Risk factors analysis and stratification for microscopically positive resection margin in gastric cancer patients

CURRENT STATUS: UNDER REVISION

yuta kumazu
Kanagawa Kenritsu Gan Center
ORCiD: https://orcid.org/0000-0003-2522-6295

Tsutomu Hayashi
Email: tsuhayas@ncc.go.jp
Corresponding Author
ORCiD: https://orcid.org/0000-0002-9193-8269

Takaki Yoshikawa
National Cancer Center Hospital

Takanobu Yamada
Kanagawa Kenritsu Gan Center

Kentaro Hara
Kanagawa Kenritsu Gan Center

Yota Shimoda
Kanagawa Kenritsu Gan Center

Masato Nakazono
Kanagawa Kenritsu Gan Center

Shinsuke Nagasawa
Kanagawa Kenritsu Gan Center

Manabu Shiozawa
Kanagawa Kenritsu Gan Center

Soichiro Morinaga
Kanagawa Kenritsu Gan Center

Yasushi Rino
DOI: 10.21203/rs.3.rs-15866/v1

SUBJECT AREAS
General Surgery  Surgery

KEYWORDS
Resection margins, Gastric cancer, Risk factors, Intraoperative frozen section, Gastrectomy
Abstract

Background: Cancer cells are often found postoperatively at surgical resection margins (RM) in patients with gastric cancer because of submucosal infiltration or hesitation to secure adequate RM. This study was designed to evaluate risk factors for microscopic positive RM and to clarify which patients should undergo intraoperative frozen section diagnosis (IFSD).

Methods: Patients who underwent R0/1 gastrectomy for gastric adenocarcinoma between 2000 and 2018 in a single cancer center in Japan were studied. We divided the patients into a positive RM group and negative RM group according to the results of definitive histopathological examinations. We performed multivariate analysis to analyze risk factors for positive RM by and used the identified risk factors to risk stratify the patients.

Results: A total of 2757 patients were studied, including 49 (1.8%) in the positive RM group. The risk factors significantly associated with positive RM were remnant gastric cancer (odds ratio [OR] 4.7), esophageal invasion (OR 6.3), tumor size ≥80 mm (OR 3.9), and a histopathological diagnosis of undifferentiated type (OR 3.6), macroscopic type 4 (OR 3.7), or pT4 disease (OR 4.6). On risk stratification analysis, the incidence of positive RM was 0.1% without any risk factors, increasing to 0.4% with one risk factor, 3.1% with two risk factors, 5.3% with three risk factors, 21.3% with four risk factors, and 85.7% with five risk factors.

Conclusions: The risk of macroscopically positive RM increased in patients who have risk factors. IFSD should be performed in patients who have four or more risk factors.

Background

Gastric cancer, a leading cause of cancer-related death worldwide, remains the fourth most common malignancy [1]. Complete tumor removal is essential to effectively treat resectable gastric cancer. To achieve complete resection, it is necessary to secure a sufficient tumor-free margin as confirmed macroscopically or endoscopically. However, tumors progress horizontally, sometimes spreading as submucosal infiltration while maintaining an apparently normal mucosa, which can mislead surgeons or endoscopists. Even if surgeons make careful efforts to achieve complete resection with negative margins, tumor cells are occasionally observed in resection margins (RM) on postoperative
histological examinations, and such margins are diagnosed as microscopically positive (R1).

The correlation between microscopically positive RM and outcomes in patients with gastric cancer remains an issue of debate [2, 3]. However, several previous studies have reported that microscopically positive RM had an unfavorable impact on survival [4–7]. In other words, decreasing the risk of microscopically positive RM might benefit patients. Intraoperative frozen section diagnosis (IFSD) with a diagnostic accuracy of 93–100% has been used to detect tumor cells at the resection margin [8–10]. Although IFSD can decrease unanticipated positive RM, the procedure requires time, costs, and labor. Therefore, it should be performed in specific patients at high risk for positive RM. Stratification of patients based on the risk of positive RM is meaningful in terms of conserving resources.

The aims of this study were to evaluate risk factors for microscopic positive RM and to clarify which patients should undergo IFSD.

Methods
Patients
This retrospective cohort study examined 2757 patients who underwent surgery for gastric cancer or esophagogastric junction cancer from January 2000 through March 2018 in Kanagawa Cancer Center, Japan. All tumors fulfilled the following criteria: (1) histologically proven adenocarcinoma, (2) R0 or R1 resection, and (3) Gastric cancer and adenocarcinoma of the esophagogastric junction. The exclusion criteria were as follows: (1) stage IV cancer without ascites cytology positive reaction (2) R2 resection, and (3) Type I adenocarcinoma of the esophagogastric junction according to the Siewert classification [11]. Cancer staging was based on the Union for International Cancer Control TNM classification, 7th edition [12].
Surgical procedure
i) Selection of gastrectomy

Gastrectomy and lymph node dissection were performed in accordance with the JGCA guidelines[13–15]. In short, distal gastrectomy or proximal gastrectomy was performed when a sufficient proximal or distal RM could be obtained, if not so, total gastrectomy was performed. In principle, D1 or D1 + lymphadenectomy was indicated for cT1N0 tumors, and D2 lymphadenectomy
was indicated for cT2-4 or cN + tumors.

ii) Determination of resection line of gastrectomy

The resection line was determined to secure a tumor-free margin as defined in the Japanese gastric cancer guidelines. In principle, a tumor-free margin larger than 3 cm was secured in cases of macroscopic type 1 or type 2 advanced cancer, while a tumor-free margin larger than 5 cm was secured in cases of macroscopic type 3, type 4, or type 5 advanced cancer as classified according to the classification of JGCA[14]. If tumor invasion spread across the pylorus or cardia, the resection line was determined as the line expected to be tumor-free on intraoperative macroscopic examination. On the other hand, in early cancer, endoscopic marking with clips was performed as required before surgery to indicate the location of the tumor and resection line.

Finally, surgeons opened specimens of the removed stomachs and confirmed negative RM macroscopically during operation. If tumor invasion beyond the resection edge was suspected macroscopically, IFSD was performed, and additional resection was done as necessary.

Evaluation and Statistical Analysis

The patients were divided into a positive RM group and negative RM group on the basis of the microscopic findings of the longitudinal margins on definitive histopathological examinations.

The baseline characteristics were compared between the positive and negative RM groups by Pearson’s chi-squared tests or Fisher’s exact tests for categorical variables, as appropriate, and by the Mann-Whitney U tests for continuous variables. Risk factors for positive RM were analyzed by binomial logistic regression analysis. We used risk factors identified by logistic regression analysis to risk stratify the patients.

IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp. Released 2016. Armonk, N.Y.) software was used for statistical analysis. P-values of less than 0.05 were considered to indicate statistical significance.

Results

Patient Characteristics

The demographic and clinicopathological characteristics of the patients are shown in Table 1. Forty-nine patients (1.8%) with positive RM were studied. Among the 49 patients, there are no patients who
received IFSD, and 27 (55.1%) had positive proximal RM, 15 (30.6%) had positive distal RM, and 7 (14.3%) had positive bilateral RM. Patients in the positive RM group received neoadjuvant chemotherapy (14.3% vs. 4.5%, p = 0.007) and total gastrectomy (59.2% vs. 35.4%, p < 0.001) more frequently than those in the negative RM group. Remnant gastric cancer (12.2% vs. 2.5%, p = 0.002) and esophageal invasion (18.4% vs. 3.5%, p < 0.001) were more frequent in the positive RM group than in the negative RM group. Tumor size in the positive RM group was larger than that in the negative RM group (105 mm vs. 41 mm, p < 0.001). The proportions of tumors with undifferentiated type of histopathology (89.8% vs. 54.2%, p < 0.001) and macroscopic type 4 (49% vs. 3.7%, p < 0.001) were higher in the positive RM group than in the negative RM group. Patients in the positive RM group had more advanced tumors in terms of pT category, pN category, and pTNM stage. No patient had pT1 tumors in the positive RM group.

Table 1
Baseline demographic, clinicopathological characteristics in patients with positive margin (PM) and negative margin (NM). IQR; Interquartile Range, BMI, body mass index; ASA-PS, American Society of Anesthesiologists physical status classification system.

| Risk Factor | Number of positive resection margin (Incidence rate) | Univariate OR (95% CI) | P-value | Multivariate OR (95% CI) | P-value |
|-------------|------------------------------------------------------|------------------------|---------|--------------------------|---------|
| Neoadjuvant chemotherapy | No | 42 (1.6%) | 1.0 | 1.0 |
| | Yes | 7 (5.4%) | 3.5 (1.5-8.0) | 0.002 | 0.7 (0.2-1.9) | 0.593 |
| Remnant cancer | No | 43 (1.6%) | 1.0 | 1.0 |
| | Yes | 6 (8.1%) | 5.41 (2.2-13.1) | < 0.001 | 4.7 (1.6-13.3) | 0.003 |
| Esophageal invasion | No | 41 (1.5%) | 1.0 | 1.0 |
| | Yes | 8 (7.8%) | 5.42 (2.4-11.8) | < 0.001 | 6.3 (2.45-16.5) | < 0.001 |
| Tumor size <80 mm | 15 (0.6%) | 1.0 | 1.0 |
| ≥80 mm | 34 (10.4%) | 18.60 (10.01-34.56) | < 0.001 | 3.9 (1.78-8.7) | 0.001 |
| Histopathological type | Differentiated | 5 (0.4%) | 1.0 | 1.0 |
| | Undifferentiated | 44 (2.9%) | 7.44 (2.9-18.8) | < 0.001 | 3.6 (1.3-10.0) | 0.012 |
| Macroscopic type | Other than Type4 | 25 (0.9%) | 1.0 | 1.0 |
| | Type4 | 24 (19.2%) | 25.02 (13.8-45.3) | < 0.001 | 3.7 (1.7-8.0) | 0.001 |
| pT category | T1-3 | 9 (0.4%) | 1.0 | 1.0 |
| | T4 | 40 (7.1%) | 18.6 (8.9-38.6) | < 0.001 | 4.6 (1.9-10.9) | < 0.001 |

Incidence rates and odds ratios of risk factors
Table 2 shows the incidence rates and odds ratios of risk factors for positive RM on univariate and multivariate analyses. The incidence rates of positive RM were higher than 10% in patients who had tumor measuring ≥ 80 mm or macroscopic type 4 disease (10.4%, 19.2%, respectively). On multivariate analysis, the independent significant risk factors associated with the incidence of positive RM were remnant gastric cancer (odds ratio [OR] 4.7, 95% CI 1.6–13.3), esophageal invasion (OR 6.3, 95% CI 2.4–16.5), tumor size ≥ 80 mm (OR 3.9, 95% CI 1.7–8.7), undifferentiated type of histopathology (OR 3.6, 95% CI 1.3–10.0), macroscopic type 4 (OR 3.7, 95% CI 1.7-8.0), and pT4 (OR 4.6, 95% CI 1.9–10.9).

Table 2
Incidence rates of positive resection margin and odds ratios in univariate and multivariate analysis. OR, odds ratio; CI, confidence interval.

| Risk factors | Number of positive resection margin (Incidence rate) | Univariate OR | 95% CI          | P value |
|--------------|---------------------------------------------------|---------------|-----------------|---------|
| 0            | 1/956 (0.1%)                                       | 0.038         | 0.005–0.277     | 0.001   |
| 1            | 5/1242 (0.4%)                                      | 0.135         | 0.053–0.342     | < 0.001 |
| 2            | 10/325 (3.1%)                                      | 1.94          | 0.96–3.93       | 0.064   |
| 3            | 7/132 (5.3%)                                       | 3.44          | 1.51–7.81       | 0.003   |
| 4            | 20/94 (21.3%)                                      | 24.5          | 13.2–45.3       | < 0.001 |
| 5            | 6/7 (85.7%)                                        | 377.5         | 44.4–3203.8     | < 0.001 |

Patient risk stratification
The identified six risk factors for positive RM on multivariate analysis were used to risk stratify the patients (Table 3). In patients who had no risk factors for positive RM, incidence rate of positive RM was 0.1%. The incidence rate increased to 0.4% with one risk factor, 3.1% with two risk factors, 5.3% with three risk factors, 21.3% with four risk factors and 85.7% with five risk factors. No patient had all six risk factors. The incidence of positive RM increased progressively with an increase in the number of risk factors. The presence or three or more risk factors was associated with a significant increase in the odds ratio.

| Risk factors | Number of positive resection margin (Incidence rate) | Univariate OR | 95% CI          | P value |
|--------------|---------------------------------------------------|---------------|-----------------|---------|
| 0            | 1/956 (0.1%)                                       | 0.038         | 0.005–0.277     | 0.001   |
| 1            | 5/1242 (0.4%)                                      | 0.135         | 0.053–0.342     | < 0.001 |
| 2            | 10/325 (3.1%)                                      | 1.94          | 0.96–3.93       | 0.064   |
| 3            | 7/132 (5.3%)                                       | 3.44          | 1.51–7.81       | 0.003   |
| 4            | 20/94 (21.3%)                                      | 24.5          | 13.2–45.3       | < 0.001 |
| 5            | 6/7 (85.7%)                                        | 377.5         | 44.4–3203.8     | < 0.001 |

Table 3. Risk stratification. Incidence rate of positive resection margin by each number of the risk
Discussion

The incidence of positive RM in the entire cohort was not high (1.8%), as compared with that in previous studies evaluating macroscopically positive RM in patients with gastric cancer (1.8%-8.2%) [4–7, 16]. The results suggest that our surgical procedures and methods for evaluating appropriate resection lines were at high levels. Nevertheless, surgeons misdiagnosed the resection lines of specimens as being tumor-free, despite the fact that tumor cells remained. Therefore, to prevent unexpected microscopically positive RM, resection with wider excision or IFSD should be performed in cases with a high risk of positive RM.

Our study showed that remnant gastric cancer, esophageal invasion, a tumor size of ≥ 80 mm, undifferentiated type of histopathology, macroscopic type 4, and pT4 were risk factors for positive RM in patients with gastric cancer. Moreover, the risk of positive RM increased in patients with three or more of those risk factors. In particular, owing to high-risk associated with four (21.3%) or five factors (85.7%), IFSD should be considered in patients who have four risk factors and is mandatory in patients who have five risk factors. On the other hand, patients without any risk factors do not have to undergo IFSD, because the incidence rate of positive RM is extremely low (0.1%).

Previous studies have similarly reported that advanced T category, advanced N category, total gastrectomy, larger tumor, EGJ location, diffuse histology, and linitis plastica, which is also referred to as macroscopic type 4, [3, 17, 18] are risk factors for positive RM. On the basis of these studies, we chose candidate risk factors for preoperatively predicting clinicopathological features. We did not adopt N category as one of the risk factors because it was not appropriate to substitute pN for cN owing to the low preoperative diagnostic accuracy (63.6%) [19]. Remnant gastric cancer, which remains an unknown risk factor for positive RM in previous studies, was more frequent in the PM group of our study and was therefore adopted as a risk factor for remnant gastric cancer. Finally, six clinicopathological features were identified as risk factors on multivariate analysis in our study, although the factors other than remnant cancer have been reported previously. An innovation of our study was to stratify patients who have risk factors and to show how often microscopically positive
RM occurred in patients with such risk factors. To our knowledge, no previous study has reported on risk stratification of positive RM in patients with gastric cancer.

Our study had some limitations that should be taken into consideration when interpreting the results. First, we used the pathological T category, not the clinical T category, as an indicator of the invasion depth of tumor. There were no pT1 tumors with positive RM in our study. To utilize this result, accurate preoperative diagnosis is essential. The preoperative diagnosis of cT1 gastric cancer is reported to have 92.4–95.4% accuracy [20–22]. The invasion depth was underestimated in 4.6–7.6% of the patients, who actually had advanced cancer. If the tumor is diagnosed to be being somewhat shallower than the actual depth, the actual resection margin distance would be shorter than the essential margin, which suggests that the incidence rate of positive RM may increase.

A second limitation was that we could not evaluate the margin distance between the tumor and resection line, which was shown to be a risk factor in other studies [17]. Risk stratification in our study was useful for preoperatively estimating the risk of positive RM and for deciding whether IFSD should be performed. However, the estimated risk would change depending on the actual secured margin distance. A multicenter Italian study reported that a RM distance of less than 2 cm was a risk factor for T1 tumors, and a RM distance of less than 3 cm was a risk factor for T2 to T4 Lauren intestinal pattern tumors [18]. The required margin distance should be evaluated in patients who have high-risk factors for positive RM in future studies.

Conclusions

In conclusion, patients with gastric cancer who have four or more risk factors from among remnant gastric cancer, esophageal invasion, tumor size ≥ 80 mm, undifferentiated type of histopathology, macroscopic type 4, and pT4, have a high risk of macroscopically positive RM despite macroscopically negative margins as evaluated on intraoperative examination. In such patients, resection with wider excision should be performed as much as possible, or IFSD should be performed to confirm whether the resection margin is negative or positive.

Declarations

Funding
Not applicable.

**Authors’ contributions**

YK, TH, TY1, TY2, KH, YS, MN, SN, MS, SM, YR, MM, TO1 and TO2 contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. All authors have read and approved the manuscript.

**Ethics approval and consent to participate**

This study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of Kanagawa Cancer Center Hospital, and written informed consent was obtained before surgery from all patients.

**Availability of data and materials**

We can not share the data and materials, because the Ethics Committee of Kanagawa Cancer Center Hospital prohibit publication of raw data base including patients clinical data even if identifying/confidential data was not included.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdical claims in published maps and institutional affiliations.

**Open Access**

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article,
unless otherwise stated.

References

1. Observatory TGC (2019) Cancer Today. World Health Organization. http://gco.iarc.fr/today/data/factsheets/cancers/7-Stomach-fact-sheet.pdf. Accessed Jun 3, 2019 2019

2. Postlewait LM, Maithel SK (2016) The importance of surgical margins in gastric cancer. J Surg Oncol 113 (3):277-282. doi:10.1002/jso.24110

3. Raziee HR, Cardoso R, Seevaratnam R, Mahar A, Helyer L, Law C, Coburn N (2012) Systematic review of the predictors of positive margins in gastric cancer surgery and the effect on survival. Gastric Cancer 15 Suppl 1:S116-124. doi:10.1007/s10120-011-0112-7

4. Sun Z, Li DM, Wang ZN, Huang BJ, Xu Y, Li K, Xu HM (2009) Prognostic significance of microscopic positive margins for gastric cancer patients with potentially curative resection. Ann Surg Oncol 16 (11):3028-3037. doi:10.1245/s10434-009-0624-0

5. Morgagni P, Garcea D, Marrelli D, De Manzoni G, Natalini G, Kurihara H, Marchet A, Saragoni L, Scarpi E, Pedrazzani C, Di Leo A, De Santis F, Panizzo V, Nitti D, Roviello F (2008) Resection line involvement after gastric cancer surgery: clinical outcome in nonsurgically retreated patients. World J Surg 32 (12):2661-2667. doi:10.1007/s00268-008-9747-x

6. Wang SY, Yeh CN, Lee HL, Liu YY, Chao TC, Hwang TL, Jan YY, Chen MF (2009) Clinical impact of positive surgical margin status on gastric cancer patients undergoing gastrectomy. Ann Surg Oncol 16 (10):2738-2743. doi:10.1245/s10434-009-0616-0

7. Liang Y, Ding X, Wang X, Wang B, Deng J, Zhang L, Liang H (2015) Prognostic value of surgical margin status in gastric cancer patients. ANZ J Surg 85 (9):678-684. doi:10.1111/ans.12515
8. Ferreiro JA, Myers JL, Bostwick DG (1995) Accuracy of frozen section diagnosis in surgical pathology: review of a 1-year experience with 24,880 cases at Mayo Clinic Rochester. Mayo Clinic proceedings 70 (12):1137-1141. doi:10.1016/s0025-6196(11)63438-1

9. Spicer J, Benay C, Lee L, Rousseau M, Andalib A, Kushner Y, Marcus V, Ferri L (2014) Diagnostic Accuracy and Utility of Intraoperative Microscopic Margin Analysis of Gastric and Esophageal Adenocarcinoma. Annals of Surgical Oncology 21 (8):2580-2586. doi:10.1245/s10434-014-3669-7

10. Matsusaka S, Nagareda T, Yamasaki H, Kitayama Y, Okada T, Maeda S (2003) Immunohistochemical evaluation for intraoperative rapid pathological assessment of the gastric margin. World J Surg 27 (6):715-718. doi:10.1007/s00268-003-6792-3

11. Siewert JR, Stein HJ (1998) Classification of adenocarcinoma of the oesophagogastric junction. Br J Surg 85 (11):1457-1459. doi:10.1046/j.1365-2168.1998.00940.x

12. Biondi A, Hyung WJ (2011) Seventh edition of TNM classification for gastric cancer. J Clin Oncol 29 (32):4338-4339; author reply 4340-4332. doi:10.1200/JCO.2011.36.9900

13. Nakajima T (2002) Gastric cancer treatment guidelines in Japan. Gastric Cancer 5:1-5

14. Association. JGC (2011) Japanese gastric cancer treatment guidelines 2010 (ver. 3). Gastric Cancer 14 (2):113-123. doi:10.1007/s10120-011-0042-4

15. Association JGC (2017) Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer 20 (1):1-19. doi:10.1007/s10120-016-0622-4

16. Cho BC, Jeung HC, Choi HJ, Rha SY, Hyung WJ, Cheong JH, Noh SH, Chung HC (2007) Prognostic impact of resection margin involvement after extended (D2/D3) gastrectomy for advanced gastric cancer: a 15-year experience at a single institute. J Surg Oncol 95 (6):461-468. doi:10.1002/jso.20731
17. Blackham AU, Swords DS, Levine EA, Fino NF, Squires MH, Poultsides G, Fields RC, Bloomston M, Weber SM, Pawlik TM, Jin LX, Spolverato G, Schmidt C, Worhunsky D, Cho CS, Maithel SK, Votanopoulos KI (2015) Is Linitis Plastica a Contraindication for Surgical Resection: A Multi-Institution Study of the U.S. Gastric Cancer Collaborative. Annals of Surgical Oncology 23 (4):1203-1211. doi:10.1245/s10434-015-4947-8

18. Bissolati M, Desio M, Rosa F, Rausei S, Marrelli D, Baiocchi GL, De Manzoni G, Chiari D, Guarneri G, Pacelli F, De Franco L, Molfino S, Cipollari C, Orsenigo E (2017) Risk factor analysis for involvement of resection margins in gastric and esophagogastric junction cancer: an Italian multicenter study. Gastric Cancer 20 (1):70-82. doi:10.1007/s10120-015-0589-6

19. Fukagawa T, Katai H, Mizusawa J, Nakamura K, Sano T, Terashima M, Ito S, Yoshikawa T, Fukushima N, Kawachi Y, Kinoshita T, Kimura Y, Yabusaki H, Nishida Y, Iwasaki Y, Lee SW, Yasuda T, Sasako M, Stomach Cancer Study Group of the Japan Clinical Oncology G (2018) A prospective multi-institutional validity study to evaluate the accuracy of clinical diagnosis of pathological stage III gastric cancer (JCOG1302A). Gastric Cancer 21 (1):68-73. doi:10.1007/s10120-017-0701-1

20. Sano T, Okuyama Y, Kobori O, Shimizu T, Morioka Y (1990) Early gastric cancer. Endoscopic diagnosis of depth of invasion. Dig Dis Sci 35 (11):1340-1344

21. Yanai H, Matsumoto Y, Harada T, Nishiaki M, Tokiyama H, Shigemitsu T, Tada M, Okita K (1997) Endoscopic ultrasonography and endoscopy for staging depth of invasion in early gastric cancer: a pilot study. Gastrointestinal endoscopy 46 (3):212-216

22. Tsujimoto H, Sugasawa H, Ono S, Ichikura T, Yamamoto J, Hase K (2010) Has the accuracy of preoperative diagnosis improved in cases of early-stage gastric cancer? World J Surg 34 (8):1840-1846. doi:10.1007/s00268-010-0587-0
