JBUON 2015; 20(2): 421-427
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Associated risk factor analysis for positive resection margins after endoscopic submucosal dissection in early-stage gastric cancer

Jing Wen, En-Qiang Linghu, Yun-Sheng Yang, Qing-Sen Liu, Jing Yang, Zhong-Sheng Lu
Department of Gastroenterology, Chinese PLA General Hospital, Beijing 100853, China

Summary

Purpose: *To investigate the associated risk factors and the prognostic impact of positive resection margins after endoscopic submucosal dissection (ESD) of early-stage gastric cancer.*

Methods: *A retrospective analysis of prospectively collected data was performed on 319 consecutive lesions in 316 patients who underwent ESD. Age, gender, surgeons, lesion location, maximum diameter of resected specimens, macroscopic type, depth of tumor invasion and tumor differentiation were evaluated as potential risk factors.*

Results: *A total of 27 (8.5%) patients exhibited positive resection margins after ESD. Among 25 successfully followed-up patients 13 were subjected to gastrectomy, 1 was administered chemotherapy, 2 underwent additional endoscopic resection and 9, who were initially followed-up during a median period of 11.7 months (range 1-40), had neither recurrence nor metastasis. Univariate analysis revealed that age, lesion location, depth of tumor invasion, macroscopic type and tumor differentiation were correlated with positive resection margin. By contrast, multivariate logistic regression analysis showed that only age, tumor differentiation and depth of tumor invasion were independent risk factors of positive resection margins.*

Conclusion: *Age, tumor differentiation and depth of tumor invasion were independent risk factors for post-ESD positive resection margins. This result suggests that older patients, undifferentiated lesions and a greater depth of invasion increase the risk for post-ESD positive resection margins.*

Key words: *early gastric cancer, endoscopic mucosal dissection, resection margins, risk factors*

Introduction

ESD has been widely accepted as an alternative to conventional procedures for complete resection of gastric lesions [1]. With the wide applications of high-definition endoscopy, chromoendoscopy, narrow-band imaging and magnifying endoscopy, the detection rate of gastric cancer has gradually increased in China [2,3]. Similar to the traditional resection of oesophageal cancer, ESD also has the risk of positive resection margins. Given the difficulties in early-stage cancer detection and limitations of minimally invasive endoscopic treatment, the risk of positive resection margins may be even higher in ESD. The borders of colorectal or oesophageal lesions can be clearly recognised by chromoendoscopy with indigo carmine or Lugol staining, whereas the borders of early gastric cancer (EGC) are difficult to confirm when the background mucosa is affected by acute or chronic inflammation. Positive resection margins after endoscopic resection of oesophageal mucosal lesions directly affect the protocol chosen for treatment and prognosis of the disease. Several reports have investigated the risk factors of a positive margin in EGC [4,5]. Most

Correspondence to: Dr. Zhong-Sheng Lu, MD. Department of Gastroenterology, Chinese PLA General Hospital, No.28 Fuxing Road, Haidian District, Beijing 100853, China. Tel: +86 010 66937820, Fax: +86 010 66937820, E-mail: zhongshenglucn@163.com
Received: 23/06/2014; Accepted: 02/11/2014

reports have been published in Japan and Korea. There is no consensus of the final management for positive resection margins and no multi-centre, large-sample trials with long-term follow-up have been reported. To improve the prognosis of EGC treated with ESD further, this study sought to identify the associated risk factors of positive resection margins by analysing data on multiple aspects.

Methods

Patients

We conducted a retrospective analysis of the clinical, endoscopic and pathological data of patients who underwent ESD for the treatment of EGC from January 2006 to January 2013 at the endoscopic centre of Chinese PLA General Hospital. The inclusion criteria were as follows: 1) complete removal of mucosal lesion; 2) post-ESD pathological diagnosis of gastric high-grade intraepithelial neoplasia, intramucosal carcinoma or submucosal carcinoma; and 3) integral records of data.

A total of 316 cases met the inclusion criteria. The main clinical symptoms were stomach ache, abdominal distension or upper abdominal discomfort. This study was conducted in accordance with the declaration of Helsinki after approval from the Ethics Committee of China PLA General Hospital. Written informed consent was obtained from all participants.

ESD procedure

Erbotom ICC200 or VIO®200D (Erbe,Germany) was applied as a high-frequency electrosurgical unit. The lesions were located by endoscopy, followed by narrow band imaging (NBI) magnification and methylene blue staining around the periphery of the lesion. At 0.3-0.5 cm from the margin of the lesion, thermal coagulation markers were made around the lesion at 0.5-cm intervals. The submucosal injection was applied along the margin of the lesion to lift the mucosa. A circumferential mucosal incision was performed approximately 0.5 cm outside the mucosal markings. Dissection was performed along the submucosa, and continued until the mucosa of the lesion was completely dissected. A dual knife or IT-2 knife (forced co 40 w) was used for coagulation haemostasis of small blood vessels, whereas electric haemostatic forceps were applied to stop the bleeding from large ruptured blood vessels.

Pathological examination

The resected specimens were promptly laid out and measured. The lesion was then fixed in 10% formalin. The specimens were sliced into continuous sections at intervals of 2 mm from top to bottom, and embedded in paraffin. Three sections were prepared from each tissue, followed by haematoxylin and eosin staining. The characteristics of the tumor and lateral or vertical resection margins were evaluated by an experienced pathologist (WHK) to avoid misinterpretation. All pathological slices were observed, starting from the top of the specimen to identify the characteristics of the lesion and grade of differentiation, and determine whether the margins, substrate and vasculature were involved. Positive resection margins were defined as the presence of atypical cells (low-grade intraepithelial neoplasia, high-grade intraepithelial neoplasia or invasive cancer) at the lateral and/or vertical resection margins. If the damage of coagulation hindered the pathologic interpretation of atypical cells at the cutting edge of the substrate, the margins were considered to be positive.

Statistics

Variables, such as gender, age, surgeons, lesion location, maximum diameter of resected specimens, macroscopic type, depth of tumor invasion and tumor differentiation, were analysed as potential risk factors. The normally distributed data were expressed as mean ± SD, whereas the non-normal data were expressed as median. Statistical analysis was performed using x^2 and Mann-Whitney *U* tests. Associations between the potential risk factors and positive margins were analysed using binary logistic regression models. A probability level of $p < 0.05$ was set for statistical significance. Data processing was performed using SPSS software package (SPSS Inc., Chicago, Ill).

Results

General data

This study was a retrospective analysis of prospectively collected data from 316 patients with 319 lesions who met the inclusion criteria. Of the patients 234 (74.1%) were males. The male-to-female ratio was 2.85:1. The age of the patients ranged from 35 to 83 years (mean 63±8.6 years). Three patients underwent ESD for the treatment of 2 lesions.

Positive resection margins and follow-up data

All ESD procedures were performed by two skilled endoscopists. The mean maximum diameter was 3.3±1.3 cm (range 0.8-8). Twenty seven (8.5%) lesions after ESD exhibited positive resection margins, among which 19 (6%) were lateral and 8 (2.5%) were vertical resection margins. Four (2.5%) patients had vascular invasion or submucosal lymph node metastasis.

The range of the resections were double-checked by reviewing the surgical videos of the 19 patients with lateral resection margins based on the pathol-

ogy findings. The patients whose resection extent could be exactly determined were re-examined one month after the operation. The patients with uncertain edges based on the videos required further endoscopic evaluation, followed by ESD or endoscopic mucosal resection (EMR) when suspicious positive resection margins were detected and a re-examination one month later. Patients without positive margins had endoscopy at 3 months, 6 months, and 1 year after ESD in parallel with abdominal CT at 6 months and 1 year. Patients with positive vertical resection margins underwent gastrectomy or chemotherapy. All treatment protocols were selected according to the patients' condition, and performed according to the patients' or their relatives' willingness. Among 27 patients, 25 (92.6%) were successfully followed-up for one to 78 months with an average of 23.2 months, and all remain alive. Two patients were lost to follow-up. More detailed data on positive resection margins and follow-up are shown in Table 1.

Table 1. Resection margins and follow-up data

Patient no.	*Location*	*Differentiation (depth)*	*Positive margins*	*Supplementary treatment*	*Follow-up (months)*
1	Prepyloric region	Differentiated (M3)	Vertical resection margin	Surgery	41
2	Cardia	Differentiated (SM1)	Vertical resection margin	Surgery	32
3	Lesser curvature	Differentiated (SM2)	Vertical resection margin	Lost	
4	Sinuses ventriculi	Differentiated (SM2)	Vertical resection margin	Chemotherapy	12
5	Lesser curvature	Undifferentiated (SM1)	Vertical resection margin	Follow-up	5
6	Cardia	Undifferentiated (SM2)	Vertical resection margin	Surgery	6
7	Gastric body	Undifferentiated (SM1)	Vertical resection margin	Surgery	2
8	Sinus body junction	Undifferentiated (SM1)	Vertical resection margin	Surgery	2
9	Cardia	Differentiated (M3)	Lateral resection margin	Surgery	78
10	Sinus body junction	Differentiated (M2)	Lateral resection margin	Surgery	68
11	Greater curvature of antrum	Differentiated (M2)	Lateral resection margin	Surgery	48
12	Anterior wall of antrum	Differentiated (M1)	Lateral resection margin	Follow-up	40
13	Lesser curvature of fundus	Differentiated (M2)	Lateral resection margin	Supplementary ESD	41
14	Angulus	Differentiated (M3)	Lateral resection margin	Supplementary ESD	41
15	Lesser curvature of cardia	Differentiated (SM1)	Lateral resection margin	Surgery	40
16	Greater curvature of antrum	Undifferentiated (M2)	Lateral resection margin	Surgery	32
17	Posterior wall of fundus	Differentiated (M1)	Lateral resection margin	Follow-up	24
18	Lesser curvature of body	Undifferentiated (M3)	Lateral resection margin	Surgery	24
19	Angulus	Differentiated (M1)	Lateral resection margin	Follow-up	6
20	Antrum	Differentiated (M3)	Lateral resection margin	Follow-up	2
21	Angulus	Differentiated (M2)	Lateral resection margin	Follow-up	12
22	Antrum	Differentiated (SM1)	Lateral resection margin	Surgery	3
23	Cardia	Differentiated (SM1)	Lateral resection margin	Lost	
24	Cardia	Differentiated (M1)	Lateral resection margin	Follow-up	1
25	Sinus body junction	Differentiated (M1)	Lateral resection margin	Follow-up	9
26	Angulus	Differentiated (M1)	Lateral resection margin	Follow-up	6
27	Posterior wall of fundus	Differentiated (M3)	Lateral resection margin	Surgery	4

M1: intramucosal cancer; M2; lamina propria; M3: muscularis mucosa; SM1: submucosal<0.50cm; SM2: submucosal≥0.50cm

Risk factor analysis

Factors assessed for association with positive resection margins included age, gender, surgeons, lesion location, maximum diameter of resected specimens, macroscopic type, depth of tumor invasion and tumor differentiation. Univariate analysis showed that gender, surgeons, and maximum diameter of the resected specimens were not significantly associated with positive resection margins, whereas age, macroscopic type, lesion location, depth of invasion and tumor differentiation were significantly associated with positive resection margins (Table 2). To eliminate confounding factors, multivariate logistic regression analysis was performed and showed that only age, tumor differentiation and depth of invasion were risk factors for post-ESD positive resection margins (Table 3).

Discussion

The therapeutic endoscopy (ESD) was originally developed for *en bloc* resection of large-sized or complex-shaped lesions. Compared with conventional EMR, ESD has a higher en bloc resection rate and complete resection rate, regardless of tumor size [6]. For ESD of patients with EGC, the incidence of positive margins reported in previous studies varied largely from 5.3 to 12.6% [7-9]. With the definition of positive margins as the presence of low-grade intraepithelial neoplasia, a positive rate of 8.5% was obtained. If we limit the criteria for positive margins as invasive cancer,

Table 2. Risk factors of endoscopic submucosal dissection positive margins

Potential risk factors	*Positive resection margins (N=27) N (%)*	*Control (N=292) N (%)*	*p value*
Age, years, mean (±SD)	66.6 ± 10.8	62.6 ± 10.1	0.048
Gender	Female: 5 (1.6)	Female: 77 (24.1)	0.51
	Male: 22 (6.9)	Male: 215 (67.4)	
Maximum diameter (cm) median (range)	3.5 (1-7)	3.0 (0.8-8)	0.13
Location			
Upper	10 (3.1)	58 (18.2)	
Middle	10 (3.1)	94 (29.5)	0.045
Lower	7 (2.2)	140 (43.9)	
Macroscopic type			
I	1 (0.3)	17 (5.3)	
IIa	5 (1.6)	105 (32.9)	
IIb	4 (1.3)	7 (2.2)	0.0008
IIC	5 (1.6)	89 (27.9)	
IIa+IIC	10 (3.1)	70 (21.9)	
III	2 (0.6)	4 (1.3)	
Surgeon			
A	15 (4.7)	213 (66.8)	0.09
B	12 (3.7)	79 (24.8)	
Differentiation			
Differentiated	19 (5.9)	279 (87.5)	0.0001
Undifferentiated	8 (2.5)	13 (4.1)	
Depth of invasion			
M1	10 (3.1)	244 (76.5)	
M2	3 (0.9)	7 (2.2)	
M3	3 (0.9)	36 (11.3)	0.0002
SM	7 (2.2)	4 (1.3)	
SM	4 (1.3)	1 (0.3)	

For abbreviations see footnote of Table 1

Table 3. Multivariate logistic regression analysis of the risk factors for postoperative positive resection margins

Parameter	*p value*	*OR*	*95%CI*
Gender	0.4253	0.62	0.192-2.007
Age	0.0303	1.05	1.005-1.099
Maximum diameter	0.3645	1.18	0.821-1.710
Location	0.2577	0.72	0.406-1.273
Macroscopic type	0.5048	1.10	0.838-1.432
Depth of invasion	0.0000	1.85	1.410-2.426
Surgeon	0.1043	2.15	0.854-5.425
Differentiation	0.0147	4.32	1.333-14.018

OR: odds ratio, CI: confidence internal

the rate falls to 5.6%. We also analysed the confounding factor of different surgeons, and neither univariate nor multivariate analysis showed statistically significant difference.

Univariate analysis revealed that age, macroscopic type, lesion location, depth of invasion and tumor differentiation were significantly associated with positive resection margins. By contrast, logistic regression analysis showed that only age, tumor differentiation and depth of invasion were risk factors for post-ESD positive resection margins. The data were consistent with those of previous reports [4,5]. Age was determined as an independent risk factor in our study, which has never been reported before. Isomoto et al. [9] concluded that the complete resection rate is significantly lower in elderly patients than in non-elderly patients. Several possibilities can explain this finding. Along with aging, the probability of mucosal atrophy, intestinal metaplasia and dysplasia gradually increases, whereas inflammation creates obstacles on the judgment of the circumferential incision. In some flat lesions, no color difference exists to distinguish the lateral margin of tumor from normal mucosa, so conventional endoscopy or even NBI may miss it. Moreover, many elderly patients who cannot tolerate surgery for organ dysfunction have to choose a minimally invasive endoscopic treatment.

Isomoto et al. [8] reported that tumor size had no significant effect on curative resection, which is consistent with our results. However, tumor size is significantly associated with piecemeal resection, which yields significantly lower curative resection rates than *en bloc* resection [8,10]. In our study, all non-complete cases were excluded by criteria, which directly resulted in an unrelated conclusion. The initial indication of ESD has gradually expanded with the development of ESD technology, and the complication rate is declining [11,12]. Therefore, dealing with large lesions in early-stage cancers ESD has become manageable.

The grade of tumor differentiation was also previously reported as an independent risk factor [4,13]. Undifferentiated tumors are more likely to invade earlier the vasculature and lymph nodes or cause deep infiltration. In this study, only 13 (4.7%) poorly differentiated lesions were diagnosed in 279 specimens showing negative margins. By contrast, the ratio in margin-positive specimens (8 poorly differentiated lesions in 27 specimens (29.6%) was significantly higher than that in margin-negative ones ($p < 0.05$). These data suggest that conventional surgery is the better choice for reducing unnecessary damage and avoiding economic waste if an undifferentiated tumor is confirmed by biopsy.

One of the most important factors to prevent vertical positive resection margins is the evaluation of the depth of invasion and comprehensive indication of ESD before the operation [13]. The mucosal boundary can be discerned by magnification using NBI, and the depth of invasion can be estimated by the irregular microvascular pattern as well as the irregular microsurface pattern and demarcation line [14,15]. Endoscopic ultrasonography (EUS) can be used to assess the range and depth of the lesion and lymph node metastasis, if necessary [16]. The accuracy of EUS for gastric cancer ranges from 50 to 90% for nodal staging [17,18], which is greater than that of CT [18,19]. Furthermore, to prevent vertical positive resection margins, the surgeon should remove a certain thickness of submucosa while avoiding excessive thermal coagulate injury to the mucosa. Of the 8 patients with vertical positive resection margins, 1 was lost to follow-up, 4 were subjected to supplementary surgery and 1 received chemotherapy. For the 2 remaining patients, 1 was subjected to supplementary surgery after reviewing the operation videos, and the other one was put on close follow-up. None of the patients exhibited recurrence or relapse during the follow-period of 2-41 months (mean 14.3).

To prevent lateral positive resection margins, we accurately determined the extent of disease using a combination of NBI magnification and methylene blue staining. Among the 19 patients who had lateral resection margin positive, one was lost to follow-up, 8 underwent supplementary

surgery and 2 underwent secondary ESD. Low- or high-level neoplastic lesions were found in 8 patients. After adequate communication with the patients, no additional treatment was advised, except frequent follow-up, in which the longest follow-up was 40 months without any recurrence. Whether surgery should be actively applied on lateral positive resection margins without any follow-up guidelines has been a subject of much controversy [20-22]. We suggest frequent follow-up and the introduction of chemotherapy, supplementary ESD or surgery if necessary. If the operation video reveals that the resection size was appropriate, the patient should be scheduled for follow-up one month postoperatively when the operation-induced ulcers and inflammation would have completely healed to reduce the incidence of inflammatory response-induced dysplasia. Endoscopic iodine staining and NBI magnification must be performed during the re-examination.

This study has some limitations as it was a single-centre retrospective analysis, and bias factors could not be eliminated.

Our study analysed risk factors associated with positive resection margins, and the prognosis of EGC patients who underwent ESD. Our data indicate that positive resection margins and recurrence can be prevented if the grade of tumor differentiation, depth of invasion, age of the patients and related factors can be addressed before a logical ESD is properly conducted.

References

1. Soetikno R, Kaltenbach T, Yeh R, Gotoda T. Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract. J Clin Oncol 2005;23:4490-4498.
2. Yoshida T, Inoue H, Usui S, Satodate H, Fukami N, Kudo SE. Narrow-band imaging system with magnifying endoscopy for superficial esophageal lesions. Gastrointest Endosc 2004;59:288-295.
3. Kodashima S, Fujishiro M, Takubo K et al. Ex-vivo study of high-magnification chromoendoscopy in the gastrointestinal tract to determine the optimal staining conditions for endocytoscopy. Endoscopy 2006;38:1115-1121.
4. Kakushima N, Ono H, Tanaka M, Takizawa K, Yamaguchi Y, Matsubayashi H. Factors related to lateral margin positivity for cancer in gastric specimens of endoscopic submucosal dissection. Dig Endosc 2011;23:227-232.
5. Yoon H, Kim SG, Choi J et al. Risk factors of residual or recurrent tumor in patients with a tumor-positive resection margin after endoscopic resection of early gastric cancer. Surg Endosc 2013;27:1561-1568.
6. Park YM, Cho E, Kang HY, Kim JM. The effectiveness and safety of endoscopic submucosal dissection compared with endoscopic mucosal resection for early gastric cancer: a systematic review and meta-analysis. Surg Endosc 2011;25:2666-2677.
7. Goto O, Fujishiro M, Kodashima S, Ono S, Omata M. Outcomes of endoscopic submucosal dissection for early gastric cancer with special reference to validation for curability criteria. Endoscopy 2009;41:118-122.
8. Isomoto H, Shikuwa S, Yamaguchi N et al. Endoscopic submucosal dissection for early gastric cancer: a large-scale feasibility study. Gut 2009;58:331-336.
9. Isomoto H, Ohnita K, Yamaguchi N et al. Clinical outcomes of endoscopic submucosal dissection in elderly patients with early gastric cancer. Eur J Gastroenterol Hepatol 2010;22:311-317.
10. Chung IK, Lee JH, Lee SH et al. Therapeutic outcomes in 1000 cases of endoscopic submucosal dissection for early gastric neoplasms: Korean ESD Study Group multicenter study. Gastrointest Endosc 2009;69:1228-1235.
11. Yamaguchi N, Isomoto H, Fukuda E et al. Clinical outcomes of endoscopic submucosal dissection for early gastric cancer by indication criteria. Digestion 2009:80:173-181.
12. Lee H, Yun WK, Min BH et al. A feasibility study on the expanded indication for endoscopic submucosal dissection of early gastric cancer. Surg Endosc 2011;25:1985-1993.
13. Lee TH, Cho JY, Chang YW et al. Appropriate indications for endoscopic submucosal dissection of early gastric cancer according to tumor size and histologic type. Gastrointest Endosc 2010;71:920-926.
14. Hirata I, Nakagawa Y, Ohkubo M, Yahagi N, Yao K. Usefulness of magnifying narrow-band imaging endoscopy for the diagnosis of gastric and colorectal lesions. Digestion 2012;85:74-79.
15. Tao G , Xing-Hua L, Ai-Ming Y et al. Enhanced magnifying endoscopy for differential diagnosis of superficial gastric lesions identified with white-light endoscopy. Gastric Cancer 2014;17:122-129.
16. Fujishiro M, Kodashima S, Goto O et al. Endoscopic submucosal dissection for esophageal squamous cell neoplasms. Dig Endosc 2009;21:109-115.
17. Akahoshi K, Misawa T, Fujishima H et al. Preoperative evaluation of gastric cancer by endoscopic ultrasound. Gut 1991;32:479-482.

18. Habermann CR, Weiss F, Riecken R et al. Preoperative staging of gastric adenocarcinoma: comparison of helical CT and endoscopic US. Radiology 2004;230:45-71.
19. Ziegler K, Sanft C, Zimmer T et al. Comparison of computed tomography, endosonography, and intraoperative assessment in TN staging of gastric carcinoma. Gut 1993;34:604-610.
20. Song KY, Hyung WJ, Kim HH et al. Is gastrectomy mandatory for all residual or recurrent gastric cancer following endoscopic resection? A large scale Korean multi-center study. J Surg Oncol 2008;98:6-10.
21. Jung H, Bae JM, Choi MG, Noh JH, Sohn TS, Kim S. Surgical outcome after incomplete endoscopic submucosal dissection of gastric cancer. Br J Surg 2011;98:73-78.
22. Kusano C, Iwasaki M, Kaltenbach T, Conlin A, Oda I, Gotoda T. Should elderly patients undergo additional surgery after non-curative endoscopic resection for early gastric cancer? Long-term comparative outcomes. Am J Gastroenterol 2011;106:1064-1069.