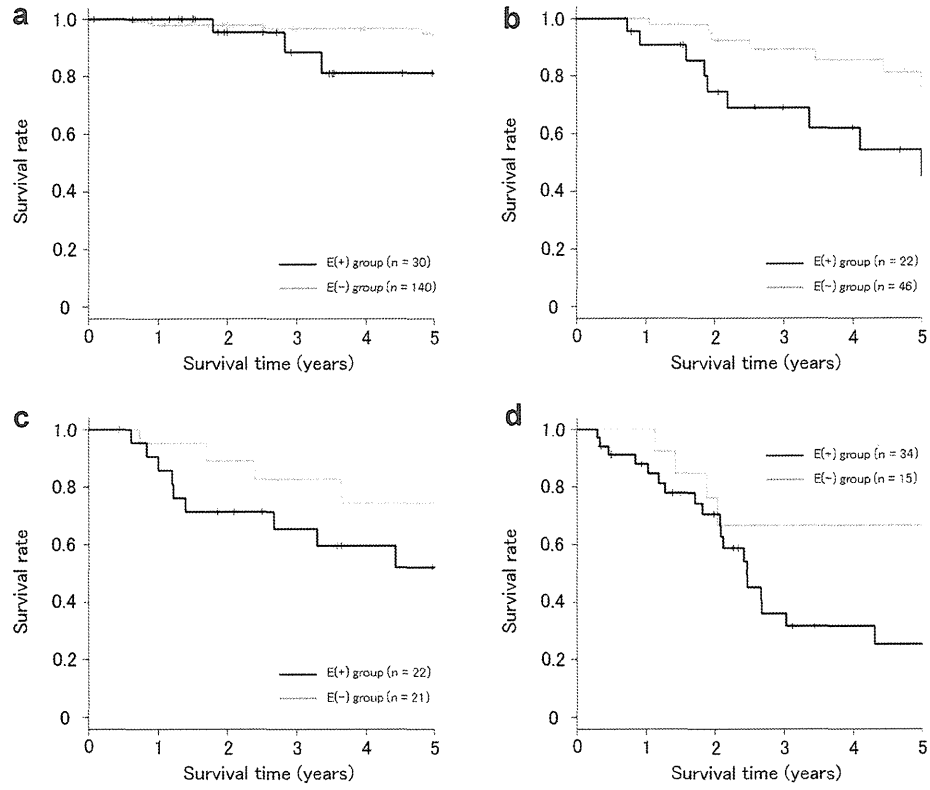

Fig. 3. Overall survival curves of patients stratified by nodal status. **a**: Overall survival curves of N0 patients. The 5-year overall survival rate is 81.0% in patients with esophageal invasion and 94.9% in those without esophageal infiltration ($P = 0.111$). **b**: Overall survival curve of N1 patients. The 5-year overall survival rate is 45.2% in patients with esophageal infiltration and 73.3% in those without esophageal infiltration ($P = 0.050$). **c**: Overall survival curves of N2 patients. The 5-year overall survival rate is 52.1% in patients with esophageal infiltration and 74.4% in those without esophageal infiltration ($P = 0.144$). **d**: Overall survival curves of N3 patients. The 5-year overall survival rate is 25.3% in patients with esophageal infiltration and 66.6% in those without esophageal infiltration ($P = 0.124$).

The survival curves of patients who underwent R0 or R1 gastrectomy are shown in Figure 1. The 5-year survival rate was significantly better in the E (−) group (86.8%) than in the E (+) group (48.7%, $P < 0.001$). Survival curves were also stratified by the pathological stage and nodal status. In the survival analysis, we tentatively adopted classifications systems for gastric cancer to both groups.

The survival curves stratified by pathological stage using the classification for gastric cancer are shown in Figure 2a–d. In addition, the survival curves stratified by nodal status are shown in Figure 3a–d. The 5-year survival curves for patients tend to be better in the E (−) group than in the E (+) group except for patients with stage IV disease.

The results of the multivariate analysis are shown in Table III. Esophageal invasion (hazard ratio; 3.323, 95% Confidential Interval; 1.815–6.082) was selected as an independent prognostic factor. In addition, histology, curability, tumor depth, nodal status, and splenectomy were also selected as independent prognostic factors.

TABLE III. Result of Multivariate Analysis

	Hazard ratio	95% Confidential interval	*P*-value
Age (≥65 vs. <65 years)	1.443	0.976–2.132	0.066
Sex (male vs. female)	0.855	0.489–1.493	0.581
Location of the epicenter (within 20 mm vs. 20–50 mm from the EGJ)	0.816	0.486–1.369	0.44
Histology (diffentiated vs. undiffentiated)	0.58	0.365–0.923	0.022
Tumor depth (T2, T3, T4 vs. T1)	3.719	1.506–9.182	0.004
Lymph node status (N+ vs. N−)	3.221	1.427–7.273	0.005
Curability (R1, R2/R0)	4.272	2.479–7.360	<0.001
Esophageal invasion (+/−)	3.323	1.815–6.082	<0.001
Tumor diameter (≥50 mm vs. <50 mm)	0.829	0.561–1.223	0.343
Type of surgery (TG vs. PG)	0.668	0.293–1.525	0.338
Splenectomy (yes vs. no)	1.853	1.125–3.049	0.015

EGJ, esophagogastric junction.

DISCUSSION

This study demonstrates that patients without esophageal invasion [E (−) group] have better overall survival than those with esophageal invasion [E (+) group]. Tumor diameter was larger and pathological T and N stage were more advanced in the E (+) group than in the E (−) group. Accordingly, curative resection was achieved less frequently in the E (+) group than in the E (−) group, which may be responsible for the poor survival outcome in the E (+) group. The same trend was observed even after stratification by pathological stage and nodal status in patients undergoing R0 or R1 surgery. Multivariate analysis also identified esophageal invasion as an independent prognostic factor.

One possible reason for the poor long-term outcome in the E (+) group compared to the E (−) group is the complicated anatomical structures around the EGJ. Surgery is generally difficult in patients with esophageal invasion because thoracostomy is sometimes required to secure the surgical margin. In the present study, proximal resection margin was shorter in the E (+) group (10 mm) than in the E (−) group (20 mm) presumably due to difficulty in securing resection margin in the E (+) group. In addition, difficulty in surgical procedure might be associated with higher local recurrence rate in the E (+) group (4.5%) than in the E (−) group even after R0 or R1 gastrectomy (data not shown).

A recent study revealed the existence of lymphatic flow from the lower esophagus into the lower mediastinal node [3]. This lymphatic flow could adversely affect the disease outcome for patients through metastatic spread to the lower mediastinal lymph node although the therapeutic value of mediastinal lymph node dissection remains controversial [9–11]. In the present study, few patients in the E (−) group received lower mediastinal lymph node dissection, therefore the actual incidence and therapeutic value of lower mediastinal lymph node dissection could not be determined.

Different tumor diameter between the groups is another contributing factor for the poor long-term outcome in the E (+) group. Bando et al. [12] reported magnitude of serosal change was associated with peritoneal metastasis and affected long-term outcome of patients. In the present study, median tumor diameter was longer and positive peritoneal lavage cytology was more frequently observed in E the (+) group than in the E (−) group; thus we included tumor diameter as a covariate in the subsequent multivariate analysis (cut-off value of 50 mm was selected as a median tumor diameter of all patients). However, it was not selected as an independent prognostic factor.

Our investigations revealed that patients who had an epicenter within 2 cm of the EGJ were more frequently observed in the E (+) group than in the E (−) group. The distribution and prognostic value of Siewert type classification have been investigated in both Western and Eastern countries [5,13–16]. Siewert type I AEG is extremely rare in Eastern countries, and most AEGs have been classified as type II or III [5,9,16–18]. The survival rate among each Siewert type is still contentious with the worst long-term outcome reported in Siewert type III AEG followed by type II, and type I [13]. Other investigations have reported there is either no significant difference [5,9,16,17] or a better survival rate in type III AEG [15,18]. In this study, the location of tumor epicenter was not selected as an independent prognostic factor.

Thoracostomy was required in 6% of patients in the E (+) group, whereas it was required only in one patient in the E (−) group. In addition, splenectomy was frequently performed in the E (+) group. These procedures are associated with increased intraoperative blood loss, prolonged operation time, and increased incidence of postoperative morbidity [9,13,15,17,19–21]. The aggressive surgery that was frequently performed in the E (+) group is one of the contributing factors that led to poor early surgical outcomes in the present study.

The present retrospective study has some limitations. Firstly, patient's background data and selected treatment, including surgical procedure and postoperative adjuvant chemotherapy, may be different between the groups. Secondly, we assessed the epicenter of the tumor and the location of the EGJ retrospectively with formalin-fixed specimen, and it is sometimes difficult particularly in patients with far advanced disease. To overcome these limitations, prospective data collection with determined treatment strategy by the protocol will be necessary.

In conclusion, patients in the E (+) group represented a more advanced stage and a poorer long-term outcome. Furthermore, esophageal invasion was identified as an independent prognostic factor. The establishment of multimodal treatment is necessary to improve the survival outcome of these patients. Further study is necessary to clarify whether patients with esophageal invasion should be classified using the system for esophageal cancer or by another method.

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ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Poor Survival Rate in Patients with Postoperative Intra-Abdominal Infectious Complications Following Curative Gastrectomy for Gastric Cancer

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ABSTRACT

Background. The impact of postoperative complications on recurrence rate and long-term outcome has been reported in patients with colorectal and esophageal cancer, but not in patients with gastric cancer. This study evaluated the impact of postoperative intra-abdominal infectious complications on long-term survival following curative gastrectomy.

Methods. This study included 765 patients who underwent curative gastrectomy for gastric cancer between 2002 and 2006. Patients were divided into 2 groups: with (C-group, $n = 81$) or without (NC-group, $n = 684$) intra-abdominal infectious complications. Survival curves were compared between the groups, and multivariate analysis was conducted to identify independent prognostic factors.

Results. Male patients were dominant, and total gastrectomy was frequently performed in the C-group. The pathological stage was more advanced and D2 lymph node dissection and splenectomy were preferred in the C-group. The 5-year overall survival (OS) rate was better in the NC-group (86.8 %) than in the C-group (66.4 %; $P < .001$). The 5-year relapse-free survival (RFS) rate was also better in the NC-group (84.5 %) than in the C-group (64.9 %; $P < .001$). This trend was still observed in stage II and III patients after stratification by pathological stage. Multivariate analysis identified intra-abdominal infectious complication as an independent prognostic factor for OS (hazard ratio, 2.448; 95 % confidence interval [95 % CI], 1.475–4.060) and RFS (hazard ratio, 2.219; 95 % CI, 1.330–3.409) in patients with advanced disease.

Conclusions. Postoperative intra-abdominal infectious complications adversely affect OS and RFS. Meticulous surgery is needed to decrease the complication rate and improve the long-term outcome of patients following curative gastrectomy.

Gastrectomy with R0 resection is inevitable to cure patients with gastric cancer.[1,2] However, even after R0 resection, a significant number of patients suffer from recurrence, particularly after surgery for advanced gastric cancer.[3–5] Tumor depth and lymph node status are well-known prognostic factors, and patient age and performance status have also been reported as having an impact on the long-term outcome of patients.[1,2, 6,7]

In Japan, gastrectomy with D2 lymph node dissection has been the standard treatment for advanced gastric cancer.[8–11] However, Western randomized trials have failed to prove the efficacy of D2 lymph node dissection, presumably because of the increased incidence of postoperative morbidity, which results in increased in-hospital deaths following D2 lymph node dissection.[12–14] Moreover, postoperative morbidity may adversely affect long-term, as well as short-term outcomes in patients.

Previously, the impact of postoperative complications on recurrence rate and long-term outcome has been reported in patients with colorectal cancer and esophageal or esophagogastric junction cancer.[15–23] In the case of colorectal cancer, anastomotic leakage is generally associated with a high local recurrence rate, as well as a poor long-term survival rate.[15–18] Additionally, a strong correlation between postoperative complications and poor long-term outcome has been reported for esophageal and esophagogastric junction cancer.[19,21,23] However, contradictory studies have also been published. Branagan and Finnis [15] reported that

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anastomotic leakage does not result in poor survival following colorectal surgery. After esophagogastrectomy, Junemann-Ramirez et al.[22] reported that anastomotic leakage does not correlate with poor survival, and Ancona et al.[20] reported that surgical complications themselves do not affect patients' long-term outcomes although survival of patients with both surgical and medical complications was poor.

In patients with gastric cancer, there have been limited reports assessing the relationship between postoperative complications and long-term outcome. Sierzega et al.[7] reported that anastomotic leakage as well as deeper tumor depth, lymph node metastasis, distant metastasis, and poor performance status were found to be independent prognostic factors following total gastrectomy for gastric adenocarcinoma. Their study included 690 patients from 7 university surgical centers in Poland. However, the impact of other postoperative complications on long-term outcome was not investigated, and their study included patients whose surgery was not curative. Moreover, it is unclear whether their results can be adopted by East-Asian countries where the incidence of gastric cancer is high and the reported incidence of postoperative complications is low compared with Western countries.[13,24–26]

The aim of the present study was to clarify the impact of postoperative intra-abdominal infectious complications on the long-term survival rate of patients undergoing curative gastrectomy in one of the highest-volume centers in Japan.

PATIENTS AND METHODS

A total of 765 patients who underwent curative gastrectomy (R0 resection) for gastric cancer at the Shizuoka Cancer Center between September 2002 and October 2006 were included in the present study. Patients who received neoadjuvant or adjuvant chemotherapy, patients who had other cancers and patients who underwent surgery for gastric stump carcinoma were excluded. Patients were also excluded if the histology of the primary lesion was not adenocarcinoma.

The patients' characteristics and pathological and surgical findings were collected from our database records and individual patient electronic medical records. The postoperative clinical course of each patient, including the incidence and severity of intra-abdominal infectious complications, was collected from individual electronic medical records. The data collection and analysis were approved by the institutional review board of the Shizuoka Cancer Center.

Pathological tumor depth, nodal status, and curability of surgery were assigned according to the International Union Against Cancer (UICC) classification, Seventh edition.[27]

Histological type was classified according to the Japanese Gastric Cancer Association (JGCA) classification system, in which tubular and papillary adenocarcinoma are defined as differentiated adenocarcinoma, while poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and mucinous adenocarcinoma are defined as undifferentiated adenocarcinoma.[28]

Definition of Postoperative Intra-abdominal Complications

In this study, the Clavien–Dindo (CD) classification was adopted to classify each patient's postoperative intra-abdominal complication.[29,30] According to the CD classification, patients were classified as having grade II complications if antibiotics were administered. They were classified as grade IIIa or IIIb if surgical intervention was indicated. If patients required admission to the intensive care unit, they were regarded as having grade IVa or IVb complications. Postoperative mortality was defined as a grade V complication. If multiple complications occurred in a single patient, the highest grade was used.

Comparison Between Patients With and Without Complications

Clinicopathological characteristics were compared between patients with postoperative intra-abdominal infectious complications (C-group, $n = 81$) and those without complications (NC-group, $n = 684$). Overall survival time and relapse-free survival time were also compared between the groups.

Statistical Analyses

All continuous variables are presented as the median (range). Statistical analyses were performed using the Fisher exact test, t test, and Mann–Whitney test. The 5-year survival rates were calculated using the Kaplan–Meier method, and the log-rank test was used to compare the groups. Independent prognostic factors were identified using the Cox proportional hazards model. In the analysis, each patient's age, sex, histology, type of surgery, degree of lymph node dissection, intraoperative blood loss, operation time, pathological stage, and postoperative intra-abdominal infectious complication were included as covariates. $P < .05$ was considered significant. All statistical analyses were conducted using R Statistics version 2.13.1.

RESULTS

The clinicopathological characteristics of all the patients are shown in Table 1. There was no difference in age between the C-group and NC-group. Male patients

TABLE 1 Clinicopathological characteristics of patients in both groups

	C-group	NC-group	*P* value
Sex (*n*)			.001
Male	68	452	
Female	13	232	
Age (years)			.061
Median	66	64	
Range	31–83	24–88	
Performance status (ECOG)			.545
0 or 1	80	678	
2 or 3	1	6	
Hemoglobin (g/dL)			.577
Median	13.7	13.7	
Range	7.5–16.4	6.3–17.5	
Albumin (g/dL)			.090
Median	4.3	4.3	
Range	2.4–5.0	1.8–5.3	
Lymphocyte count[a]			.352
Median	1920	1700	
Range	870–3450	620–3960	
Surgical procedure (*n*)			<.001
Total gastrectomy	44	142	
Partial gastrectomy	37	542	
Splenectomy (*n*)			<.001
Performed	38	67	
Not performed	43	617	
Lymph node dissection			<.001
<D2	25	431	
≥D2	56	253	
Operation time (min)			<.001
Median	244	186	
Range	125–733	50–725	
Intraoperative blood loss (mg)			<.001
Median	454	250	
Range	50–2650	0–1800	
Postoperative hospital stay (days)			<.001
Median	23	11	
Range	12–308	7–56	
Histology (*n*)			.347
Differentiated	47	355	
Undifferentiated	34	329	
Tumor depth (*n*)			<.001
T1	29	430	
T2	10	70	
T3	29	150	
T4a	11	31	
T4b	2	3	
Lymph node status (*n*)			.004
N0	39	449	
N1	10	88	
N2	17	77	
N3	15	70	
Pathological stage (*n*)			<.001
I	29	440	
II	27	120	
III	21	115	
IV	4	9	

ECOG Eastern Cooperative Oncology Group

[a] Lymphocyte count was measured in 27 patients in the C-group and 189 patients in the NC-group

predominated in both groups and total gastrectomy was frequently performed in the C-group. Preoperative serum albumin level, hemoglobin level, and lymphocyte count were not different between the groups. D2 lymph node dissection and splenectomy were also preferred in the C-group. Operation time was longer and intraoperative blood loss was higher in the C-group than in the NC-group ($P < .001$). More advanced gastric cancer was observed in the C-group than in the NC-group ($P < .001$).

The type and severity of complications are shown in Table 2. Intra-abdominal infectious complications were observed in 11 % (81 of 765) of patients. Pancreas-related infections were the most frequently observed intra-abdominal infectious complication, followed by intra-abdominal abscess and anastomotic leakage. We found 33 % of patients recovered well with medication (grade II), and surgical intervention with local or general anesthesia was required in 62 and 1 % of patients, respectively. One patient died following deterioration of a postoperative intra-abdominal infectious complication. In every pathological stage, grade IIIa complications were the most frequently observed, followed by grade II complications.

We also investigated the number of patients who required readmission because of postgastrectomy syndromes, which included bowel obstruction, cholecystitis, and insufficient oral intake. If patients had a recurrence, admission after the recurrence was not counted. In the C-group, 7 of 81 patients (9 %) required readmission because of a postgastrectomy syndrome. In the NC-group, readmission was required in 32 of 684 patients (5 %; $P = .174$). The most frequent reason for readmission was bowel obstruction in both groups (4 patients in the C-group, and 20 patients in the NC-group; $P = .308$). We investigated serum albumin levels of patients without recurrence 1 year after the surgery to assess nutritional status. There was no difference in the serum albumin level change between the groups ($P = .330$).

Details of the initial recurrence site following gastrectomy are listed in Table 3. Recurrence was observed in 21 of 81 patients (26 %) in the C-group, and 83 of 684 patients (12 %) in the NC-group ($P = .002$). In the NC-group, peritoneal metastasis was the most frequent recurrence pattern followed by lymph node metastasis and liver metastasis. In the C-group, lymph node metastasis was the most frequently observed site of recurrence. Locoregional recurrence was not observed in any of the patients in the C-group even after anastomotic leakage. The pattern of recurrence was not different between the 2 groups ($P = .401$).

In the median follow-up period of survivors of 63 months, the 5-years overall survival rate was better in the NC-group (86.8 %) than in the C-group (66.4 %; $P < .001$). The 5-years relapse-free survival rate was also better in the NC-group (84.5 %) than in the C-group (64.9 %; $P < .001$).

Overall and relapse-free survival curves stratified by pathological stage were compared between the groups (Figs. 1a, b, 2a, b). In patients with stage I early gastric cancer, there were no differences between the groups. Conversely, in patients with stage II and III gastric cancer, overall and relapse-free survival rates were significantly better in the NC-group than in the C-group, except for relapse-free survival time in patients with stage III gastric cancer. In patients with stage III gastric cancer, the 5-years relapse-free survival rate still tended to be better in the NC-group (55.1 %) than in the C-group (41.3 %); however, the difference did not reach significance ($P = .11$).

Table 4 shows the results of the Cox-proportional hazards model used to identify independent prognostic factors for overall survival. In this analysis, only patients with stage II or more advanced disease were included because the survival analysis did not show a survival difference between the 2 groups in patients with pathological stage I disease. Pathological stage (hazard ratio [HR], 2.564; 95 % CI, 1.681–3.912) and intra-abdominal infectious complications (HR, 2.448; 95 % CI, 1.475–4.060) were found to be independent prognostic factors. The same independent prognostic factors were identified for relapse-free survival (pathological stage [HR, 2.657; 95 % CI, 1.782–3.962], and intra-abdominal infectious complications [HR, 2.219; 95 % CI, 1.330–3.409], Table 5).

Figure 3 shows hazard ratio for death among subgroups. The overall survival was analyzed according to sex, age, type of surgery, splenectomy, degree of lymph node dissection, intraoperative blood loss, operation time, histology, pathological tumor depth, and pathological nodal status.

TABLE 2 Details of postoperative intra-abdominal complications

	Grade of CD classification						Total
	II	IIIa	IIIb	IVa	IVb	V	
Type of complication							
Pancreas-related complication	15	27	0	0	0	0	42
Anastomotic leakage	1	14	1	1	1	0	18
Intra-abdominal abscess	11	9	0	0	0	1	21
Pathological stage							
I	11	17	0	1	0	0	29
II	10	17	0	0	0	0	27
III	6	12	1	0	1	1	21
IV	0	4	0	0	0	0	4
Total	27	50	1	1	1	1	81

CD Clavien–Dindo

TABLE 3 Site of initial recurrence after surgery

	C-group			C-group total	NC-group
	Pancreas-related infection	Anastomotic leakage	Intra-abdominal abscess		
Peritoneal metastasis	4	1	1	6	35
Liver metastasis	5	0	1	6	19
Locoregional recurrence	0	0	0	0	5
Lymph node metastasis	8	1	4	13	31
Lung	1	1	0	2	6
Bone	0	0	0	0	5
Other	0	0	0	0	3
Unknown	0	0	0	0	1
Number of cases with recurrence[a]	12	3	6	21	83

[a] Patients with multiple recurrence sites are included for each recurrence site

DISCUSSION

The present study revealed that postoperative complications were strongly associated with poor overall survival time and relapse-free survival time. This trend was also observed even after stratification by pathological stage.

To investigate the prognostic value of postoperative complications, appropriate assessment of the incidence and severity of complications is mandatory. In 2004, Clavien and Dindo proposed the CD classification, which is a treatment-oriented, objective criteria for postoperative complications.[29,30] Recently, a number of reports, including those concerning postgastrectomy morbidities, have

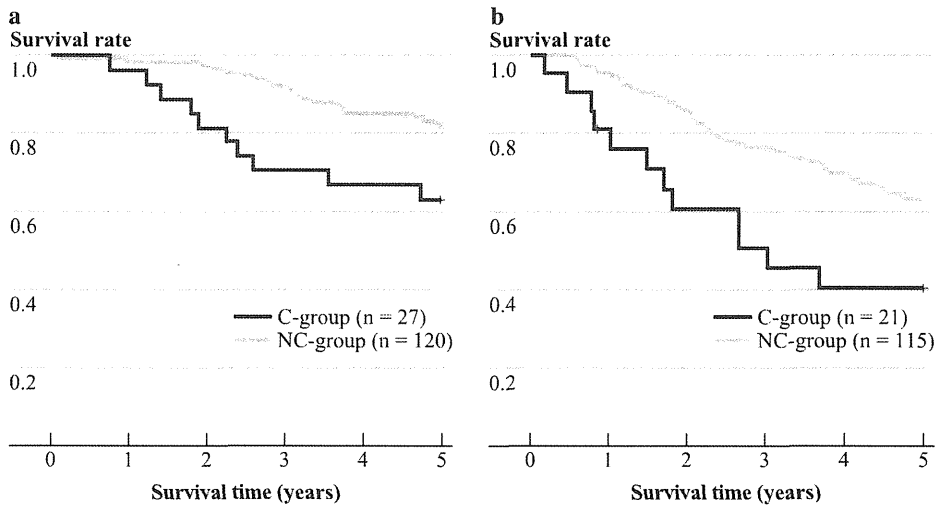

FIG. 1 **a** Overall survival curves of 147 stage II patients who underwent curative gastrectomy for gastric cancer. The 5-year overall survival rate is significantly better in the group of patients without postoperative intra-abdominal infectious complications (NC-group, 81.1 %) than in the group with complications (C-group, 63.0 %; $P = .02$). **b** Overall survival curves of 136 stage III patients who underwent curative gastrectomy for gastric cancer. The 5-year overall survival rate is significantly better in the NC-group (63.3 %) than in the C-group (40.5 %; $P = .03$)

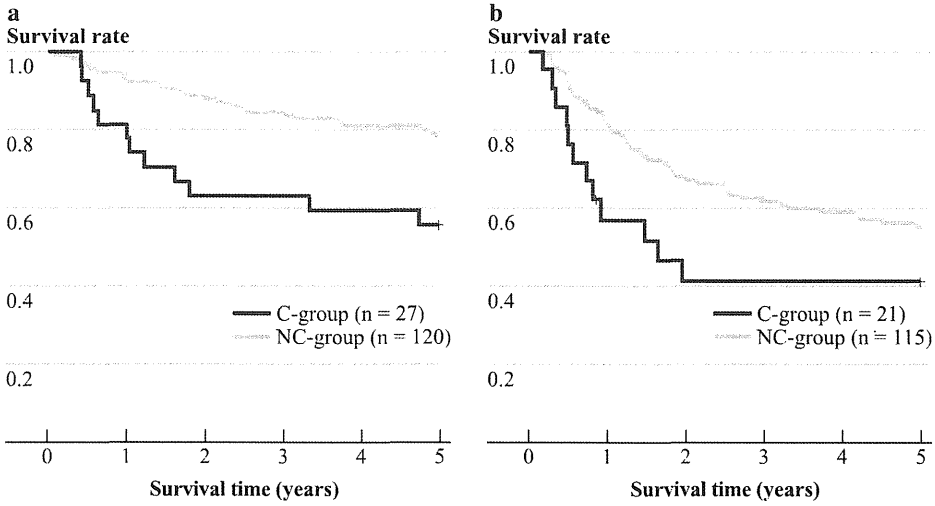

FIG. 2 **a** Relapse-free survival curves of 147 stage II patients who underwent curative gastrectomy for gastric cancer. The 5-year relapse-free survival rate is significantly better in the group of patients without postoperative intra-abdominal infectious complications (NC-group, 78.0 %) than in the group with complications (C-group, 55.6 %; $P = .02$). **b** Relapse-free survival curves of 136 stage III patients who underwent curative gastrectomy for gastric cancer. The 5-year relapse-free survival rate tends to be better in the NC-group (55.1 %) than in the C-group (41.3 %), although the difference is not significant ($P = .11$)

adopted the CD classification to evaluate postoperative problems.[31,32] In contrast, previous studies that investigated the effect of complications on long-term outcomes following surgeries generally used their own criteria to grade the severity of the complications, making it difficult to evaluate the results of the study[15–23] In the present study, to overcome this potential problem, we used the CD classification to assess the severity of complications. In the present study, patients with grade II or more severe intra-abdominal infection were regarded as having complications since we considered these complications to cause systemic inflammatory response syndrome, resulting in excess surgical trauma and tissue damage.

Administration of perioperative chemotherapies has been accepted as it increases the survival rate of patients with advanced gastric cancer.[33–36] In Japan, postoperative administration of S-1 for 1 year after curative surgery has been a standard treatment in patients with advanced gastric cancer since the results of a prospective randomized controlled trial were reported in October 2006.[33] Therefore, in the present study, we only included patients who underwent surgery before 2006 and excluded patients who received neoadjuvant chemotherapy to eliminate the effects of perioperative chemotherapies.

It is unclear why postoperative intra-abdominal infectious complications affect the long-term outcome of

TABLE 4 Results of multivariate analysis to identify independent prognostic factors for overall survival

Covariates	*P* value	Hazard ratio (HR)	95 % CI
Age (≥ 65 vs < 65 years)	.138	1.241	.933–1.651
Sex (male vs female)	.683	1.099	.700–1.725
Surgery (total gastrectomy vs partial gastrectomy)	.496	1.165	.751–1.806
Histology (differentiated vs undifferentiated)	.162	1.340	.889–2.022
pStage (III, IV vs II)	<.001	2.564	1.681–3.912
Duration of surgery (≥ 200 vs < 200 min)	.773	.949	.666–1.353
Intraoperative blood loss (≥ 300 vs < 300 mL)	.057	.726	.523–1.009
Intra-abdominal infectious complications (yes vs no)	<.001	2.448	1.475–4.060
Lymph node dissection (≥ D2 vs < D2)	.248	.761	.478–1.210

CI confidence interval

TABLE 5 Results of multivariate analysis to identify independent prognostic factors for relapse-free survival

Covariates	*P* value	Hazard ratio (HR)	95 % CI
Age (≥ 65 vs < 65 years)	.213	1.187	.906–1.555
Sex (male vs female)	.590	1.127	.729–1.743
Surgery (total gastrectomy vs partial gastrectomy)	.747	.933	.614–1.419
Histology (differentiated vs undifferentiated)	.375	1.191	.810–1.751
pStage (III, IV vs II)	<.001	2.657	1.782–3.962
Duration of surgery (≥ 200 vs < 200 min)	.492	1.123	.807–1.562
Intraoperative blood loss (≥ 300 vs < 300 mL)	.140	.795	.586–.178
Intra-abdominal infectious complications (yes vs no)	.002	2.219	1.330–3.409
Lymph node dissection (≥ D2 vs < D2)	.135	.716	.462–1.110

CI confidence interval

patients. Following colorectal surgery, it was reported that anastomotic leakage increased the rate of local recurrence presumably due to viable colorectal cancer cells being deposited extraluminally into the pelvis.[16–18] However, in the present study the incidence of local recurrence did not increase even after anastomotic leakage; thus, we consider implantation of cancer cells into the abdominal cavity not a contributing factor in the present series.

Another possible factor promoting metastatic growth and early recurrence is immune suppression.[37,38] Specifically, cell-mediated immunity, in particular natural killer cells and cytotoxic T lymphocytes, is compromised, and the degree of suppression is considered to be related to the extent of surgical trauma and tissue damage. Goldfarb et al. reported treatment aimed at perioperative enhancement of cell-mediated immunity with simultaneous inhibition of excessive catecholamine and prostaglandin responses could be successful in limiting postoperative immune suppression and metastatic progression.[38] In the C-group, postoperative intra-abdominal infectious complications increased surgical stress and caused severe tissue damage due to local and generalized inflammatory reactions, resulting in more severe immune suppression than in the NC-group. We consider, therefore, that the difference in the degree of immune suppression between the groups is a possible contributing factor to the survival difference between the groups.

The present retrospective study has limitations. Firstly, backgrounds were different between patients with and without complications. Of different backgrounds, pathological stage is assumed to be the strongest prognostic factor for gastric cancer following curative gastrectomy.[1,2,6] Therefore, we stratified patients by their pathological stage, and multivariate analysis was conducted. Even after stratification, the same trend, better survival outcomes in patients without intra-abdominal infectious complications, was still observed in stage II and III patients. Multivariate analysis also identified intra-abdominal infectious complications as an independent prognostic factor. In addition, we investigated hazard ratio for death among subgroups. In each subgroup, long-term outcome tended to be better in the NC-group than in the C-group. Secondly, the degree of immune suppression was not assessed in this study. This should be examined in a future trial to clarify whether our hypothesis, that patients with intra-abdominal infectious complications have severe immune suppression resulting in high recurrence rates and poor overall and relapse-free survival rates, is correct or not.

D2 lymph node dissection and splenectomy were frequently performed in the C-group, and these procedures were thought to increase the incidence of intra-abdominal infectious complications. We also investigated the effect of D2 lymph node dissection on long-term survival rate by

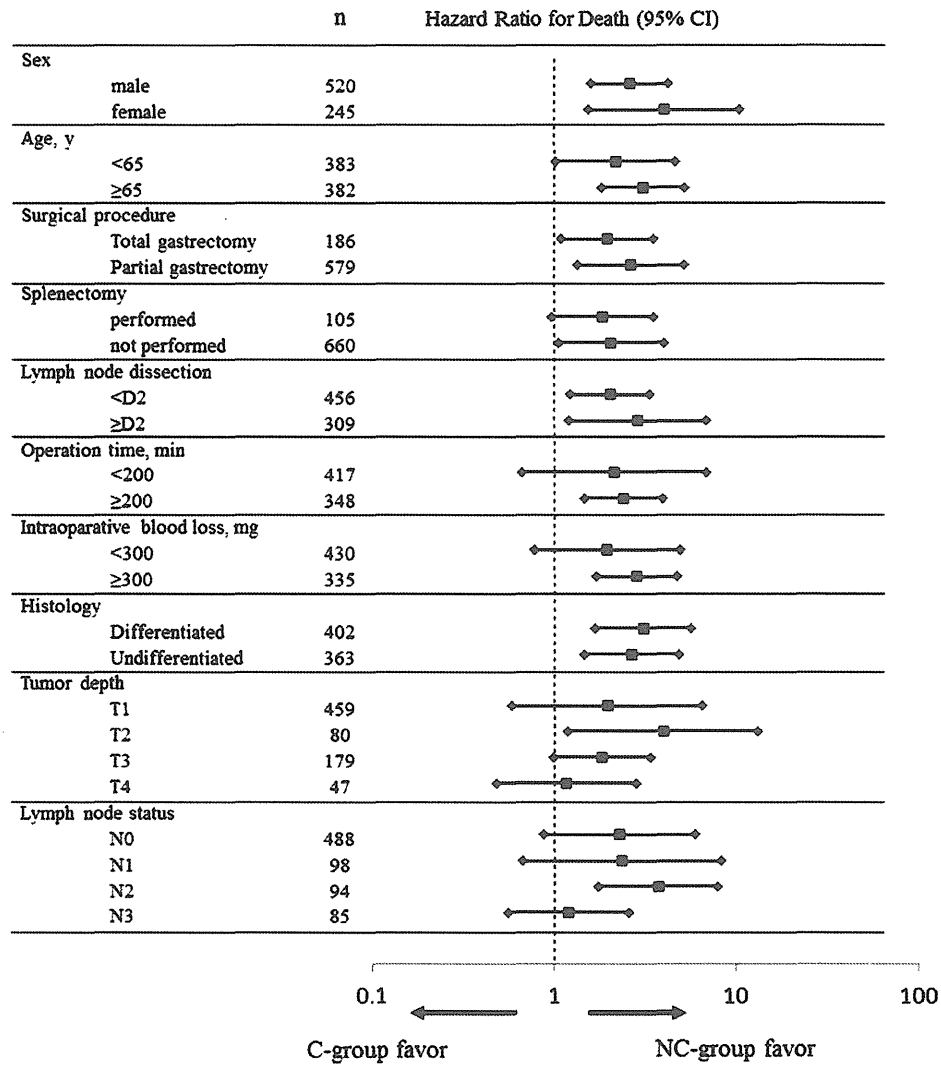

FIG. 3 Hazard ratio for death among subgroups. Long-term survival is better in NC-group than in C-group in most subgroups

multivariate analysis, and it was not identified as an independent prognostic factor. In addition, splenectomy was not identified as an independent prognostic factor even when we included it as a covariate instead of D2 lymph node dissection (data not shown). In Western countries, the most recent European Society for Medical Oncology clinical practice guidelines recommend a D2 gastrectomy as the standard procedure for curable advanced gastric cancer.[39,40] However, in their guidelines, splenectomy is only indicated if there is direct invasion, presumably due to the increased morbidity and mortality seen in 2 European randomized controlled trials.[12–14] In Japan, splenectomy is still a standard treatment for patients with upper-third advanced gastric cancer, although early results from a randomized clinical trial investigating the efficacy of splenectomy showed an increased incidence of postoperative pancreas-related infections. The effect of splenectomy on the long-term survival rate is still unclear even in Japan, and we have to wait for the final results of the randomized clinical trial.[41]

Perhaps surgeons have the urge to decrease postoperative complications in order to improve early surgical outcomes. However, the results of the present study show there are also poor long-term outcomes in patients with postoperative intra-abdominal infections. Therefore, surgeons must perform the surgery with extreme precision, not only to decrease postoperative complications, but also to improve long-term outcomes for patients.

In conclusion, postoperative intra-abdominal infectious complications adversely affect the overall and relapse-free survival of patients with stage II and III advanced gastric cancer. Surgeons have to perform the surgery with meticulous care in order to decrease the complication rate and improve the long-term outcome of patients following curative gastrectomy.

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Clinical Cancer Research

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Imaging, Diagnosis, Prognosis

Impact of Expression of Human Epidermal Growth Factor Receptors EGFR and ERBB2 on Survival in Stage II/III Gastric Cancer

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Abstract

Purpose: EGF receptor (EGFR) and HER2 positivity are considered to be negative prognostic factors in gastric cancer. Biomarker analysis was conducted to evaluate the impact of EGFR and HER2 expression on the outcome of patients enrolled in the Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer (ACTS-GC), a randomized controlled trial comparing postoperative adjuvant S-1 therapy with surgery alone in 1,059 patients with stage II/III gastric cancer.

Experimental Design: Formalin-fixed, paraffin-embedded surgical specimens were retrospectively examined in 829 patients (78.3%). The effects of EGFR and HER2 positivity on survival were analyzed on the basis of the 5-year survival data from the study. EGFR positivity was defined as an immunohistochemistry (IHC) score of 3+, and HER2 positivity as an IHC score of 3+ or an IHC score of 2+ with a positive dual-color *in situ* hybridization status.

Results: EGFR and HER2 were positive in 75 (9.0%) and 113 (13.6%) patients, respectively. The overall and relapse-free survival rates were significantly lower in EGFR-positive patients than in EGFR-negative patients, whereas they were similar in HER2-positive and HER2-negative patients. Multivariate analysis showed that EGFR positivity correlated with poor outcomes [HR = 1.504; 95% confidence interval (CI) = 1.020–2.149; $P = 0.040$]. Treatment with S-1 improved survival compared with surgery alone, irrespective of EGFR and HER2 status.

Conclusions: EGFR positivity, but not HER2 positivity, was associated with poor patient outcomes after curative resection of stage II/III gastric cancer. There was no interaction between S-1 and EGFR or HER2 status with respect to survival outcome. *Clin Cancer Res; 18(21); 5992–6000. ©2012 AACR.*

Introduction

Gastric cancer is the second leading cause of cancer-related deaths worldwide, and the highest mortality rates have been reported in East Asia, including Japan, Korea, and China (28.1 per 100,000 males, 13.0 per 100,000 females; ref. 1). The mainstay of treatment of gastric cancer is surgery; however, in stage II (excluding T1 disease) and stage III (moderately advanced) disease, many patients suffer recurrence, even after curative resection. Various regimens for adjuvant chemotherapy have been implemented to prevent this.

S-1 (TS-1; Taiho Pharmaceutical Co. Ltd.) is an oral fluoropyrimidine preparation, combining tegafur, gimeracil, and oteracil potassium (2). The Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer (ACTS-GC), which was a prospective randomized phase III trial, showed that S-1 was more effective than surgery alone in East Asian patients with stage II/III gastric cancer (3, 4). However, the 5-year overall survival (OS) rate in patients with stage IIIB disease was 50.2% in the S-1 group in a subset analysis, suggesting room for improvement (4). There is a need to evaluate the effectiveness of intensive preoperative and/or postoperative chemotherapy with multiple agents, including some new biologic agents, in patients at high risk of relapse.

The type I HER family has 4 homologous members: HER1/erbB1 [EGF receptor (EGFR)], HER2/erbB2 (HER2), HER3/erbB3, and HER4/erbB4. All members share a

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Translational Relevance

The clinical significance of EGF receptor (EGFR) and HER2 overexpression remains to be fully defined because not all previous studies have shown an association between overexpression of these receptors and poor outcomes of patients with gastric cancer. We studied archived specimens obtained from 829 patients enrolled in the Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer (ACTS-GC) trial at 65 centers. All specimens were evaluated by standard methods and unified criteria in a central laboratory. The results provide compelling evidence that an EGFR 3+ status on immunohistochemical analysis, but not HER2 positivity, is significantly associated with poor outcomes after curative resection of stage II/III gastric cancer. There was no apparent interaction between S-1 and EGFR or HER2 status with respect to survival.

common structure, with an extracellular ligand-binding domain, a transmembrane domain, and an intracytoplasmic tyrosine kinase domain. Ligand binding to these receptors induces the formation of receptor homodimers and heterodimers, and the activation of downstream signaling pathways. The HER family might therefore contribute to malignant progression. In gastric cancers, overexpressions of EGFR and HER2 are considered prognostic factors, and have been targeted by novel biologic agents (5–10). Recently, the first phase III Trastuzumab for Gastric Cancer (ToGA) trial showed that trastuzumab enhanced the efficacy of chemotherapy in HER2-positive advanced gastric cancer, indicating that HER2 expression might predict the response to anti-HER2 agents even in gastric cancer (11). However, the clinical significance of EGFR and HER2 overexpression remains to be fully defined because not all studies have shown an association with poor outcomes (12, 13).

The present study therefore explored the protein expression of EGFR and HER2 using immunohistochemical analysis and gene amplification of *HER2* by dual-color *in situ* hybridization (dual-ISH) in gastric cancer tissues obtained from patients enrolled in the ACTS-GC. We retrospectively evaluated the impact of the expression of these receptors on treatment outcomes.

Materials and Methods

Patients and sample collection

Tumor tissue was collected from patients enrolled in the ACTS-GC. The inclusion criteria and the treatment protocol were as described previously (3, 4).

The present biomarker study was designed retrospectively after the completion of the first interim analysis of the ACTS-GC. Archived formalin-fixed, paraffin-embedded (FFPE) specimens obtained by surgical resection were available for 829 (78.3%) of the 1,059 patients who were enrolled in the ACTS-GC at 65 centers. The specimens were shipped to the National Cancer Center Hospital East (Kashiwa, Japan), where immunohistochemical and dual-ISH analyses were conducted, and the results were evaluated. The protocol of this biomarker study was approved by the ethics committee of the Japanese Gastric Cancer Association and the Institutional Review Board of each participating hospital.

IHC

All of the reagents and instruments for IHC were manufactured by Ventana Medical Systems, Inc. FFPE sections (thickness = 3–5 μm) were automatically stained with Ventana BenchMark ULTRA using primary antibodies against EGFR (CONFIRM EGFR 3C6) and HER2 (I-VIEW PATHWAY anti-HER2/neu 4B5), and a Ventana iView DAB Universal Kit, according to the manufacturer's protocol. Staining was evaluated using light microscopy and was interpreted by 2 independent pathologists (K. Kitada and A. Ochiai) who were blinded to all clinical information. Tumor cell-membrane immunostaining was scored using a 4-grade scale (0, 1+, 2+, or 3+). EGFR reactivity was scored as 0 if there was no membranous reactivity within the tumor, or as 1+, 2+, or 3+ depending on the intensity above the background level (7). We followed the consensus panel recommendations for HER2 scoring in gastric cancer (14).

Dual-ISH

All reagents and instruments for dual-ISH were manufactured by Ventana Medical Systems, Inc. Dual-ISH analyses for *HER2* were carried out for specimens with IHC scores of 2+ or 3+ with Ventana Benchmark ULTRA, using DNA cocktail probes [*HER2* and *CEP17* (centromeric probe 17)] according to the manufacturer's protocol. For each specimen, the numbers of *HER2* signals (silver ISH, black) and *CEP17* signals (red ISH, red) were counted for 20 nuclei, and the *HER2/CEP17* ratio was calculated by dividing the total number of *HER2* signals by the total number of *CEP17* signals. Negativity for *HER2* gene amplification was defined as an *HER2/CEP17* ratio of less than 1.8, whereas positivity was defined as an *HER2/CEP17* ratio of more than 2.2. If the *HER2/CEP17* ratio was in the equivocal range (1.8–2.2), the number of *HER2* and *CEP17* signals was counted for 20 additional nuclei, and the *HER2/CEP17* ratio was calculated from the results of 40 nuclei. Eventually, amplification of *HER2* was defined as an *HER2/CEP17* ratio of 2.0 or more, based on a partially modified version of the *HER2* scoring system for breast cancer (15).

Definition of positivity

For EGFR, an IHC score of 3+ was defined as positive, and IHC scores of 0, 1+, and 2+ were defined as negative. For HER2, an IHC score of 3+ or an IHC score of 2+ with a dual-ISH *HER2/CEP17* ratio of 2.0 or more was defined as positive, and IHC scores of 0 and 1+ or a score of IHC 2+ with a dual-ISH *HER2/CEP17* ratio of less than 2.0 were defined as negative (14).

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Reverse-transcription PCR

Representative hematoxylin and eosin–stained slides of FFPE specimens were reviewed by a pathologist to estimate tumor load per sample. Slide sections 10 μm in thickness were then stained with nuclear fast red (Sigma-Aldrich) for manual microdissection. Tumor tissue was selected at a magnification of 5 to 10 times and dissected from the slide using a scalpel, as described previously (16).

RNA isolation from tumor tissue and the cDNA preparation steps were conducted as described previously (17), with a slight modification in the extraction step, using RNeasy Mini Elute spin-columns (Qiagen).

Gene expression levels of *EGFR* and *HER2* were determined by means of TaqMan real-time PCR (Life Technologies) as described previously (17). β-Actin was used as an endogenous reference gene. The detection of amplified cDNA results in a cycle threshold (C_t) value, which is inversely proportional to the amount of cDNA. Gene expression values (relative mRNA levels) are expressed as ratios (differences between the C_t values) between the gene of interest (*EGFR* or *HER2*) and a reference gene (β-actin). This reference gene provides a baseline measurement for the amount of RNA isolated from a specimen.

Statistical analysis

Survival curves were estimated using the Kaplan–Meier product-limit method, and the statistical significance of differences between survival curves was assessed using the log-rank test. Univariate and multivariate survival analyses were conducted using a Cox proportional hazards model. Categorical data analysis was conducted using the χ^2 test. Either the Wilcoxon test or the Kruskal–Wallis test was used to assess correlations between groups. Results were considered statistically significant at $P < 0.05$. All statistical analyses were carried out with the SAS software package version 9.1 and JMP software version 8.01 (SAS Institute Inc.).

We estimated what minimum difference in survival would be required with EGFR- or HER2-positive cancers to show a survival difference as compared with EGFR- or HER2-negative cancers, respectively. We assumed that patients with EGFR- or HER2-positive tumors would have poorer outcomes. Given a positivity rate of 10%, 15%, or 20%, demonstration of a statistically significant difference in survival between patients with positive tumors and those with negative tumors would require HRs of at least 1.624, 1.520, and 1.465, respectively, assuming a 2-sided $\alpha = 0.05$ and a power = 80% in a proportional hazards model.

Results

Patients and sample collection

When the biomarker population of this study was compared with the total population of ACTS-GC as previously reported (3), there was no significant difference between these groups (Table 1). The IHC results were obtained for both EGFR and HER2 expression in all 829 specimens as follows: EGFR grade 0, 204 (24.6%); EGFR grade 1+, 372 (44.9%); EGFR grade 2+, 178 (21.5%); EGFR grade 3+, 75 (9.0%); HER2 grade 0, 443 (53.4%); HER2 grade 1+, 210 (25.3%); HER2 grade 2+, 101 (12.2%); and HER2 grade 3+, 75 (9.0%). Representative examples of immunostaining for EGFR and HER2 are shown in Supplementary Fig. S1 and S2.

Dual-ISH analyses were conducted on 176 specimens with a HER2 IHC score of 2+ or 3+. The IHC score and dual-ISH status for HER2 were as follows: IHC 2+/dual-ISH negative, 63 (7.6%); IHC 2+/dual-ISH positive, 38 (4.6%); IHC 3+/dual-ISH negative, 2 (0.2%); and IHC 3+/dual-ISH positive, 72 (8.7%). Dual-ISH could not be determined in one specimen, but this was classified as HER2-positive because the IHC score was 3+. IHC 3+ scores were generally consistent with dual-ISH positive status (72/74 cases; 97.3%), whereas IHC 2+ scores were not (38/101 cases; 37.6%).

We also measured the relative gene-expression levels of *EGFR* and *HER2* by reverse-transcription PCR (RT-PCR) analysis in tumor tissue dissected from FFPE specimens. The IHC scores for EGFR and HER2 significantly correlated with their gene-expression levels ($P < 0.001$, Kruskal–Wallis test; Supplementary Fig. S3).

Eventually, we classified 75 cases (9.0%) as positive for EGFR and 113 (13.6%) as positive for HER2. The groups were well balanced with respect to EGFR and HER2 status and other factors (Table 1). Both EGFR and HER2 positivities were more common among differentiated type than undifferentiated type tumors (EGFR, 58.7%, $P < 0.001$; HER2, 75.2%, $P < 0.001$ [χ^2-test]). HER2 positivity was associated with male gender ($P < 0.001$), older age ($P = 0.0052$), and lower tumor stage ($P < 0.001$), whereas EGFR positivity was not (Supplementary Table S1). Eighteen cases (2.2%) were positive for both EGFR and HER2, 57 (6.9%) were positive for EGFR alone, and 95 (11.5%) were positive for HER2 alone.

Effects of EGFR and HER2 expressions on survival

Five-year OS and relapse-free survival (RFS) were 73.6% [95% confidence interval (CI) = 69.3%–77.9%] and 66.7% (95% CI = 62.1%–71.3%), respectively, in the S-1 group, compared with 61.9% (95% CI = 57.1%–66.7%) and 53.7% (95% CI = 48.8%–58.7%) in the surgery-only group, respectively. These figures were similar to the ACTS-GC 5-year follow-up data (4).

EGFR-positive status was significantly associated with worse outcomes in the study group as a whole (Table 2, Fig. 1A; Kaplan–Meier curves for the OS of patients according to the EGFR IHC score are shown in Supplementary Fig. S4). The results for 5-year RFS were similar to those for 5-year OS (Table 2). EGFR-positive status was also associated with worse outcomes in both the S-1 group and the surgery-only group (Table 2). Irrespective of EGFR status, the 5-year OS in the S-1 group was longer than that in the surgery-only group (Fig. 1B and C).

In contrast, there was no correlation between HER2 status and patient outcomes in the study group as a whole (Table 2, Fig. 2A). The 5-year RFS was similar to the 5-year OS (Table 2). HER2-positive status was not associated with outcomes in either the S-1 group or the surgery-only group

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Table 1. Characteristics of the patients

	Entire population of ACTS-GC			Biomarker study population of ACTS-GC		
	S-1 (n = 529)	Surgery only (n = 530)	P value[a]	S-1 (n = 415)	Surgery only (n = 414)	P value[a]
Sex, n (%)			0.98			0.90
Male	367 (69.4)	369 (69.6)		282 (68.0)	283 (68.4)	
Female	162 (30.6)	161 (30.4)		133 (32.0)	131 (31.6)	
Age, n (%)			0.86			0.72
<60	199 (37.6)	195 (36.8)		160 (38.6)	158 (38.2)	
60–69	193 (36.5)	215 (40.6)		149 (35.9)	161 (38.9)	
70–80	137 (25.9)	120 (22.6)		106 (25.5)	95 (22.9)	
Median, y	63	63		63	62	
Range, y	27–80	33–80		27–80	33–80	
Tumor stage, n (%)			0.81			0.93
T1	1 (0.2)	0 (0)		1 (0)	0 (0)	
T2	289 (54.6)	286 (54.0)		222 (53.5)	223 (53.9)	
T3	225 (42.5)	232 (43.8)		180 (43.4)	182 (44.0)	
T4	14 (2.6)	12 (2.3)		12 (2.9)	9 (2.2)	
Nodal stage, n (%)[b]			0.72			0.52
N0	51 (9.6)	64 (12.1)		40 (9.6)	52 (12.6)	
N1	296 (56.0)	281 (53.0)		233 (56.1)	222 (53.6)	
N2	182 (34.4)	185 (34.9)		142 (34.2)	140 (33.8)	
N3	0 (0)	0 (0)		0 (0)	0 (0)	
Lymph-node metastases, n (%)		0.37			0.18	
0	51 (9.6)	64 (12.1)		40 (9.6)	52 (12.6)	
1–6	331 (62.6)	325 (61.3)		254 (61.2)	254 (61.4)	
7–15	117 (22.1)	113 (21.3)		97 (23.4)	85 (20.5)	
≥16	30 (5.7)	28 (5.3)		24 (5.8)	23 (5.6)	
Cancer stage, n (%)[c]			0.78			0.48
II	236 (44.6)	238 (44.9)		183 (44.1)	189 (45.7)	
IIIA	202 (38.2)	207 (39.1)		159 (38.3)	162 (39.1)	
IIIB	90 (17.0)	85 (16.0)		73 (17.6)	63 (15.2)	
IV	1 (0.2)	0 (0)		0 (0)	0 (0)	
Histologic type, n (%)[d]			0.73			0.91
Differentiated	214 (41.6)	209 (40.3)		166 (40.0)	166 (40.1)	
Undifferentiated	301 (58.4)	307 (59.7)		249 (60.0)	245 (59.2)	
EGFR status, n (%)			—			0.54
Negative	—	—		380 (91.6)	374 (90.3)	
Positive	—	—		35 (8.4)	40 (9.7)	
HER2 status, n (%)			—			0.77
Negative	—	—		357 (86.0)	359 (86.7)	
Positive	—	—		58 (14.0)	55 (13.3)	

NOTE: Characteristics of the patients in entire population of ACTS-GC was referred by ref. 3.

[a]P values for sex, EGFR status, and HER2 status were calculated with the use of the χ^2 test. P values for age, tumor stage, nodal stage, number of lymph-node metastases, cancer stage (Japanese classification), and histologic type were calculated with the use of the Wilcoxon test.

[b]Nodal stages according to the Japanese classification were defined as follows: N0, no evidence of lymph node metastasis; N1, metastasis to group 1 lymph nodes; N2, metastasis to group 2 lymph nodes; N3, metastasis to group 3 lymph nodes. Groups 1, 2, and 3 are regional lymph node classifications defined according to the location of the primary tumor and are based on the results of studies of lymphatic flow at various tumor sites and the observed survival associated with metastasis at each nodal station (i.e., position in relation to primary node).

[c]Cancer stages according to the Japanese classification were defined as follows: stage IA, T1N0; stage IB, T1N1 or T2N0; stage II, T1N2, T2N1, or T3N0; stage IIIA, T2N2, T3N1, or T4N0; stage IIIB, T3N2 or T4N1; stage IV, T4N2, any T stage with N3, or distant metastasis.

[d]In entire population of ACTS-GC, histologic type was classified among eligible patients (n = 1,034). In the surgery-only group of biomarker study population, cancers could not be classified as differentiated or undifferentiated in 3 patients.

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Table 2. Univariate analysis of OS and RFS according to the status of EGFR and HER2

				OS			RFS		
Marker	Group	Status	Number of patients	5-year survival (%)	HR (95% CI)	*P* value (log-rank)	5-year survival (%)	HR (95% CI)	*P* value (log-rank)
EGFR									
	All	Negative	754	69.0	1		61.3	1	
		Positive	75	55.4	1.642 (1.139–2.366)	0.007	49.9	1.451 (1.030–2.045)	0.033
	S-1	Negative	380	74.9	1		68.2	1	
		Positive	35	60.0	1.787 (1.018–3.134)	0.043	51.4	1.773 (1.066–2.950)	0.027
	Surgery only	Negative	374	63.1	1		54.3	1	
		Positive	40	51.2	1.514 (0.936–2.449)	0.091	48.7	1.219 (0.767–1.939)	0.402
HER2									
	All	Negative	716	68.3	1		60.0	1	
		Positive	113	64.5	1.155 (0.822–1.624)	0.406	62.3	0.991 (0.716–1.371)	0.955
	S-1	Negative	357	74.2	1		66.5	1	
		Positive	58	69.9	1.170 (0.697–1.965)	0.552	68.2	1.000 (0.609–1.643)	1.000
	Surgery only	Negative	359	62.4	1		53.5	1	
		Positive	55	58.8	1.167 (0.742–1.833)	0.504	56.0	0.997 (0.649–1.530)	0.988

(Table 2). Similarly, there was no correlation between the 75 patients with IHC 3+ and patient outcomes (5-year OS in the IHC 3+ and in the IHC 0/1+/2+ were respectively 64.7% and 68.1%, HR = 1.178, 95% CI = 0.807–1.720, log-rank $P = 0.396$; and 5-year RFS in the IHC 3+ and in the IHC 0/1+/2+ were respectively 62.2% and 60.1%, HR = 0.942, 95% CI = 0.625–1.418, log-rank $P = 0.773$). Irrespective of HER2 status, the 5-year OS in the S-1 group was longer than that in the surgery-only group (Fig. 2B and C).

Multivariate analysis in overall study population

The prognostic relevance of EGFR and HER2 was assessed using a multivariate proportional hazards model adjusted for the following established clinical prognostic factors: treatment arm, gender, age, cancer stage (Japanese classification of gastric carcinoma, 2nd English edition; ref. 18), and histologic type (Table 3). Although treatment arm and cancer stage were the strongest prognostic factors, EGFR status was also an independent prognostic factor.

Subgroup analysis

The OS in the study group as a whole was analyzed according to gender, age, cancer stage, histologic type, and EGFR/HER2 status; no interaction was found between S-1 treatment and any of these factors (Fig. 3). Kaplan–Meier estimates of OS plotted according to EGFR (Fig. 1B and C) and HER2 status (Fig. 2B and C) revealed that S-1 treatment improved survival irrespective of EGFR or HER2 status.

Discussion

The present study retrospectively evaluated the influence of EGFR and HER2 expression on the outcomes of patients enrolled in the ACTS-GC. EGFR positivity was found to be associated with worse outcomes, in agreement with earlier findings (5–7, 9). Although most previous studies defined EGFR positivity as an IHC score of 2+ and 3+, no consensus definition has been reached. To the best of our knowledge, this is the first study to show that EGFR IHC 3+ status correlates significantly with poor outcome in patients with gastric carcinoma.

Kim and colleagues reported a similar distribution of EGFR protein-expression IHC scores to those of the present study in 511 specimens of gastric carcinoma tissue (7). They also reported that 13 (61.9%) of 21 cases with IHC scores of 3+ showed *EGFR* gene amplification or high polysomy on FISH, whereas this was observed in only 14 (11.8%) of 119 cases with scores of 2+. Our present study confirmed that the EGFR IHC scores significantly correlated with *EGFR* gene-expression levels. Moreover, the median *EGFR* gene expression for cases with IHC scores of 3+ was higher than that for cases with scores of 0, 1+, and 2+ (Supplementary Fig. S3A), suggesting that a score of 3+ could be a new criterion for defining EGFR positivity in gastric cancer. This was strongly linked to EGFR overexpression and poor outcomes for patients with gastric carcinoma in this study (Supplementary Fig. S4).

Multivariate analysis revealed that an IHC score of EGFR 3+ was an independent predictor of unfavorable outcomes. As well as being a prognostic marker, EGFR positivity might be a predictor of response to EGFR-targeted therapy in gastric cancer. A phase II study showed a significant association between increased *EGFR* gene copy number (≥4.0) and OS in a subset of patients with gastric and esophagogastric junction cancer who received cetuximab combined with oxaliplatin/leucovorin/5-fluorouracil (19, 20). In addition, among 58 patients with metastatic colorectal carcinoma (mCRC) who received panitumumab in a previous study, 6 of 20 patients with an *EGFR* gene copy number more than 2.47 had an objective response, whereas no tumor response was observed in patients with copy numbers below this ($P = 0.0009$; ref. 21). Similarly, an increased *EGFR* copy number was significantly associated with response to cetuximab therapy in patients with mCRC (22), although the relationship between EGFR

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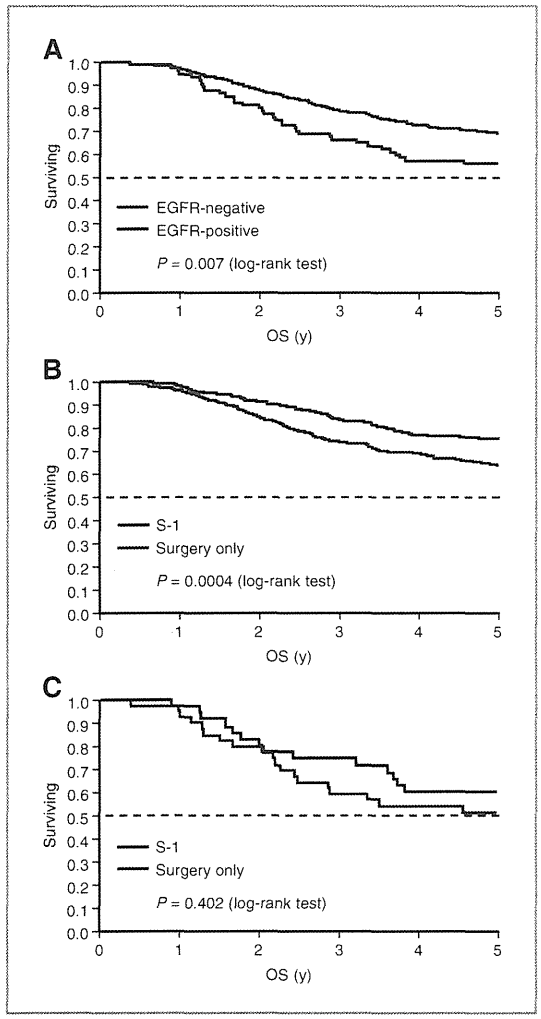

Figure 1. Kaplan–Meier curves for OS according to EGFR status. For EGFR, an IHC score of 3+ was defined as positive, and IHC scores of 0, 1+, and 2+ were defined as negative. A, OS for all patients ($n = 829$): EGFR-negative ($n = 754$) versus EGFR-positive ($n = 75$). B, OS for patients with EGFR-negative tumors: S-1 group ($n = 380$) versus surgery-only group ($n = 374$). C, OS for patients with EGFR-positive tumors: S-1 group ($n = 35$) versus surgery-only group ($n = 40$).

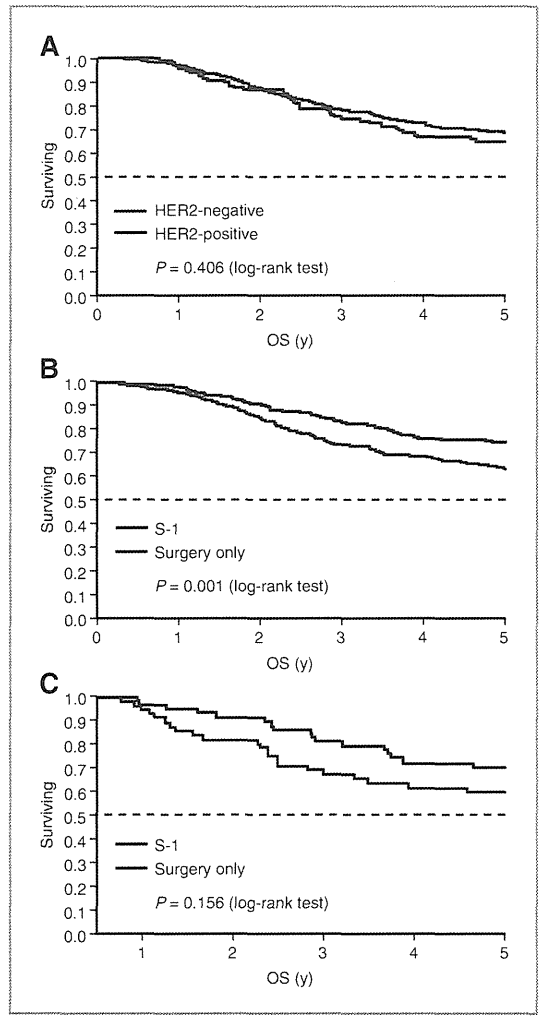

Figure 2. Kaplan–Meier curves for OS according to HER2 status. A, OS for all patients ($n = 829$): HER2-negative ($n = 716$) versus HER2-positive ($n = 113$). B, OS for patients with HER2-negative tumors: S-1 group ($n = 357$) versus surgery-only group ($n = 359$). C, OS for patients with HER2-positive tumors: S-1 group ($n = 58$) versus surgery-only group ($n = 55$).

overexpression on IHC and the response to cetuximab remains controversial (23).

Although *KRAS* mutation status is used as a negative predictive marker for EGFR-targeted agents in colorectal cancer, the frequency of *KRAS* mutations in gastric cancer seems to be relatively low (3%–21%; ref. 24). Several phase III trials of combined chemotherapy with EGFR-targeted agents, such as cetuximab, panitumumab, and lapatinib are ongoing in patients with unresectable advanced gastric cancer (10); detailed information on alterations of the EGFR protein or gene in these trials is needed to predict the response to anti-EGFR therapy in gastric cancer more accurately (19).

The frequency of EGFR overexpression on IHC in gastric carcinoma ranges from 2% to 30% (7, 8, 25). Possible reasons for this wide variation include differences in fixation techniques, antibodies, scoring systems, subjectivity of pathologist interpretation, and intratumoral staining heterogeneity. To improve the accuracy of assessing EGFR positivity, additional gene-amplification analysis might be useful, as conducted for HER2, and standardized EGFR testing procedures should be established.

The prevalence of HER2 overexpression on IHC in the present study fell within the previously reported range (median positive rate = 18%; range = 4%–53%; ref. 12).

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Table 3. Cox regression multivariate analysis of prognostic factors for OS in all patients

Factor	Group	Number of patients	5-year survival (%)	HR (95% CI)	*P* value
Arm	Surgery only	414	61.9	1	
	S-1	415	73.6	0.617 (0.481–0.790)	<0.001
Sex	Male	565	67.2	1	
	Female	264	69.0	0.988 (0.757–1.301)	0.932
Age, y	<60	318	69.5	1	
	60–69	310	72.2	1.242 (1.057–1.460)	
	70–80	201	58.4	1.544 (1.118–2.132)	0.009
Cancer stage (Japanese classification)	II	372	77.0	1	
	IIIa	321	63.7	1.683 (1.431–1.979)	
	IIIb	136	52.2	2.833 (2.048–3.918)	<0.001
Histologic type	Differentiated	332	65.1	1	
	Undifferentiated[a]	497	69.6	0.894 (0.684–1.171)	0.412
EGFR status	Negative	754	69.0	1	
	Positive	75	55.4	1.504 (1.020–2.149)	0.040
HER2 status	Negative	716	68.3	1	
	Positive	113	64.5	1.068 (0.736–1.514)	0.722

[a]Including 3 patients with gastric cancer categorized into neither differentiated nor undifferentiated type.

Consistent with the results of Begnami and colleagues (8), the concordance between IHC (scores 2+ and 3+) and dual-ISH (positive) was 62.9% in the present study; most IHC 3+ results corresponded with dual-ISH positive status (98.6%), whereas IHC 2+ tumors showed relatively low concordance between IHC score and dual-ISH status (37.6%). The present results are also in agreement with the finding that HER2 positivity is more prevalent among differentiated-type tumors than undifferentiated-type tumors (6, 8, 11). Consequently, we consider our present evaluation of HER2 status to be realistic.

The role of HER2 as a prognostic factor in gastric cancer remains controversial. A recent systematic review assessing the impact of HER2 overexpression on survival found that 20 studies (57%) reported no difference in OS, 2 (6%) showed significantly longer OS in patients with HER2 overexpression, and 13 (37%) found significantly worse OS in patients with HER2 overexpression (12). To the best of our knowledge, the present investigation is the first large biomarker study to evaluate the influence of HER2 positivity on the postoperative outcomes of patients with gastric cancer enrolled in a randomized phase III trial. Trastuzumab was not administered to these patients until the completion of the 5-year follow-up, because it had not been approved at that time. The present results therefore provide strong evidence that HER2 status does not influence outcomes after D2 dissection for locally advanced gastric cancer in East Asian patients, in contrast to breast cancer.

Although it is unclear why EGFR overexpression was a prognostic marker in this study and HER2 overexpression was not, it might be partially explained by the fact that gastric cancer is a heterogeneous disease. A recent study reported that patients with HER2-positive gastric tumors have longer OS than those with HER2-negative tumors. This finding was based on an analysis of 381 patients with metastatic gastric/gastroesophageal junction cancer. On subgroup analysis, similar differences in OS according to HER2 status were seen in the subgroup of patients with intestinal-type cancer but not in those with diffuse-type cancer (26). Because the subgroup of patients with intestinal-type cancer includes a higher proportion of HER2-positive cases than EGFR-positive cases, as shown in Table 2, the association between intestinal-type and good outcomes may mask potential prognostic effects of HER2 positivity. Further understanding of the molecular biologic and pathologic characteristics of gastric cancer is considered necessary to improve EGFR and HER2 targeting in this disease.

Neither EGFR nor HER2 was associated with the efficacy of S-1; this was not surprising because neither one is thought to have an appreciable impact on the metabolism or mechanism of action of S-1. In several preclinical studies on mice, the antitumor activity of S-1 combined with trastuzumab, lapatinib, or cetuximab was greater than that of either drug alone on xenografts of gastric cancer cells overexpressing HER2 or EGFR. This enhancement of activity was considered to be mediated by thymidylate synthase (27, 28). These experimental results suggest that S-1 combined with an EGFR- or HER2-targeted agent (or both) is a promising regimen for patients with EGFR/HER2-positive gastric cancer.

In conclusion, the current study provides compelling evidence that EGFR 3+ status, but not HER2 status, on IHC is significantly associated with worse patient outcomes after curative resection of stage II/III gastric cancer. Furthermore, there is no apparent interaction between S-1 and EGFR or HER2 status with respect to survival. We therefore propose