distress is composed of the following symptom items: sense of food sticking, postprandial fullness and early satiation. As a result of the Kocher maneuver, the duodenum is moved to the left of its normal position, is elongated [13] and lies on the abdominal side when the patient is in a supine position. In other words, the duodenum locates higher than the gastric remnant. Such a morphological physical change may affect gastric emptying and other functions of the gastric remnant and increase meal-related distress. However, the specific mechanisms are currently unknown. In the future, elucidation of this mechanism through the gastrointestinal function tests such as a gastric emptying study would be informative. On the other hand, it is currently difficult to provide a proper explanation for why the Kocher maneuver leads to poor scores on the quality of ingestion SS that is composed of appetite, hunger, and satiety, as well as on PCS and MCS. It is possible that factors other than the explanatory variables assessed in this study may be involved. This issue will require further investigation. The Kocher maneuver is a useful technique when there is tension on the sutures of the anastomotic site of a B–I reconstruction; however, PGS could be worsened by employing this technique, therefore it should be applied minimally when it is required.

Regarding the extent of resection of the gastric wall, the Japanese gastric cancer treatment guidelines [15] stipulate "Standard gastrectomy involves resection of at least two-thirds of the stomach with D2 lymph node dissection". The guidelines also mention non-standard gastrectomy in which the extent of gastric resection and/or lymphadenectomy is altered according to the tumor characteristics. In cases of early-stage gastric cancer modified surgery is performed, in which the extent of gastric resection and/or lymph node dissection is reduced. However, few studies have investigated the relationship between the size of the gastric remnant and PGS. Nomura et al. reported that in cases of early-stage gastric cancer, compared with the 1/2 and 1/3 gastric remnant DGBI patient group, patients in the 1/2 gastric remnant group showed improved food intake, little postoperative weight loss and few abdominal symptoms such as diarrhea and abdominal pain [16]. It is believed that in cases of B–I reconstruction if the gastric remnant is large the gastric reservoir capacity is preserved, which helps to maintain a nutritional status and body composition. In the present study the larger the gastric remnant the lower was the percentage of weight loss. However, this result was not statistically significant. In the study by Nomura et al. weight measurements were taken <1 year after surgery; however, in the present study, weight measurements were taken on average >3 years after surgery. The present study includes data showing improvement in postoperative weight loss for a long postoperative period; therefore, we believe that there was no significant difference in the percentage of weight loss because of the size of the gastric remnant.

On the other hand, patients with larger gastric remnants showed improved scores on the necessity for additional meals and the meal dissatisfaction SS. Scores on the diarrhea SS were also significantly better. Our comparison of the $\geq 1/2$ gastric remnant group and the $\leq 1/4$ gastric remnant group showed a moderate effect on the diarrhea SS scores. There are multiple explanations regarding the causes of postgastrectomy diarrhea [17]. One of these is rapid gastric emptying. Because foods with high osmolality immediately flow directly into the small intestine after ingestion in postgastrectomy patients it is believed that intestinal peristalsis is accelerated and intestinal contents increase, resulting in diarrhea [18]. It is therefore highly possible that smaller gastric remnants and a reduced gastric reservoir capacity result in increased diarrhea. The influence of these factors is considered to result in persistent diarrhea for a relatively long postoperative period. The results of this study thus show that it is possible for patients with larger gastric remnants to have satisfactory PGS scores over a long term when DGBI is performed as long as there are no oncological problems such as early-stage gastric cancer.

Advances in automatic suturing devices and the widespread use of laparoscopic surgery have led to development and use of a variety of anastomosis methods even when B–I reconstruction is performed after distal gastrectomy. However, there is no clear evidence indicating whether hand sewing or machine sewing is preferable or end-to-end or side-to-end anastomosis is preferable and debate over these issues continues. Few studies have investigated the influence of the method or type of anastomosis (side-to-side vs. end-to-end) on PGS in cases of B–I reconstruction after distal gastrectomy. Kaiho et al. [19] compared side-to-end anastomosis with end-to-end anastomosis and reported that although side-to-end anastomosis showed satisfactory motility in the gastric remnant, there was no difference in gastric emptying. Takahashi et al. [20] compared mechanically stapled anastomosis (side-to-end) with hand-sewn anastomosis (end-to-end) and found that, although there was more residual stomach content in cases of mechanical stapled anastomosis when investigated using the Residue, Gastritis, Bile (RGB) classification via endoscopy 1 year after surgery, there was no significant difference in the frequency of gastritis and bile reflux. Endoscopic findings were not investigated in this study and the time from surgery until completion of the questionnaire was long, with an average of >3 years after surgery. A long time after surgery, the effect of the anastomotic technique on PGS, living status and QOL is either non-existent or minimal.

This study has some limitations. Since the nature of this study was a retrospective explanatory study rather than a randomized controlled trial there was a certain imbalance in the number of patients and also a possible difference in patient characteristics among groups. Another limitation is that it was difficult to provide rational explanations for all results. PGS varies widely among individuals and is influenced by a variety of physical and functional factors. However, there have been no multi-institutional collaborative studies that have attempted to examine as many symptoms as this study for a single surgical procedure. Because the results of this study are generally consistent with our clinical experience we believe the results are reliable to a certain extent. In addition, the data from this study are the results of assessments of symptoms $\geq$1 year after surgery and indicate PGS observed over a relatively long period of $\geq$3 years after surgery. We believe that these data are extremely valuable in the quest to understand the long-term postoperative effects of techniques using DGBI. To determine the most appropriate gastrectomy technique from a PGS perspective investigation of the underlying pathophysiology via gastrointestinal function tests, such as a gastric emptying study, and further prospective studies, such as a randomized controlled trial, focusing on these issues may be required.

In conclusion, we performed an assessment of post-DGBI long-term symptoms using the PGSAS-45. The results of this study indicate that in cases of DGBI the performance of the Kocher maneuver increases meal-related distress, reduces the quality of ingestion and imparts a negative effect on QOL. In addition, the data suggest that larger gastric remnants may reduce diarrhea and improve meal-related scores. On the other hand, we found no evidence that differences in the method or type of anastomosis had an effect on postgastrectomy disorders.

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ORIGINAL ARTICLE

Long-term outcomes and prognostic factors of patients with advanced gastric cancer treated with S-1 plus cisplatin combination chemotherapy as a first-line treatment

Shigenori Kadowaki · Azusa Komori · Yukiya Narita · Sohei Nitta · Kazuhisa Yamaguchi · Chihiro Kondo · Hiroya Taniguchi · Daisuke Takahari · Takashi Ura · Masashi Ando · Kei Muro

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Abstract
Background The long-term outcomes of advanced gastric cancer (AGC) patients treated with S-1 plus cisplatin (SP) combination chemotherapy remain unclear. Therefore, we sought to evaluate these outcomes to identify the prognostic factors affecting patient survival.
Methods We retrospectively analyzed 153 AGC patients treated with SP at a single institution between January 2005 and July 2011.
Results Median overall survival (OS) was 15.0 months [95 % confidence interval (CI), 12.5–17.9 months]. Three independent prognostic factors affecting poor survival were identified: performance status (PS) $\geq$ 1 [hazard ratio (HR) = 2.39, 95 % CI, 1.58–3.62); >1 metastatic site (HR = 1.57, 95 % CI, 1.10–2.26], and elevated alkaline phosphatase levels (HR = 1.70, 95 % CI, 1.16–2.49). A simple prognostic index was generated using three risk groups: good (no risk factor), moderate (one or two risk factors), and poor (three risk factors). The median OS for good-, moderate-, and poor-risk groups was 28.6, 14.8, and 7.3 months, respectively (log-rank test; $P < 0.0001$). Among the twelve 3-year survivors, 9 (75 %) had a PS of 0 and 8 (67 %) had only one metastatic site.
Conclusions Three prognostic factors were identified in AGC patients treated with SP. Using a simple prognostic index, the patients were divided into three risk groups, in which the survival differences were markedly significant, suggesting that patients with good PS and only one metastatic site may have a higher chance of long-term survival than those with poor PS and multiple metastatic sites.

Keywords Gastric cancer · S1 plus cisplatin chemotherapy · Prognostic factors · Long-term survival

Introduction

Among malignant tumors, gastric cancer is the second leading cause of death worldwide [1]. Although the mortality rate associated with gastric cancer is decreasing, this disease claims approximately 50,000 lives in Japan every year. Several randomized trials have revealed that overall survival (OS) in advanced gastric cancer (AGC) is improved with systemic chemotherapy compared with the best supportive care [2–4]; however, several recent phase III trials reported that the prognosis for AGC remained poor, with a median survival time of 9.2–13.0 months [5–7]. In AGC, the outcome of systemic chemotherapy depends on patient and tumor characteristics. Recently studies have shown that poor performance status (PS) [8–13], multiple metastatic sites [11–13], peritoneal metastasis [9, 13], bone metastasis [8, 13], liver metastasis [9], no prior gastrectomy [8, 10], and elevated alkaline phosphatase (ALP) levels [9] were independent prognostic factors of poor OS.

A randomized phase III trial conducted by the Japan Clinical Oncology Study Group (JCOG) demonstrated that OS after S-1 (an oral fluoropyrimidine) monotherapy was not inferior to that after infused 5-fluorouracil (5-FU) [14]. Furthermore, a phase III trial of S-1 alone versus S-1 plus cisplatin in a randomized controlled trial in the treatment of

S. Kadowaki (✉) · A. Komori · Y. Narita · S. Nitta · K. Yamaguchi · C. Kondo · H. Taniguchi · D. Takahari · T. Ura · M. Ando · K. Muro
Department of Clinical Oncology, Aichi Cancer Center Hospital, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya, Aichi 464-8681, Japan
e-mail: skadowaki@aichi-cc.jp

stomach cancer (SPIRITS) demonstrated that S-1 plus cisplatin (SP) was superior to S-1 [7]. A phase III First-Line Advanced Gastric Cancer Study conducted mainly among Western countries demonstrated that SP was not inferior to 5-FU plus cisplatin, with SP having lower toxicities [15]. On the basis of these results, SP combination chemotherapy was established as a standard first-line treatment for AGC in Japan. In clinical settings, however, little is known regarding the prognostic factors and long-term outcomes of AGC patients treated with SP. Hence, the aim of the present retrospective study was to analyze the prognostic factors affecting OS and to evaluate the characteristics of long-term survival in AGC patients treated with SP as a first-line treatment in a clinical setting.

Materials and methods

Patients and treatment schedule

The present retrospective study analyzed the outcomes of 153 AGC patients treated with SP as first-line chemotherapy between January 2005 and July 2011 at the Aichi Cancer Center Hospital (Aichi, Japan). All patients were diagnosed with histologically confirmed inoperable adenocarcinoma. Most patients received SP according to a standard schedule (S-1 at 80 mg/m^2 per day for 3 weeks followed by a 2-week rest; cisplatin at 60 mg/m^2 intravenous infusion on day 8; 5-week cycles). Only 7 patients received SP in a thrice-weekly schedule (S-1 at 80 mg/m^2 per day for 2 weeks followed by a 1-week rest; cisplatin at 60 mg/m^2 intravenous infusion on day 1; 3-week cycles). Treatment was continued until disease progression, intolerable toxicity, or patient refusal. Tumor response was assessed on the basis of the Response Evaluation Criteria in Solid Tumors version 1.0 [16]. Confirmation of treatment response was not required for this study. Written informed consent was obtained from all patients before administration of the first-line treatment.

Statistical analysis

Progression-free survival (PFS) was calculated from the date of SP initiation to the date of disease progression or death from any cause, whichever occurred first. OS was calculated from the date of SP initiation to the date of death from any cause. PFS and OS were estimated using the Kaplan–Meier method. Of the 153 patients, 6 (4 %) were lost to follow-up. Survival status was updated in April 2013. Univariate analyses were performed using the log-rank test with the following variables: (1) age (<65 vs. ≥65 years); (2) gender (male vs. female); (3) Eastern Cooperative Oncology Group performance status (ECOG PS) (0 vs. 1–2); (4) histology (diffuse vs. intestinal type); (5) peritoneal metastasis (no vs. yes); (6) liver metastasis (no vs. yes); (7) lymph node metastasis (no vs. yes); (8) number of metastatic sites (1 vs. ≥2); (9) history of gastrectomy (no vs. yes); and (10) ALP levels (normal vs. high). A multivariate analysis of prognostic factors using the stepwise Cox proportional hazard model was performed with these categorical variables to calculate the hazard ratio (HR) and associated 95 % confidence intervals (CIs). A two-sided P value less than 0.05 was considered statistically significant. All statistical calculations were performed using Dr. SPSS II software (SPSS Japan, Tokyo, Japan).

Results

Patient characteristics

Pretreatment patient characteristics are summarized in Table 1. As shown, the median patient age was 62.0 years (range 28–83 years), and 91 (60 %) were men. Among the 153 patients, 102 did not undergo gastrectomy before initial chemotherapy because of unresectable disease, 20 underwent resection of the primary site but had residual disease, and 31 had recurrent disease after previous curative gastrectomy. Of the 102 patients who had initially unresectable disease, 2 underwent curative surgery after achieving a good response with the first-line treatment and 1 underwent palliative gastrectomy for pyloric stenosis. A total of 120 (78 %) patients received second-line chemotherapy: 75 (63 %), 32 (27 %), and 13 (11 %) received taxane-based regimens, irinotecan-based regimens, and other, respectively. Only 2 (1 %) patients were actively receiving SP as the first-line treatment at the time of this analysis.

Tumor response and survival

The overall response rates are summarized in Table 2. Of the 94 patients with measurable disease, 5 (5 %) were not evaluated for unacceptable toxicities or clinical disease progression. The overall response rate was 41 % (95 % CI, 31–52 %); 2 (2 %) patients achieved complete response and 37 (39 %) achieved partial response. The median follow-up period was 26.8 months (range 4.9–69.5 months), the median PFS was 5.9 months (95 % CI, 5.0–6.8 months), and the median OS was 15.0 months (95 % CI, 12.5–17.9 months) (Fig. 1a, b). The 1-, 2-, and 3-year survival rates were 61, 29, and 12 %, respectively.

Table 1 Patient characteristics

Characteristic	$n = 153$	Percent (%)
Age, years		
Median (range)	62.0 (28–83)	
Gender		
Male	91	59
Female	62	41
ECOG PS		
0	48	31
1	98	64
2	7	5
Histological type		
Diffuse type	113	74
Intestinal type	40	26
Disease status		
Unresectable	122	80
Recurrent	31	20
Prior gastrectomy		
Yes	51	33
No	102	67
Metastatic sites		
Peritoneum	92	60
Lymph node	84	55
Liver	33	22
Bone	14	9
Lung	12	8
Number of metastatic sites		
1	76	50
2	55	36
≥3	22	14

ECOG Eastern Cooperative Oncology Group, *PS* performance status

Table 2 Tumor response in patients with measurable lesions

Response	$n = 94$	%
CR	2	2
PR	37	39
SD	30	32
PD	20	21
NE	5	5

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable

Analysis of prognostic factors

The results of univariate survival analysis are listed in Table 3. PS, peritoneal metastasis, number of metastatic sites, prior gastrectomy, and elevated ALP levels significantly affected OS. Patients with ECOG PS grade 0 had better OS than those with ECOG PS grades 1 or 2 ($P < 0.0001$). Patients with only one metastatic site had

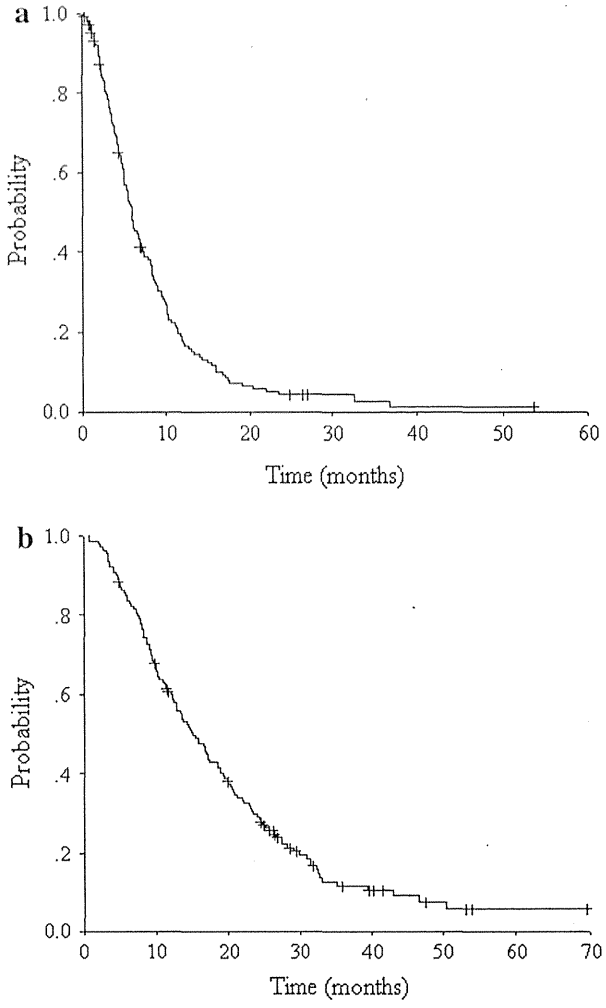

Fig. 1 **a**, **b** Kaplan–Meier survival curves of progression-free survival (PFS) and overall survival (OS) for all patients ($n = 153$). **a** Median PFS was 5.9 months (95 % CI, 5.0–6.8 months). **b** Median OS was 15.0 months (95 % CI, 12.5–17.9 months)

better OS compared with those with multiple metastatic sites ($P = 0.003$). Multivariate analyses showed that PS ≥ 1 (HR, 2.39; 95 % CI, 1.58–3.62; $P < 0.001$), multiple metastatic sites (HR, 1.57; 95 % CI, 1.10–2.26; $P = 0.014$), and elevated ALP levels (HR, 1.70; 95 % CI, 1.16–2.49; $P = 0.007$) were independent prognostic factors (Table 4) that were observed in almost all patients, although ALP data were unavailable in one patient. All three factors had similar orders of magnitude in all patients, and those with one ($n = 53$) or two ($n = 51$) prognostic factors had similar OS (log-rank test, $P = 0.46$; 15.9 vs. 12.8 months and 66 vs. 62 % for median survival time and 1-year survival rate, respectively). Therefore, we categorized patients into prognostic risk groups as follows: patients with no prognostic factor as the good-risk group ($n = 25$), those with one or two prognostic factors as the moderate-risk group ($n = 104$), and those with three

Table 3 Univariate prognostic factor analysis for overall survival

Variable	*n*	Median OS (months)	*P* value
Age (years)			
<65	92	13.7	0.15
≥65	61	18.5	
Gender			
Male	91	14.8	0.21
Female	62	15.8	
Performance status			
0	48	27.4	<0.0001
≥1	105	12.3	
Histology			
Diffuse	113	15.8	0.60
Intestinal	40	14.3	
Metastatic sites			
Peritoneum			
−	61	17.1	0.01
+	92	14.3	
Liver			
−	120	15.8	0.68
+	33	13.8	
Lymph node			
−	69	15.9	0.27
+	84	14.3	
Bone			
−	139	15.5	0.07
+	14	12.8	
Lung			
−	141	15.0	0.30
+	12	17.3	
Number of metastatic sites			
1	76	19.9	0.003
≥2	77	12.6	
Prior gastrectomy			
No	102	12.8	0.001
Yes	51	21.2	
ALP[a]			
Normal	109	17.3	0.0004
High	43	10.5	

OS overall survival, *ALP* alkaline phosphatase

[a] No measurement in one patient

prognostic factors as the poor-risk group ($n = 23$). Figure 2 shows the overall survival curves for these three risk groups. There were significant differences in OS among the three risk groups (log-rank test, $P < 0.0001$). The median survival time for the good-, moderate-, and poor-risk groups was 28.6, 14.8, and 7.3 months, respectively, and the 3-year survival rate was 38, 8, and 0 %, respectively. We found that the moderate-risk group had a 3.1-fold-increased risk of death (HR, 3.12; 95 % CI, 1.78–5.47; $P < 0.001$) and that the poor-risk group had a 7.9-fold-increased risk of death (HR, 7.93; 95 % CI, 3.99–15.76; $P < 0.001$) compared with the good-risk group. The median PFS for the good-, moderate-, and poor-risk groups was 9.7, 5.7, and 3.2 months, with significant differences (log-rank test, $P = 0.001$). Of patients who received taxanes and irinotecan as subsequent therapy, 19 (76 %) and 15 (60 %) were in the good-risk group, 71 (68 %) and 44 (42 %) in the moderate-risk group, and 13 (57 %) and 7 (30 %) in the poor-risk group, respectively.

Table 4 Multivariate analysis of overall survival using the stepwise Cox's model

	Hazard ratio	95 % CI	*P* value
Performance status	2.39	1.58–3.62	<0.001
Number of metastatic sites	1.57	1.10–2.26	0.014
ALP	1.70	1.16–2.49	0.007

CI confidence interval

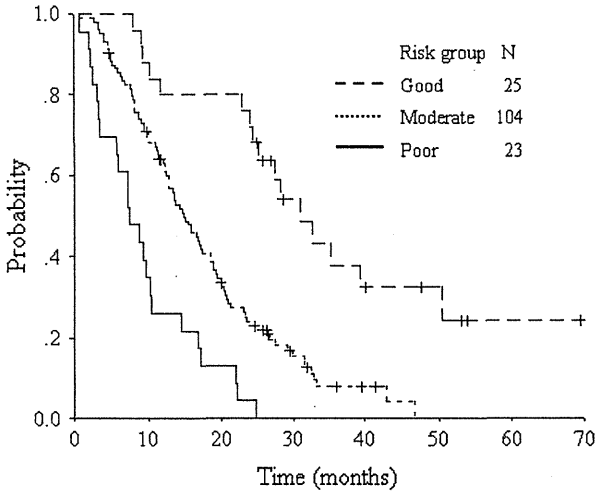

Fig. 2 Overall survival curves by prognostic index. Median survival time for the good-risk (*dashed line*), moderate-risk (*dotted line*), and poor-risk (*solid line*) groups were 28.6, 14.8, and 7.3 months, respectively, and the 3-year survival rate was 38, 8, and 0 %, respectively

Characteristics of long-term survivors

Characteristics of the 12 patients who survived more than 3 years are summarized in Table 5. Nine (75 %) of the 12 patients had a PS of 0, and 8 (67 %) had prior gastrectomy. Eight (67 %) patients had only one metastatic site and 3 (25 %) achieved a complete response (CR); 1 patient achieved CR following first-line chemotherapy and 2 achieved CR following second-line chemotherapy.

Table 5 Characteristics of 3-year survivors

Age (years)	G	PS	PGA	MS	Response		PFS	Risk group	Survival	Status
					1st	2nd				
41	F	1	No	P, LN	SD	NE	13.2	Moderate	36.1	A
57	F	0	Yes	P	IR/SD	NE	4.4	Good	39.4	D
66	F	1	No	LN	PR	PR	5.7	Moderate	39.5	A
73	F	0	Yes	Li	SD	PR	10.0	Good	40.6	D
72	M	0	No	LN, Lu	PR	PR	9.9	Moderate	41.4	A
71	F	1	Yes	P, LN	PR	SD	11.3	Moderate	42.9	D
68	M	0	No	LN, Li, Lu	PR	PR	19.0	Moderate	46.7	D
70	F	0	Yes	Lu	PR	CR	3.9	Good	47.6	A
70	M	0	Yes	LN	SD	SD	8.3	Good	50.4	D
65	M	0	Yes	LN	PR	SD	36.7	Good	53.0	A
61	F	0	Yes	Li	CR	–	53.8	Good	53.8	A, NED
55	F	0	Yes	P	IR/SD	CR	20.3	Good	69.5	A, NED

G gender, *M* male, *F* female, *PS* performance status, *PGA* prior gastrectomy, *MS* metastatic site, *P* peritoneum, *LN* lymph node, *Li* liver, *Lu* lung, *NE* not evaluable, *PFS* progression-free survival, *A* alive, *D* dead, *NED* no evidence of disease

The median PFS (10.7 months; range 3.9–53.8 months) in the 3-year survivors was longer than that in the entire population. Seven patients were alive during the follow-up period, of which 4 were classified in the good-risk group.

Discussion

In the present study, we retrospectively investigated the prognostic factors and long-term outcomes of AGC patients treated with SP at a single institution. We found that PS $\geq$1, multiple metastatic sites, and elevated ALP levels were prognostic factors of decreased OS. Using these factors, we developed a simple prognostic index to classify patients into three risk groups.

Consistent with previous studies [8–13], we found that PS was an independent predictor of OS and only one metastatic site was an independent prognostic factor of improved OS. This finding is also consistent with several other reports [11–13]. In two Korean studies, initial metastatic state (no prior gastrectomy) was a significant prognostic factor of poor survival [8, 10]. One reasonable explanation for these results is that multiple metastatic sites and initial metastatic state may reflect larger tumor volumes; however, metastatic sites such as the liver, peritoneum, and bone were not significantly associated with OS, suggesting that tumor volume may be a more important prognostic variable than metastatic lesions in the outcome of AGC patients treated with chemotherapy. Consistent with the findings of Chau et al. [9], we determined that elevated ALP level was a prognostic factor of poor OS; however, this association was unclear in our series.

Our simple prognostic model identified three different risk groups, and because all three prognostic factors can easily be evaluated before treatment, it can help clinicians and patients make clinical treatment decisions and help in further research regarding risk stratification in AGC. To date, several prognostic models have been developed to predict the outcome of AGC patients treated with palliative chemotherapy [8, 9, 13, 17]. However, in the prognostic index validated by Chau et al. [9], 27 % of the included patients had esophageal cancer and 22 % had locally advanced disease. In the report by the Korean group, a prognostic model developed using a training set of 1,870 patients was validated in sets of 935 AGC patients [17]. This model had good applicability to AGC patients who received a 3-week SP regimen [8], whereas the more complex model comprised eight prognostic factors and therefore may not be useful in clinical application. Our proposed index has not been validated and has several limitations inherent to retrospective analyses, but proposes information valuable to clinical practice and will be worthy of validation in future.

In the present study, the median PFS and OS of patients receiving SP were 5.9 and 15.0 months, which were similar to those observed in the SPIRITS trial (median PFS and OS, 6.0 and 13.0 months, respectively) [7]. Regarding patient background, a higher proportion of our patients had poorer PS (ECOG PS $\geq$ 1; 69 vs. 28 %), peritoneal metastasis (60 vs. 34 %), and only one metastatic site (50 vs. 28 %). The proportion of our patients receiving second-line chemotherapy in the present study was approximately equal to that in the SPIRITS trial (78 vs. 74 %). The median OS of patients with peritoneal metastasis was 14.3 months, similar to that in the entire population. The

decrease in survival, indicated by poorer PS, may be counteracted by the small number of involved organs involved.

To our knowledge, this is the first report on the characteristics of long-term survivors of AGC treated with SP, although there have been a few reports of long-term survivors of AGC treated with palliative chemotherapy [11, 12]. Yoshida et al. [12] reported that 11 of 497 (2 %) patients achieved 5-year survival in four phase II trials and one phase III trial conducted by JCOG. All patients had good PS (0 or 1), and 10 of 11 (91 %) had only one metastatic site: 8 (73 %) involving the abdominal lymph nodes and 2 (18 %) involving the liver. A recent study by Hosokawa et al. [11] reported that 9 of 92 (10 %) patients who had received S-1 monotherapy survived for more than 3 years. All 9 patients had a PS of 0 or 1, and 8 (89 %) had only one metastatic site: 6 (67 %) involving the peritoneum and 2 (22 %) involving the abdominal lymph nodes. In our study, 12 of 104 (12 %) patients with a follow-up period of at least 3 years survived for more than 3 years. Similar to previous studies, all 12 patients had good PS (0 or 1) and 8 (67 %) had only one site of metastasis, including the peritoneum in 2, abdominal lymph nodes in 3, liver in 2, and lung in 1 patient. Interestingly, 4 of 7 (57 %) patients who were alive during the follow-up period were classified into the good-risk group and had prior gastrectomy.

In conclusion, we identified three prognostic OS factors in AGC patients receiving SP as first-line chemotherapy in a clinical setting. A simple prognostic index was developed with different OS rates among the three risk groups. In AGC patients with good PS, the involvement of only one metastatic site increased the patient's probability of achieving long-term survival.

Conflict of interest The authors declare that they have no conflict of interest.

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Perioperative Risk Assessment for Gastrectomy by Surgical Apgar Score

Yuichiro Miki, MD, Masanori Tokunaga, MD, Yutaka Tanizawa, MD, Etsuro Bando, MD, Taiichi Kawamura, MD, and Masanori Terashima, MD, FACS

Division of Gastric Surgery, Shizuoka Cancer Center, Shizuoka, Japan

ABSTRACT

Background. Recently, a simple and easy complication prediction system, the surgical apgar score (SAS) calculated by three intraoperative parameters (estimated blood loss, lowest mean arterial pressure, and lowest heart rate), has been proposed for general surgery. In this study, we evaluated the predictability of the original SAS (oSAS) for severe complications after gastrectomy. In addition, the predictability of a modified SAS (mSAS) was evaluated, in which the cutoff value for blood loss was slightly modified.

Methods. We investigated 328 patients who underwent gastrectomy at the Shizuoka Cancer Center in 2010. Clinical data, including intraoperative parameters, were collected retrospectively. Patients with postoperative morbidities classified as Clavien–Dindo grade IIIa or more were defined as having severe complications. Univariate and multivariate analyses were performed to elucidate factors that affected the development of severe complications.

Results. Thirty-six patients (11.0 %) had severe complications postoperatively. Univariate analyses showed that the oSAS ($p = 0.007$) and mSAS ($p < 0.001$), as well as sex, preoperative chemotherapy, cStage, type of operation, thoracotomy, surgical approach, operation time, and extent of lymph node dissection, were associated with severe complications. Multivariate analysis showed that an mSAS ≤ 6 was found to be an independent risk factor for severe complication, while an oSAS ≤ 6 was not.

Conclusions. The oSAS was not found to be a predictive factor for severe complications following gastrectomy in Japanese patients. A slightly modified SAS (i.e. the mSAS) is considered to be a useful predictor for the development of severe complications in elective surgery.

Gastrectomy with lymph node dissection is the mainstay of treatment for patients with gastric cancer to achieve a cure,[1] even though several chemotherapy regimens have been shown to be effective.[2,3] The morbidity after gastrectomy with lymph node dissection has been reported to range from 14.3 to 34.0 %.[4–6] The most frequently observed complications are pancreatic fistula, anastomotic leakage, and abdominal abscess, and these complications are sometimes fatal. This is why it is important to predict the occurrence of these complications soon after surgery, and determine the appropriate postoperative care.

Previously, several scoring models have been reported to be useful for predicting complications. However, previously reported scoring models such as the Physiologic and Operative Severity Score for the Enumeration of Mortality (POSSUM),[7] the National Surgical Quality Improvement Programme (NSQIP),[8,9] and the Estimation of Physiologic Ability and Surgical Stress (E-PASS),[10] require complex calculations using numerous perioperative variables not readily available at the bedside.

Recently, a simple and easy complication prediction system, the so-called Surgical Apgar Score (SAS), was proposed for general surgery.[11] It consists of three intraoperative parameters: estimated blood loss (EBL), lowest intraoperative mean arterial pressure (L-MAP), and lowest intraoperative heart rate (L-HR), and has been validated in

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M. Tokunaga, MD
e-mail: m.tokunaga@scchr.jp

some specific types of surgeries.[12–14] However, of these three intraoperative parameters, the appropriate cutoff values for EBL may not be identical between surgeries because the amount of blood loss differs between different types of surgeries. Indeed, different cutoff values for EBL were proposed for patients undergoing total cystectomy, and this modified SAS (mSAS) proved to correlate with short-term morbidity and mortality.[14] However, the significance of the SAS and the appropriate cutoff values for EBL after gastrectomy, have not yet been evaluated.

Our aim in this study was to evaluate the predictability of the SAS for severe complications after gastrectomy. Furthermore, the predictability of an mSAS, in which the cutoff value for EBL was slightly adjusted, was evaluated.

METHODS

We investigated 328 patients who underwent gastrectomy with D1–2 lymph node dissection for primary gastric cancer at the Shizuoka Cancer Center in 2010. Clinicopathological data were collected from the database of our hospital. Intraoperative L-HR and L-MAP were collected from electronic medical records. This retrospective study was approved by the Human Ethics Review Committee of the Shizuoka Cancer Center.

The parameters evaluated in this study included intraoperative L-HR, L-MAP, and EBL. The original SAS (oSAS) was calculated by these three parameters (Table 1).[11] In addition, we developed an mSAS, in which the cutoff values for blood loss were set by the quartile values of the 328 patients in this study (Table 2). The median EBL was 274 mL (range 0–2,067 mL), and the interquartile range was 146–524 mL.

Other variables evaluated included age, sex, body mass index, preoperative albumin, type of surgery, thoracotomy, surgical approach, extent of lymph node dissection, operation time, tumor size, macroscopic type, cStage, and American Society of Anesthesiologists (ASA) score. The macroscopic type and cStage were determined according to the Japanese Gastric Cancer Association classification (3rd English edition).[15] We performed D2 lymph node dissection for patients with Stage IB or higher, while we performed D1+lymph node dissection for patients with Stage IA disease.

Evaluation of Complication Grade

We defined postoperative complications as any morbidities observed within 30 days after the first discharge. The severity of complication was graded using the Clavien–Dindo classification.[16] Patients with a Clavien–Dindo classification of grade IIIa or higher were defined as having severe complications.

Statistical Analysis

In this study, the Chi-square test was used for categorical variables, and the *t* test or Wilcoxon test was used for numerical variables, as appropriate.

Clinical characteristics and surgical findings were compared between patients with and without severe complications.

The receiver operating characteristic (ROC) analyses of the oSAS and mSAS were used to identify an appropriate cutoff level to predict severe complications.

All statistical analyses were performed with JMP software, version 8.0 (SAS Institute, Cary, NC, USA). p Values <0.05 were considered statistically significant, and all tests were two-sided.

RESULTS

Patient Characteristics

The mean age of the study population was 66.8 years, and about two-thirds of the patients were male (66.3 %). Approximately 60 % of patients were classified as Stage I. The most frequently performed operation was distal

TABLE 1 Evaluation of the original Surgical Apgar Score

	0 point	1 point	2 points	3 points	4 points
Estimated blood loss (mL)	>1,000	>600, ≤1,000	>100, ≤600	≤100	–
Lowest mean arterial pressure (mm Hg)	<40	≥40, <55	≥55, <70	≥70	–
Lowest heart rate (beats/min)	>85	>75, ≤85	>65, ≤75	>55, ≤65	≤55

TABLE 2 Cutoff values for estimated blood loss which were used in the modified Surgical Apgar Score

	0 point	1 point	2 points	3 points	4 points
Estimated blood loss (mL)	≥525	≥274, <525	≥147, <274	<147	–

gastrectomy (51.4 %), while total gastrectomy was performed in 97 patients (29.5 %). A D2 lymph node dissection was performed in 163 patients (49.7 %). Splenectomy was also performed in 47 patients (14.3 %).

Complications

There was no mortality within 30 days after the first discharge. Of the 328 patients included in our analysis, severe postoperative complications were observed in 36 patients (11.0 %). The most frequently observed severe complication was pancreatic fistula (n = 15; 4.6 %), followed by anastomotic leakage (n = 7; 2.1 %), pleural effusion (n = 6; 1.8 %), bowel obstruction (n = 5; 1.5 %), abdominal abscess (n = 3; 0.9 %), bleeding (n = 2; 0.6 %), pneumonia (n = 2; 0.6 %), and chylous ascites (n = 1; 0.3 %).

Distribution of Patients and Receiver Operating Characteristic (ROC) Analysis by Original Surgical Apgar Score (SAS)

The distribution of patients by the oSAS is shown in Fig. 1a. The area under the ROC curve for predicting severe complications by the oSAS was 0.65 (Fig. 2a). This analysis showed that the best cutoff line for the oSAS was

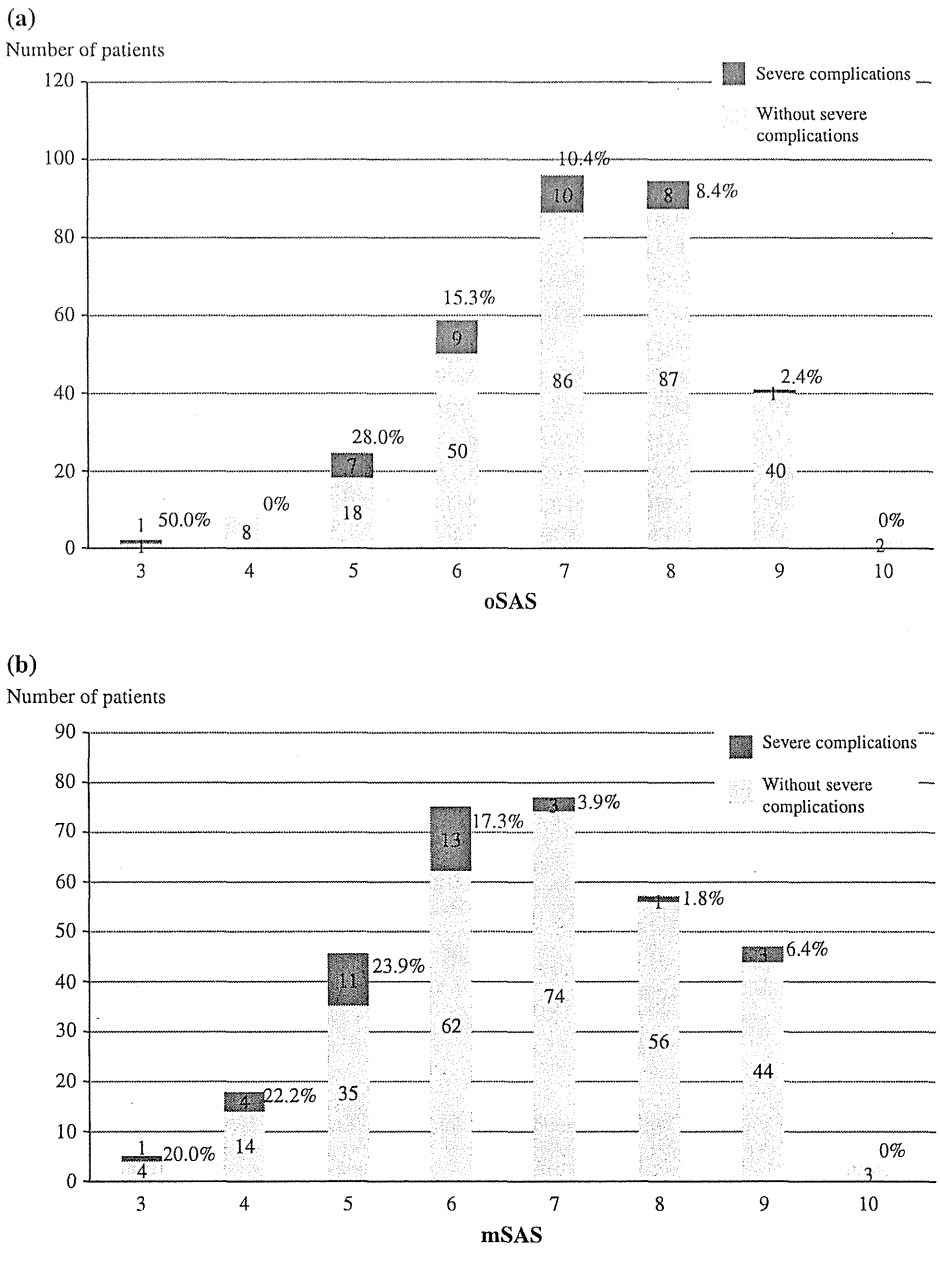

FIG. 1 Distribution of patients and proportion of severe complications by the (a) oSAS, and (b) mSAS. *oSAS* original Surgical Apgar Score, *mSAS* modified Surgical Apgar Score

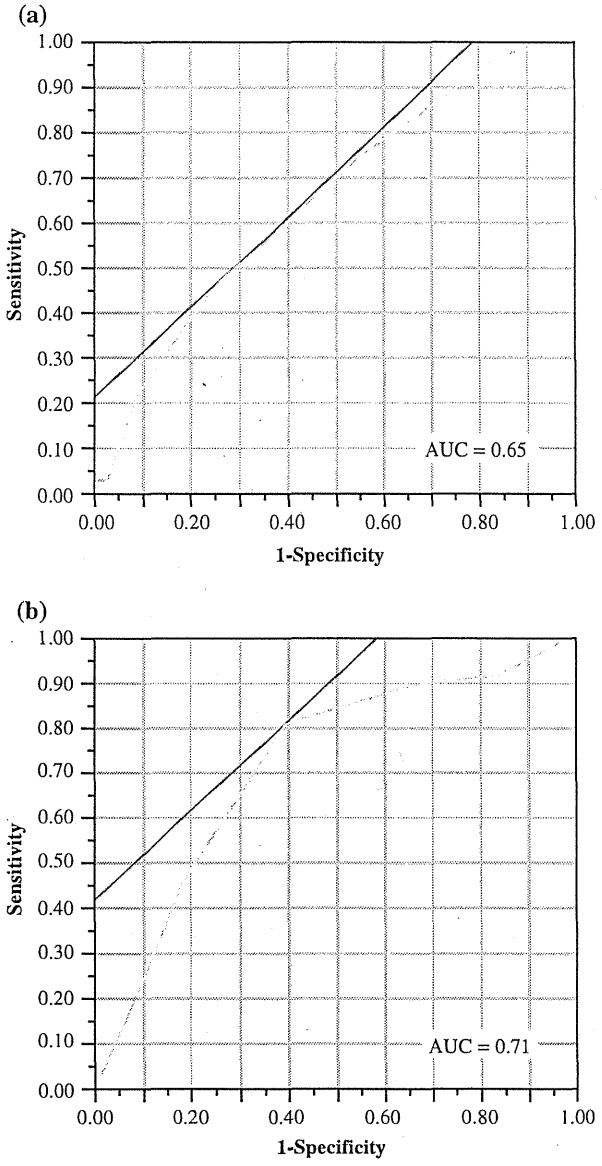

FIG. 2 Receiver operating characteristic curves for predicting severe complications by the (**a**) original SAS, and (**b**) modified SAS. *SAS* Surgical Apgar Score, *AUC* area under the curve

between six and seven points, at which sensitivity plus specificity was maximal. Sensitivity, specificity, and positive predictive value at each cutoff value for oSAS are shown in the electronic supplementary Table 1a. The number of patients under the cutoff value for the oSAS was 95 (28.9 %) (Fig. 1a).

Distribution of Patients and ROC Analysis by modified SAS

The distribution of patients by the mSAS is shown in Fig. 1b. The area under the ROC curve for predicting

TABLE 3 Association between patient characteristics and complication severity according to the Clavien–Dindo classification

Parameters	No. of patients (%) with complications		
	≤Grade II	≥Grade IIIa	*p* Value
Preoperative factors			
Sex			0.015
Male	187 (86.2)	30 (13.8)	
Female	105 (94.6)	6 (5.4)	
Age (years)			0.085
≥75	73 (83.9)	14 (16.1)	
<75	219 (90.9)	22 (9.1)	
Body mass index			0.850
≥25	69 (89.6)	8 (10.4)	
<25	223 (88.8)	28 (11.2)	
ASA score			0.506
0–1	97 (90.7)	10 (9.3)	
2–5	195 (88.2)	26 (11.8)	
Preoperative albumin (g/dL)			0.023
≥3.0	7 (63.6)	4 (36.4)	
<3.0	285 (89.9)	32 (10.1)	
Preoperative chemotherapy			0.083
Not performed	276 (89.9)	31 (10.1)	
Performed	16 (76.2)	5 (23.8)	
cStage			0.003
I, II	225 (92.2)	19 (7.8)	
III, IV	67 (79.8)	17 (20.2)	
Perioperative factors			
Operation			<0.001
Total gastrectomy	71 (73.2)	26 (26.8)	
Other	221 (95.7)	10 (4.3)	
Thoracotomy			0.016
Performed	3 (50.0)	3 (50.0)	
Not performed	289 (89.8)	33 (10.2)	
Surgical approach			0.023
Open	250 (87.7)	35 (12.3)	
Laparoscopic	42 (97.7)	1 (2.3)	
Lymph node dissection			0.021
D1, D1+	153 (92.7)	12 (7.3)	
D2	139 (85.3)	24 (14.7)	
Operation time (min)			<0.001
≥231	134 (81.3)	31 (18.7)	
<231	158 (96.9)	5 (3.1)	
oSAS			0.012
0–6	77 (82.0)	17 (18.0)	
7–10	215 (91.9)	19 (8.1)	
mSAS			<0.001
0–6	115 (79.9)	29 (20.1)	
7–10	177 (96.2)	7 (3.8)	

ASA American Society of Anesthesiologists, o*SAS*, original Surgical Apgar Score, *mSAS* modified Surgical Apgar Score

TABLE 4 Multivariate analysis of association between patient characteristics and postoperative complications rated as grade IIIa or higher by the Clavien–Dindo classification (oSAS selected as a covariate)

Variables	Odds ratio (95 % CI)	*p* Value
Sex (male)	2.07 (0.82–6.06)	0.128
Preoperative albumin (<3.0)	1.96 (0.40–9.09)	0.393
Preoperative chemotherapy (performed)	0.88 (0.24–2.90)	0.848
cStage (≥III)	0.98 (0.38–2.50)	0.976
Type of operation (TG)	5.96 (2.50–15.2)	<0.001
Extent of lymph node dissection (≥D2)	1.11 (0.45–2.75)	0.810
oSAS (≤6)	1.00 (0.42–2.34)	0.995
Operation time (≥231 min)	4.90 (1.80–15.9)	0.001

CI confidence interval, *TG* total gastrectomy, *oSAS* original Surgical Apgar Score

TABLE 5 Multivariate analysis of association between patient characteristics and postoperative complications rated as grade IIIa or higher by the Clavien–Dindo classification (mSAS selected as a covariate)

Variables	Odds ratio (95 % CI)	*p* Value
Sex (male)	2.14 (0.83–6.30)	0.115
Preoperative albumin (<3.0)	0.61 (0.14–2.94)	0.529
Preoperative chemotherapy (performed)	0.80 (0.22–2.61)	0.713
cStage (≥III)	0.95 (0.37–2.39)	0.922
Type of operation (TG)	5.25 (2.21–13.2)	<0.001
Extent of lymph node dissection (≥D2)	0.99 (0.40–2.50)	0.987
mSAS (≤6)	2.62 (1.01–7.46)	0.048
Operation time (≥231 min)	3.56 (1.26–11.8)	0.016

CI confidence interval, *TG* total gastrectomy, *mSAS* modified Surgical Apgar Score

severe complications by the mSAS was 0.71 (Fig. 2b). This analysis showed that the best cutoff line for the mSAS was between six and seven points. Sensitivity, specificity, and positive predictive value at each cutoff value for mSAS are shown in the electronic supplementary Table 1b. The number of patients under the cutoff value for the mSAS was 144 patients (43.9 %) (Fig. 1b).

Risk Factors for Severe Complications

Univariate analyses showed that the oSAS and mSAS, as well as sex, preoperative albumin, cStage, type of surgery, thoracotomy, surgical approach, operation time, and extent of lymph node dissection, were associated with severe complications (Table 3). Variables achieving a probability value <0.10 in a univariate analysis were included in a subsequent multivariate analysis to identify risk factors for severe complications. In the multivariate analysis, we did not include thoracotomy and surgical approach as covariates because these are apparent confounding factors for operative procedure; thoracotomy was used only for total gastrectomy, and laparoscopy was used only for distal gastrectomy in our institute. In the multivariate analysis, the type of surgery and operation time were selected as independent predictive factors for severe complications, while the oSAS was not (Table 4). On the other hand, when we included the mSAS as a covariate instead of the oSAS, the mSAS was selected as an independent predictive factor (Table 5).

DISCUSSION

In this study, we showed that the mSAS is a useful predictor for the development of severe complications after gastrectomy. With the mSAS, surgical teams can assess the risk of major complications immediately after surgery.

The SAS was first proposed by Gawande et al. in 2007.[11] Like the widely used obstetrical Apgar score developed by Virginia Apgar in 1953,[17] the SAS was intended for use immediately after surgery to predict patient outcomes. This surgical score reflects intraoperative hemodynamic stability, and is influenced by various factors such as the quality of surgery and anesthesia, and the patient's condition before and during surgery. At first, the SAS was validated in a cohort of patients who underwent colectomy and a cohort who underwent general and vascular surgery.[11,18,19] After that, modified versions of the SAS have been applied in other fields of surgery, including urology and neurosurgery.[12,14] However, the SAS has not yet been applied to patient cohorts who have undergone gastrectomy. To the best of our knowledge, the present study is the first to investigate the utility and significance of the SAS after gastrectomy for gastric cancer.

Several scoring models have been shown to effectively predict patient outcomes. However, they consist of many variables, which include subjective parameters, such as the severity of the patients' preoperative comorbidities, as indicated by the ASA score. The number of parameters required in the NSQIP, POSSUM, and E-PASS scores is 66, 18, and 9, respectively. Furthermore, all three scoring models need complex calculation formulae. On the contrary, the SAS uses only three intraoperative parameters, which are objective, and can be calculated easily. In addition, the area under the curve for the mSAS was not inferior to that of E-PASS or POSSUM when we calculated them with the same data set (data not shown).

Although EBL, L-MAP, and L-HR alone have been shown to be important predictors for complications in the past,[20,21] the combination of these three variables is a unique characteristic of the SAS.[11] We were able to notice intraoperative hemodynamic changes precisely using the three variables together, but not with a single variable. We

consider that intraoperative hypovolemia and hypoperfusion, reflected by an increased EBL, increased L-HR, and decreased L-MAP, lead to a lower perioperative tissue oxygenation, resulting in infectious complications.[22,23] In addition, lower L-MAP and higher L-HR might reflect intraoperative systemic inflammatory response syndrome, which has an increased postoperative complication rate.[24]

We set new cutoff values for EBL in this study. This kind of modification was also made in patients undergoing total cystectomy where a higher cutoff value for the oSAS was set because EBL during total cystectomy is usually higher than that during general surgeries. In our series, the median intraoperative blood loss was 274 mL which was thought to be lower than that in general surgery. Therefore, we adopted a new cutoff value for EBL; we used the quartile values of EBL in our mSAS. We consider appropriate cutoff values for EBL may be different depending on the type of surgery, although the cutoff values for L-MAP and L-HR should be the same in every type of surgery.

The present study showed that the mSAS is an independent risk factor for severe complications, while the oSAS is not. The oSAS was not selected as an independent risk factor even when we adopted a cutoff value other than ≤ 6 (data not shown). When we find patients with an mSAS ≤ 6, we should pay particular attention to the development of severe complications. For these patients, careful observation and intensive care are necessary.

Although the oSAS with slight modification is a useful scoring system to predict severe postoperative complications, this kind of modification reduces the universal appeal of the scoring system because different cutoff values have to be set when the SAS is used for different surgical procedures. From the point of view of general use, therefore, previously reported risk scoring models (POSSUM, E-PASS, etc.) may be superior to the SAS. Nevertheless, we still believe the mSAS is a promising scoring system because it is simple and as useful as POSSUM or E-PASS, and it can be calculated with ease at the bedside immediately after surgery. Accordingly, the SAS with slight modification can be applied easily in clinical practice if cutoff values are appropriately revised for specific types of surgery.

D2 lymphadenectomy has become a standard treatment for advanced gastric cancer in Western countries since the 15-year follow-up of the Dutch trial showed the survival benefit of D2 lymphadenectomy.[25] In Japan, although D2 lymphadenectomy has been a standard treatment for advanced gastric cancer, the Japanese guidelines allow less than a D2 lymphadenectomy for early gastric cancer. This is why we performed a D1 or D1+lymphadenectomy in 50.3 % of the patients in this study. However, Western randomized controlled trials have shown an increased incidence of intra-abdominal infection including pancreatic fistula after D2 lymphadenectomy compared with D1 lymphadenectomy, and Western guidelines recommend that D2 lymphadenectomies should only be performed at high-volume centers.[26,27] We believe the mSAS would be useful for the prediction of this potentially fatal complication.

The present retrospective study in a single institute has several limitations. First, the sample size was small to obtain any conclusive results. Second, it is unclear whether our cutoff value for EBL is also useful in Western countries. In Western countries, the average body mass index is higher than in Japan, resulting in longer operation times, higher EBL, and increased postoperative morbidity and mortality rates. Therefore, the appropriate cutoff value for EBL in Western countries may be different from that in the present study. We consider the cutoff value we set should be validated with a large number of patients, including Western patients, in the future. Third, although we believe that the appropriate cutoff value for EBL is different among different types of surgery, this concept has not been validated in surgeries other than total cystectomy. This issue should also be clarified in the future. Fourth, the SAS is not widely utilized in clinical practice, and it may be seen more as a research comparison tool. In addition, it is unclear whether appropriate control of the three variables improves patient outcome. Finally, about 60 % of patients had Stage I disease in our study, which is much higher than in Western countries. Accordingly, 50.3 % of patients underwent a D1+lymphadenectomy in our series. Therefore, for the results of the present study to be adopted in Western countries, a further validation study will be needed.

CONCLUSIONS

The oSAS was not found to be a significant predictive factor for severe complications after gastrectomy. On the contrary, our mSAS, in which the cutoff value for EBL was slightly modified, was considered to be a useful predictor for severe complications. The mSAS appears to be a useful tool for assessing the risk of severe complications immediately after surgery in patients with gastric cancer.

CONFLICTS OF INTEREST Yuichiro Miki, Masanori Tokunaga, Yutaka Tanizawa, Etsuro Bando, Taiichi Kawamura, and Masanori Terashima declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Impact of the expression of thymidylate synthase and dihydropyrimidine dehydrogenase genes on survival in stage II/III gastric cancer

Mitsuru Sasako · Masanori Terashima · Wataru Ichikawa · Atsushi Ochiai · Koji Kitada · Issei Kurahashi · Shinichi Sakuramoto · Hitoshi Katai · Takeshi Sano · Hiroshi Imamura

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Abstract

Background The efficacy of 5-fluorouracil (5FU)-based therapy, which remains the cornerstone of gastrointestinal cancer treatment, depends upon the expression of enzymes involved in pyrimidine metabolism, including thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP), and orotate phosphoribosyltransferase (OPRT). We analyzed the expression of these genes in patients enrolled in the Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer (ACTS-GC) and their possible roles as biomarkers for treatment outcomes.

Methods Formalin-fixed, paraffin-embedded specimens were available for 829 of a total of 1,059 (78.3 %) patients. TS, DPD, TP, and OPRT expression was measured by RT-PCR in manually microdissected tumor specimens and normalized to the reference gene, β-actin. The expression level of each gene was categorized as low or high using cutoffs at the 33.3rd, 50th, or 66.7th percentiles.

Results The hazard ratio (HR) for overall survival (OS) after S-1 treatment versus surgery alone was significantly lower in high (>66.7th percentile; HR = 0.370; 95 % CI 0.221–0.619) compared to low (<66.7th percentile;

For the ACTS-GC Group.

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M. Sasako (✉)
Department of Surgery, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan
e-mail: msasako@hyo-med.ac.jp

M. Terashima
Division of Gastric Surgery, Shizuoka Cancer Center, 1007 Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan

W. Ichikawa
Division of Medical Oncology, Department of Medicine, Showa University, School of Medicine, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-8555, Japan

A. Ochiai
Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

K. Kitada
Department of Surgery, National Hospital Organization, Fukuyama Medical Center, 4-14-17 Okinogami-cho, Fukuyama, Hiroshima 720-8520, Japan

I. Kurahashi
Department of Medical Informatics and Economics, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan

S. Sakuramoto
Department of Surgery, Saitama Medical University, International Medical Center, 1397-1 Yamane, Hidaka, Saitama 350-1298, Japan

H. Katai
Gastric Surgery Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo, Tokyo 104-0045, Japan

T. Sano
Department of Gastroenterological Surgery, Cancer Institute Hospital, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto, Tokyo 135-8550, Japan

H. Imamura
Department of Surgery, Toyonaka Municipal Hospital, 4-14-1 Shibahara-cho, Toyonaka, Osaka 560-8565, Japan

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HR = 0.757; 95 % CI 0.563–1.018) TS expression groups ($P = 0.015$). Similarly, the HR for OS after S-1 therapy versus surgery alone was significantly lower in high (>33.3rd percentile; HR = 0.520, 95 % CI 0.376–0.720) compared to low (<33.3rd percentile; HR = 0.848, 95 % CI 0.563–1.276) DPD expression groups ($P = 0.065$). There was no interaction between TP or OPRT expression and OS.

Conclusions This large biomarker study showed that high TS and DPD gene expression in tumors was associated with enhanced benefit from postoperative adjuvant S-1 treatment in gastric cancer. There was no interaction between TP and OPRT expression and S-1 treatment.

Keywords Stomach neoplasms · Thymidylate synthase · Dihydrouracil dehydrogenase · Chemotherapy, adjuvant · Biological markers

Introduction

Gastric cancer is the second commonest cause of cancer-related death worldwide. The mainstay of treatment for gastric cancer is surgery. However, in stage II (excluding T1) and stage III (moderately advanced) disease, many patients develop recurrence, even after curative resection. S-1 (TS-1; Taiho Pharmaceutical, Tokyo, Japan) is an oral fluoropyrimidine preparation combining tegafur, gimeracil, and oteracil potassium [1]. The Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer (ACTS-GC), a prospective randomized phase III trial, demonstrated that surgery plus S-1 treatment was more effective than surgery alone in Japanese patients with stage II/III gastric cancer [2, 3]. 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 [3]. Therefore, it is important to also evaluate the effectiveness of intensive preoperative and/or postoperative chemotherapy with multiple agents in patients at high risk of relapse. Alternatively, reliable biomarkers are needed to improve outcomes by enabling the selection of patients who would benefit from S-1 or other novel therapies. We previously reported that EGFR positivity, but not HER2 positivity, was associated with poor patient outcomes after curative resection of stage II/III gastric cancer, using archived specimens obtained from patients enrolled in the ACTS-GC [4]. Furthermore, there was no apparent interaction between S-1 and EGFR or HER2 status with respect to survival [4].

Several enzymes play key roles in fluoropyrimidine metabolism. Thymidylate synthase (TS) is the rate-limiting enzyme in the de novo synthesis of 2′-deoxy-thymidine-5′-monophosphate, which is required for DNA synthesis and repair, and is therefore the primary target of fluoropyrimidines [5]. Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in 5-fluorouracil (5FU) catabolism [6]. Thymidine phosphorylase (TP) and orotate phosphoribosyltransferase (OPRT) convert 5FU to active metabolites such as 2′-deoxy-5-fluorouridine and 5-fluorouridine-5′-monophosphate, respectively [7]. Basically, high TS, DPD, and TP expression and low OPRT expression in tumors have been thought to result in relatively low sensitivity to fluoropyrimidine-based chemotherapy [5–8]. Many studies have evaluated correlations between the expression levels of these enzymes and clinical outcomes using gastrointestinal tumor specimens, suggesting that the expression of them could allow the accurate prediction of clinical outcome in patients receiving fluoropyrimidine-based chemotherapy [9]. However, the clinical significance of the expression of these genes remains unclear, as many inconsistent results are reported in the literature, and most published reports concern retrospective analyses of data from nonrandomized or relatively small randomized studies.

In this study we have therefore measured the expression of TS, DPD, TP, and OPRT genes by RT-PCR in gastric tumor specimens obtained from patients enrolled in the ACTS-GC. We evaluated them retrospectively to determine whether their expression levels would be predictive markers for a response to S-1 and/or prognostic markers.

Materials and methods

Study population and design

Tumor tissue was collected from patients enrolled in the ACTS-GC, for which the inclusion criteria and treatment protocol have been described previously [2, 3]. This biomarker study was designed retrospectively, after the completion of the first interim analysis of the ACTS-GC, to determine any predictive value for benefit from S-1 treatment or for prognosis [4]. 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 and constituted the biomarker study population (Fig. 1). The protocol used for this biomarker study was approved by the ethics committee of the Japanese Gastric Cancer Association and the institutional review board of each participating hospital. This study also complied with REMARK guidelines [10], as shown in Table S1 of the Electronic supplementary material (ESM).

Reverse transcription PCR

Representative hematoxylin and eosin stained slides from FFPE specimens were reviewed by a pathologist to

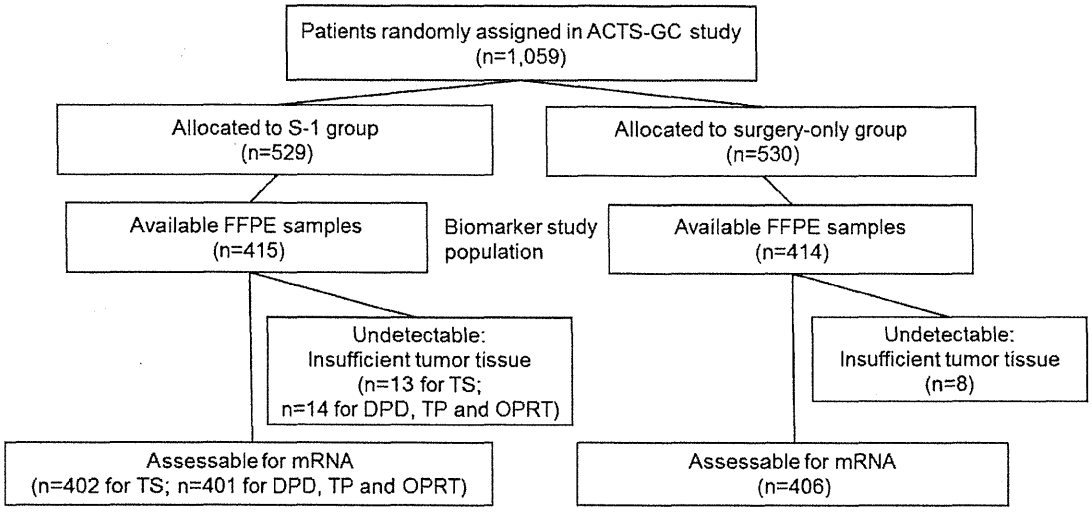

Fig. 1 Diagram of patient flow

estimate tumor load. Sections 10 μm in thickness were then stained with Nuclear Fast Red (Sigma–Aldrich, St Louis, MO, USA) for manual microdissection. Tumor tissue was selected at a magnification of 5–10× and dissected using a scalpel, as described previously [11].

RNA was isolated from tumor tissue and cDNA was prepared as described previously [12], with a slight modification to the extraction step, which used RNeasy MinElute spin columns (Qiagen, Chatsworth, GA, USA). Expression levels of the TS, DPD, TP, and OPRT genes were determined using TaqMan real-time PCR (Life Technologies, Foster City, CA, USA), as described previously [12]. β-Actin was used as an endogenous reference gene. The measurement of amplified cDNA used the cycle threshold (*Ct*) value, which is inversely proportional to the amount of cDNA. Gene expression values (relative mRNA levels) were expressed as ratios (differences between the *Ct* values) of the gene of interest (TS, DPD, TP, and OPRT) to a reference gene (β-actin). This reference gene provided a baseline measurement for the amount of mRNA isolated from a specimen. The expression levels of each gene were categorized as low or high at the 33.3rd, 50th, or 66.7th percentiles.

Immunohistochemistry

All reagents and instruments for immunohistochemistry (IHC) were purchased from Ventana Medical Systems, Inc. (Tucson, AZ, USA). FFPE, 3–5 μm thick sections were automatically stained using a Ventana BenchMark® ULTRA with primary monoclonal antibodies specific for TS, DPD, and TP and a polyclonal antibody specific for OPRT, prepared by Taiho [13–15], and an iView DAB Universal Kit, according to the manufacturer's instructions. Staining was evaluated using light microscopy by two independent pathologists (KK and AO) who were blind to all clinical information. Tumor cell immunostaining was assessed semiquantitatively in three randomly selected × 20 fields in a semiquantitative manner to reflect both the intensity of staining and percentage of cells stained. Intensity was classified as unstained (0), weakly stained (1+), moderately stained (2+), or strongly stained (3+).

Statistical analysis

Categorized data was analyzed using the chi-square test. Either the Wilcoxon test or the Kruskal–Wallis test was used to assess correlations between groups. 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 performed using a Cox proportional hazards model. Results were considered statistically significant at $P < 0.05$, except for the interaction test, for which $P < 0.1$ was considered statistically significant [16, 17]. Because this analysis was primarily exploratory, adjustments for multiple comparisons were not made [16]. All statistical analyses used the SAS software package (version 9.1) and JMP software (version 8.01; SAS Institute Inc., Cary, NC, USA).

We estimated the minimum difference in survival that would be required to show a significant survival difference between patients with tumors in which gene expression was high or low in each treatment arm. Each arm included approximately 400 patients. Given a tertile or median cutoff point, demonstrating a statistically significant difference in survival between patients with tumors with high and low gene expression would require HRs of at least 0.56

Table 1 Patient characteristics

	Total ACTS-GC population			Biomarker study subpopulation		
	S-1 ($N = 529$)	Surgery only ($N = 530$)	P value[a]	S-1 ($N = 415$)	Surgery only ($N = 414$)	P value[a]
Sex, no. (%)						
Male	367 (69.4)	369 (69.6)	0.98	282 (68.0)	283 (68.4)	0.90
Female	162 (30.6)	161 (30.4)		133 (32.0)	131 (31.6)	
Age, no. (%)						
<60 years	199 (37.6)	195 (36.8)	0.86	160 (38.6)	158 (38.2)	0.72
60–69 years	193 (36.5)	215 (40.6)		149 (35.9)	161 (38.9)	
70–80 years	137 (25.9)	120 (22.6)		106 (25.5)	95 (22.9)	
Median (years)	63	63		63	62	
Range (years)	27–80	33–80		27–80	33–80	
Tumor stage, no. (%)						
T1	1 (0.2)	0 (0)	0.81	1 (0)	0 (0)	0.93
T2	289 (54.6)	286 (54.0)		222 (53.5)	223 (53.9)	
T3	225 (42.5)	232 (43.8)		180 (43.5)	182 (44.0)	
T4	14 (2.6)	12 (2.3)		12 (2.9)	9 (2.2)	
Nodal stage, no. (%)[b]						
N0	51 (9.6)	64 (12.1)	0.72	40 (9.6)	52 (12.6)	0.52
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, no. (%)						
0	51 (9.6)	64 (12.1)	0.37	40 (9.6)	52 (12.6)	0.18
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, no. (%)[c]						
II	236 (44.6)	238 (44.9)	0.78	183 (44.1)	189 (45.7)	0.48
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, no. (%)[d]						
Differentiated	214 (41.6)	209 (40.3)	0.73	166 (40.0)	166 (40.1)	0.91
Undifferentiated	301 (58.4)	307 (59.7)		249 (60.0)	245 (59.2)	
TS expression level, no. (%)[e]						
Low	–	–	–	138 (34.3)	134 (33.0)	0.72
Intermediate	–	–		137 (34.1)	131 (32.3)	
High	–	–		127 (31.6)	141 (34.7)	
DPD expression level, no. (%)[e]						
Low	–	–	–	136 (33.9)	133 (32.8)	0.60
Intermediate	–	–		135 (33.7)	135 (33.3)	
High	–	–		130 (32.4)	138 (34.0)	
TP expression level, no. (%)[e]						
Low	–	–	–	129 (32.2)	140 (34.5)	0.80
Intermediate	–	–		131 (32.7)	139 (34.2)	
High	–	–		141 (35.2)	127 (31.3)	
OPRT expression level, no. (%)[e]						

Table 1 continued

	Total ACTS-GC population			Biomarker study subpopulation		
	S-1 ($N = 529$)	Surgery only ($N = 530$)	P value[a]	S-1 ($N = 415$)	Surgery only ($N = 414$)	P value[a]
Low	–	–	–	129 (32.2)	140 (34.5)	0.23
Intermediate	–	–		131 (32.7)	140 (34.5)	
High	–	–		141 (35.2)	126 (31.0)	

Characteristics of all ACTS-GC patients can be found in the literature [2]

[a] P values for sex and histologic type were calculated using the chi-square test. P values for age, tumor stage, nodal stage, number of lymph-node metastases, cancer stage (Japanese classifications), and gene expression level were calculated using the Wilcoxon test

[b] Nodal stages were defined according to the Japanese classification 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 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 were defined according to the Japanese classification 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 the total ACTS-GC population, histologic type was classified for eligible patients ($N = 1{,}034$). In the surgery-only group of the biomarker study population, cancers could not be classified as differentiated or undifferentiated in three patients

[e] Gene expression levels were undetectable for some of the samples, as shown in Fig. 1

and 0.58, respectively, assuming a two-sided $\alpha = 0.05$ and a power of 80 % in a proportional hazards model.

Results

Patient characteristics

There was no significant difference between the population used in this biomarker study and the total population of the ACTS-GC (Table 1), as previously reported [2]. The groups were well balanced with respect to gene expression levels and other factors.

Expression of TS, DPD, TP, and OPRT

Gene expression was assessable in 808 patients for TS and in 807 patients for DPD, TP, and OPRT, representing 97 % of the biomarker study population (Fig. 1). Histograms of the expression values for each gene showed typical normal distributions (see Fig. S1 of the ESM). Each relative mRNA level at the 33.3rd, 50th, and 66.7th percentile was as follows: 2.47, 3.03, and 3.87 for TS; 0.50, 0.69, and 0.97 for DPD; 4.19, 5.44, and 7.09 for TP; and 0.45, 0.54, and 0.67 for OPRT, respectively.

We classified patients into four groups according to TS, DPD, TP, and OPRT protein levels measured by IHC and scored as 0, 1+, 2+, and 3+. Representative examples of immunostaining for each gene product are shown in Fig. S2 of the ESM. IHC scores and gene expression levels for TS, TP, and OPRT were significantly correlated ($P < 0.001$), and there was considerable overlap between the four groups (see Fig. S3 of the ESM). On the other hand, IHC scores for DPD did not correlate with gene expression levels ($P > 0.05$), with more than half of the patients classified as 3+ by IHC.

Correlation of the expression of *TS*, *DPD*, *TP*, and *OPRT* genes on survival

In the biomarker study population, 5-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 %), respectively, in the surgery-only group. These figures were similar to the ACTS-GC 5-year follow-up data [3].

When gene expression was categorized as low or high using the 66.7th percentile, high TS expression was significantly associated with good OS and RFS in the S-1 group only (Table 2). In contrast, when gene expression was categorized as low or high using the 33.3rd percentile, high DPD expression was significantly associated with good OS and RFS in the S-1 group only (Table 3). There was no significant association of TS and DPD expression—categorized using the median—with outcomes in each group, although these figures were similar to the results obtained using the 66.7th and 33.3rd percentiles (data not shown).

There was no association between TP or OPRT expression and outcomes in either the S-1 or surgery-only groups (Tables 2, 3). Furthermore, there was no association between IHC scores for these four genes and outcomes (data not shown).