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

**ESPS Manuscript NO: 11438**

**Columns: RETROSPECTIVE STUDY**

**Clinicopathologic factors and molecular markers related to lymph node metastasis in early gastric cancer**

Jin EH *et al*. Predictive factors for lymph node metastasis

Eun Hyo Jin, Dong Ho Lee, Sung-Ae Jung, Ki-Nam Shim, Ji Yeon Seo, Nayoung Kim, Cheol Min Shin, Hyuk Yoon,Hyun Chae Jung

**Eun Hyo Jin, Ji Yeon Seo, Hyun Chae Jung**, Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul 110-744, South Korea

**Dong Ho Lee, Nayoung Kim, Cheol Min Shin, Hyuk Yoon,** Department of Internal Medicine, Seoul National University Bundang Hospital, Gyeonggi-do 463-707, South Korea

**Sung-Ae Jung, Ki-Nam Shim,** Department of Internal Medicine, Ewha Womans University College of Medicine, Seoul 158-710, South Korea

**Author contributions:** Jin EH, Lee DH designed research; Jin EH, Seo JY, Jung HC performed research; Kim N, Shin CM contributed new reagents or analytic tools; Yoon H, Shim KN analyzed data; Jin EH wrote the paper.

**Correspondence to: Dong Ho Lee, MD, PhD, Professor,** Department of Internal Medicine, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam, Gyeonggi-do 463-707, South Korea. [dhljohn@snubh.org](mailto:dhljohn@snubh.org)

**Telephone:** +82-31-7877006 **Fax:** +82-31-7874051

**Received:** May 21, 2014 **Revised:** July 9, 2014

**Accepted:** July 24, 2014

**Published online:**

**Abstract**

**AIM:** To analyze predictive factors for lymph node metastasis in early gastric cancer.

**METHODS:** We analyzed 1104 patients with early gastric cancer (EGC) who underwent a gastrectomy with lymph-node dissection from May 2003 through July 2011. The clinicopathologic factors and molecular markers were assessed as predictors for lymph node metastasis. Molecular markers such as microsatellite instability, human mutL homolog 1, p53, epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) were included. The *χ*2 test and logistic regression analysis were used to determine clinicopathologic parameters.

**RESULTS:** Lymph node metastasis was observed in 104 (9.4%) of 1104 patients. Among 104 cases of lymph node positive patients, 24 patients (3.8%) were mucosal cancers and 80 patients (16.7%) were submucosal. According to histologic evaluation, the number of lymph node metastasis found was 4 (1.7%) for well differentiated tubular adenocarcinoma, 45 (11.3%) for moderately differentiated tubular adenocarcinoma, 36 (14.8%) for poorly differentiated tubular adenocarcinoma, and 19 (8.4%) for signet ring cell carcinoma. Of 690 EGC cases, 77 cases (11.2%) showed EGFR overexpression. HER2 overexpression was present in 110 cases (27.1%) of 406 EGC patients. With multivariate analysis, female gender (odds ratio 2.281, *P* = 0.009), presence of lymphovascular invasion (odds ratio 10.950, *P* < 0.0001), diameter (≥ 20 mm, odds ratio 3.173, *P* = 0.01), and EGFR overexpression (odds ratio 2.185, *P* = 0.044) were independent risk factors for lymph node involvement.

**CONCLUSION:** Female gender, tumor size, lymphovascular invasion and EGFR overexpression were predictive risk factors for lymph node metastasis in EGC.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words**: Receptor; Epidermal growth factor; Stomach neoplasms; Carcinoma; Neoplasm metastasis; Lymph node

**Core tip:** We analyzed the factors related lymph node metastasis in early gastric cancer. The factors were not only clinicopathologic finding but also molecular biomarkers. It is unique because of the first study about biomarker related with metastatic lymph node in early gastric cancer.

Jin EH, Lee DH, Jung SA, Shim KN, Seo JY, Kim N, Shin CM, Yoon H,Jung HC.Clinicopathologic factors and molecular markers related to lymph node metastasis in early gastric cancer. *World J Gastroenterol* 2014; In press

**INTRODUCTION**

Early gastric cancer (EGC) is deﬁned as cancer invasion conﬁned to the mucosa or submucosa, irrespective of lymph node metastasis[[1](file:///C:\Users\SNUH\Dropbox\EGC_논문\새로수정한%20논문\R08386%20Jin_EGC_final-edited-cleaned.doc#_ENREF_1),[2](file:///C:\Users\SNUH\Dropbox\EGC_논문\새로수정한%20논문\R08386%20Jin_EGC_final-edited-cleaned.doc#_ENREF_2)].Radical gastrectomy with lymph node dissection is the procedure of choice for EGC. Because the prognosis of patients with EGC has improved, the treatment strategies for EGC now include the improvement of quality of life.

Recently, endoscopic mucosal resection (EMR) has been widely accepted as an alternative treatment to open surgery for early gastric cancer without lymph node metastasis (LNM)[[3](file:///C:\Users\SNUH\Dropbox\EGC_논문\새로수정한%20논문\R08386%20Jin_EGC_final-edited-cleaned.doc#_ENREF_3),[4](file:///C:\Users\SNUH\Dropbox\EGC_논문\새로수정한%20논문\R08386%20Jin_EGC_final-edited-cleaned.doc#_ENREF_4)].EMR preserves gastric function and maintains a high quality of life, while extensive surgery carries a significant risk of morbidity and mortality. However, the indications for EMR are limited to EGC with elevated lesions < 2 cm in diameter and differentiated mucosal cancer without ulceration[4]. An endoscopic technique has included endoscopic submucosal dissection (ESD) that can be used to remove a larger amount of tumor en bloc with a negative safety margin[5]. In order to apply endoscopic techniques such as EMR/ESD to treat EGC, the absence of lymph node metastasis must be confirmed. Identifying patients at high risk for LNM is important for the application of a minimally-invasive endoscopic technique.

Several molecular markers have been reported to be useful predictors for prognosis of gastric cancer. Microsatellite instability (MSI) is a form of genomic instability that is associated with defective DNA mismatch repair in tumors[6]. In gastric cancer, the frequency of a microsatellite instability-high (MSI-H) phenotype was reported to range from 8.2% to 37%[7,8]. Several studies have shown that MSI in gastric cancers was an independent predictive factor of lower LNM and improved survival[9]. In addition, MSI was directly associated with the function of a mismatch repair gene such as human mutL homolog 1 (hMLH1)[10]. A study showed that hMLH1 methylation plays a probable role in the advanced stages of tumor progression[11]. In addition, mutation of the p53 gene is one of the most frequent genetic abnormalities associated with gastric cancer; it is associated with lymph node metastasis in EGC[12]. Moreover, epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) overexpression were associated with disease recurrence and poor prognosis in gastric cancer patients[13,14]. Thus, the aim of this study was to identify the clinicopathologic factors and molecular markers related lymph node metastasis and to identify high risk patients for minimal invasive therapy.

**MATERIALS AND METHODS**

***Patients***

A retrospective review identified 1104 patients with EGC who underwent a radical gastrectomy with regional lymph-node dissection from May 2003 through July 2011 at Seoul National University Bundang Hospital (Seoul, South Korea). This study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB No. B-1308-214-101). Patients were excluded if they had a recurrence or multifocal gastric cancer. The Histologic type was classified according to the World Health Organization classification for gastric cancer. Undifferentiated gastric carcinoma included poorly differentiated tubular adenocarcinoma (PD) and signet ring cell carcinoma (SRC). Well-differentiated (WD) and moderately-differentiated tubular adenocarcinoma (MD) were classified as the differentiated type. The relationship between the various clinicopathologic factors, molecular markers and lymph node metastasis were analyzed to identify the risk factors that were predictive of lymph node metastasis. These factors included: age (< 60 years or ≥ 60 years), sex, tumor size, location (upper third, middle third, or lower third), gross type of lesion (elevated, depressed, flat, or mixed), depth of invasion, lymphatic-vascular involvement, and histological type. Molecular markers such as MSI, hMLH1, p53, EGFR and HER2 were analyzed.

The Japanese classification of gastric carcinoma was used to designate the gross type of tumor: type I (protruded), type IIa (superficial elevated), type IIb (flat), type IIc (superficial depressed), and type III (excavated)[15]. Type I, type IIa, and a combination of these two types with IIb were classified as the elevated type. Type IIb was defined as a flat type. Type IIc and III lesions, as well as the combined lesions, were defined as the depressed type. Both the elevated and depressed types, such as type IIa and IIc, were classified as mixed types.

***Microsatellite instability analysis***

DNA was obtained from formalin-fixed, paraffin-embedded surgical sections. DNA was extracted from harvested tumor cells by standard proteinase-K digestion and phenol/chloroform extraction. Normal DNA was extracted from the surrounding normal tissue. Five microsatellite markers originally recommended by a NCI workshop on MSI (BAT-25, BAT-26, D2S123, D5S346 and D17S250) were used to analyze paired normal and tumor DNA for MSI. According to the guidelines of the international workshop of NCI, tumors were classified as MSI-H when at least 2 of the 5 markers displayed novel bands, MSI-low (MSI-L) when additional alleles were found with one of the five markers, and microsatellite stable (MSS) when all microsatellite markers examined displayed identical patterns in both tumor and normal tissue.

***Immunohistochemistry***

Core tissue biopsy specimens (2 mm in greatest dimension) were obtained from individual paraffin-embedded tumors (donor blocks) and arranged in new recipient blocks (tissue microarray blocks), using a trephine apparatus (Superbiochips Laboratories, Seoul, South Korea). Three separate core samples per tumor were obtained to counter the effects of tumor heterogeneity. Sections (4 mm) were cut from each tissue microarray block, deparaffinized, and dehydrated. Immunohistochemical staining for hMLH1, p53, EGFR, HER-2 was performed as previously described[16,17]. Immunohistochemical expression of HER-2 was scored using DAKO-Hercep Test kits as follows: score 0, no membrane staining at all or membrane staining in < 10% of tumor cells; score 1+, faint/barely perceptible partial membrane staining in > 10% of tumor cells; score 2+, weak to moderate staining of entire membrane in > 10% of tumor cells; and score 3+, strong staining of entire membrane in > 10% of tumor cells. Scores of 0 and 1+ were considered negative for HER-2 overexpression, and scores of 2+ and 3+ were considered positive. EGFR immunopositivity was scored by using the instructions supplied with the EGFR PharmDx kits; scores of 2+ and 3+ indicated overexpression.

***Statistical analysis***

To identify the predictive factors of lymph node metastasis, the data were analyzed by using Pearson’s *χ*2 test and an unpaired Student’s *t*-test. Multivariate logistic regression analysis was then performed to evaluate the risk factors for LNM. *P* < 0.05 was considered to be statistically significant. Statistical calculations were performed using IBM SPSS (version 19).

**RESULTS**

Of the 1104 patients with EGC evaluation, the mean age was 58.5 years (range: 25-86 years). This study included 709 men and 395 women. The mean tumor size was 27.8 mm. Mucosal cancers were 625 (56.6%) and submucosal cancers were 479 (43.4%). According histologic classification, WD was 236 (21.4%), MD was 398 (36.1%), PD was 243 (22.0%), and SRCC was 227 (20.6%). In 104 of 1104 (9.4%) patients, pathologic specimens contained LNM (Table 1).

With molecular marker analysis, 909 (90.1%) of 1,009 EGCs showed MSS. MSI-L was observed in 3.1% and MSI-H was observed in 6.8% of EGCs. Of 764 patients, 48 (6.3%) were deemed to have loss of hMLH1, while 716 (93.7%) had expression of hMLH1. Loss of p53 was seen in 651 (62.2%) of 716 patients. Of 690 EGC cases, 77 cases (11.2%) showed EGFR overexpression. In addition, HER2 overexpression was found in 110 cases (27.1%) of 406 EGC patients (Table 2).

The respective rate of LNM was 3.8% among lesions confined to the mucosa and 16.7% among those infiltrating the submucosa (sm1 cancer, 7.3%; sm2 cancer, 21.6%; sm3 cancer, 20.3%). According to histologic evaluation, the number of lymph node metastasis found was 4 (1.7%) for WD cancer, 45 (11.3%) for MD cancer, 36 (14.8%) for PD cancer, and 19 (8.4%) for SRC cancer. Lymph node metastasis was more frequent in MD than SRC cancers.

With univariate analysis, lymph node metastasis was associated with age (≥ 60 years), female gender, tumor size (≥ 20 mm), macroscopic type, depth of invasion, lymphovascular invasion, and histological type (Table 3). Among molecular markers, EGFR overexpression was significantly associated with lymph node metastasis in early gastric cancer (Table 4). Of these factors, female gender, large tumor size (≥ 20 mm), lymphovascular invasion, and EGFR overexpression were independently associated with lymph node metastasis by multivariate logistic regression analysis (Table 5).

**DISCUSSION**

Gastric cancer is the second leading cause of cancer-related deaths worldwide[18], and the highest mortality rates of AGC have been reported in East Asia including Japan and South Korea[19,20]. In contrast, EGC has a good prognosis with surgical treatment[21]. In South Korea, the proportion of EGC increased to 47.4% of all diagnosed gastric cancers in 2004[22]. This was attributed to widely-performed upper gastrointestinal endoscopy screening programs. Because the prognosis of patients with EGC has improved with radical gastrectomy, the treatment strategies for EGC now include the improvement of quality of life. Endoscopic resection such as EMR/ESD can be applied to EGC without lymph node metastasis instead of a radical gastrectomy[3,4].

Preoperative evaluation of for lymph node metastasis is the most important consideration, when deciding on a treatment strategy for EGC[23]. A number of researchers have attempted to identify factors predictive of LNM in EGC. The size of the primary tumor, histologic type, lymphatic or venous invasion, and depth of invasion are known to be associated with regional lymph node metastases in EGC[24-27]. In addition, multi-detector computerized tomography (MDCT) and/or endoscopic ultrasound (EUS) were generally employed to detect metastatic lymphadenopathy. However, the overall diagnostic accuracy of MDCT imaging for LNM in EGC has been reported to range from 37% to 70 %, whereas that of EUS was reported to range from 39% to 90%[28-30]. Reported sensitivity and specificity of EUS to detect LNM in gastric cancer varies widely: sensitivity from 59.5% to 97.2% and specificity from 40.0% to 100%[1]. Using MDCT, studies showed a sensitivity of 84.2% and a specificity of 84.0%[1]. Preoperational accuracy of LNM staging using EUS or CT was inadequate for the prediction of the pathological N stage in order to determine the treatment plan.

Not only clinicopathologic factors but also molecular markers can be predictors for lymph node metastasis in gastric cancer patients[13,14]. The human epidermal growth factor receptor (HER) consists of four transmembrane tyrosine kinase receptors, which have a similar structure, are named ErbB1 (HER1, also known as EGFR), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4)[31]. Alterations in the expression of receptor tyrosine kinases pathways including EGFR, HER2 were proven to be critical factors for cancer cell survival[32]. EGFR expression correlated with disease recurrence and poorer survival in gastric cancer patients[13,14]. Furthermore, HER2 has predictive ability for estimating overall survival in gastric cancer patients and may be useful for determining their prognosis[14]. However, EGFR positivity, but not HER2 positivity, was associated with poor patient outcomes after a curative resection of stage II/III gastric cancer[33]. In our study, EGFR overexpression was an independent risk factor for lymph node metastasis in EGC patients. However, HER2 overexpression was not associated with lymph node metastasis. Previous studies have reported EGFR or HER2 overexpression in gastric cancer regardless of stage. Only a handful of studies were limited to early gastric cancer for EGFR or HER2 overexpression.

In this study, the clinicopathologic risk factors for lymph node metastasis were found to be female gender, the presence of lymph-vascular involvement, and tumor size > 2 cm. Lymph-vascular involvement and tumor size were consistent to those reported by previous studies. Interestingly, female gender was an independent predictive factor for LNM; this was a unique finding compared to a previous report. Male to female gender ratio was 1:1.08 among young patients (age < 40 years) and 2.5:1 in older patients (age > 40 years)[34]. Age-standardized and cumulative incidence rates of gastric cancer in males are approximately double those of females. This predominance of gastric cancer in males is related to a 10-to-15 year delay in female gastric cancer. The prevalence of gastric cancer in females is similar to that of males only after menopause[35]. This finding suggested that sex hormones (estrogens) protect woman from gastric cancer. In previous studies in South Korea, the incidence of lymph node metastasis in female EGC was higher than in male EGC and female gender is a predictive risk factor for lymph node metastasis[36,37]. However, this gender difference of lymph node metastasis in EGC was not shown in other populations. It is extremely difficult to generalize risk factors in all populations.

Some studies have reported a lower rate of LNM and better prognosis in EGC with SRC histology than cancer with PD[38,39]. Previous studies have reported a rate of LNM with SRC histology to range from 5.7% to 15%[23,38,40]. Our study found that the rate of LNM with SRC histology was lower than PD cancer and even MD (18.3% *vs* 34.6% and 18.3% *vs* 43.3%). However, the frequency of LNM in mucosal cancer with SRC histology was much higher than mucosal cancer with differentiated histology (0.0% in WD, 2.9% in MD, 10.6% in PD, and 9.6% in SRC). Based on our study, mucosal EGC with SRC histology still had a higher risk of LNM than differentiated EGC. We suggest that the application of EMR/ESD in EGC with SRC was inadequate (Table 6).

This study had some limitations. First, it was a retrospective study based on medical records in a single center. Because of its retrospective nature, we could not collect additional data such as family history, comorbidity, or life style. Second, we analyzed pathologic findings based on postoperative examination of the resected specimen. At the time of endoscopy, the endoscopist subjectively estimated tumor size and reported gross findings and the presence of ulceration; this may have caused a discrepancy between endoscopic findings and pathologic findings. Considering that the preoperative clinical decision was made by endoscopic findings, it may be difficult to apply our pathologic characteristics to determine treatment plans. However, endoscopic resection criteria including tumor size, presence of ulceration and gross finding were based on pathologic evaluation of a surgical specimen that was fixed in formalin[41]. In addition, endoscopic findings had an inter-observer variability. Third, not all surgical specimens underwent immunohistochemical staining. Finally, there is the problem of selection bias. To perform immunohistochemical staining on all the postoperative specimens in EGC is not cost effective. However, EGFR overexpression correlated with LNM and a poorer prognosis; therefore, EGFR targeted therapy may be considered as adjuvant therapy postoperatively for high risk patients with lymph node metastasis in EGC. Despite of these limitations, our study has significance because we analyzed not only clinicopathologic factors but also molecular markers for a high risk of LNM in EGC patients.

Female gender, tumor size, and lymphovascular invasion were predictive risk factors for LNM in EGC. In addition, EGFR overexpression was identified as an independent prognostic factor with multivariate analysis; thus, suggesting that EGFR overexpression is likely to be one of the potential risk factor for LNM in EGC.

**COMMENTS**

***Background***

Endoscopic resection can be an alternative treatment to a radical gastrectomy for early gastric cancer without lymph node metastasis. The possible presence of lymph node metastasis is critical for the selection of the appropriate treatment strategy for early gastric cancer.

***Research frontiers***

This study to determine the predictive factors for lymph node metastasis in early gastric cancer (EGC). This is significant because the first research showed the biomarkers were related with lymph node metastasis in early gastric cancer.

***Innovations and breakthroughs***

In this study, epidermal growth factor receptor (EGFR) overexpression is one of the potential risk factors for lymph node metastasis in EGC.

***Applications***

The results suggest that patients who had EGFR overexpression in EGC were considered as high risk group for lymph node metastasis. Physicians pay attention to decide the treatment strategy.

***Terminology***

Microsatellite instability is the condition of genetic hypermutability that results from impaired DNA mismatch repair. The EGFR is the cell-surface receptor for members of the epidermal growth factor-family of extracellular protein ligands. Human epidermal growth factor receptor 2 is a member of the EGFR/ERBB family.

***Peer review***

This study analyzed 1104 patients with early gastric cancer who underwent a gastrectomy with lymph-node dissection. The goal was to assess predictive factors for lymph node metastasis in early gastric cancer. This is a general look at a specific tumor work up. The data suggest that EGFR overexpression is likely to be one of the potential risk factors for lymph node metastasis in EGC. This information may be value in helping the management of these subjects.

**REFERENCES**

1 **Kwee RM**, Kwee TC. Predicting lymph node status in early gastric cancer. *Gastric Cancer* 2008; **11**: 134-148 [PMID: 18825308 DOI: 10.1007/s10120-008-0476-5]

2 **Japanese Gastric Cancer Association.** Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; **14**: 101-112 [PMID: 21573743 DOI: 10.1007/s10120-011-0041-5]

3 **Gotoda T**, Yamamoto H, Soetikno RM. Endoscopic submucosal dissection of early gastric cancer. *J Gastroenterol* 2006; **41**: 929-942 [PMID: 17096062]

4 **Ono H**, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S. Endoscopic mucosal resection for treatment of early gastric cancer. *Gut* 2001; **48**: 225-229 [PMID: 11156645]

5 **Miyamoto S**, Muto M, Hamamoto Y, Boku N, Ohtsu A, Baba S, Yoshida M, Ohkuwa M, Hosokawa K, Tajiri H, Yoshida S. A new technique for endoscopic mucosal resection with an insulated-tip electrosurgical knife improves the completeness of resection of intramucosal gastric neoplasms. *Gastrointest Endosc* 2002; **55**: 576-581 [PMID: 11923778]

6 **Boland CR**, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248-5257 [PMID: 9823339]

7 **Wu M**, Semba S, Oue N, Ikehara N, Yasui W, Yokozaki H. BRAF/K-ras mutation, microsatellite instability, and promoter hypermethylation of hMLH1/MGMT in human gastric carcinomas. *Gastric Cancer* 2004; **7**: 246-253 [PMID: 15616773]

8 **Seo HM**, Chang YS, Joo SH, Kim YW, Park YK, Hong SW, Lee SH. Clinicopathologic characteristics and outcomes of gastric cancers with the MSI-H phenotype. *J Surg Oncol* 2009; **99**: 143-147 [PMID: 19117018 DOI: 10.1002/jso.21220]

9 **Tamura G**, Sakata K, Nishizuka S, Maesawa C, Suzuki Y, Terashima M, Eda Y, Satodate R. Allelotype of adenoma and differentiated adenocarcinoma of the stomach. *J Pathol* 1996; **180**: 371-377 [PMID: 9014856]

10 **Li GM**. Mechanisms and functions of DNA mismatch repair. *Cell Res* 2008; **18**: 85-98 [PMID: 18157157]

11 **Moghbeli M**, Moaven O, Memar B, Raziei HR, Aarabi A, Dadkhah E, Forghanifard MM, Manzari F, Abbaszadegan MR. Role of hMLH1 and E-cadherin promoter methylation in gastric cancer progression. *J Gastrointest Cancer* 2014; **45**: 40-47 [PMID: 24022108 DOI: 10.1007/s12029-013-9548-9]

12 **Xiangming C**, Hokita S, Natsugoe S, Tanabe G, Baba M, Takao S, Kuroshima K, Aikou T. Cooccurrence of reduced expression of alpha-catenin and overexpression of p53 is a predictor of lymph node metastasis in early gastric cancer. *Oncology* 1999; **57**: 131-137 [PMID: 10461060]

13 **Galizia G**, Lieto E, Orditura M, Castellano P, Mura AL, Imperatore V, Pinto M, Zamboli A, De Vita F, Ferraraccio F. Epidermal growth factor receptor (EGFR) expression is associated with a worse prognosis in gastric cancer patients undergoing curative surgery. *World J Surg* 2007; **31**: 1458-1468 [PMID: 17516110]

14 **Chen C**, Yang JM, Hu TT, Xu TJ, Yan G, Hu SL, Wei W, Xu WP. Prognostic role of human epidermal growth factor receptor in gastric cancer: a systematic review and meta-analysis. *Arch Med Res* 2013; **44**: 380-389 [PMID: 23871709 DOI: 10.1016/j.arcmed.2013.07.001]

15 **Sano T**, Aiko T. New Japanese classifications and treatment guidelines for gastric cancer: revision concepts and major revised points. *Gastric Cancer* 2011; **14**: 97-100 [PMID: 21573921 DOI: 10.1007/s10120-011-0040-6]

16 **Choi JS**, Kim MA, Lee HE, Lee HS, Kim WH. Mucinous gastric carcinomas: clinicopathologic and molecular analyses. *Cancer* 2009; **115**: 3581-3590 [PMID: 19479974 DOI: 10.1002/cncr.24422]

17 **Kang GH**, Yoon GS, Lee HK, Kwon YM, Ro JY. Clinicopathologic characteristics of replication error-positive gastric carcinoma. *Mod Pathol* 1999; **12**: 15-20 [PMID: 9950157]

18 **Alberts SR**, Cervantes A, van de Velde CJ. Gastric cancer: epidemiology, pathology and treatment. *Ann Oncol* 2003; **14** Suppl 2: ii31-ii36 [PMID: 12810455]

19 **Inoue M**, Tajima K, Kitoh T, Sakamoto J, Yamamura Y, Sato T, Suzuki R, Koshikawa T, Nakamura S, Suchi T. Changes in histopathological features of gastric carcinoma over a 26-year period (1965-1990). *J Surg Oncol* 1993; **53**: 256-260 [PMID: 8341058]

20 **Lee HJ**, Yang HK, Ahn YO. Gastric cancer in Korea. *Gastric Cancer* 2002; **5**: 177-182 [PMID: 12378346]

21 **Sano T**, Sasako M, Kinoshita T, Maruyama K. Recurrence of early gastric cancer. Follow-up of 1475 patients and review of the Japanese literature. *Cancer* 1993; **72**: 3174-3178 [PMID: 8242540]

22 **Association TICotKGC**. 2004 Nationwide Gastric Cancer Report in Korea*. J Korean Gastric Cancer Assoc* 2007; **7**: 47-54

23 **Tong JH**, Sun Z, Wang ZN, Zhao YH, Huang BJ, Li K, Xu Y, Xu HM. Early gastric cancer with signet-ring cell histologic type: risk factors of lymph node metastasis and indications of endoscopic surgery. *Surgery* 2011; **149**: 356-363 [PMID: 20727560 DOI: 10.1016/j.surg.2010.07.006]

24 **Guadagni S**, Reed PI, Johnston BJ, De Bernardinis G, Catarci M, Valenti M, di Orio F, Carboni M. Early gastric cancer: follow-up after gastrectomy in 159 patients. *Br J Surg* 1993; **80**: 325-328 [PMID: 8472141]

25 **Wu CY**, Chen JT, Chen GH, Yeh HZ. Lymph node metastasis in early gastric cancer: a clinicopathological analysis. *Hepatogastroenterology* 2002; **49**: 1465-1468 [PMID: 12239968]

26 **Boku T**, Nakane Y, Okusa T, Hirozane N, Imabayashi N, Hioki K, Yamamoto M. Strategy for lymphadenectomy of gastric cancer. *Surgery* 1989; **105**: 585-592 [PMID: 2705096]

27 **Fujimoto A**, Ishikawa Y, Akishima-Fukasawa Y, Ito K, Akasaka Y, Tamai S, Maehara T, Kiguchi H, Ogata K, Nishimura C, Miki K, Ishii T. Significance of lymphatic invasion on regional lymph node metastasis in early gastric cancer using LYVE-1 immunohistochemical analysis. *Am J Clin Pathol* 2007; **127**: 82-88 [PMID: 17145628]

28 **Park SR**, Lee JS, Kim CG, Kim HK, Kook MC, Kim YW, Ryu KW, Lee JH, Bae JM, Choi IJ. Endoscopic ultrasound and computed tomography in restaging and predicting prognosis after neoadjuvant chemotherapy in patients with locally advanced gastric cancer. *Cancer* 2008; **112**: 2368-2376 [PMID: 18404697 DOI: 10.1002/cncr.23483.]

29 **Habermann CR**, Weiss F, Riecken R, Honarpisheh H, Bohnacker S, Staedtler C, Dieckmann C, Schoder V, Adam G. Preoperative staging of gastric adenocarcinoma: comparison of helical CT and endoscopic US. *Radiology* 2004; **230**: 465-471 [PMID: 14752188]

30 **Li B**, Zheng P, Zhu Q, Lin J. Accurate preoperative staging of gastric cancer with combined endoscopic ultrasonography and PET-CT. *Tohoku J Exp Med* 2012; **228**: 9-16 [PMID: 22864063]

31 **Jimeno A**, Hidalgo M. Blockade of epidermal growth factor receptor (EGFR) activity. *Crit Rev Oncol Hematol* 2005; **53**: 179-192 [PMID: 15718144]

32 **Ciardiello F**, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001; **7**: 2958-2970 [PMID: 11595683]

33 **Terashima M**, Kitada K, Ochiai A, Ichikawa W, Kurahashi I, Sakuramoto S, Katai H, Sano T, Imamura H, Sasako M. Impact of expression of human epidermal growth factor receptors EGFR and ERBB2 on survival in stage II/III gastric cancer. *Clin Cancer Res* 2012; **18**: 5992-6000 [PMID: 22977193 DOI: 10.1158/1078-0432]

34 **Eguchi T**, Takahashi Y, Yamagata M, Kasahara M, Fujii M. Gastric cancer in young patients. *J Am Coll Surg* 1999; **188**: 22-26 [PMID: 9915238]

35 **Sipponen P**, Correa P. Delayed rise in incidence of gastric cancer in females results in unique sex ratio (M/F) pattern: etiologic hypothesis. *Gastric Cancer* 2002; **5**: 213-219 [PMID: 12491079]

36 **Hwang JY**, Lee HJ, Ryu SW, Kim IH, Sohn SS. Preoperative Predictive Factors of Lymph Node Metastasis in Early Gastric Cancer*. J Korean Surg Soc* 2005; **68**: 457-463

37 **Hyung WJ**, Cheong JH, Kim J, Chen J, Choi SH, Noh SH. Analysis of prognostic factors and gastric cancer specific survival rate in early gastric cancer patients and its clinical implication*. J Korean Surg Soc* 2003; **65**: 309-315

38 **Ha TK**, An JY, Youn HK, Noh JH, Sohn TS, Kim S. Indication for endoscopic mucosal resection in early signet ring cell gastric cancer. *Ann Surg Oncol* 2008; **15**: 508-513 [PMID: 18071825]

39 **Lee JH**, Choi IJ, Kook MC, Nam BH, Kim YW, Ryu KW. Risk factors for lymph node metastasis in patients with early gastric cancer and signet ring cell histology. *Br J Surg* 2010; **97**: 732-736 [PMID: 20235088 DOI: 10.1002/bjs.6941.]

40 **Kim HM**, Pak KH, Chung MJ, Cho JH, Hyung WJ, Noh SH, Kim CB, Lee YC, Song SY, Lee SK. Early gastric cancer of signet ring cell carcinoma is more amenable to endoscopic treatment than is early gastric cancer of poorly differentiated tubular adenocarcinoma in select tumor conditions. *Surg Endosc* 2011; **25**: 3087-3093 [PMID: 21487870 DOI: 10.1007/s00464-011-1674-5.]

41 **Gotoda T**, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer* 2000; **3**: 219-225 [PMID: 11984739]

**P-Reviewer:** Gong JP, Ko S, Li YZ, Stanojevic GZ **S-Editor:** Gou SX

**L-Editor: E-Editor:**

**Table 1 Baseline characteristics of patients with early gastric cancer (*n* = 1104) *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | | **Value** | |
| Age (yr) | |  | |
|  | < 60 | 546 (49.5) | |
|  | ≥ 60 | 558 (50.5) | |
|  | mean ± SD | 58.49 ± 11.63 |
| Gender |  |  | |
|  | Male | 709 (64.2) | |
|  | Female | 395 (35.8) | |
| Size of tumor (mm) | |  | |
|  | < 20 mm | 397 (34.3) | |
|  | ≥ 20 mm | 725 (65.7) | |
|  | mean ± SD | 27.8 ± 17.8 |
| Location |  |  | |
|  | Upper third | 125 (11.3) | |
|  | Middle third | 325 (29.4) | |
|  | Lower third | 654 (59.2) | |
| Macroscopic type | |  | |
|  | Elevated (I, IIa, I + IIa, IIa + IIb) | 86 (7.8) | |
|  | Flat (IIb) | 81 (7.3) | |
|  | Depressed (IIc, III, IIb + III) | 815 (73.8) | |
|  | Mixed | 122 (11.1) | |
| Depth of invasion | |  | |
|  | Mucosa | 625 (56.6) | |
|  | Submucosa |  | |
|  | Sm 1 | 150 (13.6) | |
|  | Sm 2 | 157 (14.2) | |
|  | Sm 3 | 172 (15.6) | |
| Ulcer | |  | |
|  | Absent | 958 (86.8) | |
|  | Present | 146 (13.2) | |
| Lymphovascular invasion | |  | |
|  | Absent | 955 (86.5) | |
|  | Present | 149 (13.5) | |
| Histological type | |  | |
|  | Well differentiated | 236 (21.4) | |
|  | Moderate differentiated | 398 (36.1) | |
|  | Poorly differentiated | 243 (22.0) | |
|  | Signet ring cell | 227 (20.6) | |
| Lymph-node metastasis | |  | |
|  | Negative | 1000 (90.6) | |
|  | Positive | 104 (9.4) | |

Data are expressed as absolute numbers (percentage) or mean ± SD. SM1: Upper third; SM2: Middle third; SM3: Lower third.

**Table 2 Molecular markers of patients with early gastric cancer *n* (%)**

|  |  |  |
| --- | --- | --- |
| **Molecular markers** | | **Value** |
| Microsatellite instability | |  |
|  | MSS | 909 (90.1) |
|  | MSI-L | 31 (3.1) |
|  | MSI-H | 69 (6.8) |
| hMLH1 |  |  |
|  | Loss | 48 (6.3) |
|  | Expression | 716 (93.7) |
| p53 |  |  |
|  | Negative | 651 (62.2) |
|  | Positive | 396 (37.8) |
| EGFR overexpression | |  |
|  | Negative | 613 (88.8) |
|  | positive | 77 (11.2) |
| HER2 overexpression | |  |
|  | Negative | 296 (72.9) |
|  | Positive | 110 (27.1) |

MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high; hMLH1: Human mutL homolog 1; EGFR: Epidermal growth factor receptor; HER2: Human epidermal growth factor receptor 2

**Table 3 Univariate analysis of potential risk factors for lymph node metastasis *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Lymph node metastasis** | | **Presence (*n* = 104)** | **Absence (*n* = 1000)** | ***P* value** |
| **Factor** |  |
| Age (yr) | |  |  | 0.049 |
|  | < 60 | 61 (58.7) | 485 (48.5) |  |
|  | ≥ 60 | 43 (41.3) | 515 (51.5) |  |
| Gender | |  |  | 0.003 |
|  | Male | 53 (51.0) | 656 (65.6) |  |
|  | Female | 51 (49.0) | 344 (34.4) |  |
| Size of tumor (mm) | |  |  | < 0.0001 |
|  | < 20 mm | 9 (8.7) | 370 (37.0) |  |
|  | ≥ 20 mm | 95 (91.3) | 630 (63.0) |  |
| Location | |  |  | 0.389 |
|  | upper third | 9 (8.7) | 116 (11.6) |  |
|  | middle third | 36 (34.6) | 289 (28.9) |  |
|  | lower third | 59 (56.7) | 595 (59.5) |  |
| Macroscopic type | |  |  | < 0.0001 |
|  | Elevated | 7 (6.7) | 78 (7.8) |  |
|  | Flat | 1 (1.0) | 80 (8) |  |
|  | Depressed | 72 (69.2) | 743 (74.3) |  |
|  | Mixed | 24 (23.1) | 98 (9.8) |  |
| Depth of invasion | |  |  | < 0.0001 |
|  | Mucosa | 24 (23.1) | 601 (60.1) |  |
|  | Submucosa | 80 (77.0) | 399 (39.9) |  |
|  | SM1 | 11 (10.6) | 139 (13.9) |  |
|  | SM2 | 34 (32.7) | 123 (12.3) |  |
|  | SM3 | 35 (33.7) | 137 (13.7) |  |
| Ulceration | | |  | 0.222 |
|  | Absent | 86 (9.0) | 872 (91.0) |  |
|  | Present | 18 (12.1) | 128 (85.9) |  |
| Lymphovascular invasion | | |  | < 0.0001 |
|  | Absent | 44 (42.3) | 911 (91.1) |  |
|  | Present | 60 (57.7) | 89 (8.9) |  |
| Histological type | |  |  | < 0.0001 |
|  | Well differentiated | 4 (3.8) | 232 (23.2) |  |
|  | Moderate differentiated | 45 (43.3) | 352 (35.2) |  |
|  | Poorly differentiated | 36 (34.6) | 208 (20.8) |  |
|  | Signet ring cell | 19 (18.3) | 208 (20.8) |  |

SM1: Upper third; SM2: Middle third; SM3: Lower third.

**Table 4 Univariate analysis of predictive molecular markers for lymph node metastasis *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Lymph node metastasis** | **Presence** | **Absence** | ***P* value** |
| Microsatellite instability |  |  | 0.412 |
| MSS | 89 (90.8) | 820 (90.0) |  |
| MSI-L | 1 (1.0) | 30 (3.3) |  |
| MSI-H | 8 (8.2) | 61 (6.7) |  |
| hMLH1 |  |  | 0.703 |
| negative | 5 (7.4) | 43 (6.2) |  |
| positive | 63 (92.6) | 653 (93.8) |  |
| p53 |  |  | 0.773 |
| Negative | 59 (60.8) | 592 (62.3) |  |
| Positive | 38 (39.2) | 358 (37.7) |  |
| EGFR overexpression |  |  | 0.001 |
| negative | 55 (77.5) | 558 (90.1) |  |
| positive | 16 (22.5) | 61 (9.9) |  |
| HER2 overexpression |  |  | 0.084 |
| negative | 33 (84.6) | 263 (71.7) |  |
| positive | 6 (15.4) | 104 (28.3) |  |

MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high; hMLH1: Human mutL homolog 1; EGFR: Epidermal growth factor receptor; HER2: Human epidermal growth factor receptor 2.

**Table 5 Multivariate analysis of potential risk characteristics for lymph node metastasis**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics** | **Odds ratio** | **95%CI** | ***P*-value** |
| Gender (female) | 2.281 | 1.228-4.235 | 0.009 |
| Lymphovascular invasion | 10.950 | 5.418-22.134 | < 0.0001 |
| Diameter (≥ 20 mm) | 3.173 | 1.324-7.603 | 0.01 |
| EGFR | 2.185 | 1.020-4.683 | 0.044 |

EGFR: Epidermal growth factor receptor.

**Table 6 Lymph node metastasis by depth of invasion and histological type *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Lymph node metastasis** | | |
|  | **Presence (*n* = 104)** | **Absence *(n* = 1000)** | **Total** |
| Well differentiated |  |  |  |
| Mucosa | 0 (0.0) | 176 (17.6) | 176 (0.0) |
| Submucosa | 4 (3.8) | 56 (5.6) | 60 (6.7) |
| Moderate differentiated |  |  |  |
| Mucosa | 3 (0.0) | 165 (1.8) | 168 (1.8) |
| Submucosa | 42 (40.4) | 187 (18.7) | 229 (18.3) |
| Poorly differentiated |  |  |  |
| Mucosa | 11 (10.6) | 112 (11.2) | 123 (8.9) |
| Submucosa | 25 (24.0) | 96 (9.6) | 121 (20.7) |
| Signet ring cell |  |  |  |
| Mucosa | 10 (9.6) | 147 (14.7) | 157 (6.4) |
| Submucosa | 9 (8.7) | 61 (6.1) | 70 (12.9) |