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The clinicopathological characteristics and genetic alterations of gastric cancer patients according to the Lauren classification
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Abstract:
Objective
The Lauren classification is an important histological classification of gastric cancer (GC) with different biological behaviors between histological types.

Background
To date, there are few reports on the genetic alterations and survival differences between different histological types according to the Lauren classification.

Methods
In total, 433 GC patients undergoing surgery were enrolled. The clinicopathological features, prognoses, and genetic alterations of the different Lauren types were compared.

Results
Diffuse-type GC was associated with a younger age, female predominance, more Borrmann type 3 and 4 tumors, more advanced pathological tumor (T) and node (N) categories, more tumor recurrences (especially peritoneal recurrence), and worse 5-year overall survival and disease-free survival rates than intestinal-type GC and mixed-type GC. Regarding genetic alterations, mixed-type GC was associated with more TP53 mutations than intestinal-type GC and diffuse-type GC. Multivariate analysis demonstrated the following independent prognostic factors: age, Lauren classification, and pathological T and N categories. Regarding mixed-type GC, diffuse-type major tumors were associated with more lymphovascular invasion, a more advanced N category and TNM stage, and fewer PI3K/AKT pathway mutations than intestinal-type major tumors.

Conclusions

Diffuse-type GC had unfavorable clinicopathological features and a worse prognosis than intestinal-type GC. For mixed-type GC, the clinicopathological features and genetic alterations were different between intestinal-type major tumors and diffuse-type major tumors.
The clinicopathological characteristics and genetic alterations of gastric cancer patients according to the Lauren classification

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Running title: Genetic alterations in different Lauren types of gastric cancer

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Conflict of interest

The authors declare that they have no conflict of interest.

Source of support

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Results: Diffuse-type GC was associated with a younger age, female predominance, more Borrmann type 3 and 4 tumors, more advanced pathological tumor (T) and node (N) categories, more tumor recurrences (especially peritoneal recurrence), and worse 5-year overall survival and disease-free survival rates than intestinal-type GC and mixed-type GC. Regarding genetic alterations, mixed-type GC was associated with more TP53 mutations than intestinal-type GC and diffuse-type GC. Multivariate analysis demonstrated the following independent prognostic factors: age, Lauren classification, and pathological T and N categories. Regarding mixed-type GC, diffuse-type major tumors were associated with more lymphovascular invasion, a more advanced N category and TNM stage, and fewer PI3K/AKT pathway mutations than intestinal-type major tumors.

Conclusions: Diffuse-type GC had unfavorable clinicopathological features and a worse prognosis than intestinal-type GC. For mixed-type GC, the clinicopathological features and genetic alterations were different between intestinal-type major tumors and diffuse-type major tumors.

Keywords: gastric cancer; Lauren classification; clinicopathological feature; genetic
alteration; prognosis
Introduction

Gastric cancer (GC) is the sixth most common cancer and the second most common cause of cancer-related deaths worldwide.\(^1\) Surgical resection with curative intent remains the major therapeutic treatment for GC.

According to the Lauren classification proposed since 1965,\(^2\) gastric adenocarcinoma is divided into two major histological types: intestinal-type and diffuse-type. The two histological types have distinct clinicopathological and molecular features.\(^3\)-\(^5\) Diffuse-type GC is associated with a younger age, female predominance, a more advanced pathological tumor, node, metastasis (TNM) stage, and a worse prognosis than intestinal-type GC.\(^3\),\(^4\) A meta-analysis with 61,468 patients enrolled demonstrated that diffuse-type GC is an independent prognostic factor, which is not altered by race, stage, and exposure to chemotherapy.\(^6\) Regarding molecular differences, TP53 is the only mutated gene that occurs in both types of GC, while microsatellite instability (MSI) is more common in intestinal-type GC than in diffuse-type GC.\(^5\)

Some GC tissues, the so-called mixed-type, exhibit histological heterogeneity and consist of a mixture of intestinal and diffuse types. Our previous study\(^4\) showed that the clinicopathological features and prognosis of mixed-type GC were similar to those of diffuse-type GC. The survival rate of mixed-type GC was even reported to be worse than that of intestinal-type or diffuse-type GC.\(^7\) However, whether the major histological component has an impact on the clinicopathological and molecular features in mixed-type GC patients is still unknown.

In this study, we hypothesized that the major histological component in mixed-type GC might be associated with clinicopathological and molecular features. To verify our hypothesis, we divided patients with mixed-type GC into two groups
according to the major histological component: the intestinal-type major and diffuse-type major groups. The clinicopathological and molecular features were compared between the two subtypes. In addition, we compared the clinicopathological features, recurrence patterns, prognoses, and genetic alterations of the three histologic types, namely intestinal-type, diffuse-type, and mixed-type GC, according to the Lauren classification.

Methods

Patients and sample collection

A total of 433 patients who underwent gastrectomy for gastric adenocarcinoma between 2005 and 2010 were enrolled. Patients who had gastric stump cancer or a history of previous gastric surgery were excluded. Subtotal gastrectomy was performed for distal or middle third lesions, while total gastrectomy was performed for proximal third lesions. According to the Lauren classification, the enrolled patients were separated into three groups: intestinal-type, diffuse-type and mixed-type GC. For mixed-type GC, we divided the patients into two subgroups according to the histologic type: intestinal-type major (more than 50% of cancer cells were intestinal-type) and diffuse-type major (more than 50% of cancer cells were diffuse-type). All surgical specimens were examined by experienced pathologists.

The tumor tissues and normal gastric mucosa tissues were collected and stored in a biobank at our institution. The study was approved by the Ethical Committee of Taipei Veterans General Hospital. The study was performed in accordance with the Declaration of Helsinki. Written informed consent before tumor tissue collection was obtained from all study participants. The pathological staging of the GC was performed according to the 8th American Joint Committee on Cancer (AJCC)/Union
for International Cancer Control (UICC) TNM classification system.  

Follow-up

Follow-up examinations were performed at our outpatient department every 3 months. Tumor recurrence was diagnosed by biopsies or by imaging studies when biopsies were not obtained. Tumor recurrence was classified as locoregional, hematogenous, distant lymphatic, or peritoneal. Tumor recurrence in the hepatoduodenal ligament, celiac axis, or peripancreatic region was defined as locoregional recurrence. Remote lymphatic metastasis (in the para-aortic, Virchow’s, and inguinal nodes) and pulmonary lymphangitic spread were defined as distant lymphatic recurrence.

Analysis of Helicobacter pylori infections, Epstein-Barr virus infections, microsatellite instability status, and genetic alterations

DNA extraction from tissue specimens was performed using the QIAamp DNA Tissue Kit (Qiagen, Valencia, CA) according to a previous report.  

Both tumor and nontumor tissues were assessed for Helicobacter pylori (HP) infection with the polymerase chain reaction (PCR) method. The reference sequence of the HP reference genome (GenBank: AE000511.1) was used as described in a previous report.  

Epstein-Barr virus (EBV) DNA assays were carried out using the Sequenom MassARRAY system (Sequenom, San Diego, CA).  

For analysis of microsatellite instability (MSI) status, five reference microsatellite markers, D5S345, D2S123, D17S250, BAT25 and BAT26, were used to determine MSI status. MSI-high (MSI-H) was defined as ≥ 2 loci of instability with
5 markers, while MSI-low/stable (MSI-L/S) was defined as one locus or without MSI loci.¹¹

A MassARRAY system (Agena, San Diego, CA) was used to identify mutations in 8 GC-related genes (TP53, ARID1A, PTEN, PIK3CA, AKT1, AKT2, AKT3, and BRAF).¹⁰ Among them, PI3K/AKT pathway genetic mutations were defined as mutations identified in PIK3CA, PTEN, AKT1, AKT2, or AKT3.

The copy number of the PIK3CA gene was analyzed by quantitative real-time PCR, and the primer sequences of the long interspersed nuclear element-1 (LINE1 element) were used as an internal reference target.¹²

**Statistical analysis**

IBM SPSS Statistics 25.0 was used for statistical analyses. A χ² test with Yates correction or Fisher’s exact test was used to compare the categorical data. Overall survival (OS) was defined from the date of surgery to the date of death or last follow-up. The Kaplan–Meier method was used to perform the survival analysis and draw survival curves for OS. Univariate analysis of the covariates (prognostic factors) of OS was performed first. The covariates with P value <0.05 were selected for the entry of Cox proportional hazards model. Multivariate analysis using Cox proportional hazards model with likelihood ratio (forward stepwise) test for several steps of iteration was performed. A P value < 0.05 was defined as statistically significant.

**Results**

**Clinicopathological features**

As shown in Table 1, diffuse-type GC was associated with a younger age, female predominance, more Borrmann type 3 and 4 tumors, and more pathological T4
category than intestinal-type and mixed-type GCs. Intestinal-type GC were associated with fewer pathological N3 category than diffuse-type and mixed-type GCs.

**Initial recurrence patterns**

As shown in Table 2, patients with diffuse-type GC had more tumor recurrences than patients with intestinal-type or mixed-type GC (39.7% vs. 28.0% vs. 22.6%, \(P=0.019\)). Regarding the initial recurrence patterns, patients with diffuse-type GC had more distant metastases than patients with intestinal-type or mixed-type GC (34.7% vs. 25.2% vs. 19.4%, \(P=0.036\)), and peritoneal recurrences were especially notable (24.0% vs. 9.8% vs. 7.5%, \(P<0.001\)). There were no differences in locoregional recurrence or distant lymphatic recurrence between the three groups.

**Analysis of genetic alterations**

As shown in Table 3, patients with mixed-type GC had more TP53 mutations than those with intestinal-type or diffuse-type GC (18.4% vs. 8.4% vs. 7.9%, \(P=0.010\)). There were no significant differences in MSI phenotype or other genetic alterations between the three different histologic types.

**Survival analysis**

As shown in Figure 1A, the 5-year OS (51.2% vs. 45.2% vs. 38.1%, \(P=0.035\)) rates were significantly higher in intestinal-type GC, followed by mixed-type GC and diffuse-type GC.

The univariate analysis demonstrated that age, gender, tumor location, lymphovascular invasion, Lauren classification, and pathological T and N categories were significantly associated with OS. The aforementioned seven covariates were included in the multivariate analysis. The multivariate analysis using Cox proportional hazards model demonstrated that age, Lauren classification, and pathological T and N categories were independent prognostic factors (Table 4).
Analysis of the clinicopathological features and genetic alterations of mixed-type GC according to the major histological component

As shown in Table 5, regarding mixed-type GC, diffuse-type major GC was associated with more lymphovascular invasion, a more advanced pathological N category and a higher TNM stage than intestinal-type major GC.

As shown in Figure 1B, the 5-year OS (50.1% vs. 51.2%, P=0.636) rates were not significant different between the intestinal-type major tumors and the intestinal-type GC, while the 5-year OS (41.3% vs. 38.1%, P=0.294) rates were not significantly different between the diffuse-type major tumors and the diffuse-type GC.

Regarding genetic alterations (Table 3), diffuse-type major GC was associated with fewer PI3K/AKT pathway mutations (4.8% vs. 17.6%, P=0.026) than intestinal-type major GC.

Discussion

Our results showed that diffuse-type GC had unfavorable clinicopathological features and a worse prognosis than intestinal-type GC or mixed-type GC. Mixed-type GC was associated with more TP53 mutations than intestinal-type or diffuse-type GC. Regarding mixed-type GC, diffuse-type major GC was associated with more lymphovascular invasion, a more advanced N category and TNM stage, and fewer PI3K/AKT pathway mutations than intestinal-type major GC.

Diffuse-type GC was reported to be associated with a worse prognosis than intestinal-type GC,\(^3,4\) which is similar to our results. In addition, similar clinicopathological features and prognoses were observed between mixed-type GC and intestinal-type GC in the present study. Our previous study\(^4\) showed that the clinicopathological features and prognosis of mixed-type GC were similar to those of
diffuse-type GC; both aforementioned histological types had unfavorable clinicopathological features and worse prognoses than intestinal-type GC. Mixed-type GC was even reported to have worse survival than intestinal-type and diffuse-type GC. We hypothesized that the discrepant results between the present study and our previous study and other series might be due to differences in patient numbers and the major component of the histological type in mixed-type GC. To verify our hypothesis, we divided mixed-type GC into intestinal-type major and diffuse-type major. The 5-year OS rate of mixed-type GC was slightly lower for patients with diffuse-type major tumors than for those with intestinal-type major tumors, with no significant difference (41.3% vs. 50.1%, $P=0.386$), and patients with diffuse-type major tumors had significantly more lymphovascular invasion, a more advanced pathological N category and a higher TNM stage than patients with intestinal-type major tumors. In addition, as shown in Figure 1B, the survival curve for intestinal-type major tumors was close to that for intestinal-type GC, while the survival curve for diffuse-type major tumors was close to that for diffuse-type GC. It is reasonable that the biological behavior of mixed-type GC might be related to the major histologic component of either intestinal-type or diffuse-type tumors.

It was reported that in a subset of patients, diffuse-type GC that developed from intestinal-type GC had $PIK3CA$ mutations and that these tumors were susceptible to $mTOR$ inhibitors. Our novel findings demonstrated that for mixed-type GC, patients with intestinal-type major GC had more $PI3K/AKT$ pathway mutations than patients with diffuse-type major GC (17.6% vs. 4.8%, $P=0.026$). In addition, intestinal-type GC was associated with a slightly higher frequency of $PI3K/AKT$ pathway mutations than diffuse-type and mixed-type GC (17.4% vs. 10.5% vs. 10.5%). We hypothesized that $PI3K/AKT$ pathway mutations might play an important role in the development of
intestinal-type GC, and even in the intestinal-type major category of mixed-type GC. Targeted therapy might be beneficial for this subgroup of patients and further studies are required to validate our hypothesis. To date, there has been no report regarding the prognostic impact of the major histologic component on the mixed-type GC. Our results might provide useful information for future studies and management of this subtype of GC.

To date, it has been reported that genetic mutations are distinct between intestinal-type and diffuse-type of GC; only TP53 genetic mutations occur regularly in both intestinal and diffuse-type of GC.\(^5\) In the present study, the frequency of TP53 mutation was comparable between intestinal-type and diffuse-type (8.4% vs. 7.9%), which was significantly lower than mixed-type GC (18.4%). Whether more TP53 mutations are associated with the development of mixed-type GC has not yet been reported. Further in vitro and in vivo studies and studies enrolling more patients are required to validate our results.

There are some limitations in the present study. First, this is a retrospective study and selection bias exists. Second, the patient number is small in some subgroup analyses, and a study with more patients is required to validate our results. Third, the genetic panel was limited in the present study, as current practice NGS panels are more readily available. Although the expression of some genetic mutations was significantly different between different Lauren classifications of GC in the present study, a comprehensive study of genetic alterations is required to better understand gastric carcinogenesis and provide useful information for GC treatment in the future.

**Conclusions**

Diffuse-type GC had unfavorable clinicopathological features and a worse
prognosis than intestinal-type GC. For mixed-type GC, the clinicopathological features and genetic alterations were different depending on the major histological component of either intestinal-type or diffuse-type tumors.

**Declarations**

**Informed consent policy**

This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (IRB No.: 2020-06-001BC) and in accordance with the Ethical Principles for Medical Research Involving Human Subjects, as outlined in The Declaration of Helsinki.

**Availability of data and material**

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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**Conflict of interest**

All the authors have no conflict of interest in relation with the manuscript.

**Author’s Contributions**

WLF and KHH analyzed and interpreted the patient data regarding the clinicopathological features and survival analysis. HFC prepared the original draft.
WLF reviewed and edited the manuscript. AFL performed the histological examinations of gastric cancer. All authors read the approved the final manuscript.
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**Figure Legends:**

Figure 1. (A) The 5-year OS (51.2% vs. 45.2% vs. 38.1%, P=0.035) rates were significantly higher in intestinal-type GC, followed by mixed-type GC and diffuse-type GC. Mixed-type GC was divided into two groups: intestinal-type major tumors and diffuse-type major tumors. (B) The 5-year OS (50.1% vs. 51.2%, P=0.636) rates were not significant different between the intestinal-type major tumors and the intestinal-type GC, while the 5-year OS (41.3% vs. 38.1%, P=0.294) rates were not significantly different between the diffuse-type major tumors and the diffuse-type GC.
Table 1. Clinical profiles in GC patients with different Lauren types.

| Variables                      | Total patients | Intestinal-type | Diffuse-type | Mixed-type | P value |
|--------------------------------|----------------|-----------------|--------------|------------|---------|
|                                | n=433          | n=167           | n=152        | n=114      |         |
|                                | n (%)          | n (%)           | n (%)        | n (%)      |         |
| Age                            |                |                 |              |            | <0.001  |
| <65 years                      | 185 (42.7)     | 53 (31.7)       | 91 (59.9)    | 41 (36.0)  |         |
| ≥65 years                      | 248 (57.3)     | 114 (68.3)      | 61 (40.1)    | 73 (64.0)  |         |
| Gender                         |                |                 |              |            | <0.001  |
| Male                           | 303 (70.0)     | 132 (79.0)      | 87 (57.2)    | 84 (73.7)  |         |
| Female                         | 130 (30.0)     | 35 (21.0)       | 65 (42.8)    | 30 (26.3)  |         |
| Tumor size                     |                |                 |              |            | 0.202   |
| <5 cm                          | 158 (36.5)     | 67 (40.1)       | 47 (30.9)    | 44 (38.6)  |         |
| ≥5 cm                          | 275 (63.5)     | 100 (59.9)      | 105 (69.1)   | 70 (61.4)  |         |
| Tumor location                 |                |                 |              |            | 0.576   |
| Upper stomach                  | 85 (19.6)      | 35 (21.0)       | 31 (20.4)    | 19 (16.7)  |         |
| Middle stomach                 | 149 (34.4)     | 46 (27.3)       | 65 (42.8)    | 38 (33.3)  |         |
| Lower stomach                  | 186 (43.0)     | 86 (51.5)       | 48 (31.6)    | 52 (45.6)  |         |
| Whole stomach                  | 13 (3.0)       | 0               | 8 (5.3)      | 5 (4.4)    |         |
| Gross appearance               |                |                 |              |            | 0.004   |
| Superficial type               | 50 (11.5)      | 17 (10.2)       | 19 (12.5)    | 14 (12.3)  |         |
| Borrmann type 1                | 24 (5.5)       | 12 (7.2)        | 4 (2.6)      | 8 (7.0)    |         |
| Borrmann type 2                | 100 (23.1)     | 45 (26.9)       | 23 (15.1)    | 32 (28.1)  |         |
| Borrmann type 3                | 192 (44.3)     | 76 (45.5)       | 69 (45.4)    | 47 (41.2)  |         |
| Borrmann type 4                | 67 (15.5)      | 17 (10.2)       | 37 (24.3)    | 13 (11.4)  |         |
| Lymphovascular invasion        | 307 (70.9)     | 117 (70.1)      | 102 (67.1)   | 88 (77.2)  | 0.191   |
| HP infection                   | 226 (52.2)     | 81 (48.5)       | 80 (52.6)    | 65 (57.0)  | 0.370   |
| EBV infection                  | 57 (13.2)      | 19 (11.4)       | 23 (15.1)    | 15 (13.2)  | 0.612   |
| Pathological T category        |                |                 |              |            | 0.020   |
| T1                             | 64 (14.8)      | 27 (16.2)       | 23 (15.1)    | 14 (12.3)  |         |
| T2                             | 58 (13.4)      | 31 (18.6)       | 8 (5.3)      | 19 (16.7)  |         |
| T3                             | 154 (35.6)     | 50 (29.9)       | 54 (35.5)    | 50 (43.9)  |         |
| T4                             | 157 (36.3)     | 59 (35.3)       | 67 (44.1)    | 31 (27.2)  |         |
| Pathological N category        |                |                 |              |            | <0.001  |
| N0                             | 114 (26.3)     | 56 (33.5)       | 30 (19.7)    | 28 (24.6)  |         |
| N1                             | 67 (15.5)      | 32 (19.2)       | 23 (15.1)    | 12 (10.5)  |         |
| N2                             | 104 (24.0)     | 45 (26.9)       | 35 (23.0)    | 24 (21.1)  |         |
| N3                             | 148 (34.2)     | 34 (20.4)       | 64 (42.1)    | 50 (43.9)  |         |
| Pathological TNM Stage         |                |                 |              |            | 0.117   |
| I                              | 83 (19.2)      | 40 (24.0)       | 22 (14.5)    | 21 (18.4)  |         |
| II                             | 97 (22.4)      | 44 (26.3)       | 28 (18.4)    | 25 (21.9)  |         |
| III                            | 216 (49.9)     | 71 (42.5)       | 87 (57.2)    | 58 (50.9)  |         |
| IV                             | 37 (8.5)       | 12 (7.2)        | 15 (9.9)     | 10 (8.8)   |         |

T: Tumor; N: Node; TNM: Tumor, Node, Metastasis; bold: statistically significant
Table 2. The initial recurrence pattern in GC patients with different Lauren types.

|                        | Total patients  | Intestinal-type | Diffuse-type | Mixed-type | P value |
|------------------------|-----------------|-----------------|--------------|------------|---------|
| Total patients with recurrence | 109 (30.5) | 40 (28.0) | 48 (39.7) | 21 (22.6) | 0.019   |
| Locoregional recurrence | 40 (11.2) | 16 (11.2) | 16 (13.2) | 8 (8.6) | 0.569   |
| Distant metastasis     | 96 (26.9) | 36 (25.2) | 42 (34.7) | 18 (19.4) | 0.036   |
| Peritoneal dissemination| 50 (14.0) | 14 (9.8) | 29 (24.0) | 7 (7.5) | <0.001  |
| Hematogenous metastasis| 44 (12.3) | 20 (14.0) | 13 (10.7) | 11 (11.8) | 0.717   |
| Liver                  | 31 (8.7) | 17 (11.9) | 8 (6.6) | 6 (6.5) | 0.213   |
| Lung                   | 6 (1.7) | 2 (1.4) | 1 (0.8) | 3 (3.2) | 0.345   |
| Bone                   | 8 (2.2) | 4 (2.8) | 2 (1.7) | 2 (2.2) | 0.695   |
| Brain                  | 1 (0.3) | 0 | 0 | 1 (1.1) | 0.155   |
| Adrenal                | 1 (0.3) | 0 | 1 (0.8) | 0 | 0.861   |
| Skin                   | 4 (1.1) | 1 (0.7) | 2 (1.7) | 1 (1.1) | 0.725   |
| Distant lymphatic recurrence | 22 (6.2) | 8 (5.6) | 12 (9.9) | 2 (2.2) | 0.060   |

Some patients had more than one recurrence pattern; bold: statistically significant
| Variables                        | Total n=433 | Lauren’s classification | Mixed type GC |
|---------------------------------|-------------|-------------------------|---------------|
|                                 |             | Intestinal-type n=167   | Intestinal-type major n=51 |
|                                 |             | Diffuse-type n=152      | Diffuse-type major n=63 |
|                                 |             | Mixed-type n=114        | P value        |
|                                 | n (%)       | n (%)                   | n (%)          |
|                                 |             | Intestinal-type         | Diffuse-type   |
|                                 |             | major n=51              | major n=63     |
|                                 |             | n (%)                   | n (%)          | P value |
| MSI status                      |             |                         | 0.117          | 0.648   |
| MSI-H                           | 40 (9.2)    | 12 (7.2)                | 8 (15.7)       |
| MSI-L/S                         | 393 (90.8)  | 155 (92.8)              | 43 (84.3)      |
| PIK3CA amplification            | 153 (35.3)  | 53 (31.7)               | 19 (37.3)      |
| Genetic mutations               |             |                         |               |
| *PI3K/AKT* pathway              | 57 (13.2)   | 29 (17.4)               | 9 (17.6)       |
| *TP53*                          | 47 (10.9)   | 14 (8.4)                | 11 (21.6)      |
| *ARID1A*                        | 36 (8.3)    | 16 (9.6)                | 6 (11.8)       |
| *BRAF*                          | 1 (0.2)     | 1 (0.6)                 | 0              |
| MSI: microsatellite instability; MSI-H: MSI-high; MSI-L/S: MSI-low/stable; HP: Helicobacter pylori; EBV: Epstein-Barr virus; bold: statistically significant
Table 4. Univariate and multivariate analysis of factors affecting OS of GC patients.

|                         | Univariate analysis |           |           | Multivariate analysis |           |           |
|-------------------------|---------------------|-----------|-----------|-----------------------|-----------|-----------|
|                         | HR                  | 95%CI     | P value   | HR                    | 95%CI     | P value   |
| **Age**                 |                     |           |           |                       |           |           |
| <65 years               | 1.00                |           |           | 1.00                  |           |           |
| ≥65 years               | 1.47                | 1.145-1.884 | <0.001    | 1.79                  | 1.381-2.331 | <0.001    |
| **Gender**              |                     |           | 0.013     |                       |           |           |
| Male                    | 1.00                |           |           |                       |           |           |
| Female                  | 0.71                | 0.535-0.928 |           |                       |           |           |
| **Tumor location**      |                     |           | 0.002     |                       |           |           |
| Upper stomach           | 1.00                |           |           |                       |           |           |
| Middle stomach          | 0.64                | 0.453-0.897 |           |                       |           |           |
| Lower stomach           | 0.89                | 0.648-1.209 |           |                       |           |           |
| Whole stomach           | 1.91                | 0.9989-3.639 |           |                       |           |           |
| **Lymphovascular invasion** |                 |           | <0.001    |                       |           |           |
| No                      | 1.00                |           |           |                       |           |           |
| Yes                     | 2.77                | 2.204-3.788 |           |                       |           |           |
| **Lauren’s type**       |                     |           | 0.036     | 0.036                 |           |           |
| Intestinal-type         | 1.00                |           |           | 1.00                  |           |           |
| Diffuse-type            | 1.40                | 1.061-1.836 |           | 1.26                  | 0.945-1.669 |           |
| Mixed-type              | 1.03                | 0.756-1.405 |           | 0.83                  | 0.600-1.154 |           |
| **Pathological T category** |                 |           | <0.001    | <0.001                |           |           |
| T1                      | 1.00                |           |           | 1.00                  |           |           |
| T2                      | 1.40                | 0.771-2.556 |           | 1.08                  | 0.580-2.019 |           |
| T3                      | 2.68                | 1.645-4.355 |           | 1.43                  | 0.839-2.445 |           |
| T4                      | 6.17                | 3.850-9.900 |           | 3.10                  | 1.828-5.261 |           |
| **Pathological N category** |               |           | <0.001    | <0.001                |           |           |
| N0                      | 1.00                |           |           | 1.00                  |           |           |
| N1                      | 1.41                | 0.894-2.220 |           | 1.18                  | 0.738-1.884 |           |
| N2                      | 2.08                | 1.418-3.054 |           | 1.39                  | 0.920-2.110 |           |
| N3                      | 6.36                | 4.467-9.068 |           | 4.99                  | 3.307-7.525 |           |
| **MSI status**          |                     |           | 0.104     |                       |           |           |
| MSI-L/S                 | 1.00                |           |           |                       |           |           |
| MSI-H                   | 0.47                | 0.924-2.349 |           |                       |           |           |
| **PI3K/AKT pathway mutation** |             |           | 0.861     |                       |           |           |
| No                      | 1.00                |           |           |                       |           |           |
| Yes                     | 0.97                | 0.688-1.367 |           |                       |           |           |
| **ARID1A mutation**     |                     |           | 0.443     |                       |           |           |
| No                      | 1.00                |           |           |                       |           |           |
| Yes                     | 0.85                | 0.551-1.298 |           |                       |           |           |
| **TP53 mutation**       |                     |           | 0.722     |                       |           |           |
| No                      | 1.00                |           |           |                       |           |           |
| Yes                     | 1.07                | 0.739-1.547 |           |                       |           |           |
| **PIK3CA amplification** |                 |           | 0.420     |                       |           |           |
| No                      | 1.00                |           |           |                       |           |           |
| Yes                     | 0.90                | 0.704-1.157 |           |                       |           |           |

HR: hazard ratio; MSI: microsatellite instability; MSI-L/S: microsatellite instability-low/stable; MSI-H: microsatellite instability-high; T: Tumor; N: Node; bold: statistically significant.
Table 5. Clinical profiles of mixed-type GC with intestinal-type major tumors and diffuse-type major tumors.

| Variables                      | Intestinal-type major n=51 | Diffuse-type major n=63 | P value |
|--------------------------------|-----------------------------|--------------------------|---------|
|                                | n (%)                       | n (%)                    |         |
| Age <65 years                  | 19 (37.3)                   | 22 (34.9)                | 0.796   |
| Age ≥65 years                  | 32 (62.7)                   | 41 (65.1)                |         |
| Gender                         |                             |                          | 0.857   |
| Male                           | 38 (74.5)                   | 46 (73.0)                |         |
| Female                         | 13 (25.5)                   | 17 (27.0)                |         |
| Tumor size <5 cm               | 23 (45.1)                   | 21 (33.3)                | 0.200   |
| Tumor size ≥5 cm               | 28 (54.9)                   | 42 (66.7)                |         |
| Tumor location                 |                             |                          | 0.605   |
| Upper stomach                  | 10 (19.6)                   | 9 (14.3)                 |         |
| Middle stomach                 | 15 (29.4)                   | 23 (36.5)                |         |
| Lower stomach                  | 25 (49.0)                   | 27 (42.9)                |         |
| Whole stomach                  | 1 (2.0)                     | 4 (6.3)                  |         |
| Gross appearance               |                             |                          | 0.027   |
| Superficial type               | 8 (15.7)                    | 6 (9.5)                  |         |
| Borrmann type 1                | 5 (9.8)                     | 3 (4.8)                  |         |
| Borrmann type 2                | 16 (31.4)                   | 16 (25.3)                |         |
| Borrmann type 3                | 20 (39.2)                   | 27 (42.9)                |         |
| Borrmann type 4                | 2 (3.9)                     | 11 (17.5)                |         |
| Lymphovascular invasion        | 34 (66.7)                   | 54 (85.7)                | 0.016   |
| HP infection                   | 29 (56.9)                   | 36 (57.1)                | 0.976   |
| EBV infection                  | 7 (13.7)                    | 8 (12.7)                 | 0.872   |
| Pathological T category        |                             |                          | 0.077   |
| T1                             | 10 (19.6)                   | 4 (6.3)                  |         |
| T2                             | 10 (19.6)                   | 9 (14.3)                 |         |
| T3                             | 17 (33.3)                   | 33 (52.4)                |         |
| T4                             | 14 (27.5)                   | 17 (27.0)                |         |
| Pathological N category        |                             |                          | 0.004   |
| N0                             | 20 (39.2)                   | 8 (12.7)                 |         |
| N1                             | 3 (5.9)                     | 9 (14.3)                 |         |
| N2                             | 12 (23.5)                   | 12 (19.0)                |         |
| N3                             | 16 (31.4)                   | 34 (54.0)                |         |
| Pathological TNM Stage         |                             |                          | 0.009   |
| I                              | 16 (31.4)                   | 5 (7.9)                  |         |
| II                             | 10 (19.6)                   | 15 (23.8)                |         |
| III                            | 21 (41.2)                   | 37 (58.7)                |         |
| IV                             | 4 (7.8)                     | 6 (9.5)                  |         |

T: Tumor; N: Node; TNM: Tumor, Node, Metastasis; bold: statistically significant
