Predictors of Lymph Node Metastasis and Differences Between Pure and Mixed Histologic Types of Early Gastric Signet-ring Cell Carcinomas

Yuning Chu, MM,* Tao Mao, MD,* Xiaoyu Li, MD,* Xue Jing, MD,* Minghan Ren, MM,* Zhen Huang, MM,† Xiao-Bin Zhou, MD,‡ Yunqing Chen, MD,§ and Zibin Tian, MD*  

Abstract: The aim of this study was to investigate predictors of lymph node metastasis (LNM) in early gastric signet-ring cell carcinoma (SRCC) and determine clinicopathologic and prognostic differences of different histologic subtypes. We retrospectively analyzed 13,661 gastric cancer patients; 231 were eligible for inclusion. The aim of this study was to investigate predictors of LNM in early gastric SRCC. Univariate and multivariate analyses revealed SM2 invasion (odds ratio [OR] = 5.070, P = 0.003), lymphovascular invasion (LVI) (OR = 14.876, P < 0.001), pathologic pattern of mixed SRCC (OR = 3.226, P = 0.026), ulcer presence (OR = 3.340, P = 0.019) and lesion size over 20 mm (OR = 2.823, P = 0.015) as independent risk factors for LNM. Compared with pure SRCC, the mixed subtype was associated with older age, larger lesion size, higher LVI frequency, more frequent perineural invasion, and most importantly, higher LNM incidence. Patients with pure SRCC showed significantly longer overall survival (P = 0.004) and disease-specific survival (P = 0.002) than mixed SRCC patients. Pathologic subtype differences of different histologic subtypes. We retrospectively analyzed 13,661 gastric cancer patients; 231 were eligible for inclusion. The aim of this study was to investigate predictors of LNM in early gastric SRCC. Univariate and multivariate analyses revealed SM2 invasion (odds ratio [OR] = 5.070, P = 0.003), lymphovascular invasion (LVI) (OR = 14.876, P < 0.001), pathologic pattern of mixed SRCC (OR = 3.226, P = 0.026), ulcer presence (OR = 3.340, P = 0.019) and lesion size over 20 mm (OR = 2.823, P = 0.015) as independent risk factors for LNM. Compared with pure SRCC, the mixed subtype was associated with older age, larger lesion size, higher LVI frequency, more frequent perineural invasion, and most importantly, higher LNM incidence. Patients with pure SRCC showed significantly longer overall survival (P = 0.004) and disease-specific survival (P = 0.002) than mixed SRCC patients. Pathologic subtype

Between Pure and Mixed Histologic Types of Early Gastric Signet-ring Cell Carcinomas

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MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the Ethics Committee of the Affiliated Hospital of Qingdao University (QYFY WZLL 2019-04-04). Our study was also performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

Patients

A total of 13,661 patients who underwent gastrectomy with lymphadenectomy for histologically proven gastric adenocarcinoma at the Affiliated Hospital of Qingdao University from June 2002 to June 2014 were reviewed retrospectively. The following EGC cases were excluded: (1) advanced-stage gastric cancer (n = 12,019); (2) intestinal metaplasia or intraepithelial neoplasia (n = 92); (3) metastatic gastric cancer or multiple carcinomas (n = 58); (4) lymphoma (n = 47); (5) gastric stump carcinoma (n = 56); and (6) other life-threatening diseases (n = 37). A total of 1352 EGC patients were selected for careful pathologic analysis. Ultimately, 231 early gastric SRCC patients were eligible for inclusion in this study.

Data Collection

According to the fourth edition of the Japan Gastric Cancer Association (JGCA) treatment guidelines,5 gastrectomy with lymph node dissection was performed on the enrolled patients. The specimens were serially sectioned into 3-mm-thick slices after gross examination, and 2 experienced pathologists individually examined the histologic slides retrieved and investigated each case blindly without the knowledge of clinical and endoscopic findings. For difficult cases, several specialists reached a consensus through discussion.

According to the World Health Organization (WHO) diagnostic criteria,14 pure SRCC was defined as a predominant component (>50%) of isolated carcinoma cells containing intracytoplasmic mucin, and mixed SRCC was defined as adenocarcinoma with a minor component (10% to 50%) of isolated carcinoma cells containing intracytoplasmic mucin (Fig. 1). Lymphovascular invasion (LVI) was identified immunohistochemically using the D2-40 antibody (Dako-Cytomation, Glostrup, Denmark); the S100 protein was detected to diagnose perineural invasion. The diagnostic criterion for LNM was the presence of cancerous tissue inside the lymph node capsule. In accordance with the JGCA

FIGURE 1. Representative pathologic images. A, Pure signet-ring cell carcinoma. B, Mixed signet-ring cell carcinoma. C, Pure signet-ring cell carcinoma. D, Mixed signet-ring cell carcinoma.
classification, when multiple lesions were present, the tumor with the most advanced T category (or the largest lesion when the T stages were identical) was classified.\textsuperscript{15} On the basis of the Paris endoscopic classification, the macroscopic features of EGC were divided into the following 5 subtypes: type 0-I (protruded), type 0-IIa (superficial elevated), type 0-IIb (flat), type 0-IIc (superficial depressed), and type 0-III (excavated).\textsuperscript{16} The tumors were also graded as small (\(\leq 20\) mm) and large (\(>20\) mm) to allow for the re-evaluation of endoscopic submucosal dissection (ESD) indications. Regarding invasion depth, submucosal lesions were classified into 2 groups: SM1 (\(\leq 500\) \(\mu\)m depth of invasion) and SM2 (\(>500\) \(\mu\)m depth of invasion).

Data for the clinical features, endoscopic and pathologic characteristics of all included patients were collected, including age, sex, incidence of hypertension, heart disease

\begin{table}
\centering
\caption{LNM Risk According to Clinicopathologic Parameters in Early Gastric SRCC}
\begin{tabular}{lcccc}
\hline
 & Total (N = 231) & LNM Negative (N = 194) & LNM Positive (N = 37) & Univariate OR (95\% CI) \\
\hline
\hline
Age (y) & & & & \\
\leq 60 & 155 (67.1) & 131 (67.5) & 24 (64.9) & 1 \\
> 60 & 76 (32.9) & 63 (32.5) & 13 (35.1) & 1.126 (0.538, 2.358) \\
\hline
Sex & & & & \\
Male & 138 (59.7) & 116 (59.8) & 22 (59.5) & 1 \\
Female & 93 (40.3) & 78 (40.2) & 15 (40.5) & 0.986 (0.482, 2.019) \\
\hline
Hypertension & & & & \\
Absence & 178 (77.1) & 154 (79.4) & 24 (64.9) & 1 \\
Presence & 53 (22.9) & 40 (20.6) & 13 (35.1) & 2.085 (0.976, 4.456) \\
\hline
Heart disease & & & & \\
Absence & 219 (94.9) & 184 (94.8) & 35 (94.6) & 1 \\
Presence & 12 (5.1) & 10 (5.2) & 2 (5.4) & 1.051 (0.221, 5.007) \\
\hline
Diabetes mellitus & & & & \\
Absence & 217 (93.9) & 184 (94.8) & 33 (89.2) & 1 \\
Presence & 14 (6.1) & 10 (5.2) & 4 (10.8) & 0.977 (0.570, 1.765) \\
\hline
BMI & & & & \\
\leq 28 & 205 (88.7) & 172 (88.7) & 33 (89.2) & 1 \\
> 28 & 26 (11.3) & 22 (11.3) & 4 (10.8) & 0.948 (0.307, 2.929) \\
\hline
Family history & & & & \\
Absence & 175 (75.8) & 149 (76.8) & 26 (70.3) & 1 \\
Presence & 56 (24.2) & 45 (23.2) & 11 (29.7) & 1.401 (0.642, 3.055) \\
\hline
CEA & & & & \\
Negative & 136 (58.9) & 120 (61.9) & 16 (43.2) & 1 \\
Positive & 95 (41.1) & 74 (38.1) & 21 (56.8) & 2.128 (1.044, 4.338) \\
\hline
Lesion size & & & & \\
Small (\(\leq 20\) mm) & 186 (80.5) & 163 (84.0) & 23 (62.2) & 1 \\
Large (\(>20\) mm) & 45 (19.5) & 31 (16.0) & 14 (37.8) & 3.201 (1.486, 6.894) \\
\hline
Macroscopic type & & & & \\
0-IIb (flat) & 44 (19.0) & 39 (20.1) & 5 (13.5) & 1 \\
0-IIa (elevated) & 8 (3.5) & 8 (4.1) & 0 (0.0) & 0.830 (0.729, 0.944) \\
0-IIc (depressed) & 88 (38.1) & 71 (36.6) & 17 (45.9) & 1.868 (0.640, 5.450) \\
0-I (protruded) & 8 (3.5) & 6 (3.1) & 2 (5.4) & 2.600 (0.408, 16.559) \\
0-III (excavated) & 83 (35.9) & 70 (36.1) & 13 (35.1) & 1.449 (0.481, 4.366) \\
\hline
No. tumors & & & & \\
Single & 221 (95.7) & 185 (95.4) & 36 (97.3) & 1 \\
Multitude & 10 (4.3) & 9 (4.6) & 1 (2.7) & 0.571 (0.070, 4.647) \\
\hline
Ulcus & & & & \\
Absence & 119 (51.5) & 107 (55.2) & 12 (32.4) & 1 \\
Presence & 112 (48.5) & 87 (44.8) & 25 (67.6) & 2.562 (1.217, 5.393) \\
\hline
Invasion depth & & & & \\
M & 126 (54.5) & 119 (61.3) & 7 (18.9) & 1 < 0.001 \\
SM1 & 38 (16.5) & 32 (16.5) & 6 (16.2) & 3.187 (2.748, 7.176) < 0.001 \\
SM2 & 67 (29) & 43 (22.2) & 24 (64.9) & 5.903 (3.862, 9.024) < 0.001 \\
\hline
Histologic type & & & & \\
Pure SRCC & 116 (50.2) & 108 (55.7) & 8 (21.6) & 1 < 0.001 \\
Mixed SRCC & 115 (49.8) & 86 (44.3) & 29 (78.4) & 4.552 (1.980, 10.465) < 0.001 \\
\hline
LVI & & & & \\
Absence & 200 (86.6) & 184 (94.8) & 16 (43.2) & 1 < 0.001 \\
Presence & 31 (13.4) & 10 (5.2) & 21 (56.8) & 24.150 (9.721, 59.994) < 0.001 \\
\hline
Perineural invasion & & & & \\
Absence & 205 (88.7) & 174 (89.7) & 31 (83.8) & 1 < 0.001 \\
Presence & 26 (11.3) & 20 (10.3) & 6 (16.2) & 1.684 (0.626, 4.528) < 0.001 \\
\hline
\end{tabular}
\end{table}

CI indicates confidence interval; M, tumor confined within the mucosal layer; SM1, tumor invading the superficial \(<0.5\) mm in depth\) submucosa; SM2, tumor invading the deep \(>0.5\) mm in depth\) submucosa.
and diabetes mellitus, body mass index (BMI), family history, carcinoembryonic antigen (CEA) level, lesion size, macroscopic type, depth of invasion, number of tumors, presence of ulcers, LVI, perineural invasion, and LNM.

Postoperative Follow-up

Postresection outcomes were investigated through the routine scheduled outpatient service at 3-month intervals in the first 2 years and every 6 months thereafter for clinical examination, including gastroscopy, chest x-ray, abdominal and pelvic ultrasound or computed tomography, and tumor markers. Telephone interviews by the investigators were performed to assess the general situation of each patient. Overall survival (OS) was defined from the date of the operation to the date of death or the cutoff date (August 31, 2019).

Statistical Analysis

Continuous variables were translated into categorical variables. For age, we set 60 years old as the cutoff value. According to the criteria for obesity, the enrolled patients were divided into a nonobesity group (BMI ≤ 28) and an obesity group (BMI > 28). Statistical analyses were conducted with SPSS software (version 23.0; SPSS Inc., Chicago, IL). Differences among categorical variables associated with predictors and LNM were assessed using a χ² test or Fisher exact test, and variables that were significant in the univariate analysis were subsequently entered into a multivariate logistic regression model for the analysis of independent risk factors for LNM. The associations between variables and LNM are described by odds ratios (ORs) and 95% confidence intervals. Survival rates was calculated by the Kaplan-Meier method, and the difference between the survival curves was analyzed using a log-rank test. The Cox regression model was applied to evaluate prognostic factors. P-value <0.05 (2 sided) was considered statistically significant.

The statistical methods and analyses of this study were reviewed by professor Xiaobin Zhou from the Department of Health Statistics, Qingdao University.

RESULTS

The incidence of early gastric SRCC was 17.1% (231/1352) in all EGCs. The prevalence of LNM in early SRCC was 16.0% (37/231) overall, 6.9% (8/116) in patients with pure SRCC, and 25.2% (29/115) in those with mixed SRCC.

Clinicopathologic Features of Early Gastric SRCC According to LNM

Early gastric SRCCs were more common in young (younger than 60 y old) patients than elderly patients and in males than in females (ratio: 1.48:1). Patients with CEA values that exceeded the normal level were more likely to have LNM, and this association was statistically significant (P = 0.035). However, the other clinical parameters, such as age, sex, underlying diseases, and family history, were not significantly associated with LNM.

With regard to the endoscopic features, the macroscopic type and number of tumors failed to reach statistical significance. Ulcerative lesions were significantly related to LNM (P = 0.011). Regarding tumor size, LNM occurred more frequently in patients with large tumors than in those with small tumors (P = 0.002).

LNM was found in 37 patients (16.0%). Regarding lesion depth, 126 (54.5%) patients had tumors limited to the mucosal (M) layer, 38 had superficial submucosal (SM1) tumors (16.5%), and 67 had deep submucosal (SM2) tumors (29.0%). The percentages of LNM positivity in these 3 groups were 5.6%, 15.8%, and 35.8%, respectively (P < 0.001). Among the LNM-positive cases, 78.4% (29/37) of the EGCs were mixed SRCC histologic type, while 55.7% (108/194) of the LNM-negative EGCs were pure SRCC histologic type (P < 0.001). LNM was found significantly more frequently in patients with LVI (P < 0.001) than in patients without invasion. However, the perineural invasion was not significantly associated with LNM in our cohort. Additional detailed clinicopathologic features of early SRCC according to LNM status are summarized in Table 1.

Risk Factors for LNM in Early SRCC by Univariate and Multivariate Analyses

The univariate analyses demonstrated that high CEA levels, tumor size (> 20 mm), the presence of ulcers, histologic type, depth of invasion (SM1 and SM2), and LVI differed significantly between patients with and without LNM. On the basis of the stepwise multivariate analysis, the significant independent risk factors for LNM in early SRCC were SM2 invasion (OR = 5.070, P = 0.003), LVI (OR = 14.876, P < 0.001), the pathologic pattern of mixed SRCC (OR = 3.226, P = 0.026), the presence of ulcers (OR = 3.340, P = 0.019) and a lesion size over 20 mm (OR = 2.823, P = 0.015). The independent risk factors are listed in Table 2.

Comparison of the Clinicopathologic Features Between Pure SRCC and Mixed SRCC

As shown in Table 3, the following differences in clinicopathologic characteristics between pure SRCC and mixed SRCC groups were significant: (1) the LNM rate was much higher in patients with mixed SRCC (25.2%) than those with pure SRCC (6.9%) (P < 0.0001); (2) LVI was found in a significantly higher proportion of cases of mixed SRCC (20.9%) than cases of pure SRCC (6.0%) (P < 0.001);

| TABLE 2. Multivariate Logistic Regression Analysis of LNM in Early Gastric SRCC |
| Factors | OR (95% CI) | P |
|---|---|---|
| Mixed SRCC histologic type | 3.226 (1.152, 9.034) | 0.026 |
| Ulcer | 3.340 (1.223, 9.122) | 0.019 |
| Large lesion size (> 20 mm) | 2.823 (1.225, 6.505) | 0.015 |
| LVI | 14.876 (5.272, 41.980) | < 0.001 |
| Invasion depth | 2.823 (0.559, 8.922) | 0.013 |
| SM1 | 5.070 (1.707, 15.055) | 0.003 |

CI indicates confidence interval; SM1, invading the superficial (< 0.5 mm in depth) submucosa; SM2, invading the deep (> 0.5 mm in depth) submucosa.
perineural invasion was observed more frequently in the mixed SRCC group (16.5%) than in the pure SRCC group (6.0%) \((P = 0.012)\); (4) SM1 and SM2 invasion were less frequent in the pure SRC group than in the mixed SRCC group \((P = 0.009)\); and (5) age was the only statistically significant clinical characteristic \((P = 0.045)\) in this comparison. However, the differences in sex, basic diseases, obesity, family history, CEA level, size, macroscopic type, lesion number or ulcerative findings were not statistically significant between the 2 groups.

**Differences in LNM and OS Between Pure and Mixed Early Gastric SRCC According to Invasion Depth**

According to Table 4, LNM occurred more frequently in patients with mixed early gastric SRCC than those with

| TABLE 3. Comparison of Clinicopathologic Parameters Between Pure and Mixed Early Gastric SRCC |
|-----------------------------------------------|
| **n (%)**                                    |
| **Total (N = 231)** | **Pure SRCC (N = 116)** | **Mixed SRCC (N = 115)** | **Univariate OR (95% CI)** | **P** |
|---|---|---|---|---|
| **Age (y)** | | | | |
| ≤ 60 | 155 (67.1) | 85 (73.3) | 70 (60.9) | 1 |
| > 60 | 76 (32.9) | 31 (26.7) | 45 (39.1) | 1.763 (1.011, 3.074) | 0.045 |
| **Sex** | | | | |
| Male | 138 (59.7) | 67 (57.6) | 71 (61.7) | 1.180 (0.697, 1.998) | 0.537 |
| Female | 93 (40.3) | 49 (42.2) | 44 (38.3) | 1 |
| **Hypertension** | | | | |
| Absence | 136 (58.9) | 72 (62.1) | 64 (55.7) | 1.304 (0.771, 2.205) | 0.322 |
| Presence | 95 (41.1) | 44 (37.9) | 51 (44.3) | 1.304 (0.771, 2.205) | 0.322 |
| **Diabetes mellitus** | | | | |
| Absence | 217 (93.9) | 112 (96.6) | 105 (91.3) | 1 |
| Presence | 14 (6.10) | 4 (3.4) | 10 (8.7) | 1.010 (0.447, 2.284) | 0.981 |
| **BMI** | | | | |
| ≤ 28 | 205 (88.7) | 103 (88.8) | 102 (88.7) | 1 |
| > 28 | 26 (11.3) | 13 (11.2) | 13 (11.3) | 2.667 (0.811, 8.763) | 0.515 |
| **Family history** | | | | |
| Absence | 175 (75.8) | 90 (76.6) | 85 (73.9) | 1 |
| Presence | 56 (24.2) | 26 (22.4) | 30 (26.1) | 1.222 (0.668, 2.323) | 0.322 |
| **CEA** | | | | |
| Absence | 136 (58.9) | 72 (62.1) | 64 (55.7) | 1 |
| Presence | 95 (41.1) | 44 (37.9) | 51 (44.3) | 1.304 (0.771, 2.205) | 0.322 |
| **Lesion size** | | | | |
| Small (≤ 20 mm) | 186 (80.5) | 95 (81.9) | 91 (79.1) | 1 |
| Large (> 20 mm) | 45 (19.5) | 21 (18.1) | 24 (20.9) | 1.193 (0.621, 2.291) | 0.966 |
| **Macroscopic type** | | | | |
| 0-II (flat) | 44 (19.0) | 19 (16.4) | 25 (21.7) | 1 |
| 0-II (elevated) | 8 (3.5) | 4 (3.4) | 4 (3.5) | 1.558 (0.746, 3.254) | 0.238 |
| 0-IIc (depressed) | 88 (38.1) | 46 (39.7) | 42 (36.5) | 1.184 (0.277, 5.057) | 0.819 |
| 0-I (protruded) | 8 (3.5) | 2 (1.7) | 6 (5.2) | 1.081 (0.593, 1.972) | 0.799 |
| 0-III (excavated) | 83 (35.9) | 45 (38.8) | 38 (33.0) | 3.553 (0.677, 18.638) | 0.134 |
| **No. tumors** | | | | |
| Single | 221 (95.7) | 113 (97.4) | 108 (93.9) | 1 |
| Multitude | 10 (4.3) | 3 (2.6) | 7 (6.1) | 2.441 (0.615, 9.685) | 0.210 |
| **Ulcer** | | | | |
| Absence | 119 (51.5) | 55 (47.4) | 64 (55.7) | 1 |
| Presence | 112 (48.5) | 61 (52.6) | 51 (44.3) | 0.718 (0.428, 1.206) | 0.009 |
| **Invasion depth** | | | | |
| M | 126 (54.5) | 75 (64.7) | 51 (44.3) | 1 |
| SM1 | 38 (16.5) | 15 (12.9) | 23 (20.0) | 2.255 (1.074, 4.733) | 0.032 |
| SM2 | 67 (29) | 26 (22.4) | 41 (35.7) | 2.319 (1.264, 4.254) | 0.007 |
| **LNM** | | | | |
| Absence | 194 (84.0) | 108 (93.1) | 86 (74.8) | 1 |
| Presence | 37 (16.0) | 8 (6.9) | 29 (25.2) | 4.552 (1.980, 10.465) | <0.001 |
| **LVI** | | | | |
| Absence | 200 (86.6) | 109 (94.0) | 91 (79.1) | 1 |
| Presence | 31 (13.4) | 7 (6.0) | 24 (20.9) | 4.107 (1.692, 9.968) | <0.001 |
| **Perineural invasion** | | | | |
| Absence | 205 (88.7) | 109 (94.0) | 96 (83.5) | 1 |
| Presence | 26 (11.3) | 7 (6.0) | 19 (16.5) | 3.082 (1.242, 7.648) | 0.012 |

CI indicates confidence interval; M, tumor confined within the mucosal layer; SM1, tumor invading the superficial (<0.5 mm in depth) submucosa; SM2, tumor invading the deep (>0.5 mm in depth) submucosa.
pure histologic type no matter in which layer. Except for SM2 lesions ($P=0.024$), this difference failed to reach statistical significance in the M and SM1 groups.

With regard to the survival analyses for early gastric SRCC, patients with pure SRCC showed higher 5-year survival rates than patients with mixed histologic type in all 3 layers. However, this association was not statistically significant in these subgroups (Table 5).

**Table 4. LNM in Pure and Mixed Early Gastric SRCC According to Invasion Depth**

| Invasion     | Total (N = 231) | LNM Negative (N = 194) | LNM Positive (N = 37) | Univariate OR (95% CI) | $P$  |
|--------------|-----------------|------------------------|-----------------------|------------------------|------|
| M invasion   |                 |                        |                       |                        |      |
| Pure SRCC    | 75 (59.5)       | 73 (61.3)              | 2 (28.6)              | 1                      | 0.086|
| Mixed SRCC   | 51 (40.5)       | 46 (38.7)              | 5 (71.4)              | 3.967 (0.739, 21.305)   | 0.213|
| SM1 invasion |                 |                        |                       |                        |      |
| Pure SRCC    | 15 (39.5)       | 14 (43.8)              | 1 (16.7)              | 1                      |      |
| Mixed SRCC   | 23 (60.5)       | 18 (56.2)              | 5 (83.3)              | 3.889 (0.407, 37.185)   |      |
| SM2 invasion |                 |                        |                       |                        |      |
| Pure SRCC    | 26 (38.8)       | 21 (48.8)              | 5 (20.8)              | 1                      | 0.024|
| Mixed SRCC   | 41 (61.2)       | 22 (51.2)              | 19 (79.2)             | 3.627 (1.146, 11.483)   |      |

CI indicates confidence interval; M, tumor confined within the mucosal layer; SM1, tumor invading the superficial (<0.5 mm in depth) submucosa; SM2, tumor invading the deep (>0.5 mm in depth) submucosa.

**DISCUSSION**

Numerous reports have identified SRCC as an independent predictor of poor prognosis due to specific characteristics such as a high incidence of LNM accompanied by a high rate of peritoneal carcinomatosis and low sensitivity to chemotherapy, especially during the period when the vast majority of these tumors were diagnosed at an advanced stage.

Owing to the popularization of gastroscopy, a large proportion of gastric cancers are diagnosed at an early stage, and precise histopathologic evaluations of EGC are now possible because of en bloc resection using ESD. The early detection of SRCC by an endoscopic examination and biopsy may be attributed to a high proportion of depressed lesions and easily detectable histologic features such as a high incidence of LNM associated with a high rate of peritoneal carcinomatosis and low sensitivity to chemotherapy.

**Table 5. OS Among the Patients With Pure and Mixed Early Gastric SRCC According to Invasion Depth**

| Invasion     | M 5-Year OS (%) | SM1 5-Year OS (%) | SM2 5-Year OS (%) | $P$ |
|--------------|-----------------|-------------------|-------------------|------|
| Pure SRCC    | 98.7            | 96.2              | 93.3              | 0.783|
| Mixed SRCC   | 98.0            | 90.2              | 87.0              | 0.512|

M indicates tumor confined within the mucosal layer; SM1, tumor invading the superficial (<0.5 mm in depth) submucosa; SM2, tumor invading the deep (>0.5 mm in depth) submucosa.

**Table 6. LNM in Early Gastric SRCC According to Therapeutic Criteria**

| Indication | Expanded ESD | Beyond ESD |
|------------|--------------|------------|
|            | Total LNM    | LNM (%)    | Total LNM | LNM (%) | $P$  |
| Pure SRCC  | 26           | 0          | 90        | 8       | 0.196|
| Mixed SRCC | 19           | 5.3       | 96        | 28      | 0.040|
| Total      | 45           | 2.2       | 186       | 36      | 0.003|
for LNM and a more favorable prognosis than other types.\textsuperscript{8,20,21,23–25} Hence, SRCC is not currently identified as a predictor of poor prognosis. Technical advances in the endoscopic treatment of EGC have created unprecedented opportunities to treat EGC patients who are expected to have an extremely low probability of LNM. Therefore, the prediction of the biological behavior, especially LNM risk, of early SRCCs is an important issue in selecting the treatment modality.\textsuperscript{26} However, there are no conclusive guidelines for early gastric SRCC, which accounts for a large proportion of EGC.

We investigated the clinicopathologic factors and prognostic outcomes of early gastric SRCCs. Consistent with previous studies, early gastric SRCCs had a higher prevalence in young patients and were predominantly macroscopically depressed lesions.\textsuperscript{8} Sugihara et al\textsuperscript{27} suggested that SRCC forms more frequently in the intramucosal section than the extramucosal section of the lesion, which is in line with our results. This phenomenon was also reported in other series.\textsuperscript{20,22} The risk factors varied among relevant studies, but LVI, invasion depth and tumor size were found to be significantly related to LNM in almost every study. To further investigate the relationship between various clinicopathologic features and LNM, we performed univariate and multivariate analyses and confirmed that large tumor size, LVI, the presence of ulcers, the mixed SRCC histologic pattern and SM2 invasion depth were independent predictors of LNM.

By comparing patients’ clinical, endoscopic, and histopathologic characteristics and prognoses, we observed that compared with those with pure SRCC, those with mixed SRCC were older, had deeper invasion depth, more frequently had LVI and perineural invasion, and most importantly, had a higher incidence of LNM despite unremarkable significant differences. Furthermore, our study showed a distinctive tendency toward a difference in survival between mixed and pure SRCC patients, which is in accordance with other studies.\textsuperscript{2,22} Moreover, according to this research, the histologic type was an independent factor predicting prognosis in early gastric SRCC patients.

There is a continuous discussion concerning the indications for the endoscopic treatment of EGC. Numerous studies have reevaluated the risk of LNM in EGC following the introduction of expanded indications for ESD. A meta-analysis involving 9798 EGC patients showed that expanding the indications for ESD to include undifferentiated lesions <20 mm still requires careful investigation.\textsuperscript{28} However, many researchers recommend endoscopic resection for early gastric SRCC because it had favorable behavior compared with non-SRCC types in their studies.\textsuperscript{4,6,8,24,25} When we narrowed our cohort into a subgroup with expanded indications for ESD that had tumors <2 cm, no LVI, no ulcerations, and invasion confined to the M level, 45 cases (26 pure SRCCs and 19 mixed SRCCs) were included. The LNM incidence was 0% (0/26) for pure SRCCs and 5.3% (1/19) for mixed SRCCs. This also suggests that ESD is more feasible for pure SRCCs than for mixed SRCCs. Hence, a more accurate application of the SRCC classification could improve the ESD criteria.

However, the cellular histology of SRCC has been proven to be an important risk factor for LNM in EGC.\textsuperscript{20} Kim et al\textsuperscript{26} reported that compared with pure SRCC, the mixed SRCC subtype was associated with a higher frequency of LVI, more frequent intestinal metaplasia in the adjacent mucosa, and most importantly, a higher incidence of LNM. Imamura et al\textsuperscript{21} also stated that EGC

\begin{figure}
\centering
\includegraphics[width=\textwidth]{survival_curves.png}
\caption{Survival curves. A, OS of EGC patients with pure signet-ring cell carcinoma (pSRCC) and mixed signet-ring cell carcinoma (mSRCC) in months ($P=0.004$). B, Disease-specific survival of EGC patients with pSRCC and mSRCC in months ($P=0.002$).}
\end{figure}
TABLE 7. Univariate and Multivariate Analyses of the Factors Affecting OS Among the Patients With Early Gastric SRCC

|                        | Univariate Analysis | Multivariate Analysis |
|------------------------|---------------------|-----------------------|
|                        | 5-Year OS (%) | *P*       | Hazard Ratio | 95% CI | *P* |
| Age (y)                | <0.001             |           |            |       |     |
| ≤ 60                   | 98.7               | 1         |              |       |     |
| > 60                   | 88.2               | 5.246     | 1.938     | 14.197 |     |
| Sex                    | 0.037              | 1         | 0.154      |       |     |
| Female                 | 97.6               | 1         | 2.572     | 0.702  | 9.418 |
| Male                   | 92.8               | 0.033     | 1         |       |     |
| CEA                    | 96.1               | 1         | 1.439     | 0.500  | 4.144 |
| Positive               | 92.6               |           |           |       |     |
| Invasion depth         | 0.049              |           |           |       |     |
| M                      | 98.4               | 1         |           |       |     |
| SM1                    | 92.5               | 6.192     | 1.320     | 29.054 | 0.023 |
| SM2                    | 89.5               | 7.529     | 1.328     | 42.667 | 0.021 |
| LNM                    | <0.001             |           |           | <0.001 |     |
| Absence                | 96.9               | 1         |           |       |     |
| Presence               | 86.5               | 5.352     | 1.969     | 14.551 |     |
| LVI                    | 0.002              |           |           | 0.682  |     |
| Absence                | 97.0               | 1         |           |       |     |
| Presence               | 83.9               | 0.765     | 0.212     | 2.764  |     |
| Histologic type        | 0.047              |           |           |       |     |
| Pure SRCC              | 97.4               | 1         |           |       |     |
| Mixed SRCC             | 93.0               | 3.682     | 1.015     | 13.354 |     |

CI indicates confidence interval; M, tumor confined within the mucosal layer; SM1, tumor invading the superficial (<0.5 mm in depth) submucosa; SM2, tumor invading the deep (>0.5 mm in depth) submucosa.

with a mixed SRC histology exhibited more aggressive behavior than purely tubular adenocarcinoma or pure SRCC. Zheng et al considered highly aggressive behavior, such as proliferation, apoptosis, angiogenesis, mucin secretion, and cell adhesion due to the increased expression of proteins such as Ki-67, extracellular matrix metalloproteinase inducer, and vascular endothelial growth factor; to be a possible reason for the aggressive features of mixed-type gastric cancer. Piessen et al also proved that mixed-type gastric cancer frequently showed cytosine-phosphate-guanine (CpG) island hypermethylation. Furthermore, Kim et al found that pure SRCCs might show a gastric phenotype, whereas mixed SRCCs could be associated with an intestinal immunophenotype.

On the basis of our findings, we propose that the presence of a pure or mixed SRCC component should be reported in daily pathologic practice, especially in cases of EGC, and such findings should be taken into consideration in the assessment of curable resection for ESD specimens. It should be noted that this was a retrospective study at a single institution, which was the major limitation. Therefore, a well-designed multicentric prospective cohort study is essential. Furthermore, we speculate that a subgroup analysis of mixed SRC histology samples categorized by the rate of minor components of isolated carcinoma cells containing mucin may reveal a significant difference in LNM frequency and survival rate. Hence, further research may be needed to investigate different subtypes more precisely in the future.

CONCLUSIONS

The independent risk factors for LNM in early SRCCs were SM2 invasion, LVI, the pathologic pattern of mixed SRCC, ulcer presence and a lesion size over 20 mm. Furthermore, compared with pure SRCC, the mixed SRCC subtype was associated with older age, larger lesion size, higher frequency of LVI, more frequent perineural invasion, and most importantly, a higher incidence of LNM and worse prognosis. Therefore, early SRCCs should be further classified by the purity of the SRCC component.

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