Results Of the 311 patients enrolled, 14 were ineligible and 27 failed to start the protocol treatment. The CD-DST failed in 64 other patients, and survival analyses were conducted with the remaining 206 patients (39 stage II disease, 155 stage III disease, and 12 stage IV disease). The outcome of patients who were determined to be responders was significantly superior to that of nonresponders regardless of the 5-fluorouracil concentrations, although no differences in clinicopathologic characteristics were observed between the two groups, except for age.
Conclusions The CD-DST identified those who benefit from adjuvant chemotherapy. It deserves further evaluation in the setting of a prospective randomized trial.
ClinicalTrials.gov identifier: NCT00287755

Keywords Chemosensitivity test · Relapse-free survival · Appropriate cutoff values · Responder · Nonresponder

Introduction

The outcome of patients with resectable gastric cancer has improved owing to the development of technologies making possible earlier diagnosis, as well as the continued progress in surgical techniques and multidisciplinary treatments. However, the outcome remains unsatisfactory in patients with advanced or recurrent disease. Recently, several anticancer agents have been newly introduced, and have raised hope for an improved outcome after chemotherapy. S-1 (TS-1, Taiho Pharmaceutical, Tokyo, Japan) is an oral anticancer drug that combines tegafur (a prodrug of 5-fluorouracil; 5-FU) with 5-chloro-2,4-dihydropyrimidine (CDHP) and potassium oxonate in a molar ratio of 1:0.4:1. A phase III study comparing surgical treatment alone with surgery plus adjuvant S-1 chemotherapy in patients who underwent curative resection of stage II and stage III gastric cancer (Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer; ACTS-GC) demonstrated that postoperative adjuvant chemotherapy with S-1 significantly improved survival [1, 2]. However, human tumors of even a similar histopathologic category may have markedly different drug sensitivity profiles [3–6]. In vitro drug sensitivity tests have thus been developed to individualize chemotherapy for cancer patients [7–15]. We hypothesize that personalized therapy guided by adequate chemosensitivity testing may lead to a better outcome than conventional empirical therapy. Since the publication of ACTS-GC, orally administered S-1 has become the standard drug for postoperative adjuvant chemotherapy for gastric cancer in Japan [16]. However, this implies that S-1 is also given to patients whose tumors are not sensitive to 5-FU. To address this problem, we organized a research group designated Gastric Cancer 04 (GC-04), consisting of 32 surgical institutions distributed nationwide, in the Japan Clinical Cancer Research Organization (JACCRO). GC-04 conducted this exploratory phase II trial to evaluate the clinical value of chemosensitivity testing of 5-FU in patients who received S-1 postoperatively. Our main goal was to verify whether survival is better in patients whose tumors are sensitive to 5-FU in vitro than in those insensitive to 5-FU in vitro. The primary end point was relapse-free survival (RFS). Secondary end points included 3-year overall survival (OS) and safety. The study was performed from December 2005 to December 2013.

Materials and methods

The trial was conducted in accordance with the World Medical Association Declaration of Helsinki and Japanese Ethical Guidelines for Clinical Studies. The protocol was approved by the institutional review board of each participating hospital. Written informed consent was obtained from all patients. All members of the steering committee and the sponsor jointly designed the trial and collected the data, which were managed by the independent JACCRO GC-04 Data Center. The data were analyzed by an independent data and safety monitoring committee.

Eligibility criteria

The eligibility criteria were as follows: (1) presence of histologically proven stage II, stage IIIA, or stage IIIB gastric cancer, and stage IV gastric cancer with N3 but without hepatic, peritoneal, or distant organ metastasis; (2) treatment by D2 or more extensive lymph node dissection; (3) an age of 20–80 years; (4) no previous treatment for cancer; and (5) adequate organ function (a leukocyte count of at least 4,000/ml; a platelet count of at least 100,000/ml; a total bilirubin level of no more than 1.5 mg/dl, aspartate aminotransferase and alanine aminotransferase levels of no more than 2.5 times the upper limit of the normal range; and a serum creatinine level no greater than the upper limit of the normal range). Tumor stage classification and D classification were in accordance with the Japanese Classification of Gastric Carcinoma (second English edition) [17]. For patients to be included in the final analysis, the in vitro sensitivity of tumor tissue to 5-FU had to be successfully evaluated by chemosensitivity testing.

Drug sensitivity test

The collagen gel droplet embedded culture drug sensitivity test (CD-DST) was used to assess in vitro sensitivity to 5-FU because it is the only commercially available method

distributed as a kit, and various studies have demonstrated its usefulness in evaluating the in vitro chemosensitivity of fresh human tumors [18–24]. The CD-DST was performed as described previously [25, 26].

Briefly, a portion of each resected tumor specimen was excised and thinly sliced. Each sample was treated with dispersion enzyme cocktail EZ (Kurabo Industries, Osaka, Japan). The resulting cell suspension was transferred to collagen-coated flasks (CG-flask; Kurabo Industries) and cultured in preculture medium containing 10 % fetal bovine serum at 37 °C in 5 % CO_2 overnight. The collagen gel was digested with 0.05 % collagenase (type I; Sigma-Aldrich Japan, Tokyo, Japan) to obtain viable cancer cells. The cancer cell suspension prepared was added to reconstructed type I collagen solution (Cellmatrix type CD; Kurabo Industries) to obtain a final cell density of 1×10^5/ml. Three drops of the collagen–cell mixture (30 µl per droplet) were placed in each well of a six-well plate on ice and allowed to gel at 37 °C in a CO_2 incubator overnight. Subsequently, the tumor cells in the collagen gel droplet were exposed to 5-FU at concentrations corresponding to the area under the drug concentration–time curve in patients and were incubated for 120 h. The 5-FU concentrations used were 0.2, 0.4, 1.0, and 2.0 µg/ml. After removal of the medium containing 5-FU, each well was rinsed twice with 3 ml of Hanks balanced salt solution each time, overlaid with 4 ml of PCM-2 medium (serum-free medium; Kurabo Industries), and incubated for 7 days. At the end of the incubation, neutral red was added to each well at a final concentration of 50 µg/ml, and the colonies in the collagen gel droplet were fixed in 10 % neutral-buffered formalin, washed with water, air-dried, and quantified by optical density image analysis. In vitro sensitivity was expressed as the *T*/*C* ratio, where *T* is the optical density of the 5-FU-treated samples on day 7 and *C* is the optical density of nontreated controls on day 7. The growth inhibition rate (GIR) was calculated as $1 - T/C$.

In the pilot study using 31 fresh gastric cancers that were provided to the central laboratory in the initial stage of this study, we could not find any significant difference in GIR between 5-FU alone and 5-FU with CDHP, which is included in S-1, i.e., GIR of 57.5 ± 22.5 % and 64.3 ± 17.8 % for 5-FU alone at 1.0 and 2.0 µg/ml, respectively, versus GIR of 58.0 ± 20.3 % and 66.4 ± 20.9 % for 5-FU at 1.0 and 2.0 µg/ml with CDHP, respectively. As a result, in vitro sensitivity to 5-FU was used as a surrogate of in vivo sensitivity to S-1 in this study.

RNA extraction, complementary DNA synthesis, and real-time quantitative reverse transcription polymerase chain reaction

The effect of S-1 can be modulated by the expression levels of 5-FU-related metabolic enzymes, including dihydropyrimidine dehydrogenese (DPD) [27], thymidine synthetase (TS) [28], thymidine phosphorylasa (TP), and orotate phosphoribosyltransferase (OPRT) [29].

Expression levels of TS, DPD, TP, and OPRT messenger RNA (mRNA) were measured as previously [30]. Briefly, total RNA of primary gastric cancer cells was extracted using an Isogen kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. Complementary DNA was derived from each sample, and target complementary DNA sequences were amplified by quantitative polymerase chain reaction (PCR) using a fluorescence-based real-time detection method [ABI PRISM 7900 sequence detection system (TaqMan); Applied Biosystems, Foster City, CA, USA]. The PCR conditions were 50 °C for 10 s and 95 °C for 10 min, followed by 42 cycles at 95 °C for 15 s and 60 °C for 1 min. TS, DPD, TP, and OPRT mRNA levels were quantified as ratios between two measurements (gene of interest/β-actin).

Definition of the appropriate cutoff values

Tumors with a GIR equal to the cutoff value or higher were classified as in vitro sensitive (responders), and those with lower GIRs were classified as in vitro insensitive (nonresponders). After a median follow-up time of 3 years, the hazard ratio (HR) for relapse in the responder group as compared with the nonresponder group was calculated by plotting cutoff values of the in vitro GIR from 10 to 90 % with 10 % increments for each of the four different in vitro 5-FU concentrations. Appropriate cutoff values were defined when the HR for relapse and the log-rank *P* value were at their minimum.

Study design and treatment

Patients were enrolled within 6 weeks after surgery via a Web-based electronic data capture system (FLADS; Takt Systems, Tokyo, Japan) into the JACCRO GC-04 Data Center. Enrolled patients received two oral doses of 40 mg of S-1 per square meter of body-surface area per day for 4 weeks, followed by 2 weeks of no chemotherapy (Fig. 1). During the treatment weeks, the dosages of S-1 were assigned according to body-surface area as follows: less than 1.25 m^2, 80 mg daily; 1.25 m^2 or greater to less than 1.5 m^2, 100 mg daily; and 1.5 m^2 or greater, 120 mg daily. This 6-week cycle treatment was repeated for 1 year after surgery. If patients had grade 3 or grade 4 hematologic toxicity or grade 2, 3, or 4 nonhematologic toxicity, the daily dose of S-1 was reduced, from 120 to 100 mg, from 100 to 80 mg, or from 80 to 50 mg, respectively.

Patients were followed up for 3 years postoperatively. Adverse events were assessed according to the Common Toxicity Criteria (version 2.0) of the National Cancer Institute.

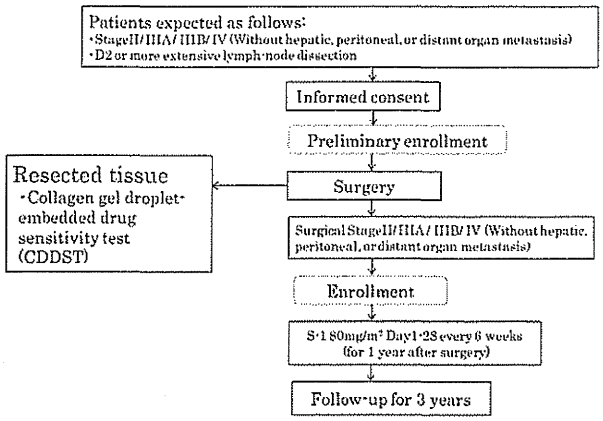

Fig. 1 Study schema

Follow-up

Patients underwent hematologic tests and clinical examinations every 2 weeks. Adverse events were evaluated every 3 months for 1 year after surgery.

The presence of recurrence was determined by means of imaging studies, including ultrasonography, computed tomography (CT), gastrointestinal radiography, and endoscopy. Patients underwent at least one type of imaging study, usually CT, at 6-month intervals during the first 2 years after surgery and then at 1-year intervals until 3 years after surgery. Peritoneal relapse was diagnosed when CT or ultrasonography identified cytology-positive ascites. Case-report forms, which included the results of follow-up tests and evaluations and the survival status of patients, were submitted to the JACCRO GC-04 Data Center 0.5, 1, 1.5, 2, and 3 years after surgery.

Statistical analysis

Our previous retrospective study of 128 patients with gastric cancer demonstrated that survival of the responders as determined by chemosensitivity testing, the histoculture drug-response assay [9], was significantly superior to that of the nonresponders [31, 32]. Taking into consideration the possibilities of failure of chemosensitivity testing and inclusion of ineligible patients, we estimated that a total enrollment of 300 patients would be sufficient to reproduce similar results in the present prospective study. Because the number of days from surgery to enrollment was likely to differ among patients, we decided to calculate the OS and RFS from the date of surgery. All statistical analyses were performed using JMP 8.0 and SAS 9.2 statistical software programs (SAS, Cary, NC, USA). The 3-year RFS and OS rates were estimated using the Kaplan–Meier method, and the log-rank test was used to compare the survival curves. A Cox proportional hazards model was used to calculate the HRs. *P* values less than 0.05 indicated statistical significance.

Results

Patients and procedures

Between December 2005 and December 2013, 311 patients were enrolled at 32 centers in Japan (Fig. 2). At enrollment, 14 patients were found to be ineligible for the following reasons: no tumor specimens available for chemosensitivity testing (ten patients), T1 cancer (two patients), enrollment before approval of the institutional review board (one patient), and laboratory test values at enrollment that did not meet the protocol requirements (one patient). In addition, 27 patients did not receive the protocol treatment of S-1. Among the tumors from the remaining 270 patients, in vitro chemosensitivity was not successfully assessed in 64 tumors for the following reasons: insufficient number of tumor cells for assay (30 patients), bacterial contamination (29 patients), low tumor cell viability (two patients), and insufficient cell growth (three patients). As a result, survival and safety were analyzed in 206 patients in whom chemosensitivity testing was successful.

Characteristics of the 206 patients

The 206 patients consisted of 151 men and 55 women with a median age of 65 years. Distribution of the disease stage, T stage, N stage, extent of lymph node dissection, ECOG performance status, type of gastrectomy, and tumor histologic type are shown in Table 1.

Adverse events and treatment compliance

Among the 206 patients who received the protocol S-1 treatment, adverse events were evaluated and classified as grade 1, 2, 3, or 4 according to the Common Toxicity Criteria (version 2.0) of the National Cancer Institute. Grade 3 or

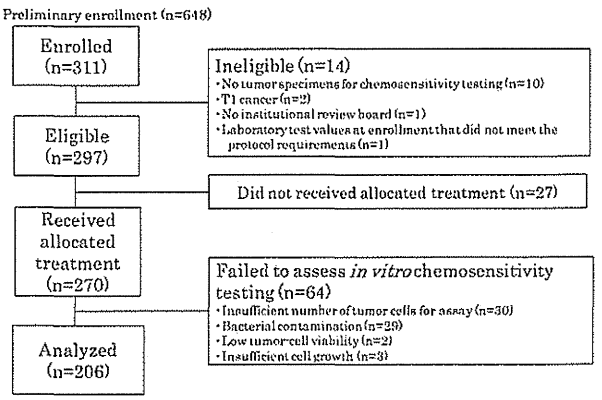

Fig. 2 CONSORT diagram

Table 1 Patient characteristics of the 206 patients

Gender	
Male	151 (73.3 %)
Female	55 (26.7 %)
Age	
Median	65 years
Range	32–79 years
Mean	63.5 years
Body surface area (m^2)	
<1.25	4 (1.9 %)
≥1.25, <1.5	67 (32.5 %)
≥1.5	135 (65.5 %)
Cancer stage[a]	
II	39 (18.9 %)
IIIA	97 (47.1 %)
IIIB	58 (28.2 %)
IV	12 (5.8 %)
T stage[a]	
T1	2 (1.0 %)
T2	52 (25.2 %)
T3	135 (65.5 %)
T4	17 (8.3 %)
N stage[a]	
N0	11 (5.3 %)
N1	109 (52.9 %)
N2	79 (38.3 %)
N3	7 (3.4 %)
Lymph node dissection[a]	
D2	197 (95.6 %)
D3	9 (4.4 %)
ECOG PS	
0	150 (72.8 %)
1	56 (27.2 %)
Type of gastrectomy	
Distal	124 (60.2 %)
Total	82 (39.8 %)
Tumor histology	
Intestinal type	81 (39.3 %)
Diffuse type	123 (59.7 %)
Neuroendocrine cell carcinoma	1 (0.5 %)
Unknown	1 (0.5 %)

ECOG PS Eastern Cooperative Oncology Group performance status

[a] Japanese Classification of Gastric Cancer 13th edition

grade 4 adverse events included neutropenia (10.7 %), diarrhea (5.9 %), mechanical ileus (4.1 %), leukopenia (2.6 %), anorexia (2.6 %), anemia (2.2 %), skin rash (2.2 %), and stomatitis (2.2 %). S-1 treatment was continued for at least 3 months in 183 patients (88.8 %), for at least 6 months in 154 patients (74.8 %), for at least 9 months in 139 patients (67.0 %), and for 12 months in 99 patients (48.1 %). The dose of S-1 was decreased in 76 (36.9 %) of the 206 patients who received the protocol treatment. Of the 99 patients who received the treatment for 12 months, the dose was decreased in 41 patients (41.4 %).

OS and RFS

On the basis of follow-up data updated as of December 31, 2013, the median follow-up from the time of surgery was 3.2 years in the 206 patients. Forty-seven patients had died. The causes of death were relapse in 39 patients, other cancer in two patients, causes other than cancer in four patients, and unknown causes in two patients. Recurrent diseases occurred in 51 patients. The OS and RFS rates in the 206 patients were 96.1 % and 86.8 %, respectively, at 1 year, 87.7 % and 76.9 %, respectively, at 2 years, and 80.6 % and 71.9 %, respectively, at 3 years.

Messenger RNA levels of TS, DPD, TP, and OPRT

Gene expression levels of TS, DPD, TP, and OPRT were successfully measured in these 206 tumors. However, mRNA levels of none of these biomarkers correlated with the GIR induced by 5-FU in each of four different 5-FU concentrations (data not shown).

Association between in vitro sensitivities to 5-FU and survival of patients who received S-1 treatment

One of the main purposes of this study was to investigate appropriate cutoff values for classifying patients as likely responders or nonresponders. As described in "Materials and methods," four different in vitro 5-FU concentrations, which were comparable to clinically achievable plasma 5-FU concentrations, were used to assess the in vitro sensitivity of tumor cells to 5-FU in the CD-DST. The correlation between in vitro chemosensitivity and survival outcome after a 3-year follow-up period is summarized with the forest plot in Fig. 3 in relation to the HR, 3-year RFS, and log-rank *P* value between the responder and nonresponder groups. As shown in Fig. 3, an HR of less than 0.4 with narrow 95 % confidence intervals (CIs) and significant *P* values strongly suggested that appropriate cutoff values for dividing patients into responders and nonresponders were in vitro GIRs of 20–30 % at an in vitro 5-FU concentration of 0.2 μg/ml, 30–40 % at 0.4 μg/ml, 50–60 % at 1.0 μg/ml, and 60–70 % at 2.0 μg/ml. These results indicated that the appropriate cutoff values are substantially influenced by the in vitro drug concentration and can be defined in some, albeit not narrow, ranges.

When these cutoff values were applied, as shown in Fig. 3, responders had significantly better survival than nonresponders for each of the four different in vitro 5-FU

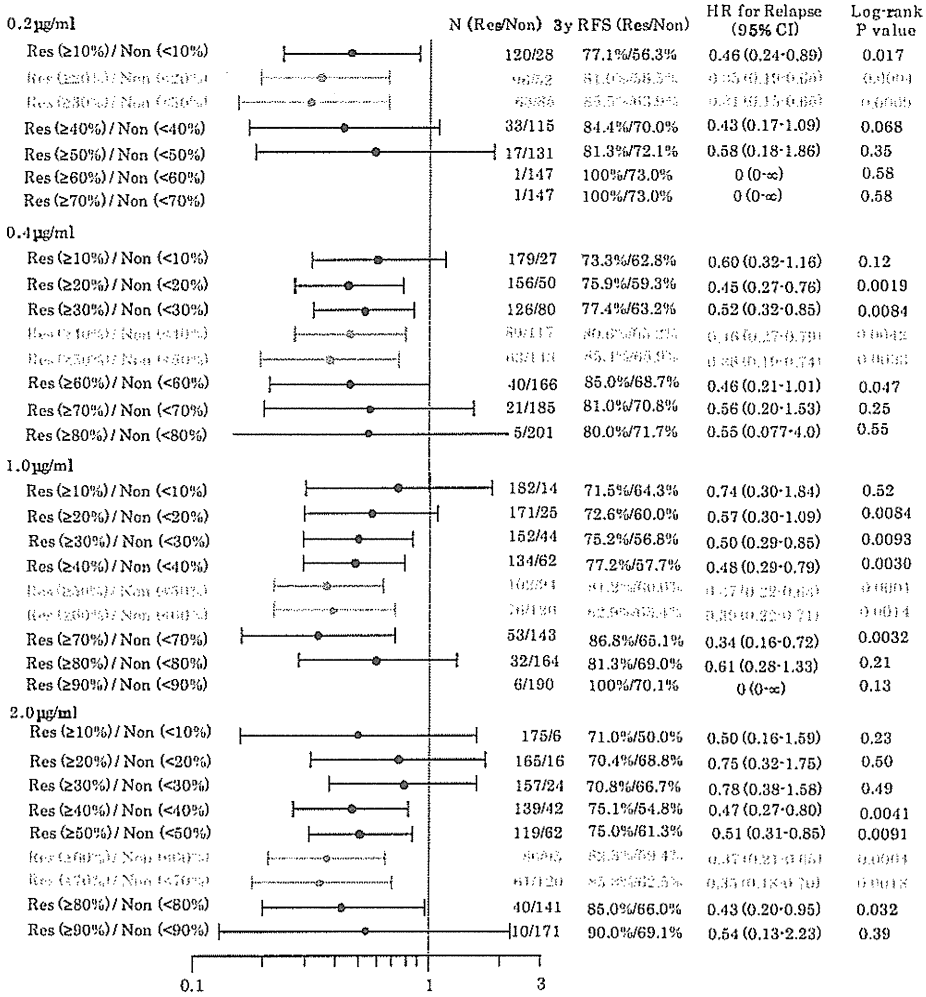

Fig. 3 Forest plot to identify appropriate cutoff values for each of the four in vitro 5-fluorouracil concentrations for a total of 206 patients. *CI* confidence interval, *HR* hazard ratio, *Non* nonresponders, *Res* responders, *RFS* relapse-free survival

concentrations, whereas no significant differences were observed in background clinical characteristics, except for age, between the responder and nonresponder groups. As an example, when an in vitro GIR of 60 % at an in vitro 5-FU concentration of 1.0 μg/ml was applied as a cutoff value, the HR for tumor relapse in the 76 responders, compared with the 120 nonresponders, was 0.39 (95 % CI 0.22–0.71; $P = 0.0014$). The 3-year RFS rate was 82.9 % (95 % CI 74.4–91.3 %) in the responder group and 63.4 % (95 % CI 54.7–72.1 %) in the nonresponder group (Fig. 4), whereas there were no significant differences in background clinical characteristics, except for age, between the responder and nonresponder groups as in Table 2. In addition, as indicated in Table 2, there was no significant difference in relapse sites between the two groups.

The HR for tumor relapse of responders compared with nonresponders was 0.24 (95 % CI 0.08–0.68) in 113 patients with N0 or N1 lymph node metastasis and 0.58

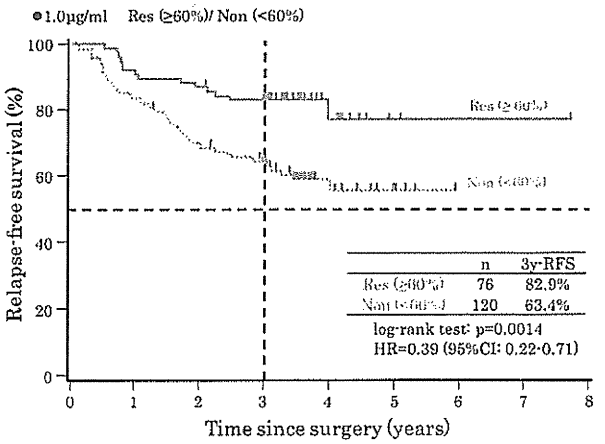

Fig. 4 Relapse-free survival (*RFS*) of responder (*Res*) and nonresponder (*Non*) groups classified by a growth inhibition rate of 60 % at an in vitro 5-fluorouracil concentration of 1.0 μg/ml. *CI* confidence interval, *HR* hazard ratio

Table 2 Comparison of background clinical characteristics of responders and nonresponders classified by a growth inhibition rate of 60 % at a 5-fluorouracil concentration of 1.0 μg/ml

Characteristic	Responders ($n = 76$)	Non responders ($n = 120$)	P
Gender			0.09
Male	61 (80.3 %)	83 (69.2 %)	
Female	15 (19.7 %)	37 (30.8 %)	
Age	62.5 years (32–78 years)	67.0 years (33–79 years)	0.01
Cancer stage[a]			0.54
II	14 (18.4 %)	23 (19.2 %)	
III	56 (73.7 %)	92 (76.7 %)	
IV	6 (7.9 %)	5 (4.2 %)	
Tumor stage[a]			0.42
T1	0 (0 %)	2 (1.7 %)	
T2	22 (28.9 %)	27 (22.5 %)	
T3	47 (61.8 %)	83 (69.2 %)	
T4	7 (9.2 %)	8 (6.7 %)	
N stage[a]			0.95
N0	4 (5.3 %)	7 (5.8 %)	
N1	38 (50.0 %)	64 (53.3 %)	
N2	31 (40.8 %)	45 (37.5 %)	
N3	3 (3.9 %)	4 (3.3 %)	
Type of lymph node dissection[a]			0.29
D2	71 (93.4 %)	116 (96.7 %)	
D3	5 (6.6 %)	4 (3.3 %)	
ECOG PS			0.18
0	51 (67.1 %)	91 (75.8 %)	
1	25 (32.9 %)	29 (24.2 %)	
RDI[b]	70.2 %	64.3 %	0.29
	(0.9–186 %)	(0.4–119 %)	
Type of gastrectomy			0.80
Total	47 (61.8 %)	72 (60.0 %)	
Distal	29 (38.2 %)	48 (40.0 %)	
Tumor histology			0.54
Intestinal type	27 (35.5 %)	52 (43.3 %)	
Diffuse type	48 (63.2 %)	67 (55.8 %)	
Unknown	1 (1.3 %)	1 (0.8 %)	
Sites of relapse[c]	$n = 12$	$n = 43$	
Local	0 (0 %)	5 (11.6 %)	0.22
Peritoneum	4 (33.3 %)	19 (44.2 %)	0.50
Liver	4 (33.3 %)	11 (25.6 %)	0.59
Distant	4 (33.3 %)	7 (16.3 %)	0.19
Lymph node	4 (33.3 %)	6 (14.0 %)	0.12

There were no significant differences in the background clinical characteristics, except for age, between the responder and nonresponder groups, even when classified by any other defined cutoff values (data not shown)

ECOG PS Eastern Cooperative Oncology Group performance status, *RDI* relative dose intensity

[a] Japanese Classification of Gastric Cancer 13th edition

[b] RDI = actual intake of doses/total protocol doses of S-1 for 1 year (%)

[c] Some patients had plural relapses

(95 % CI 0.25–1.23) in 83 patients with N2 or N3 lymph node metastasis. It was 0.18 (95 % CI 0.00–1.01) in 47 patients with stage II disease and 0.38 (95 % CI 0.18–0.74) in 148 patients with stage III disease.

Discussion

The CD-DST is a chemosensitivity test wherein isolated tumor cells are embedded in collagen droplets. This three-dimensional culture system has the following advantages over other conventional methods such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [33] and ATP [34] assays: the ability to use small specimens, the ability to assess the effect of anticancer drugs at physiological concentrations, and the ability to eliminate the masking effect caused by fibroblast contamination in culture with the aid of an image analysis system [26, 35].

The efficacy of the CD-DST in cancer treatment has previously been demonstrated in various malignant human tumors, including gastric cancer [18, 19] and other malignancies [20–24]. However, as recent controversial discussion on chemosensitivity testing for human tumor specimens has indicated [36–42], the studies with the CD-DST also had significant limitations, including small sample sizes, the lack of prospective studies, and the lack of clear cutoff values to distinguish chemotherapy sensitivity from resistance. Accordingly, we conducted this exploratory phase II trial in a multicenter setting to evaluate the clinical value of chemosensitivity testing for 5-FU in patients who received S-1 postoperatively. Our main goal was to verify whether survival is better in patients whose tumors are sensitive to 5-FU in vitro than in those with tumors insensitive to 5-FU in vitro, when appropriate cutoff values to classify the patients as responders and nonresponders were defined.

As one of the major findings of the present study, in vitro chemosensitivity testing of gastric cancer samples using the CD-DST proved to be a feasible method and yielded a success rate of 76 % (206 of 270 samples). Major reasons for unsuccessful assessment of the remaining 64 samples included insufficient number of tumor cells for assay (30 samples) and bacterial contamination (29 samples), as shown in Fig. 2. Both problems may possibly be attributed to the limitations arising from a multicenter setting, such as the inconsistent manner of the handling of tumor samples or the time to transport samples to the laboratory. As a result, there still remains some room for improvement of these technical issues. Also, the test results were obtained within 7 days in all cases, suggesting that the CD-DST may be a useful method in prospective studies to evaluate the clinical significance of sensitivity-test-guided chemotherapy in an adjuvant setting.

As one of accompanying studies in this trial, mRNA expression levels of TS, DPD, TP, and OPRT were quantified by reverse transcription PCR by use of prepared fresh tumor cells. No correlation was found between the mRNA expression of those enzymes and in vitro 5-FU sensitivity, suggesting that it is not possible to predict 5-FU sensitivity solely on the basis of gene expression of the enzymes considered in this study.

Our second finding of this trial was that appropriate cutoff values classifying patients as responders or nonresponders were able to be defined by the calculated HR for tumor relapse and log-rank *P* values. The 3-year RFS rate was significantly better in the responder group than in the nonresponder group when the defined appropriate cutoff values for each in vitro 5-FU concentration were applied. The cutoff values of 50–60 % at a 5-FU concentration of 1.0 μg/ml were already used in previously published reports [18, 19, 21], in which those values were retrospectively determined. Our results verify the finding of the previous studies that there is a direct association between in vitro sensitivity and therapy outcome.

The primary end point of this study was 3-year RFS, the same as in the CLASSIC trial, which was an adjuvant chemotherapy study recently conducted in South Korea [43]. The CLASSIC trial successfully demonstrated a significant survival benefit from adjuvant capecitabine and oxaliplatin chemotherapy compared with surgery alone in patients with stage II–IIIB gastric cancer after D2 surgery. Additionally, in ACTS-GC, whose primary end point was 5-year OS, the 3-year RFS rates were 72.4 and 61.1 % and the 5-year OS rates were 71.7 and 61.1 % in the S-1 group and the surgery-only group, respectively. These findings, in addition to the results of this study, may justify the currently controversial use of the 3-year RFS as the primary end point in clinical trials of adjuvant chemotherapy for potentially curable gastric cancer.

Since the definition of RFS is crucial and very delicate in this set of patients, the follow-up method used was the same as that in ACTS-GC. The absolute number of patients whose relapse was firstly identified was 23 during the first 1 year, 20 between 1 and 2 years, and 8 between 2 and 3 years after surgery in this study, meaning that most of the recurrence occurred within 2 years after surgery. This seems to justify the follow-up method used in the current study.

There were no significant differences between the responder and nonresponder groups in the background clinical characteristics, except for age. The responder group had younger patients than the nonresponder group. However, as also demonstrated in Table 2, the relative dose intensity was almost comparable between these two groups. As a result, the better survival in responders did not seem to be explained by S-1 treatment compliance.

The subset analysis of the HR for tumor relapse of responders compared with nonresponders with respect to tumor stages and lymph node metastases suggested a tendency for a more favorable effect of S1 treatment on patients with an earlier stage of tumor development and of extent of lymph node metastasis, as indicated by ACTS-GC. However, this was not definitely confirmed in this study, probably because of insufficient number of enrolled patients.

In conclusion, to the best of our knowledge, the present phase II study conducted in a multicenter setting is the first large clinical trial to evaluate prospectively the clinical significance of chemosensitivity testing in patients with gastric cancer. Use of the CD-DST may contribute to the proper selection of candidates for chemotherapy and may aid in the reduction of unnecessary adverse events in patients insensitive to 5-FU. This encouraging finding needs further evaluation in a randomized controlled phase III trial to prove that sensitivity-test–guided chemotherapy may provide greater survival benefit than conventional empirical chemotherapy in patients with locally advanced gastric cancer in an adjuvant setting.

Acknowledgments The present study was started by an initial principal investigator, Tetsuro Kubota, late professor of Keio University School of Medicine, Tokyo. Since he passed away halfway through this study in January 2009, all participating members have agreed to call this study the "Kubota memorial trial" in appreciation of his great contribution to the research field of anticancer drug sensitivity.

Conflict of interest The following authors have a financial or other interest that is relevant to the subject matter considered in this article:*Consultant or advisory role*: Masashi Fujii, Taiho Pharmaceutical; Toshifusa Nakajima, Taiho Pharmaceutical; Yuh Sakata, Taiho Pharmaceutical, Otsuka Holdings. *Stock ownership*: Masashi Fujii, Otsuka Holdings. *Honorarium*: Nobuhiko Tanigawa, Taiho Pharmaceutical; Hiroki Yamaue, Taiho Pharmaceutical; Shinichi Sakuramoto, Taiho Pharmaceutical; Takao Inada, Taiho Pharmaceutical; Yasuhiro Kodera, Taiho Pharmaceutical; Yuko Kitagawa, Taiho Pharmaceutical; Yuh Sakata, Taiho Pharmaceutical; Atsuhi Nashimoto, Taiho Pharmaceutical; Toshiharu Yamaguchi, Taiho Pharmaceutical; Toshifusa Nakajima, Taiho Pharmaceutical. *Research funding*: Hiroki Yamaue, Taiho Pharmaceutical; Yasuhiro Kodera, Taiho Pharmaceutical; Yuko Kitagawa, Taiho Pharmaceutical; Yuh Sakata, Taiho Pharmaceutical.

Appendix

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REVIEW ARTICLE

Gastric adenocarcinoma with para-aortic lymph node metastasis: a borderline resectable cancer?

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Abstract Dissection of the para-aortic lymph nodes (PAN) had once been enthusiastically explored at dedicated centers throughout Japan. Reflecting the results of a randomized trial, however, the current standard surgery for advanced resectable gastric cancer does not include systematic dissection of the PAN. Gastric cancer with PAN metastases, currently considered distant metastases, is classified as Stage IV, and according to the algorithm of the Japanese guidelines, is not indicated for surgery with curative intent. Historical data indicates, however, that a certain proportion of long-term survivors can be introduced among patients with PAN metastasis through D2 + PAN dissection. The Japan Clinical Oncology Group launched a series of phase II trials exploring a strategy employing neoadjuvant chemotherapy followed by D2 + PAN dissection for patients radiologically diagnosed to harbor metastases to the PAN. The campaign was successful, with 57 % of these patients surviving for 5 years after two cycles of neoadjuvant S-1/CDDP followed by surgery. This strategy is now the tentative standard, mentioned in the 4th version of the Japanese Gastric Cancer Treatment Guidelines as one of the current clinical questions, and could be replaced by a more powerful combination chemotherapy or treatment employing more or longer cycles of chemotherapy in the future. The relevance of the strategy consisting of neoadjuvant chemotherapy followed by D2 + PAN dissection and its fundamental difference from the concept of conversion therapy are discussed herein with reference to the literature.

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Keywords Gastric cancer · Para-aortic lymph nodes · Lymphadenectomy · Neoadjuvant chemotherapy

Introduction

With the advances in surgical and imaging techniques, a distinct subset of pancreatic cancer has emerged that blurs the borderline between resectable and locally advanced disease: cancers preoperatively suspected to have invaded major vessels, such as the portal vein, hepatic artery and superior mesenteric artery [1]. Although macroscopically complete resection is occasionally possible, these cases often end up with a microscopically positive resection margin that virtually nullifies the expected survival benefit of radical surgery. Thus, neoadjuvant chemo- or chemoradiotherapy is often proposed for borderline resectable pancreatic cancer, both in the hope of down-sizing the tumor and for the selection of patients who do not progress during the preoperative therapy [1]. In the current article, a proposal will be made to define gastric cancer suspected of harboring metastasis to the para-aortic lymph node as borderline resectable gastric cancer in the sense that, although deemed unresectable according to the current stage classifications and core parts of the treatment guidelines, there remain some hopes for a cure if adequate multidisciplinary treatment, including radical surgery, is administered. This proposal has been mentioned in the 4th edition of the Japanese Gastric Cancer Treatment Guidelines, not in the text, but as an answer to one of the selected clinical questions.

The outcome of unresectable gastric cancer, a commonly encountered disease worldwide, is at times as dismal as that of pancreatic cancer. It has a tendency to metastasize through various pathways and involve multiple organs [2], often developing into a disseminated disease even if

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ORIGINAL ARTICLE

Clinicopathological factors associated with HER2 status in gastric cancer: results from a prospective multicenter observational cohort study in a Japanese population (JFMC44-1101)

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Abstract

Background Human epidermal growth factor (HER) 2 positivity and its association with clinicopathological factors remain unclear in Japanese gastric cancer (GC) patients. We performed a prospective, multicenter, observational cohort study to evaluate HER2 protein expression and gene amplification in Japanese metastatic and recurrent GC patients, and explored its correlations with clinicopathological features.

Methods HER2 protein expression and gene amplification were centrally assessed in formalin-fixed, paraffin-embedded GC tissue by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Patient information was collected, and associations between clinicopathological factors and HER2 positivity (IHC score 3+ and/or FISH positive) and low HER2 expression (IHC score 0/FISH positive or IHC score 1+/FISH positive) were examined.

Results From September 2011 to June 2012, 1461 patients were registered across 157 sites, and the HER2 status of 1427 patients was evaluated. The rate of HER2 positivity was 21.2 %, whereas the rate of high HER2 expression (IHC score 2+/FISH positive or IHC score 3+) was 15.6 % and that of low HER2 expression was 7.0 %. Multiple logistic regression analysis identified intestinal type, absence of peritoneal metastasis, and hepatic metastasis as significant independent factors related to HER2 positivity. The intestinal type was confirmed to be the GC subtype predominantly associated with lower HER2 expression. Sampling conditions including number of biopsy samples, formalin concentration, and formalin-fixation time did not significantly affect HER2 positivity.

Conclusions HER2 expression in Japanese patients was comparable to that in other populations examined. Intestinal type was an independent factor related to HER2 positivity and low HER2 expression.

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ORIGINAL ARTICLE

Long-term quality-of-life comparison of total gastrectomy and proximal gastrectomy by Postgastrectomy Syndrome Assessment Scale (PGSAS-45): a nationwide multi-institutional study

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Abstract
Background Although proximal gastrectomy (PG) is widely accepted as a function-preserving operation for early upper-third gastric cancer, postoperative disorders, such as reflux or gastric stasis, have often been pointed out. From the perspective of postoperative disorder, the choice of total gastrectomy (TG) or PG for such cancers is still controversial. By using the newly developed Postgastrectomy Syndrome Assessment Scale (PGSAS)-45, the quality of life after TG and PG was compared.
Methods The PGSAS-45 consists of 45 items composed of the SF-8 and GSRS scales and 22 new items. The main outcomes are measured by seven subscales (SS) covering symptoms, physical and mental component summary (SF-8), meals (amount and quality), ability to work, dissatisfaction for daily life, and change in body weight. A total of 2,368 eligible questionnaires were acquired from 52 institutions. From these, 393 patients with TG and 193 patients with PG were selected and compared.
Results The PG was better than TG in terms of body weight loss (TG 13.8 % vs. PG 10.9 %; $p = 0.003$), necessity for additional meals (2.4 vs. 2.0; $p < 0.001$), diarrhea SS (2.3 vs. 2.0; $p = 0.048$), and dumping SS (2.3 vs. 2.0; $p = 0.043$). There were no differences in the other main outcome measures.
Conclusions Proximal gastrectomy appears to be valuable as a function-preserving procedure for early upper-third gastric cancer.

Keywords Proximal gastrectomy · Total gastrectomy · Postgastrectomy syndrome · Quality of life · Stomach cancer

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Introduction

Gastric cancer remains the second leading cause of cancer death in the world and is the most frequent malignancy in Japan, South America, and Eastern Europe [1, 2]. Long-term survivors after radical gastrectomy have been increasing as the result of better early detection and improved surgical techniques [3–5]. The better surgical outcome has led to greater interest in the quality of life (QOL) of gastrectomized patients. For prevalence of postgastrectomy disorder, the procedures used in gastrectomy for early gastric cancer are designed as function-preserving operations or various reconstructions to restore postoperative QOL [6]. Although the postgastrectomy disorders greatly influence the living condition (QOL) of gastrectomized patients, there are limits to evaluation of outpatients because of the difficulty in measuring subjective and physical symptoms. In recent years, questionnaires have been developed to create objective rating systems for QOL [7–11]. The Japan Postgastrectomy Syndrome Working Party was founded in order to investigate symptoms and lifestyle changes among patients who have undergone gastrectomy. This Working Party collaboratively developed a questionnaire to evaluate the symptoms, i.e., living status and QOL, among gastrectomized patients. Using this questionnaire, a nationwide, multi-institution surveillance study was performed.

The frequency of cancers in the upper third of the stomach and gastroesophageal junction has been increasing in both Western and Asian countries [12–15]. Total gastrectomy (TG) and proximal gastrectomy (PG) are operative options for proximal gastric cancer. In PG, the gastric fundic gland region is kept, and gastric-acid secretion and Castle intrinsic factor are maintained, but patients often suffer from reflux or gastric stasis. The choice of TG or PG has been discussed from the viewpoint of postoperative disorders, especially reflux esophagitis and nutrition. By using the newly developed Postgastrectomy Syndrome Assessment Scale (PGSAS-45), QOL after TG and PG for gastric cancer was compared.

Methods

Patients

Fifty-two institutions participated in this study. The PGSAS-45 questionnaire was distributed to 2,922 patients between July 2009 and December 2010. Of these forms, 2,520 (86.2 %) were retrieved, of which 152 were deemed ineligible because of patient age >75 years ($n = 90$), postoperative period <1 year ($n = 29$), co-resection of other organs ($n = 8$), and other factors ($n = 25$). As a result, 2,368 questionnaires (81 %) were decided as eligible for inclusion in various analyses related to the PGSAS-45. Of these, 393 patients who had undergone TG and 193 who had undergone PG were identified and retrieved for the current study (Fig. 1).

Patient eligibility criteria

Patient eligibility criteria were: (1) pathologically confirmed stage IA or IB gastric cancer; (2) first-time gastrectomy; (3) age ≥ 20 and ≤ 75 years; (4) no history of chemotherapy; (5) no known recurrence or distant metastasis; (6) gastrectomy conducted one or more years prior to the enrollment date; (7) performance status (PS) ≤ 1 on the Eastern Cooperative Oncology Group (ECOG) scale; (8) full capacity to understand and respond to the questionnaire; (9) no history of other diseases or operations that might influence the responses to the questionnaire; (10) no organ failure or mental illness; and (11) provision of written informed consent. Patients with dual malignancy or concomitant resection of other organs (with co-resection equivalent to cholecystectomy being the exception) were excluded.

QOL assessment

The PGSAS-45 is a newly developed, multidimensional QOL questionnaire (QLQ) based on the Short-Form Health Survey (SF-8) [16] and the Gastrointestinal Symptom Rating Scale (GSRS) [17–20]. The PGSAS-45 questionnaire consists of 45 questions, with eight items from the SF-8, 15 from the GSRS, and 22 clinically important items selected by the Japan Postgastrectomy Syndrome Working Party (Table 1). The PGSAS-45 questionnaire includes 23 items pertaining to postoperative symptoms (items 9–33), including 15 items from the GSRS and eight newly selected items. In addition, 12 questionnaire items pertaining to dietary intake, work, and level of satisfaction for daily life are included. Dietary intake items include five about the amount of food ingested (items 34–37 and 41) and three about the quality of ingestion (items 38–40). One questionnaire item pertains to work (item 42), while three address the level of satisfaction for daily life (items 42–45). For the 23 symptom items, a seven-grade (1–7) Likert scale is used. A five-grade (1–5) Likert scale is used for all other items except 1, 4, 29, 32, and 34–37. For items 1–8, 34, 35 and 38–40, higher scores indicate better conditions. For items 9–28, 30, 31, 33, and 41–45, higher scores indicate worse conditions. The main outcome measures were refined through consolidation and selection. Twenty-three symptom items were consolidated into seven symptom subscales by factor analysis, as listed in Tables 1 and 2. Assessment data include total symptom score, quality of ingestion subscale, level of satisfaction for daily life, physical component summary (PCS), and mental component

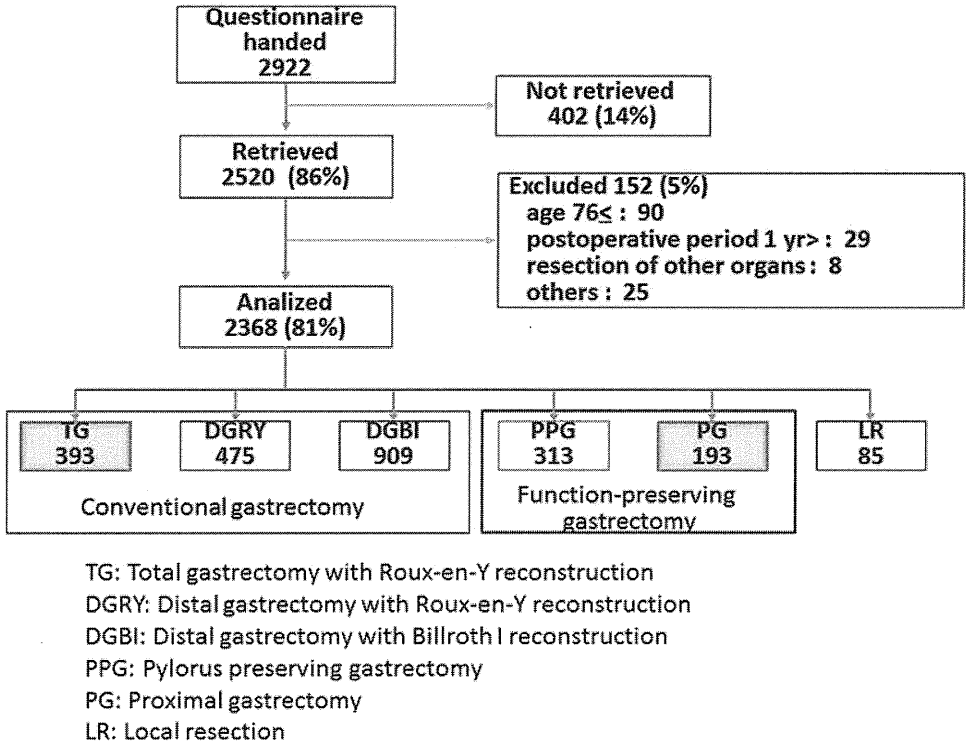

Fig. 1 Outline of the study

summary (MCS) of the SF-8 as main outcome measures. In addition, the following results were selected as main outcome measures: changes in body weight, amount of food ingested per meal, necessity for additional meals, ability to work, dissatisfaction with symptoms, dissatisfaction at the meal, and dissatisfaction at working. Each subscale score is calculated as the mean of composed items, and the total symptom score is calculated as the mean of seven symptom subscales (Table 2).

Study methods

This study utilized continuous sampling from a central registration system for participant enrollment. The questionnaire was distributed to all eligible patients as they presented to participating clinics. Patients were instructed to return completed forms to the data center. All QOL data from questionnaires were matched with individual patient data collected via case report forms.

This study was registered with the University Hospital Medical Information Network's Clinical Trials Registry (UMIN-CTR; registration number 000002116). It was approved by the ethics committees at all institutions. Written informed consent was obtained from all enrolled patients.

Statistics

In comparing patient QOLs after TG and PG, statistical methods included the t test and Chi square test. All outcome measures that exhibited significant difference in univariate analysis were further analyzed using multiple regression analysis. $p < 0.05$ was considered statistically significant. In the case of $p < 0.1$ by univariate analysis, Cohen's d was calculated. In the case of $p < 0.1$ in multiple regression analysis, standardization coefficient of regression (β), a decision coefficient (R^2), and the p value were calculated and shown in a table. Cohen's d, β, and R^2 measure effect sizes. Interpretation of effect sizes were 0.2 $\leq$ small, 0.5 $\leq$ medium, and 0.8 $\leq$ large in Cohen's d; 0.1 $\leq$ small, 0.3 $\leq$ medium, and 0.5 $\leq$ large in β; and 0.02 $\leq$ small, 0.13 $\leq$ medium, and 0.26 $\leq$ large in R^2.

StatView software for Windows Ver. 5.0 (SAS Institute Inc.) was used for all statistical analyses.

Results

Patient characteristics

Background data of both groups of patients are shown in Table 3. Reconstruction procedures were not regulated by the protocol, and depended on the principle of the institution or discretion of each surgeon. Consequently, whereas all patients treated by TG (393 patients) underwent Roux en Y reconstruction, the reconstruction after PG (193 patients) was varied and consisted of gastro-esophagostomy (115 patients), jejunal interposition (34 patients), and jejunal pouch interposition (44 patients).

Table 1 Structure of PGSAS-45

Domains	Subdomains	Items		Subscales
QOL	SF-8 (QOL)	1 Physical functioning*	Five-point or six-point Likert scale	Physical component summary*
		2 Role physical*		Mental component summary*
		3 Bodily pain*		
		4 General health*		
		5 Vitality*		
		6 Social functioning*		
		7 Role emotional*		
		8 Mental health*		
Symptoms	GSRS (symptoms)	9 Abdominal pains	Seven-point Likert scale except items 29 and 32	Esophageal reflux subscale (items 10, 11, 13, 24)
		10 Heartburn		Abdominal pain subscale (items 9, 12, 28)
		11 Acid regurgitation		Meal-related distress subscale (items 25–27)
		12 Sucking sensations in the epigastrium		Indigestion subscale (items 14–17)
		13 Nausea and vomiting		Diarrhea subscale (items 19, 20, 22)
		14 Borborygmus		Constipation subscale (items 18, 21, 23)
		15 Abdominal distension		Dumping subscale (items 30, 31, 33)
		16 Eructation		
		17 Increased flatus		Total symptom scale (above seven subscales)
		18 Decreased passage of stools		
		19 Increased passage of stools		
		20 Loose stools		
		21 Hard stools		
		22 Urgent need for defecation		
		23 Feeling of incomplete evacuation		
	Symptoms	24 Bile regurgitation		
		25 Sense of foods sticking		
		26 Postprandial fullness		
		27 Early satiation		
		28 Lower abdominal pains		
		29 Number and type of early dumping symptoms		
		30 Early dumping general symptoms		
		31 Early dumping abdominal symptoms		
		32 Number and type of late dumping symptoms		
		33 Late dumping symptoms		

Table 1 continued

Domains	Subdomains	Items	Subscales
Living status	Meals (amount) 1	34 Ingested amount of food per meal*	–
		35 Ingested amount of food per day*	
		36 Frequency of main meals	
		37 Frequency of additional meals	
	Meals (quality)	38 Appetite*	Five-point Likert scale
		39 Hunger feeling*	Quality of ingestion subscale* (items 38–40)
		40 Satiety feeling*	
	Meals (amount) 2	41 Necessity for additional meals	–
	Social activity	42 Ability for working	–
QOL	Dissatisfaction (QOL)	43 Dissatisfaction with symptoms	Dissatisfaction for daily life subscale (items 43–45)
		44 Dissatisfaction at the meal	
		45 Dissatisfaction at working	

In items or subscales with * higher score indicates better condition. In items or subscales without * higher score indicates worse condition. Each subscale is calculated as the mean of composed items or subscales (except PCS and MCS of SF-8). Items 29 and 32 do not have score. Therefore, they were analyzed separately

Table 2 Domains and main outcome measures

Domains/subdomains		Main outcome measures
Symptoms	Subscales	Seven symptom subscales
		Esophageal reflux (10, 11, 13, 24), *abdominal pain* (9, 12, 28), *meal-related distress* (25–27), *indigestion* (14–17), *diarrhea* (19, 20, 22), *constipation* (18, 21, 23), *dumping* (30, 31, 33)
	Total	*Total symptom score*
Living status	Body weight	Change in body weight (%)*
	Meals (amount)	Ingested amount of food per meal* (34)
		Necessity for additional meals (41)
	Meals (quality)	*Quality of ingestion subscale** (38–40)
	Work	Ability for working (42)
QOL	Dissatisfaction	Dissatisfaction with symptoms (43), at the meal (44), at working (45)
		Dissatisfaction for daily life subscale (43–45)
	SF-8	*Physical component summary** (1–5)
		*Mental component summary** (4–8)

Main outcome measures that are italicized are composed of more than two items. In items or subscales with *, higher score indicates better condition; in items or subscales without *, higher score indicates worse condition. Each subscale is calculated as the mean of composed items or subscales

In the PG group, the mean postoperative period was significantly longer (TG 35.0 ± 24.6 months vs. PG 40.5 ± 28.1 months; $p = 0.0163$), and the rates of celiac and pyloric branch preservation were significantly higher, while the rates of laparoscopic approaches, D2 lymph node dissection, and combined resections were significantly lower than in the TG group.

QOL assessments

The results of the main outcome measures by univariate analysis are shown in Table 4. The body weight loss (TG 13.8 % vs. PG 10.9 %; $p = 0.0001$; Cohen's $d = 0.35$), diarrhea subscale (TG 2.3 vs. PG 2.0; $p = 0.0016$; Cohen's $d = 0.29$), and dumping subscale (TG 2.3 vs. PG 2.0; $p = 0.0118$; Cohen's $d = 0.24$) in the PG group were significantly lower than those in the TG group.

The necessity for additional meals was significantly lower in the PG group than in the TG group (TG 2.4 vs. PG 2.0; $p < 0.001$; Cohen's $d = 0.40$), which indicates a better status in the PG group. However, the constipation subscale value of the PG group was significantly higher than that of the TG group (TG 2.1 vs. PG 2.3; $p = 0.0145$; Cohen's $d = 0.21$), and the quality of ingestion subscale value of the PG group was significantly lower than that of

Table 3 Patient background and operative features

Type of gastrectomy	TG Mean (SD)	PG Mean (SD)	*p* value
Number of patients	393	193	
Postoperative period (months)	35.0 (24.6)	40.5 (28.1)	0.0163
Age	63.4 (9.2)	63.7 (7.7)	>0.1
Sex (male/female)	276/113	139/53	>0.1
BMI (preoperative)	23.0 (3.3)	23.1 (3.0)	>0.1
Operation background			
Approach (laparoscopic/open)	97/293	33/159	0.0364
Celiac branch of vagus (preserved/divided)	12/371	83/105	<0.0001
Pyloric branch of vagus (preserved/divided)	4/379	120/62	<0.0001
Extent of lymph node dissection			<0.0001
D2	164	7	
D1b	192	93	
D1a	28	72	
D1	4	7	
D1>	0	6	
None	0	0	
Combined resection			<0.0001
Cholecystectomy	83	14	
Splenectomy	52	2	
Others	2	1	
None	246	162	

TG Roux en Y reconstruction (n = 393); *PG* Gastro-esophagostomy (n = 115), Jejunal interposition (n = 34), Jejunal pouch interposition (n = 44)

Table 4 Main outcome measures by univariate analysis

Measure	TG		PG		Cohen's *d*	*p* value
	Mean	SD	Mean	SD		
Change in body weight*	−13.80 %	7.90 %	−10.90 %	8.20 %	0.35	0.0001
Esophageal reflux subscale	2.0	1.0	2.0	1.0		>0.1
Abdominal pain subscale	1.8	0.8	1.7	0.7		>0.1
Meal-related distress subscale	2.6	1.1	2.6	1.1		>0.1
Indigestion subscale	2.3	0.9	2.2	0.8		>0.1
Diarrhea subscale	2.3	1.2	2.0	1.0	0.29	0.0016
Constipation subscale	2.1	0.9	2.3	1.1	0.21	0.0145
Dumping subscale	2.3	1.1	2.0	1.0	0.24	0.0118
Total symptom score	2.2	0.7	2.1	0.7		>0.1
Ingested amount of food per meal*	6.4	1.9	6.5	1.9		>0.1
Necessity for additional meals	2.4	0.8	2.0	0.8	0.40	<0.0001
*Quality of ingestion subscale**	3.8	0.9	3.6	1.0	0.20	0.0281
Ability for working	2.0	0.9	2.0	0.9		>0.1
Dissatisfaction with symptoms	2.1	1.0	2.0	0.9		>0.1
Dissatisfaction at the meal	2.8	1.1	2.7	1.1		>0.1
Dissatisfaction at working	2.1	1.1	2.0	1.1		>0.1
Dissatisfaction for daily life subscale	2.3	0.9	2.2	0.9		>0.1
*Physical component summary**	49.6	5.6	49.5	6.1		>0.1
*Mental component summary**	49.2	6.0	49.0	6.0		>0.1

Integrated subscales are italicized in the table

For outcome measures with * higher score indicates better condition; for outcome measures without * higher score indicates worse condition

the TG group (TG 3.8 vs. PG 3.6; $p = 0.0281$; Cohen's $d = 0.20$), both of which indicate worse status of the PG group.

The physical and mental component summaries were not different in the two groups.

To eliminate confounding factors, multiple regression analysis was performed by adding postoperative period, age, sex, surgical approach, and celiac branch of vagal nerve preservation as explanatory variables (Table 5). Although the effect size of the advantages in PG over TG is relatively small, comparing the type of gastrectomy, the PG group was better than the TG group in body weight loss ($\beta = 0.148$; $p = 0.003$), diarrhea ($\beta = 0.097$; $p = 0.048$), dumping ($\beta = 0.106$; $p = 0.043$), and necessity for additional meals ($\beta = 0.192$; $p < 0.001$). Constipation and quality of ingestion, which were worse in the PG group by univariate analysis, showed no difference by multivariate analysis.

Multiple regression analysis revealed that the postoperative period influenced the extent of body weight loss ($\beta = 0.097$; $p = 0.030$), diarrhea ($\beta = -0.076$; $p = 0.078$), and quality of ingestion ($\beta = 0.092$; $p = 0.0365$). This means that as the postoperative period lengthens, body weight loss and diarrhea improve.

The age influenced the constipation subscale ($\beta = 0.147$; $p = 0.001$), dumping ($\beta = -0.114$; $p = 0.010$), and the quality of ingestion ($\beta = -0.126$; $p = 0.034$). At older ages, although dumping decreased, constipation increased.

Diarrhea was often found in men ($\beta = 0.137$; $p = 0.001$), and surgical approach and celiac branch preservation had little influence on any of the main outcome measures by multiple regression analysis.

Discussion

Optimal evaluation methods for postgastrectomy disorders are important for selecting and improving the operative procedures and maintaining the high QOL for gastric cancer patients [21–23]. The Japan Postgastrectomy Syndrome Working Party developed a questionnaire to evaluate general features; i.e., symptoms, living status, and QOL, among gastrectomized patients. Using this questionnaire, a nationwide, multi-institution surveillance study was performed. This was the first nationwide survey of its type and involved 52 medical institutions throughout Japan. The necessary QOL data were collected from 2,520 patients, and the final sample size, following exclusion and participant selection, was sufficient for statistical validity of this type of study.

In recent years, a tendency to increasing numbers of proximal gastric cancers has been reported, and early detection and potentially curative operations by PG for upper-third gastric cancers have been increasing [24, 25].

Table 5 Main outcome measures by multivariate analysis

Measure	Type of gastrectomy (TG)		Postoperative period		Age		Gender (male)		Approach (laparoscopic)		Celiac branch of vagus (preserved)		R^2	p value
	β	p value	β	p value	β	p value	β	p value	β	p value	β	p value		
Change in body weight	−0.148	0.003	0.097	0.030		>0.1		>0.1		>0.1		>0.1	0.037	0.0024
Diarrhea subscale	0.097	0.048	−0.076	0.078		>0.1	0.137	0.001		>0.1		>0.1	0.045	0.0002
Constipation subscale	−0.086	0.081		> 0.1	0.147	0.001		>0.1		>0.1		>0.1	0.030	0.0108
Dumping subscale	0.106	0.043		> 0.1	−0.114	0.010		>0.1		>0.1		>0.1	0.039	0.0027
Necessity for additional meals	0.192	0.0001		> 0.1	0.085	0.045		>0.1	0.083	0.058		>0.1	0.052	< 0.0001
*Quality of ingestion subscale**		>0.1	0.092	0.037	−0.126	0.003		>0.1		>0.1		>0.1	0.033	0.0056

Integrated subscales are italicized in the table

For outcome measures with * higher score indicates better condition; for outcome measures without * higher score indicates worse condition

If β is positive, the score of the outcome measure of the patients belonging to the category in brackets is higher in cases when the factor is a nominal scale, and the score of outcome measure of the patients with larger values is higher in cases when the factor is a numeral scale

In this study, the effect of tumor progression was removed by constraining patient selection to those with pathologic Stage IA/IB disease, and it is thought that accurate QOL comparison between operative procedures is possible under these circumstances. Although QOL scores usually depend on the time after surgery, Kobayashi et al. [11] reported that the QOL after gastrectomy was impaired during a few months after surgery, but more or less stabilized at around 6 months after surgery. This is the reason that, in this nationwide survey, we chose to evaluate patients who had lived for 12 months or more after surgery. In addition, we used multiple regression analysis with time relapse after surgery as one of variables so as to adjust this problem.

Whereas the reconstruction for TG was only by the Roux-en-Y method, the reconstructions of PG could be by esophagogastrostomy, jejunal interposition, and jejunal pouch interposition [6]. Because the best reconstruction for PG has not yet been established, various procedures are performed. However, as the gastric fundic gland region is preserved in PG, gastric-acid secretion and production of Castle intrinsic factor and ghrelin, a gut hormone known increase to appetite, are maintained [26, 27].

In the PG group, the rates of celiac and pyloric branch vagal nerve preservation were significantly higher, and the rates of laparoscopic approaches, D2 lymph node dissection, and combined resection were significantly lower than in the TG group. Standard TG is composed of more D1b dissection and sacrifice of the vagal nerve, often with combined resection, such as of the spleen and gallbladder [6, 28]. On the other hand, PG, which is a function-preserving operation, usually consists of less than D1b dissection and preservation of the vagal nerve [6]. The differences in the surgical background are caused by the procedure itself. Therefore, there seems to be no problem in comparing the QOL scores of these two groups.

From the results of the main outcome measures by univariate and multivariate analysis, body weight loss, diarrhea, dumping, and necessity for additional meals were significantly lower in the PG than in the TG group. Although esophageal reflux is common after PG [29, 30], various reconstruction methods have recently been described that reduce this problem [31, 32]. In this study, there was no difference in the esophageal reflux subscale values between the groups. This result suggests that PG is not necessarily disadvantageous with regard to reflux.

As three types of reconstruction with various modifications were performed with PG reconstruction, it is necessary to compare the three procedures in future studies. Dumping symptoms, such as early dumping with systemic symptoms, early dumping with abdominal symptoms, and late dumping, were examined in detail. Late dumping was significantly less common in the PG than in the TG group. Also, a tendency toward less early dumping with abdominal symptoms was seen in the PG group (data not shown). As a result, PG performed well on the dumping subscale. Although PG reflected the storage capacity and pylorus-preserving function, in TG, solid food is passed rapidly to the jejunum because of no storage ability [33].

Although the constipation subscale results and quality of ingestion subscale values were worse with PG than with TG by univariate analysis, multivariable regression analysis revealed that there were no statistical differences in these subscales as the result of the type of gastrectomy. Body weight loss and quality of ingestion subscale improved if the postoperative period was long. This means that gastrectomized patients adapt in some ways to the anatomic changes over time, even after more than 1 year following gastrectomy.

Multivariable regression analysis showed that dumping decreased and constipation increased with advancing age. This result may reflect the known intestinal peristaltic decrease in older patients [34–37].

By multivariable regression analysis, men were more likely to have diarrhea than women. This may be a consequence of the fact that the intestinal transit time is longer in women than in men at equivalent ages [37–39]. As for the effect of the surgical approaches and celiac branch preservation, no differences were found by multivariable regression analysis.

There were no statistical differences between the groups with regard to ability to work, dissatisfaction with symptoms, dissatisfaction at working, dissatisfaction for daily life subscale, PCS, or MCS. It is suggested that daily life is largely unchanged and that statistically different post-gastrectomy disorders do not have a major effect on adaptation.

In conclusion, although the effect size of the advantages of PG over TG is relatively small, our results indicate that PG is useful as a function-preserving procedure for early upper-gastric cancer. Although this study is limited in that it is retrospective and examines a single time point, it suggests the value of PG, use of which should be encouraged. To confirm this conclusion, a randomized study to determine the most desirable reconstruction for PG to achieve a good long-term QOL will have to be conducted using the PGSAS-45 questionnaire and successive endoscopic examinations.

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