular detection of lymph node metastases in gastric cancer patients. *Gastric Cancer* 2014; **17**: 273-280 [PMID: 23743877 DOI: 10.1007/s10120-013-0271-9]

28 **Yaguchi Y**, Sugasawa H, Tsujimoto H, Takata H, Nakabayashi K, Ichikura T, Ono S, Hiraki S, Sakamoto N, Horio T, Kumano I, Otomo Y, Mochizuki H, Yamamoto J, Hase K. One-step nucleic acid amplification (OSNA) for the application of sentinel node concept in gastric cancer. *Ann Surg Oncol* 2011; **18**: 2289-2296 [PMID: 21301968 DOI: 10.1245/s10434-011-1591-9]

29 **Yasuda K**, Adachi Y, Shiraishi N, Inomata M, Takeuchi H, Kitano S. Prognostic effect of lymph node micrometastasis in patients with histologically node-negative gastric cancer. *Ann Surg Oncol* 2002; **9**: 771-774 [PMID: 12374660]

30 **Kim JH**, Park JM, Jung CW, Park SS, Kim SJ, Mok YJ, Kim CS, Chae YS, Bae JW. The significances of lymph node micrometastasis and its correlation with E-cadherin expression in pT1-T3N0 gastric adenocarcinoma. *J Surg Oncol* 2008; **97**: 125-130 [PMID: 18095267 DOI: 10.1002/jso.20937]

31 **Wang J**, Yu JC, Kang WM, Wang WZ, Liu YQ, Gu P. The predictive effect of cadherin-17 on lymph node micrometastasis in pN0 gastric cancer. *Ann Surg Oncol* 2012; **19**: 1529-1534 [PMID: 22009269 DOI: 10.1245/s10434-011-2115-3]

32 **Cao L**, Hu X, Zhang Y, Huang G. Adverse prognosis of clustered-cell versus single-cell micrometastases in pN0 early gastric cancer. *J Surg Oncol* 2011; **103**: 53-56 [PMID: 21031429 DOI: 10.1002/jso.21755]

33 **Kim JJ**, Song KY, Hur H, Hur JI, Park SM, Park CH. Lymph node micrometastasis in node negative early gastric cancer. *Eur J Surg Oncol* 2009; **35**: 409-414 [PMID: 18573635 DOI: 10.1016/j.ejso.2008.05.004]

34 **Ishii K**, Kinami S, Funaki K, Fujita H, Ninomiya I, Fushida S, Fujimura T, Nishimura G, Kayahara M. Detection of sentinel and non-sentinel lymph node micrometastases by complete serial sectioning and immunohistochemical analysis for gastric cancer. *J Exp Clin Cancer Res* 2008; **27**: 7 [PMID: 18577253 DOI: 10.1186/1756-9966-27-7]

35 **Yonemura Y**, Endo Y, Hayashi I, Kawamura T, Yun HY, Bandou E. Proliferative activity of micrometastases in the lymph nodes of patients with gastric cancer. *Br J Surg* 2007; **94**: 731-736 [PMID: 17377930 DOI: 10.1002/bjs.5604]

36 **Miyake K**, Seshimo A, Kameoka S. Assessment of lymph node micrometastasis in early gastric cancer in relation to sentinel nodes. *Gastric Cancer* 2006; **9**: 197-202 [PMID: 16952038 DOI: 10.1007/s10120-006-0378-3]

37 **Morgagni P**, Saragoni L, Scarpi E, Zattini PS, Zaccaroni A, Morgagni D, Bazzocchi F. Lymph node micrometastases in early gastric cancer and their impact on prognosis. *World J Surg* 2003; **27**: 558-561 [PMID: 12715223 DOI: 10.1007/s00268-003-6797-y]

38 **Choi HJ**, Kim YK, Kim YH, Kim SS, Hong SH. Occurrence and prognostic implications of micrometastases in lymph nodes from patients with submucosal gastric carcinoma. *Ann Surg Oncol* 2002; **9**: 13-19 [PMID: 11829425]

39 **Morgagni P**, Saragoni L, Folli S, Gaudio M, Scarpi E, Bazzocchi F, Marra GA, Vio A. Lymph node micrometastases in patients with early gastric cancer: experience with 139 patients. *Ann Surg Oncol* 2001; **8**: 170-174 [PMID: 11258783]

40 **Fukagawa T**, Sasako M, Mann GB, Sano T, Katai H, Maruyama K, Nakanishi Y, Shimoda T. Immunohistochemically detected micrometastases of the lymph nodes in patients with gastric carcinoma. *Cancer* 2001; **92**: 753-760 [PMID: 11550144]

41 **Nakajo A**, Natsugoe S, Ishigami S, Matsumoto M, Nakashima S, Hokita S, Baba M, Takao S, Aikou T. Detection and prediction of micrometastasis in the lymph nodes of patients with pN0 gastric cancer. *Ann Surg Oncol* 2001; **8**: 158-162 [PMID: 11258781]

42 **Harrison LE**, Choe JK, Goldstein M, Meridian A, Kim SH, Clarke K. Prognostic significance of immunohistochemical micrometastases in node negative gastric cancer patients. *J Surg Oncol* 2000; **73**: 153-157 [PMID: 10738269]

43 **Kubota K**, Nakanishi H, Hiki N, Shimizu N, Tsuji E, Yamaguchi H, Mafune K, Tange T, Tatematsu M, Kaminishi M. Quantitative detection of micrometastases in the lymph nodes of gastric cancer patients with real-time RT-PCR: a comparative study with immunohistochemistry. *Int J Cancer* 2003; **105**: 136-143 [PMID: 12672044 DOI: 10.1002/ijc.11031]

44 **Nicholl MB**, Elashoff D, Takeuchi H, Morton DL, Hoon DS. Molecular upstaging based on paraffin-embedded sentinel lymph nodes: ten-year follow-up confirms prognostic utility in melanoma patients. *Ann Surg* 2011; **253**: 116-122 [PMID: 21135695 DOI: 10.1097/SLA.0b013e3181fca894]

45 **Jafferbhoy S**, McWilliams B. Clinical significance and management of sentinel node micrometastasis in invasive breast cancer. *Clin Breast Cancer* 2012; **12**: 308-312 [PMID: 23039999 DOI: 10.1016/j.clbc.2012.07.012]

46 **Yanagita S**, Natsugoe S, Uenosono Y, Kozono T, Ehi K, Arigami T, Arima H, Ishigami S, Aikou T. Sentinel node micrometastases have high proliferative potential in gastric cancer. *J Surg Res* 2008; **145**: 238-243 [PMID: 17603078 DOI: 10.1016/j.jss.2007.04.037]

47 **Shimizu Y**, Takeuchi H, Sakakura Y, Saikawa Y, Nakahara T, Mukai M, Kitajima M, Kitagawa Y. Molecular detection of sentinel node micrometastases in patients with clinical N0 gastric carcinoma with real-time multiplex reverse transcription-polymerase chain reaction assay. *Ann Surg Oncol* 2012; **19**: 469-477 [PMID: 22065193 DOI: 10.1245/s10434-011-2122-4]

48 **Kinami S**, Fujimura T, Ojima E, Fushida S, Ojima T, Funaki H, Fujita H, Takamura H, Ninomiya I, Nishimura G, Kayahara M, Ohta T, Yoh Z. PTD classification: proposal for a new classification of gastric cancer location based on physiological lymphatic flow. *Int J Clin Oncol* 2008; **13**: 320-329 [PMID: 18704632 DOI: 10.1007/s10147-007-0755-x]

49 **Bok GH**, Kim YJ, Jin SY, Chun CG, Lee TH, Kim HG, Jeon SR, Cho JY. Endoscopic submucosal dissection with sentinel node navigation surgery for early gastric cancer. *Endoscopy* 2012; **44**: 953-956 [PMID: 22987216 DOI: 10.1055/s-0032-1310162]

**P-Reviewers:** Luo HS, Silva MF, Syam af **S-Editor:** Ma YJ **L-Editor:** **E-Editor:**

**Table 1 Immunohistochemical studies in gastric cancer patients with histological node negatively diagnosed by hematoxylin-eosin staining *n* (%)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **year** | **Marker** | **Depth of tumor** | **No. of**  | **No. of micrometastatic** | **5-yr**  | ***P* value** |
|  |  |  | **invasion** | **patients** | **patients** | **survival** |  |
| Maehara *et al*[4] | 1996 | CAM5.2 | T1 | 34 | 8 (23.5) | N/A | N/A |
| Cai *et al*[1] | 2000 | CAM5.2 | T1b | 69 | 17 (24.6) | 82% *vs* 100% | < 0.01 |
| Harrison *et al*[42] | 2000 | CAM5.2 | T1-T4 | 25 | 9 (36)  | 35% *vs* 66% | 0.048 |
| Nakajo *et al*[41] | 2001 | AE1/AE3 | T1-T3 | 67 | 10 (14.9) | N/A | N/A |
| Fukagawa *et al*[40] | 2001 | AE1/AE3 | T2-T3 | 107 | 38 (35.5) | 94% *vs* 89%  | 0.86 |
| Morgagni *et al*[39] | 2001 | MNF116 | T1 | 139 | 24 (17.3) | 87% *vs* 88%  | 0.6564 |
| Choi *et al*[38] | 2002 | 35βH11 | Tib | 88 | 28 (31.8) | 92.9% *vs* 95% | 0.6836 |
| Yasuda *et al*[29] | 2002 | CAM5.2 | T2-T4a | 64 | 20 (31.3) | 66% *vs* 95% | < 0.01 |
| Morgagni *et al*[37] | 2003 | MNF116 | T1 | 300 | 30 (10) | 94% *vs* 89% | 0.7794 |
| Miyake *et al*[36] | 2006 | AE1/AE3 | T1 | 120 | 27 (22.5) | N/A | N/A |
| Yonemura *et al*[35] | 2007 | AE1/AE3 | T1-T4 | 308 | 37 (12) | N/A | N/A |
| Kim *et al*[30] | 2008 | AE1/AE3 | T1-T4a | 184 | 31 (16.8) | 58.5% *vs* 91.8% | < 0.001 |
| Ishii *et al*[34] | 2008 | O.N.352 | T1b-T2 | 35 | 4 (11) | N/A | N/A |
| Kim *et al*[33] | 2009 | AE1/AE3 | T1 | 90 | 9 (10) | N/A | N/A |
| Cao *et al*[32] | 2011 | AE1/AE3 | T1 | 160 | 34 (21.3) | 55.9% *vs* 92.9% | < 0.001 |
| Wang *et al*[31] | 2011 | AE1/AE3 | T1-T3 | 191 | 54 (28.3) | 27.8% *vs* 87.1% | < 0.001 |

T1: Invasion of lamina propria or submucosa; T3: Invasion of subserosa; T4: Penetration of serosa without invading adjacent structures (T4a) or invasion of adjacent structures; N/A: not applicable.

|  |
| --- |
| **Table 2 reverse transcription-polymerase chain reaction studies in gastric cancer patients with histological node negativity diagnosed by hematoxylin-eosin staining *n* (%)** |
|
| **Ref.** | **Year** | **Markers** | **Depth of tumor invasion** | **No. of patients** | **No. of total LNs** | **No. of micrometastatic patients** | **Outcomes** |
|
|
| Okada *et al*[16] | 2001 | CEA, CK20, MAGE3 | T1-T4a | 24 | 335 | 10 (41.7) | N/A |
|
| Matsumoto *et al*[17] | 2002 | CEA | T1-T4 | 50 | 312 | 14 (28) | N/A |
| Arigami *et al*[18] | 2005 | CEA | T1-T3 | 80 | 1862 | 25 (31.3)  | N/A |
| Sonoda *et al*[24] | 2006 | MUC2, TFF1 | T1 | 33 | 310 | 11 (33) | N/A |

T1: Invasion of lamina propria or submucosa; T3: Invasion of subserosa; T4: Penetration of serosa without invading adjacent structures (T4a) or invasion of adjacent structures; N/A: not applicable, LN: Lymph node; RT-PCR: Reverse transcription-polymerase chain reaction; CEA: Carcinoembryonic antigen; CK: Cytokeratin.