Adenocarcinoma with mixed subtypes in the early and advanced gastric cancer.

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Research Article

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Abstract

Background

Based on the WHO classification, adenocarcinoma with mixed subtypes (AM) is a histological classification. We aimed to compare the prognosis among AM, classic adenocarcinoma (CA), mucinous adenocarcinoma (MAC), and signet-ring cell carcinoma (SRCC) in early and advanced gastric cancer (EGC, AGC), respectively.

Methods

We compared the clinicopathologic features and prognosis between AM and other histologic subtypes of CA, SRCC and MAC in EGC and ACG, respectively. A nomogram was established to predict the cancer-specific survival (CSS) of gastric cancer (GC) patients with AM. C-index, calibration curves, Receiver Operating Characteristic (ROC) and Decision Curve Analysis (DCA) curves were applied to examine the accuracy and clinical benefits.

Results

In the prognosis among these four histological subtypes in EGC patients, there are no differences. For AGC patients, AM had a significantly poorer prognosis compared with CA and MAC (P = 0.003, 0.029), but similar prognosis to SRCC. A nomogram based on race, T stage, N stage, M stage and surgical modalities were proposed to predict 1- and 3-year CSS for GC patients with AM (C-index: training cohort: 0.804, validation cohort: 0.748. 1-, 3-year CSS AUC: training cohort: 0.871, 0.914, validation cohort: 0.810, 0.798). 1- and 3-year CSS DCA curves showed good net benefits.

Conclusions

EGC patients with AM had similar survival to those with CA, MAC and SRCC. AM was an independent predictor of poor prognosis in AGC. A nomogram for predicting the prognosis of GC patients with AM was proposed to quantitatively assess the long-term survival.

Introduction

More than one million (1,033,701) new cases of gastric cancer (GC) were diagnosed globally in 2018, with 782,685 deaths [1]. Ninety per cent of gastric cancer is adenocarcinomas (ACs) [2]. There were multiple studies on the prognosis of GC patients with the histology of mucinous adenocarcinoma (MAC) and signet-ring cell carcinoma (SRCC) [3–6]. And clinical features and prognosis of SRCC were reported to be different between early and advanced gastric cancer (ECG, ACG) [7]. There may still be other histologic subtypes with distinct clinicopathologic and prognosis that need special concern in clinical management. Adenocarcinoma with mixed subtypes (AM) is a type of uncommon adenocarcinoma in GC based on WHO classification. There were some researches on the prognosis of GC patients with AM [8, 9]. However, there is no report on
the comparison of prognosis among AM, classic adenocarcinoma (CA), MAC and SRCC in EGC and AGC, respectively.

In the present study, we compared the prognosis among EGC and AGC patients with AM and other histologic subtypes of CA, MAC and SRCC based on Surveillance, Epidemiology, and End Results (SEER) database. In addition, we developed a predictive nomogram to quantify the survival estimates of GC patients with AM.

Material And Methods

Data collection

A retrospective review of GC with histology of AM from the SEER database between 2001 and 2016 was performed. Data of GC (site code ICD-O-3/WHO 2008 of “stomach”) patients with histology of AM (Code 8255), CA (Code 8144, 8210–8211, 8260–8263), MAC (Code 8480–8481) and SRCC (Code 8490) from 2004 to 2016 were collected from the SEER database. A total of 6679 patients were selected according to the following exclusion criteria: (1) GC wasn’t the first diagnosed primary tumor; (2) The information of race, tumor size, the American Joint Committee on Cancer (AJCC) 7th Tumor Node Metastasis (TNM) stage, cause of death and surgery were not available; (3) The time of follow-up was 0. EGC is defined as gastric cancer confined to the mucosa or submucosa regardless of the presence of lymph node metastasis (LNM). AGC is defined as gastric cancer exceeding the submucosa. The baseline characteristics collected included sex, age, race, primary site, the AJCC 7th TNM stage, surgery, tumor size. Follow-up data collected included overall survival (OS) and cancer-specific survival (CSS) and survival time.

Statistical analysis

We firstly explored the frequency, incidence and mortality of newly diagnosed GC patients with histology of AM. Rates were expressed as per 100,000 individuals and age-adjusted (2000 US Standard Population, 19 age groups). Annual percentage changes (APCs) were then calculated. The comparisons of clinicopathologic characteristics between AM and the other histologic subtypes including CA, MAC and SRCC in EGC and AGC were performed with chi-square test. Univariate and multivariate Cox analyses were performed to explore the influence of different histologic subtypes on cancer-specific death. A multivariate Gray’s competing risk regression model were then performed to adjust potential confounding factors.

Subsequently, the prognostic analysis was performed in GC patients with AM. Patients were assigned into training cohort (Alaska, Northern Plains, Pacific Coast, Southwest, n = 315) and validation cohort (East, n = 136) by geographic region of the United States. In the training cohort, univariate and multivariate Cox analyses were used to determine the independent prognostic factors. Significant prognostic factors were used to establish a nomogram to predict the 1- and 3-year CSS rates. Then, internal and external validations were performed. The validation cohorts were used for external validation. We used calibration curves and concordance index (C-index) curves to internal and external evaluate the predictive accuracy of the nomogram (bootstraps with 500 resample). Receiver operating characteristic (ROC) were used for the internal and external validation of the nomogram for 1- and 3-year CSS rates. Decision Curve Analysis (DCA) was then performed to analyze the clinical usability of the nomogram for 1- and 3-year CSS rates.
IBM SPSS 23.0 and R software version 3.6 were utilized in performing all the above statistical analyses. And two-tailed \( P < 0.05 \) were defined as statistical significance.

**Results**

**Incidence trends**

The number of newly diagnosed GC patients with AM from 2001 to 2016 was divided by age, and the most common age at diagnosis was 72–74 years old (Fig. 1A). The trend from 2001 to 2016 in age-adjusted incidence for GC patients with AM was illustrated with an APC of 3.7% [95% CI: 1.3–6.1] (Fig. 1B). The mortality rate increased from 2001 to 2012 with an APC of 5.1% [95% CI: 1.1–9.2], but decreased from 2012 to 2016 with an APC of -28.1% [95% CI: -35.4– -20.1] (Fig. 1B). In terms of genders, the incidence trend was quite different between the two groups (Fig. 1C).

**Comparisons of clinicopathologic differences between the histology of AM and other histologic subtypes in EGC and AGC patients**

In EGC patients, there were no significant differences in sex, tumor size, N stage and M stage between AM and other three histologic subtypes. AM was more common in nonwhite patients than MAC and SRCC (Table 1, \( P = 0.013, 0.033 \)). MAC was more likely to be discovered in the body of stomach than AM (\( P = 0.031 \)). No significant difference in the distribution of age was observed between AM and MAC. AM patients are older than SRCC patients, but younger than CA patients (\( P = 0.025, < 0.001 \)).
Table 1
Comparisons of the clinicopathologic features between AM and other histologic types in early gastric cancer.

| Variable          | AM<sup>a</sup> (%) N = 101 | CA<sup>b</sup> (%) N = 1110 | MAC<sup>c</sup> (%) N = 52 | SRCC<sup>d</sup> (%) N = 811 | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|-------------------|----------------------------|----------------------------|-----------------------------|----------------------------|---------|------------------|-------------------|--------------------|
| Sex               |                            |                            |                             |                             | < 0.001 | 0.303            | 0.619             | 0.286              |
| Female            | 47 (46.5%)                 | 458 (42.3%)                | 22 (41.3%)                  | 423 (52.2%)                |         |                  |                   |                    |
| Male              | 54 (53.5%)                 | 652 (57.7%)                | 30 (58.7%)                  | 388 (47.8%)                |         |                  |                   |                    |
| Age               |                            |                            |                             |                             | < 0.001 | < 0.001          | 0.546             | 0.025              |
| < 65              | 42 (41.6%)                 | 263 (23.7%)                | 19 (36.5%)                  | 433 (53.4%)                |         |                  |                   |                    |
| ≥ 65              | 59 (58.4%)                 | 847 (76.3%)                | 33 (63.5%)                  | 378 (46.6%)                |         |                  |                   |                    |
| Race              |                            |                            |                             |                             | 0.001   | 0.675            | 0.013             | 0.033              |
| White             | 53 (52.5%)                 | 631 (56.8%)                | 40 (76.9%)                  | 527 (65.0%)                |         |                  |                   |                    |
| Black             | 14 (13.9%)                 | 148 (13.3%)                | 4 (8.0%)                    | 99 (12.2%)                 |         |                  |                   |                    |
| Other             | 34 (33.7%)                 | 331 (29.8%)                | 8 (15.4%)                   | 185 (22.8%)                |         |                  |                   |                    |
| Tumor size        |                            |                            |                             |                             | 0.018   | 0.765            | 0.057             | 0.507              |
| < 3cm             | 59 (58.4%)                 | 689 (62.1%)                | 20 (38.5%)                  | 507 (62.5%)                |         |                  |                   |                    |
| 3-6cm             | 26 (25.7%)                 | 257 (23.2%)                | 18 (34.6%)                  | 168 (20.7%)                |         |                  |                   |                    |
| ≥ 6cm             | 16 (15.8%)                 | 164 (14.8%)                | 14 (26.9%)                  | 136 (16.8%)                |         |                  |                   |                    |
| Primary site      |                            |                            |                             |                             | < 0.001 | 0.148            | 0.031             | 0.589              |
| Antrum            | 27 (28.2%)                 | 418 (26.3%)                | 14 (26.9%)                  | 228 (25.9%)                |         |                  |                   |                    |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.
| Variable                        | AM\(^a\) (%) N = 101 | CA\(^b\) (%) N = 1110 | MAC\(^c\) (%) N = 52 | SRCC\(^d\) (%) N = 811 | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|--------------------------------|-----------------------|------------------------|----------------------|-------------------------|---------|------------------|---------------------|---------------------|
| Pylorus                        | 18 (13.3%)            | 146 (7.2%)             | 3 (5.8%)             | 116 (12.7%)             |         |                  |                     |                     |
| Body                           | 20 (18.0%)            | 158 (37.8%)            | 24 (46.2%)           | 133 (17.9%)             |         |                  |                     |                     |
| Cardia                         | 4 (3.1%)              | 46 (2.9%)              | 2 (3.8%)             | 27 (2.8%)               |         |                  |                     |                     |
| Fundus                         | 7 (6.0%)              | 60 (4.0%)              | 1 (1.9%)             | 58 (6.4%)               |         |                  |                     |                     |
| Lesser curve                   | 8 (9.1%)              | 126 (6.7%)             | 2 (3.8%)             | 107 (12.8%)             |         |                  |                     |                     |
| Greater curve                  | 16 (17.7%)            | 124 (13.3%)            | 6 (11.5%)            | 113 (17.6%)             |         |                  |                     |                     |
| Overlapping/not otherwise      | 1 (4.7%)              | 32 (1.9%)              | 0 (0%)               | 29 (4.1%)               |         |                  |                     |                     |
| otherwise specified            |                      |                       |                      |                         |         |                  |                     |                     |
| N stage                        |                      |                       |                      |                          | 0.017   | 0.053            | 0.672               | 0.597               |
| N0                             | 82 (81.1%)            | 992 (89.4%)            | 41 (78.8%)           | 689 (85.0%)             |         |                  |                     |                     |
| N1                             | 13 (12.9%)            | 90 (8.1%)              | 6 (11.5%)            | 88 (10.9%)              |         |                  |                     |                     |
| N2                             | 5 (5.0%)              | 20 (1.8%)              | 3 (5.8%)             | 23 (2.8%)               |         |                  |                     |                     |
| N3                             | 1 (0.9%)              | 8 (0.7%)               | 2 (3.8%)             | 11 (13.6%)              |         |                  |                     |                     |
| M stage                        |                      |                       |                      |                          | < 0.001 | 0.064            | 0.188               | 0.351               |
| M0                             | 91 (85.6%)            | 1050 (94.6%)           | 43 (82.7%)           | 704 (86.8%)             |         |                  |                     |                     |
| M1                             | 10 (14.4%)            | 60 (5.4%)              | 9 (17.3%)            | 107 (13.2%)             |         |                  |                     |                     |
| Surgery                        |                      |                       |                      |                          | < 0.001 | 0.038            | 0.043               | 0.008               |
| None                           | 17 (16.8%)            | 134 (12.1%)            | 19 (36.5%)           | 221 (27.3%)             |         |                  |                     |                     |

\(a\): adenocarcinoma with mixed subtypes; \(b\): classical adenocarcinoma, \(c\): mucinous adenocarcinoma \(d\): signet-ring cell carcinoma.
| Variable                  | AM<sup>a</sup> (%) N = 101 | CA<sup>b</sup> (%) N = 1110 | MAC<sup>c</sup> (%) N = 52 | SRCC<sup>d</sup> (%) N = 811 | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|--------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|---------|------------------|---------------------|---------------------|
| Local tumor destruction  | 5 (5.0%)                    | 110 (9.9%)                  | 1 (1.9%)                    | 10 (1.2%)                   |         |                  |                     |                     |
| Partial gastrectomy      | 57 (56.4%)                  | 708 (63.8%)                 | 25 (48.1%)                  | 419 (51.7%)                 |         |                  |                     |                     |
| Total gastrectomy        | 22 (21.8%)                  | 158 (14.2%)                 | 7 (13.5%)                   | 161 (19.9%)                 |         |                  |                     |                     |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.

In terms of AGC patients, there were no differences in the location of primary tumor between AM and SRCC. CA and MAC was found more frequently in the antrum and body of stomach, retrospectively (Table 2, P = 0.002, < 0.001). AM was more common in younger patients than CA. There was no significant difference in the distribution of sex, race and M stage between AM and CA. However, AM was significantly associated with larger tumor sizes and advanced T Stage, N Stage, proving poor prognosis, compared with CA (P = 0.018, < 0.001, < 0.001.). As for the comparison of AM and MAC, no differences were found in age, race, tumor size and M stage, while AM was more common in female, T4 and N2-3 stage patients (P = 0.021, 0.046, 0.002). When comparing AM and SRCC, AM was significantly associated with male, nonwhite, larger tumor sizes and the older (P = 0.002, 0.015, 0.002, < 0.001). On the contrary, SRCC was more likely to be found in patients with M1 stage compared with AM (P = 0.009).
### Table 2
Comparisons of the clinicopathologic features between AM and other histologic types in advanced gastric cancer.

| Variable        | AM<sup>a</sup> (%) N = 350 | CA<sup>b</sup> (%) N = 1736 | MAC<sup>c</sup> (%) N = 324 | SRCC<sup>d</sup> (%) N = 2195 | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|-----------------|-----------------------------|-----------------------------|-----------------------------|-------------------------------|---------|-------------------|---------------------|----------------------|
| Sex            |                             |                             |                             |                               | < 0.001 | 0.749             | 0.021               | 0.002                |
| Female         | 132 (37.7%)                 | 639 (36.8%)                 | 95 (29.3%)                  | 1025 (46.7%)                  |         |                   |                     |                      |
| Male           | 218 (62.3%)                 | 1097 (63.2%)                | 229 (70.7%)                 | 1170 (53.3%)                  |         |                   |                     |                      |
| Age            |                             |                             |                             |                               | < 0.001 | < 0.001           | 0.443               | < 0.001              |
| < 65           | 155 (44.3%)                 | 533 (30.7%)                 | 134 (41.4%)                 | 1270 (57.9%)                  |         |                   |                     |                      |
| ≥ 65           | 195 (55.7%)                 | 1203 (69.3%)                | 190 (58.6%)                 | 925 (42.1%)                   |         |                   |                     |                      |
| Race           |                             |                             |                             |                               | < 0.001 | 0.355             | 0.080               | 0.015                |
| White          | 221 (63.1%)                 | 1027 (59.2%)                | 229 (70.7%)                 | 1503 (68.5%)                  |         |                   |                     |                      |
| Black          | 53 (15.1%)                  | 278 (16.0%)                 | 45 (13.9%)                  | 252 (11.5%)                   |         |                   |                     |                      |
| Other          | 76 (21.7%)                  | 431 (24.8%)                 | 50 (15.4%)                  | 440 (20.0%)                   |         |                   |                     |                      |
| Tumor size     |                             |                             |                             |                               | < 0.001 | 0.018             | 0.787               | 0.002                |
| < 3cm          | 54 (15.4%)                  | 321 (18.5%)                 | 48 (14.8%)                  | 508 (23.1%)                   |         |                   |                     |                      |
| 3-6cm          | 89 (25.4%)                  | 532 (30.6%)                 | 90 (27.8%)                  | 571 (26.0%)                   |         |                   |                     |                      |
| ≥ 6cm          | 207 (59.1%)                 | 883 (50.9%)                 | 186 (57.4%)                 | 1116 (50.8%)                  |         |                   |                     |                      |
| Primary site   |                             |                             |                             |                               | < 0.001 | 0.038             | < 0.001             | 0.543                |
| Antrum         | 100 (28.6%)                 | 564 (32.5%)                 | 85 (26.2%)                  | 550 (25.1%)                   |         |                   |                     |                      |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.
| Variable                      | AM<sup>a</sup> (%) N = 350 | CA<sup>b</sup> (%) N = 1736 | MAC<sup>c</sup> (%) N = 324 | SRCC<sup>d</sup> (%) N = 2195 | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|-------------------------------|-----------------------------|-----------------------------|-----------------------------|-------------------------------|---------|-------------------|---------------------|---------------------|
| Pylorus                       | 42 (12.0%)                  | 208 (12.0%)                 | 24 (7.4%)                   | 266 (12.1%)                   | <0.001  | <0.001            | 0.046               | 0.931               |
| Body                          | 61 (17.4%)                  | 230 (13.2%)                 | 118 (36.4%)                 | 404 (18.4%)                   |         |                   |                     |                     |
| Cardia                        | 10 (2.9%)                   | 87 (5.0%)                   | 9 (2.8%)                    | 56 (2.6%)                     |         |                   |                     |                     |
| Fundus                        | 20 (5.7%)                   | 83 (4.8%)                   | 14 (4.3%)                   | 131 (6.0%)                    |         |                   |                     |                     |
| Lesser curve                  | 33 (9.4%)                   | 228 (13.1%)                 | 23 (7.1%)                   | 279 (12.7%)                   |         |                   |                     |                     |
| Greater curve                 | 64 (18.3%)                  | 253 (14.6%)                 | 44 (13.6%)                  | 416 (19.0%)                   |         |                   |                     |                     |
| Overlapping/not otherwise specified | 20 (5.7%)               | 83 (4.8%)                   | 7 (2.2%)                    | 93 (4.2%)                     |         |                   |                     |                     |
| T stage                       | <0.001                      | <0.001                      | 0.046                       | 0.931                         |         |                   |                     |                     |
| T2                            | 52 (14.9%)                  | 379 (21.8%)                 | 58 (17.9%)                  | 341 (15.5%)                   |         |                   |                     |                     |
| T3                            | 149 (42.6%)                 | 866 (49.9%)                 | 158 (48.8%)                 | 916 (41.7%)                   |         |                   |                     |                     |
| T4                            | 149 (42.6%)                 | 491 (28.3%)                 | 108 (33.3%)                 | 938 (42.7%)                   |         |                   |                     |                     |
| N stage                       | <0.001                      | <0.001                      | 0.002                       | 0.063                         |         |                   |                     |                     |
| N0                            | 113 (32.3%)                 | 806 (46.4%)                 | 138 (42.6%)                 | 854 (38.9%)                   |         |                   |                     |                     |
| N1                            | 70 (2.0%)                   | 379 (21.8%)                 | 77 (23.8%)                  | 447 (20.4%)                   |         |                   |                     |                     |
| N2                            | 67 (19.1%)                  | 274 (15.8%)                 | 50 (15.4%)                  | 341 (15.5%)                   |         |                   |                     |                     |
| N3                            | 100 (28.6%)                 | 277 (16.0%)                 | 59 (18.2%)                  | 553 (25.2%)                   |         |                   |                     |                     |
| M stage                       | <0.001                      | 0.178                       | 0.905                       | 0.009                         |         |                   |                     |                     |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.
### Variable

| Variable                  | AM<sup>a</sup> (% N = 350) | CA<sup>b</sup> (%) N = 1736 | MAC<sup>c</sup> (% N = 324) | SRCC<sup>d</sup> (% N = 2195) | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|--------------------------|-----------------------------|-----------------------------|-----------------------------|-------------------------------|---------|-------------------|-------------------|-------------------|
| M0                       | 295 (84.3%)                 | 1510 (87.0%)                | 272 (84.0%)                 | 1715 (78.1%)                  | <0.001  | 0.280             | 0.458             | <0.001            |
| M1                       | 55 (15.7%)                  | 226 (13.0%)                 | 52 (16.0%)                  | 480 (21.9%)                   |         |                   |                   |                   |
| Surgery                  |                             |                             |                             |                               |         |                   |                   |                   |
| None                     | 43 (12.3%)                  | 175 (10.1%)                 | 44 (13.6%)                  | 488 (22.2%)                   |         |                   |                   |                   |
| Local tumor destruction  | 2 (0.6%)                    | 11 (0.6%)                   | 4 (1.2%)                    | 16 (0.7%)                     |         |                   |                   |                   |
| Partial gastrectomy      | 200 (57.1%)                 | 1085 (62.5%)                | 194 (59.9%)                 | 992 (45.2%)                   |         |                   |                   |                   |
| Total gastrectomy        | 105 (30.0%)                 | 465 (26.8%)                 | 82 (25.3%)                  | 699 (31.8%)                   |         |                   |                   |                   |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.

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### The prognostic value of histologic subtypes for OS and CSS in EGC and AGC patients

1-year and 3-year OS rates for AM in EGC were 87.8% and 72.6%, respectively, and 1-year and 3-year CSS rates were 87.8% and 78.1%, respectively (Table 3). 1-year and 3-year OS rates for AM in AGC were 64.8% and 39.7%, respectively, and 1-year and 3-year CSS rates were 66.8% and 43.7%, respectively (Table 4). We firstly performed the Kaplan-Meier curves of four subtypes to investigate whether GC patients between AM and other histologic subtypes have different survival rates in EGC and AGC patients (Fig. 1D). The log-rank test displayed that EGC patients with AM had similar prognosis with CA, but was associated with better prognosis compared with MAC and SRCC (P = 0.002, 0.030). However, in AGC patients, AM was associated with poorer prognosis compared with CA and MAC (Fig. 1E, P < 0.001, = 0.022), but similar prognosis with SRCC.
### Table 3
Survival rates of patients with 4 histological subtypes in early gastric cancer.

| Pathological characteristics | 1-year OS<sup>e</sup> rate (%) | 3-year OS<sup>e</sup> rate (%) | 1-year CSS<sup>f</sup> rate (%) | 3-year CSS<sup>f</sup> rate (%) |
|-----------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|
| AM<sup>a</sup>              | 87.8% (81.3%-94.3%)           | 72.6% (63.3%-81.9%)           | 87.8% (81.3%-94.3%)           | 78.1% (69.6%-86.7%)           |
| CA<sup>b</sup>              | 83.9% (81.7%-86.1%)           | 70.9% (68.1%-73.8%)           | 89.7% (87.8%-91.5%)           | 81.3% (78.8%-83.8%)           |
| MAC<sup>c</sup>             | 68.5% (55.8%-81.3%)           | 44.0% (29.7%-58.3%)           | 72.4% (60.1%-84.8%)           | 53.8% (39.4%-68.2%)           |
| SRCC<sup>d</sup>            | 75.6% (72.5%-78.6%)           | 64.8% (61.3%-68.3%)           | 78.6% (75.6%-81.5%)           | 69.6% (66.2%-73.0%)           |

<sup>a</sup>: adenocarcinoma with mixed subtypes; <sup>b</sup>: classical adenocarcinoma, <sup>c</sup>: mucinous adenocarcinoma <sup>d</sup>: signet-ring cell carcinoma; <sup>e</sup>: overall survival; <sup>f</sup>: cancer-specific survival.

Multivariate Cox regression was performed for CSS to adjust potential confounding factors. The results revealed that the prognosis of four histologic subtypes had no difference in EGC patients (Fig. 2). In AGC patients, AM had a significantly poorer prognosis compared with CA and MAC (Fig. 3, HR: 0.782, 95%CI: 0.664–0.922, P = 0.003; HR: 0.787, 95%CI: 0.634–0.976, P = 0.029), but similar prognosis to SRCC. Taking death that is not related to GC into consideration, we also performed a multivariate Gray's competing risk regression model to adjust potential confounding factors, which exhibits the similar results about the histologic subtypes (Table 5, 6).

### Table 4
Survival rates of patients with 4 histological subtypes in early advanced gastric cancer.

| Pathological characteristics | 1-year OS<sup>e</sup> rate (%) | 3-year OS<sup>e</sup> rate (%) | 1-year CSS<sup>f</sup> rate (%) | 3-year CSS<sup>f</sup> rate (%) |
|-----------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|
| AM<sup>a</sup>              | 64.8% (59.5%-70.0%)           | 39.7% (33.9%-45.5%)           | 66.8% (61.5%-72.0%)           | 43.7% (37.7%-49.7%)           |
| CA<sup>b</sup>              | 76.2% (74.1%-78.3%)           | 49.9% (47.3%-52.5%)           | 80.3% (78.4%-82.3%)           | 55.5% (52.9%-58.2%)           |
| MAC<sup>c</sup>             | 73.7% (68.8%-78.6%)           | 44.9% (39.0%-50.8%)           | 76.3% (71.5%-81.0%)           | 50.5% (44.4%-56.6%)           |
| SRCC<sup>d</sup>            | 63.8% (61.7%-65.9%)           | 33.6% (31.4%-35.8%)           | 65.7% (63.7%-67.8%)           | 36.4% (34.1%-38.7%)           |

<sup>a</sup>: adenocarcinoma with mixed subtypes; <sup>b</sup>: classical adenocarcinoma, <sup>c</sup>: mucinous adenocarcinoma <sup>d</sup>: signet-ring cell carcinoma; <sup>e</sup>: overall survival; <sup>f</sup>: cancer-specific survival.
Table 5
Results of competing risks regression in patients with early gastric cancer.

| Variable                        | Subdistribution hazard ratio | P value |
|---------------------------------|------------------------------|---------|
| Sex                             |                              |         |
| Female                          |                              |         |
| Male                            | 1.218(1.014–1.463)           | 0.035   |
| Age                             |                              |         |
| < 65                            | 1(Reference)                 |         |
| ≥ 65                            | 1.431(1.177–1.739)           | < 0.001 |
| Race                            |                              |         |
| White                           | 1(Reference)                 |         |
| Black                           | 0.985(0.750–1.294)           | 0.910   |
| Other                           | 0.774(0.614–0.975)           | 0.029   |
| Tumor size                      |                              |         |
| < 3cm                           | 1(Reference)                 |         |
| 3-6cm                           | 1.518(1.213–1.900)           | < 0.001 |
| ≥ 6cm                           | 1.692(1.342–2.134)           | < 0.001 |
| Primary site                    |                              |         |
| Antrum                          | 1(Reference)                 |         |
| Pylorus                         | 1.027(0.761–1.384)           | 0.860   |
| Body                            | 1.539(1.169–2.026)           | 0.002   |
| Cardia                          | 2.008(1.360–2.964)           | < 0.001 |
| Fundus                          | 1.165(0.809–1.680)           | 0.410   |
| Lesser curve                    | 1.173(0.799–1.722)           | 0.410   |
| Greater curve                   | 1.306(0.962–1.771)           | 0.087   |
| Overlapping/not otherwise specified | 1.086(0.506–2.331)       | 0.830   |
| N stage                         |                              |         |
| N0                              | 1(Reference)                 |         |
| N1                              | 1.033(0.803–1.330)           | 0.800   |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.
| Variable            | Subdistribution hazard ratio     | P value  |
|---------------------|----------------------------------|----------|
| N2                  | 1.234(0.672–2.266)              | 0.500    |
| N3                  | 1.882(1.073–3.301)              | 0.027    |
| M stage             |                                  |          |
| M0                  | 1(Reference)                    |          |
| M1                  | 3.184(2.479–4.090)              | < 0.001  |
| Surgery             |                                  |          |
| None                | 1(Reference)                    |          |
| Local tumor destruction | 0.112(0.059–0.213)                | < 0.001  |
| Patial gastrectomy  | 0.156(0.120–0.205)              | < 0.001  |
| Total gastrectomy   | 0.174(0.123–0.245)              | < 0.001  |
| Pathology           |                                  |          |
| AM^a                | 1(Reference)                    |          |
| CA^b                | 1.035(0.666–1.608)              | 0.520    |
| MAC^c               | 1.794(0.697–2.045)              | 0.880    |
| SRCC^d              | 1.289(0.840–1.976)              | 0.240    |

^a: adenocarcinoma with mixed subtypes; ^b: classical adenocarcinoma, ^c: mucinous adenocarcinoma ^d: signet-ring cell carcinoma.
Table 6
Results of competing risks regression in patients with advanced gastric cancer.

| Variable               | Subdistribution hazard ratio | P value   |
|------------------------|------------------------------|-----------|
| Sex                    |                              |           |
| Female                 | 1 (Reference)                |           |
| Male                   | 1.035 (0.950–1.129)          | 0.430     |
| Age                    |                              |           |
| < 65                   | 1 (Reference)                |           |
| ≥ 65                   | 1.307 (1.198–1.427)          | < 0.001   |
| Race                   |                              |           |
| White                  | 1 (Reference)                |           |
| Black                  | 1.010 (0.887–1.149)          | 0.880     |
| Other                  | 0.804 (0.722–0.895)          | < 0.001   |
| Tumor size             |                              |           |
| < 3cm                  | 1 (Reference)                |           |
| 3-6cm                  | 1.189 (1.041–1.359)          | 0.011     |
| ≥ 6cm                  | 1.235 (1.090–1.399)          | < 0.001   |
| Primary site           |                              |           |
| Antrum                 | 1 (Reference)                |           |
| Pylorus                | 0.839 (0.720–0.977)          | 0.024     |
| Body                   | 0.930 (0.807–1.072)          | 0.320     |
| Cardia                 | 0.896 (0.713–1.126)          | 0.350     |
| Fundus                 | 0.841 (0.689–1.029)          | 0.093     |
| Lesser curve           | 0.788 (0.677–0.916)          | 0.002     |
| Greater curve          | 0.969 (0.858–1.095)          | 0.620     |
| Overlapping/not otherwise specified | 0.856 (0.696–1.053) | 0.140 |
| T stage                |                              |           |
| T2                     | 1 (Reference)                |           |
| T3                     | 1.687 (1.462–1.947)          | < 0.001   |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.
| Variable       | Subdistribution hazard ratio       | P value  |
|----------------|-----------------------------------|----------|
| T4             | 2.350 (2.022–2.732)               | < 0.001  |
| N stage        |                                   |          |
| N0             | 1(Reference)                      |          |
| N1             | 1.156 (1.028–1.300)               | 0.016    |
| N2             | 1.403 (1.231–1.599)               | < 0.001  |
| N3             | 1.889 (1.679–2.126)               | < 0.001  |
| M stage        |                                   |          |
| M0             | 1(Reference)                      |          |
| M1             | 2.094 (1.878–2.334)               | < 0.001  |
| Surgery        |                                   |          |
| None           | 1(Reference)                      |          |
| Local tumor destruction | 0.844 (0.537–1.327)       | 0.460    |
| Partial gastrectomy | 0.347 (0.304–0.397)              | < 0.001  |
| Total gastrectomy | 0.409 (0.357–0.469)               | < 0.001  |
| Pathology      |                                   |          |
| AM<sup>a</sup> | 1(Reference)                      |          |
| CA<sup>b</sup> | 0.769 (0.611–0.967)               | < 0.001  |
| MAC<sup>c</sup>| 0.729 (0.611–0.869)               | 0.025    |
| SRCC<sup>d</sup>| 1.045 (0.879–1.243)               | 0.620    |

*<sup>a</sup>: adenocarcinoma with mixed subtypes; <sup>b</sup>: classical adenocarcinoma, <sup>c</sup>: mucinous adenocarcinoma <sup>d</sup>: signet-ring cell carcinoma.

**Nomogram predicting the probability of CSS in GC patients with AM and validation**

We further explored the prognostic factors for CSS of GC patients with AM. In the training cohort, univariate and multivariate Cox regression analysis was performed. Race, T stage, N stage, M stage and surgery were considered independent prognostic factors for the CSS in those patients with AM. In the multivariate Cox model, T3-4 stage, N2-3 stage, M1 stage, no surgical modalities of partial gastrectomy and total gastrectomy were independently associated with the lower probability of CSS in GC patients with AM (Fig. 4).
The CSS nomogram was constructed by incorporating those independent prognostic predictors (Fig. 5A). The excellent C-index values (CSS in the training cohort: 0.804; CSS in the validation cohort: 0.748) and calibration curves of 1- and 3-year CSS in both internal and external validations indicated good agreements between the nomogram-predicted CSS probability and actual CSS probability (Fig. 5B-E). We further adopted ROC curves for the internal and external validations of the nomogram (Fig. 5F-I). The favorable 1- and 3-year AUC values (training cohort: AUC = 0.871, 0.914; validation cohort: AUC = 0.810, 0.798) illustrated good ability for CSS prediction in GC patients with AM. In addition, the nomogram's 1- and 3-year DCA curves demonstrated good net benefits across a range of death risk both in the training and validation cohort (Fig. 6).

**Discussion**

In this study, the incidence of GC with AM steadily increased over the past 16 years to approximately 0.1 per 100,000. To our knowledge, there are fewer studies on the incidence of GC with AM. The mortality of GC with AM increased from 2001 to 2012, but decreased from 2012 to 2016. GC with AM was more frequently discovered in men. And the male incidence showed a steady increase trend, while the female incidence kept a trend of fluctuation.

Our conclusions about the prognosis of MAC were mainly in consistency with previous reports [10–12]. The prognosis of MAC had no difference with CA both in EGC and AGC. In terms of SRCC, SRCC had comparative prognosis with CA in EGC, but poorer prognosis in AGC. Though it's widely accepted that SRCC is an independent predictor of poor prognosis in advanced gastric adenocarcinomas (GA), the prognosis of SRCC in EGC remains highly controversial [7, 13–16]. In our study, the comparison object of SRCC was CA that was different from previous studies. The reference value of previous researches for our study is questionable.

There were some studies about the clinical characteristics and prognosis of mixed-type EGC based on Lauren's classification [17–19]. According to WHO classification, the mixed carcinoma based on Lauren's classification is different from AM. Adenocarcinoma with mixed subtypes is morphologically a combination of identifiable glandular (tubular/papillary) and signet ring/poorly cohesive cellular components [20].

Our study proved that there were no differences in the aggressive tumor features, such as N stage, M stage, and tumor size between AM and other three histologic subtypes in EGC, which is not consistent with previous researches [9]. We think this is due to the fact that the comparison is not just with adenocarcinoma in the research mentioned above. The number of patients with AM in this research was small. In EGC, AM had a comparative prognosis with CA, MAC and SRCC.

To our knowledge, AM was not well documented in the literature of AGC. In AGC, AM was significantly associated with more aggressive features compared with CA, MAC and SRCC. AGC patients with AM had a poorer prognosis compared with CA and MAC, but a comparative prognosis with SRCC. AM was an independent risk factor for a poor outcome in AGC.
Furthermore, we explored the independent prognostic predictor of GC with AM. In our study, T3-4 stage, N2-3 stage, M1 stage, no surgical modalities of partial gastrectomy and total gastrectomy were proposed to be independent predictors of poor CSS of GC patients with AM. We believe that the establishment of our prognostic scoring system based on GC patients with AM is of clinical significance. We evaluated the value of this nomogram to predict 1- and 3-year CSS rates of GC patients with AM by C-index, calibration curves and ROC curves, which displayed good agreements both in training cohort and validation cohort. Furthermore, DCA demonstrated the benefits and clinical utility of the predictive power of our nomogram.

This study has some limitations. Firstly, this is a retrospective analysis of patients using SEER database. Secondly, we were unable to analyze the disease-free survival of patients and obtain data regarding radiotherapy, chemotherapy and targeted therapies received in localized and advanced disease due to limitations of the database. Thirdly, we didn’t discuss the effect of differentiation on prognosis. Fourthly, selection bias might exist after the case screening. Besides, there were no information of their genotypes, genetic data among these histologic subtypes that may be the prognosis factors, such as human epidermal-growth-factor receptor 2 (HER-2), Microsatellite instable (MSI) and Epstein–Barr Virus (EBV) [21–23].

**Conclusions**

In summary, there were no differences in the survival among those EGC patients with AM, CA, MAC and SRCC. AGC patients with AM had poorer prognosis than those with CA and MAC, but similar prognosis to those with SRCC. In addition, we established a nomogram to quantify the CSS of GC patients with AM to help doctors predict the survival of these patients, so as to determine the treatment strategies in actual clinical practice.

**Declarations**

**Ethics approval and consent to participate**

Because the SEER database is publicly accessible, this study does not require informed patient consent and was deemed exempt from ethics approval.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The dataset from SEER database generated and/or analyzed during the current study are available in the SEER dataset repository (https://seer.cancer.gov/).

**Competing interests**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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Authors’ contributions

XZ, YL, HW, JL (Jun Lin), JL (Jing Liu) and QZ contributed to the study conception and design. Material preparation, data collection and analysis were performed by XZ, YL, ZY and HZ. The first draft of the manuscript was written by XZ and YL. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Abbreviations

AM Adenocarcinoma with mixed subtypes
CA Classic adenocarcinoma
MAC Mucinous adenocarcinoma
SRCC Signet-ring cell carcinoma
EGC Early gastric cancer
AGC  Advanced gastric cancer
CSS  Cancer-specific survival
OS   Overall survival
GC   Gastric cancer
ROC  Receiver Operating Characteristic
DCA  Decision Curve Analysis
ACs  Adenocarcinomas
SEER Surveillance, Epidemiology, and End Results
AJCC American Joint Committee on Cancer
TNM  Tumor Node Metastasis
LNM  Lymph node metastasis
APCs Annual percentage changes
C-index Concordance index
HER-2 Human epidermal-growth-factor receptor 2
MSI  Microsatellite instable
EBV  Epstein-Barr Virus

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24. Data Availability Statement
25. Publicly available datasets were analyzed in this study. This data can be found here: Surveillance, Epidemiology, and End Results (SEER) database (https://seer.cancer.gov/).

Tables

Table 1: Comparisons of the clinicopathologic features between AM and other histologic types in early gastric cancer.
| Variable                | AM<sup>a</sup> (%) | CA<sup>b</sup> (%) | MAC<sup>c</sup> (%) | SRCC<sup>d</sup> (%) | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|-------------------------|---------------------|---------------------|----------------------|--------------------|---------|------------------|--------------------|---------------------|
| N stage                 |                     |                     |                      |                    |         |                  |                    |                     |
| N0                      | 82 (81.1%)          | 992 (89.4%)         | 41 (78.8%)           | 689 (85.0%)        | 0.017   | 0.053            | 0.672              | 0.597               |
| N1                      | 13 (12.9%)          | 90 (8.1%)           | 6 (11.5%)            | 88 (10.9%)         |         |                  |                    |                     |
| N2                      | 5 (5.0%)            | 20 (1.8%)           | 3 (5.8%)             | 23 (2.8%)          |         |                  |                    |                     |
| N3                      | 1 (0.9%)            | 8 (0.7%)            | 2 (3.8%)             | 11 (13.6%)         |         |                  |                    |                     |
| M stage                 |                     |                     |                      |                    | <0.001  | 0.064            | 0.188              | 0.351               |
| M0                      | 91 (85.6%)          | 1050 (94.6%)        | 43 (82.7%)           | 704 (86.8%)        |         |                  |                    |                     |
| M1                      | 10 (14.4%)          | 60 (5.4%)           | 9 (17.3%)            | 107 (13.2%)        |         |                  |                    |                     |
| Surgery                 |                     |                     |                      |                    | <0.001  | 0.038            | 0.043              | 0.008               |
| None                    | 17 (16.8%)          | 134 (12.1%)         | 19 (36.5%)           | 221 (27.3%)        |         |                  |                    |                     |
| Local tumor destruction | 5 (5.0%)            | 110 (9.9%)          | 1 (1.9%)             | 10 (1.2%)          |         |                  |                    |                     |
| Partial gastrectomy     | 57 (56.4%)          | 708 (63.8%)         | 25 (48.1%)           | 419 (51.7%)        |         |                  |                    |                     |
| Total gastrectomy       | 22 (21.8%)          | 158 (14.2%)         | 7 (13.5%)            | 161 (19.9%)        |         |                  |                    |                     |

<sup>a</sup>: adenocarcinoma with mixed subtypes; <sup>b</sup>: classical adenocarcinoma, <sup>c</sup>: mucinous adenocarcinoma <sup>d</sup>: signet-ring cell carcinoma.

Table 2: Comparisons of the clinicopathologic features between AM and other histologic types in advanced gastric cancer.
| Variable                  | AM\(^a\) (%) | CA\(^b\) (%) | MAC\(^c\) (%) | SRCC\(^d\) (%) | P value | AM vs. CA P value | AM vs. MAC P value | AM vs. SRCC P value |
|--------------------------|---------------|--------------|---------------|----------------|---------|------------------|-------------------|---------------------|
| Sex                      |               |              |               |                |         |                  |                   |                     |
| Female                   | 132 (37.7%)   | 639 (36.8%)  | 95 (29.3%)    | 1025 (46.7%)   | <0.001  | 0.749            | 0.021             | 0.002               |
| Male                     | 218 (62.3%)   | 1097 (63.2%) | 229 (70.7%)   | 1170 (53.3%)   |         |                  |                   |                     |
| Age                      |               |              |               |                |         |                  |                   |                     |
| <65                      | 155 (44.3%)   | 533 (30.7%)  | 134 (41.4%)   | 1270 (57.9%)   | <0.001  | <0.001           | 0.443             | <0.001              |
| ≥65                      | 195 (55.7%)   | 1203 (69.3%) | 190 (58.6%)   | 925 (42.1%)    | <0.001  | 0.355            | 0.080             | 0.015               |
| Race                     |               |              |               |                |         |                  |                   |                     |
| White                    | 221 (63.1%)   | 1027 (59.2%) | 229 (70.7%)   | 1503 (68.5%)   | <0.001  |                  |                   |                     |
| Black                    | 53 (15.1%)    | 278 (16.0%)  | 45 (13.9%)    | 252 (11.5%)    |         |                  |                   |                     |
| Other                    | 76 (21.7%)    | 431 (24.8%)  | 50 (15.4%)    | 440 (20.0%)    |         |                  |                   |                     |
| Tumor size               |               |              |               |                |         |                  |                   |                     |
| <3cm                     | 54 (15.4%)    | 321 (18.5%)  | 48 (14.8%)    | 508 (23.1%)    | <0.001  | 0.018            | 0.787             | 0.002               |
| 3-6cm                    | 89 (25.4%)    | 532 (30.6%)  | 90 (27.8%)    | 571 (26.0%)    |         |                  |                   |                     |
| ≥6cm                     | 207 (59.1%)   | 883 (50.9%)  | 186 (57.4%)   | 1116 (50.8%)   | <0.001  | 0.038            | <0.001            | 0.543               |
| Primary site             |               |              |               |                |         |                  |                   |                     |
| Antrum                   | 100 (28.6%)   | 564 (32.5%)  | 85 (26.2%)    | 550 (25.1%)    | <0.001  |                  |                   |                     |
| Pylorus                  | 42 (12.0%)    | 208 (12.0%)  | 24 (7.4%)     | 266 (12.1%)    | <0.001  | <0.001           | 0.464             | 0.931               |
| Body                     | 61 (17.4%)    | 230 (13.2%)  | 118 (36.4%)   | 404 (18.4%)    | <0.001  | 0.178            | 0.905             | 0.009               |
| Cardia                   | 10 (2.9%)     | 87 (5.0%)    | 9 (2.8%)      | 56 (2.6%)      | <0.001  |                  |                   |                     |
| Fundus                   | 20 (5.7%)     | 83 (4.8%)    | 14 (4.3%)     | 131 (6.0%)     |         |                  |                   |                     |
| Lesser curve             | 33 (9.4%)     | 228 (13.1%)  | 23 (7.1%)     | 279 (12.7%)    | <0.001  |                |                   |                     |
| Greater curve            | 64 (18.3%)    | 253 (14.6%)  | 44 (13.6%)    | 416 (19.0%)    |         |                  |                   |                     |
| Overlapping/not otherwise specified | 20 (5.7%) | 83 (4.8%) | 7 (2.2%) | 93 (4.2%) |         |                  |                   |                     |
| T stage                  |               |              |               |                |         |                  |                   |                     |
| T2                       | 52 (14.9%)    | 379 (21.8%)  | 58 (17.9%)    | 341 (15.5%)    | <0.001  | <0.001           | 0.046             | 0.931               |
| T3                       | 149 (42.6%)   | 866 (49.9%)  | 158 (48.8%)   | 916 (41.7%)    | <0.001  |                  |                   |                     |
| T4                       | 149 (42.6%)   | 491 (28.3%)  | 108 (33.3%)   | 938 (42.7%)    | <0.001  | <0.001           | 0.002             | 0.063               |
| N stage                  |               |              |               |                |         |                  |                   |                     |
| N0                       | 113 (32.3%)   | 806 (46.4%)  | 138 (42.6%)   | 854 (38.9%)    | <0.001  | <0.001           | 0.002             | 0.063               |
| N1                       | 70 (2.0%)     | 379 (21.8%)  | 77 (23.8%)    | 447 (20.4%)    |         |                  |                   |                     |
| N2                       | 67 (19.1%)    | 274 (15.8%)  | 50 (15.4%)    | 341 (15.5%)    |         |                  |                   |                     |
| N3                       | 100 (28.6%)   | 277 (16.0%)  | 59 (18.2%)    | 553 (25.2%)    |         |                  |                   |                     |
| M stage                  |               |              |               |                |         |                  |                   |                     |
| M0                       | 295 (84.3%)   | 1510 (87.0%) | 272 (84.0%)   | 1715 (78.1%)   | <0.001  | 0.178            | 0.905             | 0.009               |
| M1                       | 55 (15.7%)    | 226 (13.0%)  | 52 (16.0%)    | 480 (21.9%)    | <0.001  | 0.280            | 0.458             | <0.001              |
| Surgery                  |               |              |               |                |         |                  |                   |                     |
| None                     | 43 (12.3%)    | 175 (10.1%)  | 44 (13.6%)    | 488 (22.2%)    | <0.001  |                  |                   |                     |
| Local tumor destruction  | 2 (0.6%)      | 11 (0.6%)    | 4 (1.2%)      | 16 (0.7%)      |         |                  |                   |                     |
| Partial gastrectomy      | 200 (57.1%)   | 1085 (62.5%) | 194 (59.9%)   | 992 (45.2%)    |         |                  |                   |                     |
| Total gastrectomy        | 105 (30.0%)   | 465 (26.8%)  | 82 (25.3%)    | 699 (31.8%)    |         |                  |                   |                     |

\(a\): adenocarcinoma with mixed subtypes; \(b\): classical adenocarcinoma, \(c\): mucinous adenocarcinoma \(d\): signet-ring cell carcinoma.
### Table 3: Survival rates of patients with 4 histological subtypes in early gastric cancer.

| Pathological characteristics | 1-year OS<sup>e</sup> rate (%) | 3-year OS<sup>e</sup> rate (%) | 1-year CSS<sup>f</sup> rate (%) | 3-year CSS<sup>f</sup> rate (%) |
|------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **AM<sup>a</sup>**          | 87.8% (81.3%-94.3%)             | 72.6% (63.3%-81.9%)             | 87.8% (81.3%-94.3%)             | 78.1% (69.6%-86.7%)             |
| **CA<sup>b</sup>**           | 83.9% (81.7%-86.1%)             | 70.9% (68.1%-73.8%)             | 89.7% (87.8%-91.5%)             | 81.3% (78.8%-83.8%)             |
| **MAC<sup>c</sup>**          | 68.5% (55.8%-81.3%)             | 44.0% (29.7%-58.3%)             | 72.4% (60.1%-84.8%)             | 53.8% (39.4%-68.2%)             |
| **SRCC<sup>d</sup>**         | 75.6% (72.5%-78.6%)             | 64.8% (61.3%-68.3%)             | 78.6% (75.6%-81.5%)             | 69.6% (66.2%-73.0%)             |

<sup>a</sup>: adenocarcinoma with mixed subtypes; <sup>b</sup>: classical adenocarcinoma, <sup>c</sup>: mucinous adenocarcinoma <sup>d</sup>: signet-ring cell carcinoma; <sup>e</sup>: overall survival; <sup>f</sup>: cancer-specific survival.

### Table 4: Survival rates of patients with 4 histological subtypes in early advanced gastric cancer.

| Pathological characteristics | 1-year OS<sup>e</sup> rate (%) | 3-year OS<sup>e</sup> rate (%) | 1-year CSS<sup>f</sup> rate (%) | 3-year CSS<sup>f</sup> rate (%) |
|------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **AM<sup>a</sup>**          | 64.8% (59.5%-70.0%)             | 39.7% (33.9%-45.5%)             | 66.8% (61.5%-72.0%)             | 43.7% (37.7%-49.7%)             |
| **CA<sup>b</sup>**           | 76.2% (74.1%-78.3%)             | 49.9% (47.3%-52.5%)             | 80.3% (78.4%-82.3%)             | 55.5% (52.9%-58.2%)             |
| **MAC<sup>c</sup>**          | 73.7% (68.8%-78.6%)             | 44.9% (39.0%-50.8%)             | 76.3% (71.5%-81.0%)             | 50.5% (44.4%-56.6%)             |
| **SRCC<sup>d</sup>**         | 63.8% (61.7%-65.9%)             | 33.6% (31.4%-35.8%)             | 65.7% (63.7%-67.8%)             | 36.4% (34.1%-38.7%)             |

<sup>a</sup>: adenocarcinoma with mixed subtypes; <sup>b</sup>: classical adenocarcinoma, <sup>c</sup>: mucinous adenocarcinoma <sup>d</sup>: signet-ring cell carcinoma; <sup>e</sup>: overall survival; <sup>f</sup>: cancer-specific survival.

### Table 5: Results of competing risks regression in patients with early gastric cancer.
| Variable                     | Subdistribution hazard ratio | P value |
|------------------------------|------------------------------|---------|
| Sex                          |                              |         |
| Female                       |                              |         |
| Male                         | 1.218(1.014-1.463)           | 0.035   |
| Age                          |                              |         |
| <65                          | 1 (Reference)                |         |
| ≥65                          | 1.431(1.177-1.739)           | <0.001  |
| Race                         |                              |         |
| White                        | 1 (Reference)                |         |
| Black                        | 0.985(0.750-1.294)           | 0.910   |
| Other                        | 0.774(0.614-0.975)           | 0.029   |
| Tumor size                   |                              |         |
| <3cm                         | 1 (Reference)                |         |
| 3-6cm                        | 1.518(1.213-1.900)           | <0.001  |
| ≥6cm                         | 1.692(1.342-2.134)           | <0.001  |
| Primary site                 |                              |         |
| Antrum                       | 1 (Reference)                |         |
| Pylorus                      | 1.027(0.761-1.384)           | 0.860   |
| Body                         | 1.539(1.169-2.026)           | 0.002   |
| Cardia                       | 2.008(1.360-2.964)           | <0.001  |
| Fundus                       | 1.165(0.809-1.680)           | 0.410   |
| Lesser curve                 | 1.173(0.799-1.722)           | 0.410   |
| Greater curve                | 1.306(0.962-1.771)           | 0.087   |
| Overlapping/not otherwise specified | 1.086(0.506-2.331) | 0.830   |
| N stage                      |                              |         |
| N0                           | 1 (Reference)                |         |
| N1                           | 1.033(0.803-1.330)           | 0.800   |
| N2                           | 1.234(0.672-2.266)           | 0.500   |
| N3                           | 1.882(1.073-3.301)           | 0.027   |
| M stage                      |                              |         |
| M0                           | 1 (Reference)                |         |
| M1                           | 3.184(2.479-4.090)           | <0.001  |
| Surgery                      |                              |         |
| None                         | 1 (Reference)                |         |
| Local tumor destruction      | 0.112(0.059-0.213)           | <0.001  |
| Partial gastrectomy          | 0.156(0.120-0.205)           | <0.001  |
| Total gastrectomy            | 0.174(0.123-0.245)           | <0.001  |
| Pathology                    |                              |         |
| AM^a                         | 1 (Reference)                |         |
| CA^b                         | 1.035(0.666-1.608)           | 0.520   |
| MAC^c                        | 1.794(0.697-2.045)           | 0.880   |
| SRCC^d                       | 1.289(0.840-1.976)           | 0.240   |

a: adenocarcinoma with mixed subtypes; b: classical adenocarcinoma, c: mucinous adenocarcinoma d: signet-ring cell carcinoma.
Table 6: Results of competing risks regression in patients with advanced gastric cancer.

| Variable                              | Subdistribution hazard ratio | P value |
|----------------------------------------|------------------------------|---------|
| Sex                                    |                              |         |
| Female                                 | 1(Reference)                 |         |
| Male                                   | 1.035(0.950-1.129)           | 0.430   |
| Age                                    |                              |         |
| <65                                    | 1(Reference)                 |         |
| ≥65                                    | 1.307 (1.198-1.427)          | <0.001  |
| Race                                   |                              |         |
| White                                  | 1(Reference)                 |         |
| Black                                  | 1.010(0.887-1.149)           | 0.880   |
| Other                                  | 0.804(0.722-0.895)           | <0.001  |
| Tumor size                             |                              |         |
| <3cm                                   | 1(Reference)                 |         |
| 3-6cm                                  | 1.189(1.041-1.359)           | 0.011   |
| ≥6cm                                   | 1.235(1.090-1.399)           | <0.001  |
| Primary site                           |                              |         |
| Antrum                                 | 1(Reference)                 |         |
| Pylorus                                | 0.839(0.720-0.977)           | 0.024   |
| Body                                   | 0.930(0.807-1.072)           | 0.320   |
| Cardia                                 | 0.896(0.713-1.126)           | 0.350   |
| Fundus                                 | 0.841(0.689-1.029)           | 0.093   |
| Lesser curve                           | 0.788(0.677-0.916)           | 0.002   |
| Greater curve                          | 0.969(0.858-1.095)           | 0.620   |
| Overlapping/not otherwise specified    | 0.856(0.696-1.053)           | 0.140   |
| T stage                                |                              |         |
| T2                                     | 1(Reference)                 |         |
| T3                                     | 1.687(1.462-1.947)           | <0.001  |
| T4                                     | 2.350(2.022-2.732)           | <0.001  |
| N stage                                |                              |         |
| N0                                     | 1(Reference)                 |         |
| N1                                     | 1.156(1.028-1.300)           | 0.016   |
| N2                                     | 1.403(1.231-1.599)           | <0.001  |
| N3                                     | 1.889(1.679-2.126)           | <0.001  |
| M stage                                |                              |         |
| M0                                     | 1(Reference)                 |         |
| M1                                     | 2.094(1.878-2.334)           | <0.001  |
| Surgery                                |                              |         |
| None                                   | 1(Reference)                 |         |
| Local tumor destruction                | 0.844(0.537-1.327)           | 0.460   |
| Partial gastrectomy                    | 0.347 (0.304-0.397)          | <0.001  |
| Total gastrectomy                      | 0.409 (0.357-0.469)          | <0.001  |
| Pathology                              |                              |         |
| AM\textsuperscript{a}                  | 1(Reference)                 |         |
| CA\textsuperscript{b}                  | 0.769(0.611-0.967)           | <0.001  |
| MAC\textsuperscript{c}                 | 0.729(0.611-0.869)           | 0.025   |
| SRCC\textsuperscript{d}                | 1.045(0.879-1.243)           | 0.620   |

\textsuperscript{a}: adenocarcinoma with mixed subtypes; \textsuperscript{b}: classical adenocarcinoma, \textsuperscript{c}: mucinous adenocarcinoma \textsuperscript{d}: signet-ring cell carcinoma.

Figures
Figure 1

Incidence (A) of gastric cancer (GC) with adenocarcinoma with mixed subtypes (AM). The most common age at diagnosis was 72-74 years old. Both the age-adjusted incidence rate and mortality rate of GC with AM (B). This increased trend (C) was similar regardless of sex. Comparisons of cancer-specific survival (D) in histological subtypes plotted with the Kaplan-Meier method in early gastric cancer (EGC). Comparisons of cancer-specific survival (E) in histological subtypes plotted with the Kaplan-Meier method in advanced gastric cancer (AGC).
| Variable                                           | N   | HR(95%CI)          | P value |
|---------------------------------------------------|-----|--------------------|---------|
| Sex                                               |     |                    |         |
| Female                                            | 950 |                    |         |
| Male                                              | 1124| 1.163(0.967–1.401) | 0.109   |
| Race                                              |     |                    |         |
| White                                             | 1251|                    |         |
| Black                                             | 265 | 0.968(0.739–1.269) | 0.816   |
| Other                                             | 558 | 0.747(0.584–0.957) | 0.021   |
| Tumor size                                        |     |                    |         |
| <3cm                                              | 1275|                    |         |
| 3–6cm                                             | 469 | 1.550(1.236–1.946) | <0.001  |
| ≥6cm                                              | 330 | 1.766(1.397–2.231) | <0.001  |
| Primary site                                      |     |                    | 0.024   |
| Antrum                                            | 687 |                    |         |
| Pylorus                                           | 283 | 1.005(0.726–1.390) | 0.978   |
| Body                                              | 335 | 1.513(1.145–2.001) | 0.004   |
| Cardia                                            | 79  | 1.823(1.223–2.716) | 0.003   |
| Fundus                                            | 126 | 1.163(0.771–1.754) | 0.472   |
| Lesser curve                                      | 243 | 1.244(0.881–1.757) | 0.215   |
| Greater curve                                     | 259 | 1.257(0.909–1.737) | 0.166   |
| Overlapping/not otherwise specified               | 62  | 1.262(0.610–2.612) | 0.530   |
| N stage                                           |     |                    | 0.370   |
| N0                                                | 1804|                    |         |
| N1                                                | 197 | 1.038(0.810–1.329) | 0.769   |
| N2                                                | 51  | 1.221(0.681–2.189) | 0.503   |
| N3                                                | 22  | 1.898(0.890–4.049) | 0.097   |
| M stage                                           |     |                    |         |
| M0                                                | 1888|                    |         |
| M1                                                | 186 | 2.431(1.915–3.085) | <0.001  |
| Surgery                                           |     |                    | <0.001  |
| None                                              | 391 |                    |         |
| Local tumor destruction                           | 126 | 0.92(0.448–0.178)  | <0.001  |
| Partial gastrectomy                               | 1209| 0.115(0.088–0.160) | <0.001  |
| Total gastrectomy                                 | 348 | 0.125(0.089–0.176) | <0.001  |
| Pathology                                         |     |                    | 0.707   |
| AM                                                | 101 |                    |         |
| CA                                                | 1110| 1.260(0.688–2.308) | 0.455   |
| MAC                                               | 52  | 1.155(0.733–1.821) | 0.534   |
| SRCC                                              | 811 | 1.252(0.798–1.964) | 0.329   |
| Pathology                                         |     |                    | 0.707   |
| CA                                                | 1110|                    |         |
| AM                                                | 101 | 0.866(0.549–1.364) | 0.534   |
| MAC                                               | 52  | 1.091(0.695–1.712) | 0.706   |
| SRCC                                              | 811 | 1.083(0.892–1.316) | 0.419   |

**Figure 2**

Forest plot showing results of the multivariate Cox regression model for the cancer-specific survival of histologic subtypes in early gastric cancer (EGC).
Figure 3

Forest plot showing results of the multivariate Cox regression model for the cancer-specific survival of histologic subtypes in advanced gastric cancer (AGC).