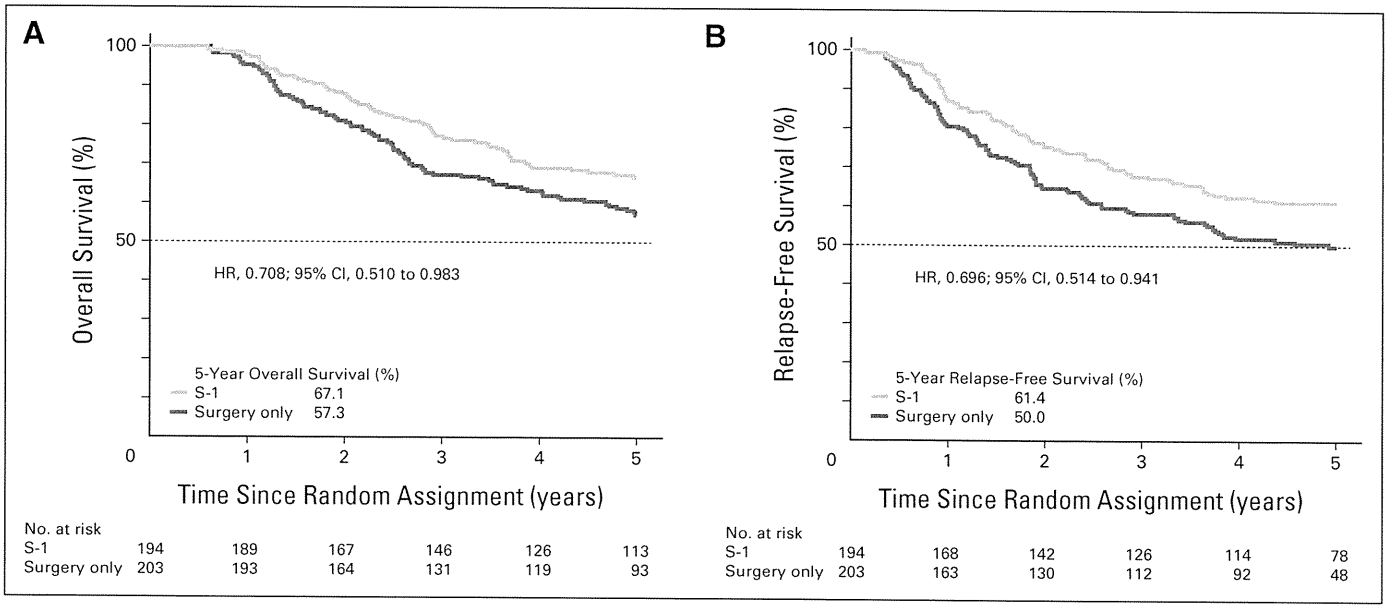

Fig 5. Kaplan-Meier estimates of (A) overall survival and (B) relapse-free survival for eligible patients with stage IIIA gastric cancer. HR, hazard ratio.

0.514 to 0.941; Fig 5B). As for stage IIIB disease, we enrolled 90 patients in the S-1 group and 85 in the surgery-only group; the 5-year OS rates were 50.2% (95% CI, 39.5% to 61.0%) in the S-1 group and 44.1% (95% CI, 33.1% to 55.0%) in the surgery-alone group, with an HR of 0.791 (95% CI, 0.520 to 1.205; Fig. 6A). Their 5-year RFS rates were 37.6% (95% CI, 27.0% to 48.2%) in the S-1 group and 34.4% (95% CI, 24.1% to 44.7%) in the surgery-alone group, with an HR of 0.788 (95% CI, 0.539 to 1.151; Fig 6B).

Site of First Relapse

Common sites of first relapse were the peritoneum, hematogenous sites, and lymph nodes (Table 1). Rates of metastasis and relapse were consistently lower in the S-1 group than in the surgery-only group for all sites. In particular, the rates of recurrence in lymph nodes and of peritoneal relapse were markedly lower in the S-1 group.

DISCUSSION

To the best of our knowledge, the ACTS-GC study is the first large clinical trial of adjuvant chemotherapy enrolling more than 1,000 patients who underwent D2 gastrectomy for gastric cancer. The results of this follow-up study showed that 1-year treatment with S-1 improved OS and RFS at 5 years compared with surgery alone, thus reconfirming the conclusions reached on early publication of the study results after a median follow-up of 3 years.

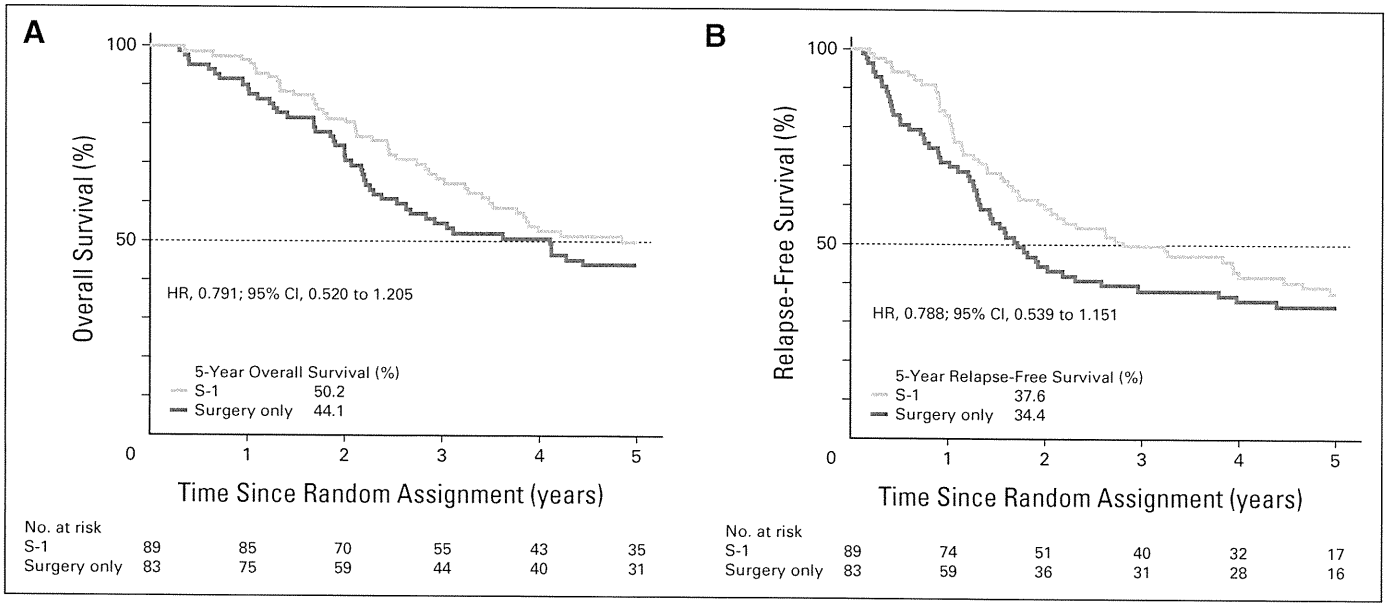

Fig 6. Kaplan-Meier estimates of (A) overall survival and (B) relapse-free survival for eligible patients with stage IIIB gastric cancer. HR, hazard ratio.

Table 1. Site of First Relapse (all randomly assigned patients)*

Site	S-1 (n = 529) No.	S-1 (n = 529) %	Surgery Only (n = 530) No.	Surgery Only (n = 530) %	HR	95% CI
Total No. of relapses	162	30.6	221	41.7	—	—
Local	11	2.1	17	3.2	0.572	0.268 to 1.221
Lymph nodes	30	5.7	54	10.2	0.505	0.323 to 0.789
Peritoneum	77	14.6	100	18.9	0.687	0.511 to 0.925
Hematogenous	61	11.5	71	13.4	0.784	0.557 to 1.105

Abbreviation: HR, hazard ratio.
*Some patients had a first relapse at more than one site.

Our present results confirmed that postoperative adjuvant chemotherapy with S-1 alone reduced the risk of death by 33.1%, thereby demonstrating that effectiveness was maintained since the previous analysis. This reduction in the risk of mortality is comparable with that obtained with combined regimens for adjuvant chemotherapy in the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial[14] and the Intergroup 0116 (INT-0116) trial.[15]

Whether the results of this study can be extrapolated to countries outside East Asia remains uncertain because of possible differences in pharmacokinetics of S-1 between whites and East Asians. If S-1 is used as adjuvant chemotherapy in whites, the dose should be carefully adjusted. A second reason is that all patients in this study underwent D2 gastrectomy although more limited surgery (D0/1) is commonly performed in the United States and some parts of Europe. In the surgery-only group, OS at 5 years was 61.1%, which was much better than that of patients undergoing D2 gastrectomy in Europe (33%) in a Dutch trial.[16] One of the reasons for this large difference may be the high level and widespread use of diagnostic technology in Japan, potentially leading to stage migration between Japan and Western countries.[17] Another important reason might be the high quality of D2 gastrectomy in Japan, whereas D0 or D1 gastrectomy remains the standard procedure in the United States and was the standard in Europe until recently. Although a Dutch trial comparing D1 with D2 gastrectomy reported negative results,[16,18] a 15-year follow-up study showed that the rate of mortality from gastric cancer was significantly lower in the D2 gastrectomy group.[19] Thus, the most recent European Society for Medical Oncology (ESMO) clinical practice guidelines recommend D2 gastrectomy as the standard procedure for curable advanced gastric cancer.[20]

The primary end point of this study was 5-year OS, although that of an ongoing adjuvant chemotherapy study in Korea and China is 3-year disease-free survival. The latter is designed to evaluate the efficacy of postoperative adjuvant chemotherapy with capecitabine and oxaliplatin compared with surgery alone. To justify the use of RFS or disease-free survival as the primary end point for adjuvant chemotherapy after curative resection of gastric cancer, more evidence is needed, but the results of this study may strongly suggest that RFS can be used as the primary end point of such studies. (In this follow-up analysis, 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 surgery-only group, respectively.)

To compare our results with those of other foreign studies, we also report the stage-specific 3- and 5-year OS and RFS according to the International Union Against Cancer (UICC) TNM Classification of Malignant Tumours, Sixth Edition. Three-year OS rates according to UICC staging in the S-1 and surgery-only groups were 91.1% and 80.9% (stage II), 77.8% and 68.3% (stage IIIA), 66.6% and 56.8% (stage IIIB), and 59.1% and 45.7% (stage IV). Three-year RFS rates were 84.3% and 73.5% (stage II), 69.1% and 56.7% (stage IIIA), 44.8% and 28.9% (stage IIIB), and 46.0% and 37.1% (stage IV). Five-year OS rates were 83.4% and 70.8% (stage II), 68.9% and 56.2% (stage IIIA), 43.7% and 40.1% (stage IIIB), and 45.1% and 42.7% (stage IV). Five-year RFS rates were 77.9% and 65.4% (stage II), 64.3% and 48.7% (stage IIIA), 35.9% and 28.9% (stage IIIB), and 26.8% and 25.0% (stage IV).

The approach for adjuvant chemotherapy differs among East Asian countries, including Japan, in which D2 gastrectomy has long been the standard procedure, and Western countries, in which D0 or D1 gastrectomy used to be or currently is standard. As Cunningham and Chua[21] stated, "surgery alone" is no longer standard treatment anywhere in the world for advanced gastric cancer. Some type of adjuvant chemotherapy, including the use of radiotherapy after D0/1 resection, can thus be considered standard treatment at present.

A meta-analysis by the Global Advanced/Adjuvant Stomach Tumor Research International Collaboration (GASTRIC) group[7] showed that some form of postoperative chemotherapy is associated with a higher survival rate than surgery alone; moreover, the use of monotherapy for postoperative adjuvant treatment resulted in good outcomes. The ACTS-GC trial demonstrated that S-1 monotherapy improved OS and RFS. In patients with early-stage (II and IIIA) tumors, the benefits of treatment with S-1 were considerable. However, the 5-year OS rate in patients with stage IIIB disease was 50.2% in the S-1 group and 44.1% in the surgery-only group, suggesting that there remains some room for improvement. Future studies should evaluate the effectiveness of intensive preoperative and/or postoperative chemotherapy with multiple agents in patients at high risk for relapse.

The results of the S-1 plus cisplatin versus S-1 in randomized controlled trial in the treatment for stomach cancer (SPIRITS) trial,[22] demonstrating that S-1 plus cisplatin is superior to S-1 alone with respect to survival in patients with unresectable or recurrent gastric cancer, and the V325 study [a randomized, multinational phase II/III trial of patients with untreated advanced gastric cancer],[23,24] showing that the addition of docetaxel to cisplatin plus fluorouracil prolongs survival, indicated that S-1 plus cisplatin and S-1 plus docetaxel are candidate regimens for postoperative adjuvant chemotherapy. These regimens were confirmed to be feasible in a postoperative setting,[25,26] and further studies should be performed to examine whether such regimens are superior to S-1 alone.

The Japan Clinical Oncology Group (JCOG) is now performing the JCOG 0501 study to compare S-1 plus cisplatin as neoadjuvant chemotherapy with surgery followed by S-1 monotherapy in patients with clinically resectable Borrmann type 4 (linitis plastica) and large type 3 gastric cancer. This trial is expected to be a landmark study, determining the future direction for preoperative chemotherapy in Japan.

The use of molecular targeted agents for gastric cancer has been studied extensively. In the Trastuzumab in Combination with Chemotherapy Versus Chemotherapy Alone for Treatment of HER2-Positive Advanced Gastric or Gastro-Esophageal Junction Cancer (ToGA) study, trastuzumab combined with cisplatin and either fluorouracil or capecitabine significantly prolonged OS in patients with HER2-positive gastric cancer.[27] The effectiveness of adjuvant chemotherapy with molecular targeted agents such as trastuzumab also needs to be assessed in patients with HER2-positive gastric cancer.

In conclusion, this 5-year follow-up study confirmed that adjuvant chemotherapy with S-1 given for 1 year after surgery improved

OS and RFS at 5 years in patients with stage II or III gastric cancer who underwent D2 gastrectomy. Postoperative chemotherapy with S-1 can be recommended for patients with stage II or III gastric cancer who undergo D2 gastrectomy, at least in Asian populations.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Mitsuru Sasako, sanofi-aventis K.K. (C), Taiho Pharmaceutical (C), Chugai Pharmaceutical (C); Atsushi Nashimoto, Taiho Pharmaceutical (C); Masashi Fujii, Taiho Pharmaceutical (C); Toshifusa Nakajima, Taiho Pharmaceutical (C); Yasuo Ohashi, Taiho Pharmaceutical (C) **Stock Ownership:** Masashi Fujii, Otsuka Holdings **Honoraria:** Mitsuru Sasako, sanofi-aventis K.K., Bayer Yakuhin, Genzyme Japan K.K., Novartis Pharma K.K., Taiho Pharmaceutical, Bristol-Myers Squibb, Yakult Pharmaceutical Industry; Shinichi Sakuramoto, Taiho Pharmaceutical; Taira Kinoshita, Taiho Pharmaceutical; Hiroshi Furukawa, Taiho Pharmaceutical; Toshiharu Yamaguchi, Taiho Pharmaceutical; Atsushi Nashimoto, Taiho Pharmaceutical; Masashi Fujii, Taiho Pharmaceutical; Toshifusa Nakajima, Taiho Pharmaceutical; Yasuo Ohashi, Taiho Pharmaceutical **Research Funding:** Mitsuru Sasako, Taiho Pharmaceutical, Bristol-Myers Squibb, Chugai Pharmaceutical, sanofi-aventis K.K.; Shinichi Sakuramoto, Taiho Pharmaceutical **Expert Testimony:** None **Other Remuneration:** None

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Final approval of manuscript: All authors

REFERENCES

1. Ferlay J, Shin HR, Bray F, et al: Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10, 2010. http://www.iarc.fr/en/publications/eresources/cancerbases/index.php
2. Hermans J, Bonenkamp JJ, Boon MC, et al: Adjuvant therapy after curative resection for gastric cancer: Meta-analysis of randomized trials. J Clin Oncol 11:1441-1447, 1993
3. Piedbois P, Buyse M: Meta-analyses need time, collaboration, and funding. J Clin Oncol 12:878-880, 1994
4. Earle CC, Maroun JA: Adjuvant chemotherapy after curative resection for gastric cancer in non-Asian patients: Revisiting a meta-analysis of randomised trials. Eur J Cancer 35:1059-1064, 1999
5. Mari E, Floriani I, Tinazzi A, et al: Efficacy of adjuvant chemotherapy after curative resection for gastric cancer: A meta-analysis of published randomised trials—A study of the GISCAD (Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente). Ann Oncol 11:837-843, 2000
6. Panzini I, Gianni L, Fattori PP, et al: Adjuvant chemotherapy in gastric cancer: A meta-analysis of randomized trials and a comparison with previous meta-analyses. Tumori 88:21-27, 2002
7. GASTRIC (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) Group, Paoletti X, Oba K, et al: Benefit of adjuvant chemotherapy for resectable gastric cancer: A meta-analysis. JAMA 303:1729-1737, 2010
8. Shirasaka T, Shimamato Y, Ohshimo H, et al: Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 7:548-557, 1996
9. Diasio RB: Clinical implications of dihydropyrimidine dehydrogenase inhibition. Oncology (Williston Park) 13:17-21, 1999
10. Sakata Y, Ohtsu A, Horikoshi N, et al: Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 34:1715-1720, 1998
11. Koizumi W, Kurihara M, Nakano S, et al: Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer: For the S-1 Cooperative Gastric Cancer Study Group. Oncology 58:191-197, 2000
12. Sakuramoto S, Sasako M, Yamaguchi T, et al: Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 357:1810-1820, 2007
13. Japanese Gastric Cancer Association: Japanese Classification of Gastric Carcinoma - 2nd English Edition. Gastric Cancer 1:10-24, 1998
14. Cunningham D, Allum WH, Stenning SP, et al: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 355:11-20, 2006
15. Macdonald JS, Smalley SR, Benedetti J, et al: Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 345:725-730, 2001
16. Bonenkamp JJ, Hermans J, Sasako M, et al: Extended lymph-node dissection for gastric cancer. N Engl J Med 340:908-914, 1999
17. Bunt AM, Hermans J, Smit VT, et al: Surgical/pathologic-stage migration confounds comparisons of gastric cancer survival rates between Japan and Western countries. J Clin Oncol 13:19-25, 1995
18. Bonenkamp JJ, Songun I, Hermans J, et al: Randomised comparison of morbidity after D1 and D2 dissection for gastric cancer in 996 Dutch patients. Lancet 345:745-748, 1995
19. Songun I, Putter H, Kranenbarg EM, et al: Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. Lancet Oncol 11:439-449, 2010
20. Okines A, Verheij M, Allum W, et al: Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 21:v50-v54, 2010
21. Cunningham D, Chua YJ: East meets West in the treatment of gastric cancer. N Engl J Med 357:1863-1865, 2007
22. Koizumi W, Narahara H, Hara T, et al: S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): A phase III trial. Lancet Oncol 9:215-221, 2008
23. Van Cutsem E, Moiseyenko VM, Tjulandin S, et al: Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: A report of the V325 Study Group. J Clin Oncol 24:4991-4997, 2006
24. Ajani JA, Moiseyenko VM, Tjulandin S, et al: Quality of life with docetaxel plus cisplatin and fluorouracil compared with cisplatin and fluorouracil from a phase III trial for advanced gastric or gastroesophageal adenocarcinoma: The V-325 Study Group. J Clin Oncol 25:3210-3216, 2007
25. Fujitani K, Tamura S, Kimura Y, et al: Phase II feasibility study of adjuvant S-1 plus docetaxel for stage III gastric cancer patients after curative D2 gastrectomy (OGSG 0604). J Clin Oncol 27, 2009 (suppl; abstr e15567)
26. Takahari D, Hamaguchi T, Yoshimura K, et al: Feasibility study of adjuvant chemothe rapy with S-1 plus cisplatin for gastric cancer. Cancer Chemother Pharmacol 67:1423-1428, 2011
27. Bang YJ, Van Cutsem E, Feyereislova A, et al: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. Lancet 376:687-697, 2010

Journal of Surgical Oncology 2012;105:38–42

Intraperitoneal Docetaxel Combined with S-1 for Advanced Gastric Cancer With Peritoneal Dissemination

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Background: Our previous phase I study indicated that combination chemotherapy with intraperitoneal docetaxel and S-1 was well tolerated by gastric cancer patients with peritoneal carcinomatosis (PC). This study evaluated the benefits of this combination chemotherapy and subsequent surgery.

Patients and Methods: Neoadjuvant Intra-Peritoneal and Systemic chemotherapy (NIPS) was introduced to gastric cancer patients with positive cytology or with PC. Two cycles of intraperitoneal chemotherapy with docetaxel combined with S-1, were administrated and gastrectomy with lymph node dissection was performed in cases without macroscopic PC at post-NIPS staging laparoscopy.

Results: Eighteen patients were enrolled in this study. Eight patients had measurable lymph node metastases by the RECIST criteria and computed tomography (CT) showed that five (62.5%) displayed a major response to the treatment. Out of 18 patients, 14 (78%) showed negative results on peritoneal cytology and no macroscopic PC, while the remaining four were cancer cell positive on peritoneal cytology or showed macroscopic PC even after NIPS. The median survival time of the entire group was 24.6 months. No treatment-related mortality was observed during NIPS and surgery.

Conclusion: This study indicated that the NIPS combined with surgery was highly active and well tolerated by advanced gastric cancer patients with PC.

J. Surg. Oncol. 2012;105:38–42. © 2011 Wiley Periodicals, Inc.

KEY WORDS: **gastric cancer; neoadjuvant chemotherapy; intra-peritoneal chemotherapy; peritoneal lavage cytology; S-1; docetaxel**

INTRODUCTION

The incidence of gastric cancer has decreased worldwide and particularly so in Western countries. Despite this, it remains the fourth-most common cancer and the second-most common cause of cancer-related deaths [1,2]. Multidisciplinary approaches to the treatment of advanced gastric cancer including chemotherapy, radiotherapy, and surgery have recently been developed and the survival benefits of such treatments are being investigated worldwide [3,4]. Furthermore, several novel chemotherapeutic agents including taxans (paclitaxel and docetaxel), irinotecan, oxaliplatin, S-1, and capecitabine have shown activity in gastric cancer [5–10].

Peritoneal carcinomatosis (PC) is the most frequent mode of recurrence and is responsible for about 60% of all deaths from gastric cancer [11]. Gastric cancer patients with PC are considered to be non-curable and are usually treated with systemic chemotherapy without surgical resection. Few clinical trials have been performed thus far for gastric cancer with PC. The Japanese Clinical Oncology Group conducted a multicenter phase III study of sequential chemotherapy with methotrexate and 5-FU (MF) compared with 5-FU continuous infusion therapy (5-FU) that included 237 gastric cancer patients with PC [12]. The median survival time (MST) was 10.6 months for MF and 9.4 months for 5-FU. Recent randomized clinical trials have proposed several standards for combination chemotherapy for non-curable gastric cancer such as docetaxel, cisplatin, and fluorouracil (DCF) in the United States, epirubicin, cisplatin, and fluorouracil (ECF) in Europe, or fluoropyrimidine (S-1) and cisplatin in Japan [13–15]. However, the MSTs in these studies were 8.9, 9.2, and 13 months, respectively, and a new-multidisciplinary approach for gastric cancer with PC is therefore needed.

S-1 is an oral fluorpyrimidine derivative consisting of tegafur, gimestat (CDHP), which has dihydropyrimidine dehydrogenase (DPD)-inhibiting activity, and otastat potassium (Oxo), which reduces its gastrointestinal toxicity. In Japan, adjuvant chemotherapy with S-1 has been a standard therapy after curative surgery for Stage II and III gastric cancer due to the multicenter phase III randomized trial [16]. We reported previously that oral intake of S-1 was highly effective against gastric PC due to the higher concentrations of 5-FU and CDHP achieved in peritoneal tumors than in plasma in a mouse model [17]. The efficacy of S-1 for gastric cancer patients with PC has also been reported [18]. Furthermore, the safety and a significant pharmacological advantage with intraperitoneal docetaxel have been proven [19]. The combination of S-1 and intravenous docetaxel has been reported as a promising therapy for advanced gastric cancer reported by Yoshida and Yamaguchi [20,21].

We recently reported the feasibility of S-1 and intraperitoneal docetaxel combination chemotherapy for patients with positive cytology on peritoneal lavage specimens or with macroscopically visible peritoneal metastasis [22]. The regimen was found to be very safe and promising for gastric cancer patients with peritoneal dissemination. The purpose of this prospective study was to investigate the efficacy of this newly developed combination chemotherapy with subsequent surgery for gastric cancer with positive peritoneal lavage cytology and/or macroscopic peritoneal dissemination.

Conflict of interest: None.

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Received 10 May 2011; Accepted 18 July 2011

DOI 10.1002/jso.22057

Published online 31 August 2011 in Wiley Online Library (wileyonlinelibrary.com).

MATERIALS AND METHODS

Patient Selection

The eligibility for this study was as follows: (i) Histopathological confirmation of gastric cancer; (ii) a positive result on peritoneal cytology or macroscopic PC diagnosed by staging laparoscopy; (iii) the absence of non-curative factors such as distant metastasis to liver or lung except of peritoneum; (iv) performance status [Eastern Cooperative Oncology Group (ECOG)] of less than two; (v) age of less than 75; (vi) no prior treatment; (vii) adequate bone marrow function (leukocyte count more than 3,000 ml^{-1} and platelet count more than 100,000 ml^{-1}); (viii) adequate liver function (serum bilirubin level less than 1.5 mg dl^{-1} and serum transaminase levels less than two times the upper limit of normal); (ix) adequate renal function (serum creatinine level less than 1.5 mg dl^{-1}; (x) no other severe medical conditions such as symptomatic infectious disease, intestinal pneumonia, active hemorrhage/bleeding, or obstructive bowel disease; (xi) not pregnant or lactating; and, (xii) provision of written informed consent in accordance with government guidelines of each institution or hospital. This study was approved by the ethics committees of Osaka University Hospital.

Treatment Strategy

Figure 1 shows the treatment strategies followed in this study. Staging laparoscopy or peritoneal lavage cytology under local anesthesia [23] was performed for gastric cancer patients with serosa-invading tumors diagnosed using multidetector-row computed tomography (CT) and 3-dimensional imaging. Two cycles of neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) was provided to patients with positive cytology and/or peritoneal deposits of cancer metastasis. The staging laparoscopy was mandatory for all patients after chemotherapy and gastrectomy with lymph node dissection was performed in patients without macroscopic peritoneal deposits of cancer metastasis.

Treatment Protocol

The abdominal cavity was irrigated with various doses of docetaxel dissolved in 1 L of saline on day 1 every three weeks; the saline was administered through a drainage tube placed for the collection of peritoneal lavage diagnosis or staging laparoscopy [22]. The dosage of docetaxel varied from 40 to 50 to 60 mg/m^2 due to the progressive inclusion of patients from the phase I study. The S-1 was administered orally at a fixed dose of 40 mg/m^2 twice daily on days 1–14 every three weeks. Patients were treated for two cycles before staging laparoscopy and subsequent gastrectomy unless unacceptable toxicity or patient unwillingness was observed.

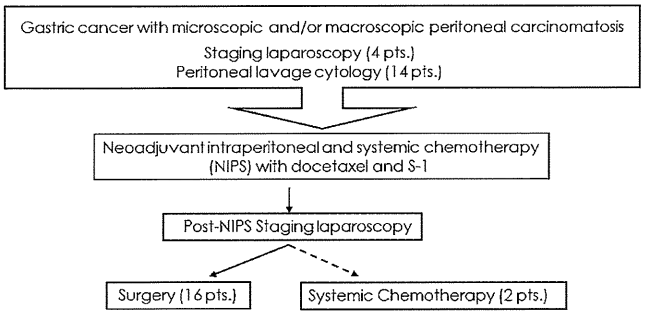

Fig. 1. Flow diagram of the treatment protocol.

Evaluation of the Disease

Before and after NIPS, conventional examinations including the multidetector-row CT were performed to assess the clinical response. The post-NIPS staging laparoscopy was mandatory to evaluate the effect of the treatment on peritoneal metastasis. Tumor response of measurable metastatic lesions was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) [24]. A complete response (CR) was defined as the disappearance of all evidence of cancer for more than four weeks. A partial response (PR) was defined as more than 50% reduction in the sum of the products of the perpendicular diameters of all lesions without any evidence of new regions or progression in any lesions. Stable disease (SD) was defined as less than a 50% reduction or less than a 25% increase in the sum of the products of the perpendicular diameters of all lesions, without any evidence of new lesions. Progressive disease (PD) was defined as more than 25% increase in more than one region or the appearance of new regions. The response in the peritoneum was evaluated by staging laparoscopy or surgery after NIPS. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 3.0 and recorded. Recurrence after surgery including such as peritoneum, liver and distant lymph nodes was diagnosed with CT, which was repeated every 3 months.

Statistical Analysis

Survival was calculated from the initial date of treatment to the occurrence of the event or to the date of the most recent follow-up visit by the Kaplan–Meier method. Univariate analysis was performed using the log-rank test, and multivariate analysis was conducted using the Cox proportional hazards model. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathological Characteristics of Patients

A total of 18 gastric cancer patients with PC were enrolled in this study representing all patients diagnosed and treated at the Department of Gastroenterological Surgery, Osaka University Hospital between July 2006 and June 2010. All 18 patients showed positive peritoneal cytology with staging laparoscopy (four cases) and peritoneal lavage cytology under local anesthesia (14 cases). Four patients performed with staging laparoscopy were also confirmed to have macroscopic PC. Table I details the clinicopathological characteristics of these patients. The patient group comprised 12 men and six women with a mean age of 62.9 years (range 51–75 years). Macroscopically, type 4 tumors accounted for 78% of the cases (14/18). Histopathologically, undifferentiated tumors including poorly differentiated and signet ring cell carcinoma were dominant (15/18, 83%). Gastrectomy with lymph node dissection was performed in 16 of the 18 patients (89%); these patients showed no macroscopic peritoneal metastasis at the post-NIPS staging laparoscopy. Surgery was not conducted on the remaining two patients because of macroscopic PC in the abdominal cavity. Of the 16 patients who underwent gastrectomy, 13 (81%) underwent total gastrectomy and one of these underwent additional splenectomy because of macroscopic lymph node metastasis in splenic hilum. Therefore, only four of 16 cases (25%) underwent D2 lymphadenectomy, while the remaining 12 had D2 lymphadenectomy without clearance of lymph nodes in splenic hilum classified as D1+ (Table I). All 16 patients who had gastrectomy after NIPS were treated with S-1. Out of two patients who had no surgery, one suffered from lethal pulmonary thrombosis and another continued systemic chemotherapy with S-1 and docetaxel after NIPS.

TABLE I. Clinicopathological Characteristics of the 18 Patients Enrolled in the Present Study

Average age, years (range)	62.9 (51–75)
Sex (Male/Female)	12/6
Tumor type	
3	4
4	14
Histology	
Diffuse type	15
Differentiated type	3
Ascites (CT)	
Present	6
Absent	12
Type of surgery (16 cases)	16/18 (88.9%)
Total gastrectomy	13
With splenectomy	1
Without splenectomy	12
Distal gastrectomy	3
Lymph node dissection	
D2	4
D1+	12

Type 3: Ulcerated carcinomas without definite limits, infiltrating into the surrounding wall; type 4: Diffusely infiltrating carcinomas in which ulceration is usually not a marked feature [33]. D2: D2 lymphadenectomy, D1+: D2 lymphadenectomy without clearance of lymph nodes in splenic hilum.

Clinical Response and Toxicity to NIPS

After the NIPS, all patients were evaluated for clinical response and toxicities. Of the 18 patients, 15 (83%) completed two cycles of the combination chemotherapy, whereas the remaining three patients were given only one cycle of the combination chemotherapy because of patient unwillingness in one case, grade 3 fever in one case, and obstruction of the catheter after one cycle in the remaining case. Eight patients showed measurable lymph node metastases according to the RECIST criteria. As shown in Table II, the CT results showed that five out of eight patients (62.5%) displayed a major response (0 CR, 5 PR) to the treatment. Out of the 18 patients, 14 (78%) showed negative results on peritoneal cytology and no macroscopic peritoneal metastasis, while the remaining four patients were still positive on peritoneal cytology after NIPS or showed macroscopic peritoneal metastasis (Table II).

Adverse events were graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events Version 4.0 (Table III). No patients showed grade 4 or higher toxicities, while one patient showed grade 3 leukopenia and neutropenia, and one showed grade 3 fever. No chemotherapy-related mortality was reported.

TABLE II. Anti-Tumor Efficacy of Neoadjuvant Intra-Peritoneal and Systemic Chemotherapy (NIPS)

RECIST criteria	n	%
Measurable disease	8	
Overall response rate (CR + PR)	5	62.5
CR	0	0
PR	5	62.5
SD	3	37.5
PD	0	0
Non-measurable disease	10	
Efficacy for peritoneal disease	18	
CY0 and P0 after NIPS	14	78
CY1 or P1 after NIPS	4	22

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CY0, peritoneal lavage cytology negative; CY1, peritoneal lavage cytology positive.

Postoperative Complications

Among the 16 patients who underwent surgery, postoperative complications occurred in three patients (19%, Table IV). Postoperative hemorrhage occurred in one patient, who required reoperation, and intra-abdominal abscess occurred in two cases. No surgery-related mortality (30 days mortality) was reported.

Survival

Figure 2 shows the overall survival time after the introduction of NIPS for all patients enrolled in this study. The MST was 24.6 months, with 76% of patients surviving for 1 year and 54% surviving for 2 years at a median follow-up time of 45 months. Fourteen patients had died by April 21, 2011. Of these, three patients died from non-cancer-related diseases, pulmonary embolism, liver dysfunction, and pneumonia. The remaining 11 patients died from peritoneal recurrence of gastric cancer.

DISCUSSION

In the present study, we conducted a prospective phase II study to evaluate the efficacy of the same NIPS regime and subsequent surgery in gastric cancer patients with microscopic or macroscopic PC. Following the NIPS treatment, 78% of enrolled patients showed negative results on peritoneal cytology and no macroscopic peritoneal metastasis. The MST of all patients was 24.6 months long. This regimen is also safe and less toxic, which might reflect the pharmacokinetics of intraperitoneally administered docetaxel, i.e., low concentration in the systemic circulation compared to a higher concentration in the abdominal cavity [22,25]. Postoperative complications were observed in three (18.8%) of 16 patients who underwent gastrectomy, which is infrequent compared to the previous reports of surgery after neo-adjuvant chemotherapy [26,27], and no surgery-related mortality was observed. Previously, we reported the feasibility and efficacy of NIPS comprising intraperitoneal mitomycin C (MMC) and cisplatin (CDDP), followed by two cycles of intravenous triplet chemotherapy of docetaxel, 5-fluorouracil (5-FU), and CDDP, with subsequent surgery [28]. Out of the 25 study

TABLE III. Toxicity Profile of Neoadjuvant Chemotherapy in 18 Patients (National Cancer Institute-Common Terminology Criteria for Adverse Events Version 4.0: NCI-CTCAE ver. 4.0)

Adverse events	n = 18				
Grade	1	2	3	Total (%)	Gr. 3%
Non-hematological toxicity					
Fatigue	4	4	0	8 (44)	0
Anorexia	4	5	0	9 (50)	0
Diarrhea	0	1	0	1 (6)	0
Rash	3	0	0	3 (17)	0
Alopecia	2	0	0	2 (11)	0
Fever	0	0	1	1 (6)	6
Hematological toxicity					
Leukopenia	1	0	1	2 (11)	6
Neutropenia	1	1	1	3 (17)	6
Anemia	2	1	0	3 (17)	0
Rise in AST	1	0	0	1 (6)	0
Rise in ALT	2	0	0	2 (11)	0
Elevated serum creatinine	1	0	0	1 (6)	0

Grade (Gr.) indicates toxicity grade according to the NCI-CTCAE ver. 4.0.

TABLE IV. Postoperative complications in 16 patients

Complications	N of Gr. 1<	(%)
Post-operative hemorrhage	1	6.3
Anastomotic insufficiency	0	0
Pancreatic fistula	0	0
Wound infection	0	0
Intra-abdominal abscess	2	12.5
Intestinal occlusion	0	0
Death resulting from complications	0	0
Any postoperative complication	3	18.8

Gr indicates toxicity grade according to the NCI-CTCAE ver. 4.0

patients, 14 (56%) showed negative results on peritoneal cytology with no macroscopic peritoneal metastasis, while the remaining 11 were cancer cell positive on peritoneal cytology or macroscopic peritoneal metastasis even after NIPS. The MST for all 25 patients was 16.7 months. On the other hand, the predominant toxicity was myelosuppression, and grade 3–4 leukopenia and neutropenia occurred in 80% of patients, requiring management by a specialized medical oncologist. These results indicated that NIPS using intraperitoneal docetaxel and S-1 could be more feasible and effective therapy for gastric cancer patients with PC.

Because the prognosis of gastric cancer with peritoneal dissemination is very poor, surgery has not been the standard therapy except for patients requiring palliation of symptom such as bleeding or obstruction. Kim et al. [29] reported the results of a randomized phase III study of S-1 alone versus S-1 plus intravenous docetaxel for unresectable and recurrent gastric cancer. The combination therapy of S-1 plus intravenous docetaxel had no apparent survival benefit overall; however, in the group of patients with no measurable disease, who were supposed to be gastric cancer patients with PC, S-1 plus intravenous docetaxel showed significant survival benefits over S-1 alone (17.5 months vs. 11.7 months, $P = 0.0389$). This indicated that the combination chemotherapy with S-1 and docetaxel is promising for gastric cancer with PC.

Intraperitoneal chemotherapy was originally developed to enhance antitumor activity against PC by maintaining a high concentration of the drug in the peritoneal cavity over a long period. The clinical effects of this approach have been verified by a number of convincing clinical trials in ovarian cancer [30,31]. Recently, intraperitoneal

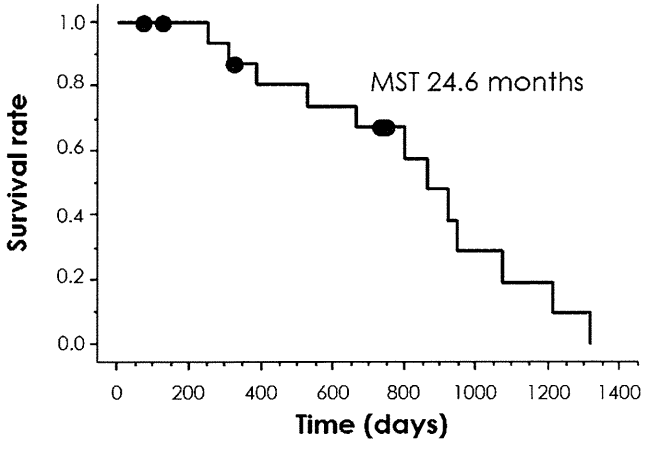

Fig. 2. Overall survival of 18 patients enrolled in this study. MST: Mean survival time. Median follow-up time was 45 months.

administration of taxans such as paclitaxel and docetaxel was also examined in gastric cancer with peritoneal dissemination to achieve higher and longer concentration of taxans in the peritoneal cavity [22,32].

In this study, out of 14 patients who had curative surgery with negative results on peritoneal cytology and no macroscopic peritoneal metastasis, eight patients died from peritoneum recurrence. The result indicates a lack of therapeutic power of this regimen to cure patients with PC. We continued monotherapy with S-1 after curative surgery and intraperitoneal chemotherapy might have been needed to continue after surgery.

Further studies are needed to define the most suitable regimen for NIPS and adjuvant chemotherapy after surgery. The efficacy of neoadjuvant chemotherapy and intraperitoneal chemotherapy for gastric cancer with PC should be examined in a phase III randomized clinical trial.

REFERENCES

1. Ferlay J, Shin HR, Bray F, et al.: Estimates of worldwide burden of cancer in 2008. Int J Cancer 2010;127:2893–2917.
2. Parkin DM, Bray F, Ferlay J, et al.: Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
3. Macdonald JS, Smalley SR, Benedetti J, et al.: Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 2001;345:725–730.
4. Cunningham D, Allum WH, Stenning SP, et al.: Perioperative chemotherapy versus surgery alone for respectable gastroesophageal cancer. N Engl J Med 2006;355:11–20.
5. Ajani JA, Fairweather J, Dumas P, et al.: Phase II study of Taxol in patients with advanced gastric carcinoma. Cancer J Sci Am 1988;4:269–274.
6. Einzig AI, Neuberg D, Remick SC, et al.: Phase II trial of docetaxel (Taxotere) in patients with adenocarcinoma of the upper gastrointestinal tract previously untreated with cytotoxic chemotherapy: The Eastern Cooperative Oncology Group (ECOG) results of protocol E1293. Med Oncol 1996;13:87–93.
7. Futatsuki K, Wakui A, Nakao I, et al.: Late phase II study of irinotecan hydrochloride (CPT-11) in advanced gastric cancer. CPT-11 Gastrointestinal Cancer Study Group. Jpn J Cancer Chemother 1994;21:1033–1038.
8. Taguchi T, Tsukagoshi S, Furue H, et al.: Phase I clinical study of oxaliplatin. Jpn J Cancer Chemother 1998;25:1899–1907.
9. Sakata Y, Ohtsu A, Horikoshi N, et al.: Late phase II study of novel oral fluoropyrimidine anticancer drug S-1(1M tegafur-0.4M Gimestat-1M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 1998;34:1715–1720.
10. Liu C, Sun Q, Hang X, et al.: Multicenter phase II study of capecitabine plus oxaliplatin as a first-line therapy in Chinese patients with advanced gastric cancer. Anticancer Drugs 2008; 19:825–831.
11. Maruyama K, Kaminishi M, Hayashi K, et al.: Gastric cancer treated in 1991 in Japan: Data analysis of nationwide registry. Japanese Gastric Cancer Association Registration Committee. Gastric Cancer 2006;9:51–66.
12. Shirao K, Boku N, Yamada Y, et al.: Randomized phase III study of 5-Fluorouracil continuous infusion (5FUci) versus methotrexate and 5-FU sequential therapy (MF) in gastric cancer with peritoneal metastasis (JCOG0106). J Clin Oncol 2009; 27:17.
13. Van Cutsem E, Moiseyenko VM, Tjulandin S, et al.: V325 Study Group Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: A report of the V325 Study Group. J Clin Oncol 2006;24:4991–4997.
14. Webb A, Cunningham D, Scarffe JH, et al.: Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus

fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer. J Clin Oncol 1997;15:261–267.
15. Koizumi W, Narahara H, Hara T, et al.: S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): A phase III trial. Lancet Oncol 2008;9:215–221.
16. Sakuramoto S, Sasako M, Yamaguchi T, et al.: ACTS-GC Group. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 2007;357:1810–1820.
17. Mori T, Fujiwara Y, Yano M, et al.: Prevention of peritoneal metastasis of human gastric cancer cells in nude mice by S-1, a novel oral derivative of 5-fluorouracil. Oncology 2003;64:176–182.
18. Ishizone S, Maruta F, Saito H, et al.: Efficacy of S-1 for patients with peritoneal metastasis of gastric cancer. Chemotherapy 2006;52:301–307.
19. Morgan RJ Jr, Doroshow JH, Synold T, et al.: Phase I trial of intraperitoneal docetaxel in the treatment of advanced malignancies primarily confined to the peritoneal cavity: Dose-limiting toxicity and pharmacokinetics. Clin Cancer Res 2003;9:5896–5901.
20. Yoshida K, Ninomiya M, Takakura N, et al.: Phase II study of docetaxel and S-1 combination therapy for advanced or recurrent gastric cancer. Clin Cancer Res 2006;12:3402–3407.
21. Yamaguchi K, Shimamura T, Hyodo I, et al.: Phase I/II study of docetaxel and S-1 in patients with advanced gastric cancer. Br J Cancer 2006;94:1803–1808.
22. Fujiwara Y, Nishida T, Takiguchi S, et al.: Feasibility study of S-1 and intraperitoneal docetaxel combination chemotherapy for gastric cancer with peritoneal dissemination. Anticancer Res 2010;30:1335–1339.
23. Makino T, Fujiwara Y, Takiguchi S, et al.: The utility of preoperative peritoneal lavage examination in serosa-invading gastric cancer patients. Surgery 2010;148:96–102.
24. Therasse P, Arbuck SG, Eisenhauer EA, et al.: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–216.
25. Fushida S, Kinoshita J, Yagi Y, et al.: Dual anti-cancer effects of weekly intraperitoneal docetaxel in treatment of advanced gastric cancer patients with peritoneal carcinomatosis: A feasibility and pharmacokinetic study. Oncol Rep 2008;19:27.
26. Yoshikawa T, Sasako M, Yamamoto S, et al.: Phase II study of neoadjuvant chemotherapy and extended surgery for locally advanced gastric cancer. Br J Surg 2009;96:1015–1022.
27. Okabe H, Ueda S, Obama K, et al.: Induction chemotherapy with S-1 plus cisplatin followed by surgery for treatment of gastric cancer with peritoneal dissemination. Ann Surg Oncol 2009;16:3227–3236.
28. Fujiwara Y, Takiguchi S, Nakajima K, et al.: Neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) for gastric cancer patients with peritoneal dissemination. Ann Surg Oncol 2011; May 17. [Epub ahead of print] PMID: 21584835.
29. Fujii M, Kim YH, Satoh T, et al.: Randomized phase III study of S-1 alone versus S-1 plus docetaxel (DOC) in the treatment for advanced gastric cancer (AGC): The START trial update, J Clin Oncol 2011 29: (suppl; abstr 4016) This is an Abstract for ASCO 2011.
30. Markman M, Bundy BN, Alberts DS, et al.: Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: An intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 2001;19:1001–1007.
31. Armstrong DK, Bundy B, Wenzel L, et al.: Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 2006; 354:34–43.
32. Ishigami H, Kitayama J, Kaisaki S, et al.: Phase II study of weekly intravenous and intraperitoneal paclitaxel combined with S-1 for advanced gastric cancer with peritoneal metastasis. Ann Oncol 2010;21:67–70.
33. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma - 2nd english edition. Gastric Cancer 1998; 1:10–24.

Postoperative Quality of Life: Development and Validation of the "Dysfunction after Upper Gastrointestinal Surgery" Scoring System

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BACKGROUND: Although postoperative quality of life is an important outcomes measure, few tools exist to evaluate patients specifically after upper gastrointestinal surgery. The previously developed Dysfunction After Upper Gastrointestinal Surgery (DAUGS)32 scoring system has been further refined to include just 20 items. This study was undertaken to validate the refined evaluation tool.

STUDY DESIGN: The study was performed as a survey, administered to patients after upper gastrointestinal resection at 3 separate institutions.

RESULTS: The DAUGS20 score after gastrectomy (n = 662) was 27.8 and that after esophagectomy (n = 221) was 36.1, showing a significant difference ($p < 0.05$). The score after distal gastrectomy (n = 282) was 25.4 and that after total gastrectomy (n = 149) was 32.0, showing a significant ($p < 0.05$) difference. The α coefficient of all items on the DAUGS20 system was 0.904 and Cronbach's α coefficients of the subscales were 0.612 to 0.856, demonstrating high reliability of this evaluation tool. In addition, 7 factors were extracted from the 20 items using definitive factor analysis, to verify validity.

CONCLUSIONS: Patient quality of life should be evaluated as an outcomes measure after surgical resection for cancer, just as overall survival is analyzed. The DAUGS20 score is reliable, has validity in the evaluation of postoperative patients, and is a valuable tool to assess patient quality of life after upper gastrointestinal surgery for cancer. (J Am Coll Surg 2011;213:508–514. © 2011 by the American College of Surgeons)

Health-related quality of life (QOL) is generally accepted to include physical, social, and psychological aspects.[1] For the patient with gastrointestinal (GI) cancer, these aspects can be assessed with a valid QOL questionnaire such as the Short Form (SF)-36,[2,3] the Functional Assessment of Cancer Therapy Scale-General Measure (FACT-G),[4] the Gastro Intestinal Quality of Life Index (GIQLI),[5] or the European Organization for Research and Treatment of Cancer Quality of Life Core-30 (EORTC QLQ-C30).[6,7] These questionnaires are designed to assess patients undergoing surgery, chemotherapy, radiotherapy, and supportive care. However, none of these tools was designed specifically to evaluate patients after surgical resection.

Until recently, no objective evaluation tools specifically designed to evaluate postoperative problems, specialized for patients after upper GI surgery (excluding the effects of chemo- and/or radiotherapy), have been available. Given the paucity of validated instruments, a dysfunction evaluation scale limited to patients after surgery for upper GI cancers was developed and consisted of 32 items (the Dysfunction After Upper Gastrointestinal Surgery-DAUGS32 Scoring System).[8-11] The DAUGS32 scoring system was validated and found to be effective in the assessment of QOL in patients undergoing surgical resection of upper GI malignancies.[11] But the instrument was rather long, at 32 items, and required further refinement, without loss of its effectiveness to assess QOL. In this study, we

Disclosure Information: Nothing to disclose.

Supported, in part, by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant No.18592394).

Received May 16, 2011; Revised July 11, 2011; Accepted July 11. 2011.

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ISSN 1072-7515/11/$36.00
doi:10.1016/j.jamcollsurg.2011.07.007

Abbreviations and Acronyms

DAUGS = Dysfunction after Upper Gastrointestinal Surgery Scoring System
EORTC QLQ = European Organization for Research and treatment of Cancer Quality of Life Questionnaire
FACT = Functional Assessment of Cancer Therapy Scale
GI = gastrointestinal
GIQLI = Gastro Intestinal Quality of Life Index
GSRS = Gastrointestinal Symptoms Rating Scale
QOL = quality of life

refined the DAUGS32 scoring system to a more sensitive scale comprised of 20 items, assessed its reliability, and verified its usefulness.

METHODS

Study participants

The ethics committee at each participating institution approved this study before its initiation. Study participants underwent surgery with curative intent for upper GI malignancies (esophageal and gastric cancers) at Jichi Medical University Hospital, Osaka University Hospital, and Osaka Medical Center for Cancer and Cardiovascular Diseases. Patients less than 1 month postsurgery, patients with previous upper GI surgery, patients who underwent adjuvant radiation or chemotherapy within 3 months, and those with signs of recurrent disease were excluded from the study. Patients with significant anemia, liver or renal dysfunction, other digestive diseases, or other malignancies, were also excluded.

Development and conduct of the survey

The 32 items from the previously developed DAUGS32 scoring system were analyzed, and the homogeneity of questions evaluated with the α coefficient. Based on these results, the survey items were reviewed by a statistician specialized in scale development, a researcher who develops scales, and a surgeon, resulting in a refined 20-item questionnaire (Table 1). The severity of symptoms was evaluated using a 6-step scale from 0 (none/never) to 5 (very severe/constant). The survey was administered from December 2007 through September 2008, with a re-evaluation from January through October 2008. A paper survey was mailed to participants after obtaining consent. The survey was repeated within 2 to 3 weeks to confirm reproducibility of the responses.

Table 1. Items Comprising the Dysfunction after Upper Gastrointestinal Surgery (DAUGS)20 Scoring System

	Question	Score					
Example:	Have you lost your appetite?	0	1	2	3	4	5
1	Do you feel more full halfway through a meal compared with how you felt before surgery?	0	1	2	3	4	5
2	Do you have a heavy sensation in your stomach after eating?	0	1	2	3	4	5
3	Do you suddenly feel bloated during a meal?	0	1	2	3	4	5
4	Do you feel a sensation of abdominal fullness after eating?	0	1	2	3	4	5
5	Have you lost your appetite?	0	1	2	3	4	5
6	Do you have difficulty in swallowing soft food?	0	1	2	3	4	5
7	Do you have a choking sensation when swallowing food?	0	1	2	3	4	5
8	Do you have difficulty in sleeping because of bitter tasting fluid regurgitating into your mouth?	0	1	2	3	4	5
9	Is any acidic fluid regurgitated into your mouth?	0	1	2	3	4	5
10	Do you vomit after meals?	0	1	2	3	4	5
11	Do you feel food retained in your chest?	0	1	2	3	4	5
12	Do you feel nauseated?	0	1	2	3	4	5
13	Do you have pain in the pit of your stomach after eating?	0	1	2	3	4	5
14	Do you have abdominal pain within 30 min of eating?	0	1	2	3	4	5
15	Do you feel fatigue or weakness within 2−3 hours after eating?	0	1	2	3	4	5
16	Do you feel sleepy within 2−3 hours after eating?	0	1	2	3	4	5
17	Do you have soft stools?	0	1	2	3	4	5
18	Do you have diarrhea?	0	1	2	3	4	5
19	Do you have less strength or a lower activity level?	0	1	2	3	4	5
20	Do you feel dizzy or unsteady when walking up stairs or slopes? 0: none/never; 1: very slight/rarely; 2: mild/occasionally; 3: moderate/often; 4: severe/very often; 5: very severe/constant	0	1	2	3	4	5

Table 2. DAUGS20 Score and Weight Loss after Upper Gastrointestinal Surgery

Variable	Gastrectomy (n = 662)	Esophagectomy (n = 221)	p Value
DAUGS20 score	27.8 ± 13.3	36.1 ± 14.2	<0.001
Weight loss after surgery, %	11.2 ± 9.3	12.9 ± 9.3	0.006
BMI after surgery, kg/m^2	20.9 ± 6.6	19.7 ± 6.8	<0.001

Data are shown as mean ± SD.
BMI, body mass index; DAUGS, Dysfunction after Upper Gastrointestinal Surgery.

Statistical analysis, reliability, and validity

SPSS V15 software was used for data analysis. The scale items were investigated by definitive factor analysis using the principal factor method and varimax rotation. The factor loading was set at 0.35 or higher. After eliminating items in the preliminary scale, factorial analysis was carried out on the remaining 20 items. Factor loadings of the 20 items were determined by the method of factor extraction, principal factor method, and varimax rotation. The final number of factors was determined to be 7, based on the criteria of an eigenvalue of ≥1 and the interpretability of factors. For factor rotation, the oblique promax rotation was used.[12,13]

Cronbach's α and the reliability coefficient of the retest method were used to assess reliability. The Cronbach coefficient was judged to achieve a confidence level of more than 0.7.[14] Construct validity was investigated using the known group method, which is applied to several groups predicted to be different in a certain attribute due to a known characteristic. In addition, the structure of subitems comprising each factor was analyzed by factor analysis, and factorial validity was reviewed by the study group.

RESULTS

Surveys were collected from 910 of 1,000 patients (response rate 91.0 %); 883 of these surveys were valid (88.3%). Subjects in the final study group included 650 men (73.6%) and 233 women (26.4%), with an average age of 65.9 ± 10.0 years (range 31 to 86 years). Of these patients, 662 patients had gastric cancer (474 men and 188 women), and 221 had esophageal cancer (176 men and 45 women).

The overall DAUGS20 score is simply the sum of the scores for the 20 items (each item scored 0 to 5), with a range from 0 to 100 points (Table 1). Lower scores indicate fewer or less frequent difficulties for the patient. The mean DAUGS20 score for gastric cancer patients was 31.3 ± 16.1 points; that for esophageal cancer was 36.1 ± 14.2 points ($p < 0.001$) (Table 2). DAUGS20 scores do not show significant variation according to the postoperative interval when evaluated (Fig. 1). The DAUGS20 scores according to surgical procedure performed are shown in Table 3. The DAUGS20 score for patients undergoing distal gastrectomy with a Billroth I reconstruction was 25.4 (n = 282), and for total gastrectomy was 32.0 (n = 149) ($p < 0.001$). Esophagectomy through a right thoracotomy

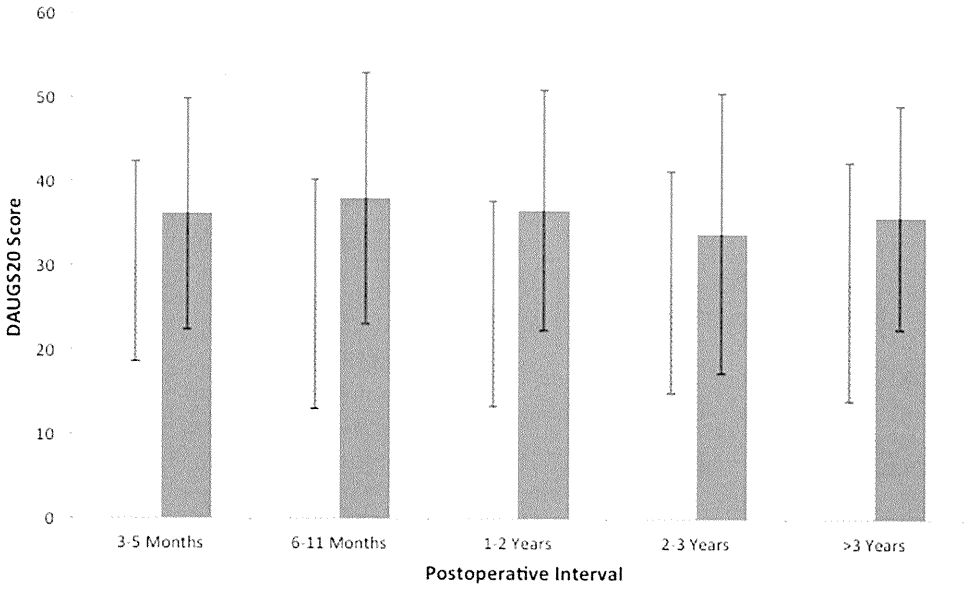

Figure 1. DAUGS20 score and postoperative interval. Average scores are shown with standard deviation (error bars) vs time interval after resection. Light gray bar, gastric cancer; dark gray bar, esophageal cancer. DAUGS, Dysfunction after Upper Gastrointestinal Surgery.

Table 3. DAUGS20 Score According to Surgical Procedure Performed

Procedure	DAUGS20 score	n	p Value
Gastrectomy			
Distal gastrectomy with B-I	25.4 ± 14.7	282	
Total gastrectomy with R-Y	32.0 ± 12.8	149	<0.001
Thoracic esophagectomy with gastric pull-up and cervical anastomosis			
Retromediastinal approach	33.5 ± 15.5	45	<0.005
Retrosternal approach	39.8 ± 13.8	38	

B-I, Billroth-I; DAUGS, Dysfunction after Upper Gastrointestinal Surgery; R-Y, Roux-en-Y.

followed by gastric pull-up and cervical anastomosis is a standard procedure for patients with esophageal cancer. The DAUGS20 score of the retrosternal approach was 39.8 (n = 38), and for the retromediastinal approach was 33.5 (n = 45). Table 4 shows the average DAUGS20 scores of the 3 institutions involved in the study.

Resultant factor loadings are shown in Table 5, and designated as: "regurgitation/reflux," "passage dysfunction immediately after eating," "limited activity due to decreased food consumption," "diarrhea-like symptoms," "reduction of gastric activity," "transfer dysfunction/deglutition disturbances," and "hypoglycemic symptoms." The Cronbach coefficients for each of these 7 factors were: 0.820, 0.762, 0.811, 0.856, 0.612, 0.789, and 0.705, respectively (Table 5).

The Cronbach coefficient for all 20 items in the scale (n = 883) was 0.904; the Cronbach coefficients for the subitems ranged from 0.612 to 0.856. The Guttman split-half reliability coefficient for the subitems, which is used to assess the uniformity of items, established stability within the range of 0.422 to 0.856.

Validity was evaluated using the "known-group" technique. The total preliminary score by surgical procedure was judged to show appropriate differences ($p < 0.0001$), so the validity of the construction was confirmed. The preliminary scale score in respondents who underwent resection of an upper gastrointestinal malignancy was based on the type of surgical procedure performed (Table 3).

DISCUSSION

There are a number of instruments to evaluate QOL in patients with upper GI malignancies. Among the most widely used are the European Organization for Research and Treatment of Cancer Quality of Life Core-30 (EORTC QLQ-C30) and the Functional Assessment of Cancer Therapy Scale-General Measure (FACT-G).[15] Disease-specific measures should be designed to particularly assess QOL of patients with a specific disease.[16] The QLQ-STO 22 instrument, administered with the QLQ-C 30, was developed to evaluate digestive symptoms in gastric cancer patients undergoing a variety of treatment approaches including surgery, chemotherapy, radiotherapy, and palliative therapy.[17] The QLQ-OES 18 is used to assess QOL in patients with esophageal cancer,[18]and the QLQ-OG25 is to assess QOL with cancer of the esophagus, esophagogastric junction, and stomach.[19] Accordingly, the QLQ scoring system includes questions regarding anxiety about the future, gustatory disorders, and alopecia. The FACT-G (www.facit.org/FACITOrg) has developed a wide range of questionnaires that assess QOL for various specific cancer types, including the "FACT-Ga" for gastric cancer patients[20] and "FACT-E" for esophageal cancer patients.[21] These FACT systems assess not only physical wellbeing but also social, family, emotional, and functional wellbeing. Another typical evaluation scale for digestive symptoms is the Rating Scale for Gastrointestinal Symptoms (GSRS), developed to evaluate postoperative digestive symptoms in patients with peptic ulcer and irritable bowel syndrome.[22]

There have been at least 20 publications regarding QOL after gastrectomy and esophagectomy using instruments such as the QLQL-C30, QLQ-STO22, OES18, OG25, FACT-Ga, FACT-E, GIQLI, and GSRS.[1,23-26] However, QLQ and FACT scoring systems may not be as useful in the assessment of QOL in patients after resection to assess postoperative dysfunction, because the aim of these surveys is to compare surgery, chemotherapy, radiotherapy, endoscopic treatment, and best supportive care. Questions in the GSRS and GIQLI instruments are based on the assumption that the stomach is present, which also might be inappropriate for patients after total or partial gastrectomy, vagotomy, or gastric pull-up. Despite the variety of assessment tools available, before development of the DAUGS32

Table 4. DAUGS20 Scores and Institution

	Gastric cancer surgery		Esophageal cancer surgery	
Institution	DAUGS20 score	n	DAUGS20 score	n
Hospital A	26.5 ± 13.5	308	33.5 ± 13.1	58
Hospital B	29.6 ± 13.5	149	36.1 ± 15.5	64
Hospital C	28.5 ± 12.5	203	37.6 ± 13.8	97
Total	27.8 ± 13.2	660	36.1 ± 14.2	219

DAUGS, Dysfunction after Upper Gastrointestinal Surgery.

Table 5. Factor Loading of 20 Items Determined by the Method of Factor Extraction, Principal Factor Method, and Varimax Rotation

	Factor loading						
Factor name	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**	**Factor 6**	**Factor 7**
Gastroesophageal reflux, $\alpha = 0.82$, r = 0.759							
Difficulty sleeping because of regurgitated bitter tasting fluid	0.884	0.102	0.075	0.049	0.093	0.048	0.012
Acidic fluid regurgitated	0.782	0.051	0.139	0.076	0.087	0.203	0.056
Choking when swallowing food	0.672	0.179	0.064	0.142	0.125	0.119	0.086
Vomiting after meals	0.438	0.340	0.091	0.033	0.169	0.064	0.113
Deglutition dysfunction, $\alpha = 0.762$, r = 0.752							
Pain in the pit of the stomach	0.127	0.725	0.089	0.092	0.120	0.125	0.125
Abdominal pain within 30 min of eating	0.120	0.672	0.150	0.205	0.109	0.061	0.180
Sensation of food retained in chest	0.202	0.516	0.158	0.060	0.152	0.243	0.205
Nausea	0.125	0.416	0.186	0.283	0.330	0.185	0.023
Limited activity due to decreased food consumption, $\alpha = 0.811$, r = 0.740							
Less strength or a lower activity level	0.134	0.125	0.825	0.128	0.212	0.065	0.199
Dizzy or unsteady	0.172	0.230	0.591	0.149	0.219	0.224	0.248
Sensation of abdominal fullness after eating	0.080	0.208	0.467	0.133	0.246	0.210	0.138
Diarrhea symptoms, $\alpha = 0.856$, r = 0.856							
Diarrhea	0.104	0.198	0.075	0.897	0.063	0.098	0.168
Soft stools	0.126	0.128	0.185	0.721	0.124	0.089	0.198
Dumping syndrome symptoms, $\alpha = 0.612$, r = 0.422							
Sudden bloating during meal	0.125	0.230	0.146	0.134	0.777	0.110	0.086
Lethargy or tiredness	0.156	0.051	0.313	0.063	0.565	0.106	0.152
Heavy sensation in stomach/nausea	0.254	0.378	0.223	0.139	0.494	0.199	0.080
Transfer dysfunction, $\alpha = 0.789$, r = 0.789							
Poor appetite	0.174	0.167	0.142	0.128	0.096	0.884	0.091
Difficulty in swallowing soft food	0.235	0.233	0.191	0.081	0.250	0.587	0.055
Hypoglycemic symptoms, $\alpha = 0.705$, r = 0.705							
Fatigue or weakness within 2–3 h of eating	0.042	0.225	0.225	0.176	0.096	0.075	0.778
Sleepiness within 2–3 h of eating	0.114	0.164	0.172	0.193	0.121	0.073	0.524

Cumulative proportion of variance explained 61.4%.
Cronbach's coefficient alpha of 20 items (α) = 0.904. Guttman split-half reliability coefficient of 20 items (r) = 0.891.

scoring system, there were no tools specifically designed to assess QOL in patients after upper GI resection of malignancies.[11]

There are various surgical approaches to the resection of gastric and esophageal cancers, including routine open resection and laparo-/thoracoscopic surgery. There are also various reconstruction methods used after gastrectomy and esophagectomy. However, evaluation and comparison of QOL after surgical procedures (eg, distal vs total gastrectomy, Billroth-I vs Roux-en-Y reconstruction, with vs without pouch reconstruction after total gastrectomy, route of gastric pull-up after esophagectomy, level of lymph-node dissection, etc) are difficult because no evaluation method specific for patients after resection of upper GI malignancies has been available. In answer to this need, the DAUGS32 scoring system was developed, referring to more than 200 references from Japan and other countries, and questions were integrated into 32 items based on statistical data for symptoms after upper GI surgery. The reliability and validity of DAUGS32 were reported, including demonstration of its clinical usefulness.[10,11] In addition to a high level of reliability and validity, most important is to follow a valid developmental scale. This was a major factor in the development of the DAUGS32 scoring system. However, after development of DAUGS32, it was desirable to further refine the scales used and to shorten the

survey instrument, which resulted in the DAUGS20 scoring system.

In order to be useful, the reliability coefficient of scales should be 0.6 or 0.7. The α coefficient of all 20 items in the DAUGS20 scoring system (n = 898) was 0.904 and Cronbach's α coefficients of the subscales were 0.612 to 0.856, showing high levels of reliability.[13,14] Regarding validity, differences in results among the surgical procedures were analyzed using the known group method, and one of the construct validities was confirmed. Surgical procedures can be roughly divided into gastric and esophageal cancer groups. The mean score after gastric cancer surgery was 27.8 and that after esophageal cancer surgery was 36.1, showing a significant difference ($p < 0.001$), similar to the tendency observed in the previous study using DAUGS32.[11] Based on these results, construct validity was confirmed.

A significant difference was noted between total and distal gastrectomy, and differences were also shown depending on the method of reconstruction after resection of esophageal cancer, suggesting that this method is not only reliable but also useful for comparing various surgical procedures, including both resection and reconstruction. Table 3 shows a comparison of the DAUGS20 score by surgical procedure. These data show that the refined DAUGS20 scale is both reliable and valid. Future studies will focus on a detailed comparison by surgical procedure for gastric and esophageal cancers.

Seven factors were extracted from the 20-item scale by definitive factor analysis (Table 5). The factors were designated as "gastro-esophageal reflux," "deglutition dysfunction," "limited activity due to decreased food consumption," "diarrhea symptoms," "dumping syndrome symptoms," "transfer dysfunction," and "hypoglycemic symptoms." The factor structure of subitems comprising each of the 7 factors was analyzed by the study group and experts in digestive surgery. As a result, the structures of the 7 factors were symptoms sufficiently representing the factor name, and further confirming the factorial validity of this scale. DAUGS20 is capable of subgroup analysis by factor, in addition to simple comparison of the total score, which is also an advantage of the DAUGS20 score.

It is interesting to note that the DAUGS20 score did not change over time, as seen in Figure 1. Similar results were observed with the DAUGS32 scoring system. In a study using the QLQ-C30 instrument, Kobayashi and colleagues[25] noted that QOL scores were different at 1 month after surgery and then returned to baseline for most parameters by 3 months. The fact that the DAUGS20 scores did not change over time may reflect the fact that the time points selected were not appropriate to show a change. In addition, patients in this study were not all followed longitudinally. Following the same cohort of patients at more time points, especially immediately after resection, may demonstrate a change in the DAUGS20 score over time, and is the design of currently ongoing studies. Future studies will examine the usefulness of the DAUGS20 scoring system in evaluating other patients, such as those undergoing pancreaticoduodenectomy, who may also suffer from symptoms related to their upper GI resection. After this validation study of the DAUGS20 instrument, it will be compared with results from other instruments such as the QLQ-C30.

CONCLUSIONS

Based on this study, evaluation of patients after resection of upper GI malignancies can be effectively performed using the DAUGS20 scoring system. One possible limitation of this system is that it was developed and tested with Japanese patients. For clinical application in other countries, strict verification by country is desirable.[23,27] However, more than 90 references from other countries were used to prepare the items included in DAUGS20, and no particular difference was noted in postoperative symptoms after upper GI surgery between Japan and other countries. Application and development of DAUGS20 in other counties may be possible, which can be demonstrated by performing international surveys. DAUGS20 is recommended to evaluate the dysfunctions after surgery for upper GI malignancies, and can effectively replace DAUGS32 as a reliable and valid tool for evaluating postoperative QOL.

Author Contributions

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Analysis and interpretation of data: Nakamura, Takemasa, Mori, Lefor
Drafting of manuscript: Nakamura, Hosoya, Lefor, Yano, Umeshita, Doki, Miyashiro, Dannoue, Kishi, Mori
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REFERENCES

1. Kaptein AA, Morita S, Sakamoto J. Quality of life in gastric cancer. World J Gastroenterol 2005;11:3189–3196.
2. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473–483.
3. Tyrväinen T, Sand J, Sintonen H, Nordback I. Quality of life in the long-term survivors after total gastrectomy for gastric carcinoma. J Surg Oncol 2008;97:121–124.

4. Cella DF, Tulsky DS, Gray G, et al. The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. J Clin Oncol 1993;11:570–579.
5. Eypasch E, Williams JI, Wood-Dauphinee S, et al. Gastrointestinal Quality of Life Index: development, validation and application of a new instrument. Br J Surg 1995;82:216–222.
6. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: A quality-of-life instrument for use in international clinical trials in oncology. J Nat Cancer Inst 1993;85:365–376.
7. Vickery CW, Blazeby JM, Conroy T, et al. Development of an EORTC disease-specific quality of life module for use in patients with gastric cancer. Eur J Cancer 2001;37:966–971.
8. Nakamura M, Kido Y, Yano M, et al. Reliability and validity of a new scale to assess postoperative dysfunction after resection of upper gastrointestinal carcinoma. Surg Today 2005;35:535–542.
9. Nakamura M, Kido Y, HosoyaY, et al. Postoperative gastrointestinal dysfunction after 2-field versus 3-field lymph-node dissection in patients with esophageal cancer. Surgery Today 2007;37: 379–382.
10. Nakamura M, Kido Y, Egawa T. Development of a 32-item scale to assess postoperative dysfunction after upper gastrointestinal cancer resection. J Clin Nurs 2008;17:1440–1449.
11. Nakamura M, Hosoya Y, Yano M, et al. Extent of gastric resection impacts patient quality of life: the Dysfunction After Upper Gastrointestinal Surgery for Cancer (DAUGS32) scoring system. Ann Surg Oncol 2010;18:314–320.
12. J Jones E. Translation of quantitative measures for use in cross-cultural research. Nurs Res 1987;36:324–327.
13. Jones EG, Kay M. Instrumentation in cross-cultural research. Nurs Res 1992;41:186–188.
14. Fayers P, Machin D. Quality of Life: the Assessment Analysis and Interpretation of Patient Reported Outcomes. Second ed. Chichester: Wiley;2007.
15. Blazeby JM, Kavadas V, Vickery CW, et al. A prospective comparison of quality of life measures for patients with esophageal cancer. Qual Life Res 2005;14:387–393.
16. Drossman DA, Patrick DL, Whitehead WE, et al. Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire. Am J Gastroenterol 2000;95:999–1007.
17. Blazeby JM, Conroy T, Bottomley A, et al. Clinical and psychometric validation of a questionnaire module, the EORTC QLQ-STO 22, to assess quality of life in patients with gastric cancer. Eur J Cancer 2004;40:2260–2268.
18. Blazeby JM, Conroy T, Hammerlid E, et al. Clinical and psychometric validation of an EORTC questionnaire module, the EORTC QLQ-OES18, to assess quality of life in patients with oesophageal cancer. Eur J Cancer 2003;39:1384–1394.
19. Lagergren P, Fayers P, Conroy T, et al. Clinical and psychometric validation of a questionnaire module, the EORTC QLQ-OG25, to assess health-related quality of life in patients with cancer of the oesophagus, the oesophago-gastric junction and the stomach. Eur J Cancer 2007;43:2066–2073.
20. Eremenco SL, Cashy J, Webster K, et al. FACT-gastric: a new international measure of QOL in gastric cancer. J Clin Oncol 2004;22(14S):8123 [2004 ASCO annual meeting proceedings (post-meeting edition)].
21. Darling G, Eton DT, Sulman J, et al. Validation of the functional assessment of cancer therapy esophageal cancer subscale. Cancer 2006;107:854–863.
22. Svedlund J, Sjödin I, Dotevall G. GSRS-a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci 1988;33:129–134.
23. Lee SS, Chung HY, Yu W. Quality of life of long-term survivors after a distal subtotal gastrectomy. Cancer Res Treat 2010;42: 130–134.
24. Adachi Y, Suematsu T, Shiraishi N, et al. Quality of life after laparoscopy-assisted Billroth I gastrectomy. Ann Surg 1999;229: 49–54.
25. Kobayashi D, Kodera Y, Fujiwara M, et al. Assessment of quality of life after gastrectomy using EORTC QLQ-C30 and STO22. World J Surg 2011;35:357–364.
26. Horváth OP, Kalmar K, Cseke L, et al. Nutritional and life-quality consequences of aboral pouch construction after total gastrectomy: A randomized, controlled study. Eur J Surg Oncol 2001;27:558–563.
27. Debb SM, Arnold B, Perez B, Cella D. Validation of the FACT-Gastric cancer quality of life questionnaire for use in Spanish-speaking countries. Psychooncology 2011;20:19–27.

Journal of Surgical Oncology 2012;105:189–194

REGIV as a Potential Biomarker for Peritoneal Dissemination in Gastric Adenocarcinoma

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Background: This study examined the clinical significance of regenerating islet-derived family member 4 (REGIV) in surgically resected gastric tumors. The potential of REGIV as a biomarker in gastric cancer was also assessed including its predictive value for prognosis and recurrence after surgery.

Methods: Immunohistochemistry was performed to assess the clinical significance of REGIV expression status in surgically resected specimens. The quantitative genetic diagnostic method, transcription-reverse transcription concerted reaction (TRC) that targeted REGIV mRNA was applied for prediction of peritoneal recurrence in gastric cancer.

Results: Positive immunostaining for REGIV was observed in 85 cases (52.5%), and correlated significantly with diffuse type histopathology ($P = 0.001$), advanced T stage ($P = 0.022$), and frequent peritoneal recurrence ($P = 0.009$). Multivariate analysis identified advanced T stage ($P < 0.001$) and REGIV expression ($P = 0.034$) as independent prognostic factors for peritoneal recurrence-free survival. Overexpression of REGIV protein was evident in the majority of peritoneal tumors (93.8%). REGIV mRNA assessed by TRC could be a predictive marker for peritoneal recurrence after curative operation.

Conclusions: REGIV overexpression is common in primary gastric tumors and a potentially suitable marker of diffuse type histopathology and peritoneal dissemination. Overexpression of REGIV mRNA, assessed by the TRC method, is a potentially suitable marker of peritoneal recurrence after curative resection.

J. Surg. Oncol. 2012;105:189–194. © 2011 Wiley Periodicals, Inc.

KEY WORDS: **gastric cancer; REGIV; peritoneal dissemination; TRC; molecular diagnosis**

INTRODUCTION

The incidence of gastric cancer has decreased worldwide and particularly so in Western countries. Despite this, it remains the fourth most common cancer and the second most common cause of cancer-related death [1,2]. The prognosis of patients with advanced gastric cancer, especially those with serosa-invading tumors, remains poor even after curative operation. In such cases, peritoneal dissemination due to seeding of free cancer cells from the primary gastric cancer is the most common type of spread [3–5]. The identification of suitable biomarkers to predict peritoneal recurrence and prognosis is therefore important to advance the treatment of patients with gastric cancer.

Regenerating islet-derived family member 4 (REGIV) belongs to a superfamily of calcium-dependent lectins [6]. REGIV is expressed in various normal tissues including the stomach, colon, small intestine, and pancreas [7,8], and is overexpressed in various tumors such as gastric, colorectal, pancreas, prostate, and gallbladder cancers [7–11]. Overexpression of REGIV was shown in colorectal adenomas with severe dysplasia and adenocarcinoma, indicating the involvement of REGIV in the early stages of colorectal carcinogenesis [12]. REGIV protein expression was also reported in goblet cells of intestinal metaplasia and goblet-like cell vesicles of gastric cancer, implicating REGIV in the differentiation of stomach cancer. A recent in vitro study further showed that the carbohydrate-recognition domain of REGIV protein is critical for colorectal cell migration and invasion [13]. Several studies have identified REGIV as a potent activator of epidermal growth factor receptor (EGFR)/Akt/activator protein-1 (AP-1). Furthermore, colon cancer cells treated with recombinant REGIV showed increased expression of Bcl-2, Bcl-xl, and survivin, suggesting a role in the inhibition of apoptosis [14–16]. Finally, REGIV expression also correlated significantly with resistance to combination chemotherapy with 5-fluorouracil (5-FU) and cisplatin [15]. Despite these data linking REGIV and human cancers, the precise biological function of REGIV overexpression in human cancer remains unclear.

In this study, we examined the expression of REGIV protein in gastric cancer tissues and assessed the correlations between REGIV expression and clinicopathological characteristics. The results showed that overexpression of REGIV protein correlated significantly with diffuse type histopathology and peritoneal recurrence after surgery. Furthermore, REGIV overexpression was observed in most peritoneal disseminated tumors obtained by surgery or staging laparoscopy. We introduce a novel, rapid, and quantitative genetic diagnostic technique that targets REGIV mRNA and called it the

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Received 29 March 2011; Accepted 16 June 2011

DOI 10.1002/jso.22021

Published online 20 July 2011 in Wiley Online Library (wileyonlinelibrary.com).

transcription-reverse transcription concerted reaction (TRC) to detect occult cancer cells in the peritoneal cavity of patients with gastric cancer. In another study, we assessed the clinical significance of the molecular diagnosis and examined the association between REGIV expression and chemoresistance to the combination chemotherapy of S-1 plus cisplatin, which is a standard regimen for gastric cancer in Japan [17].

MATERIALS AND METHODS

Patients and Specimens

We obtained gastric cancer tissues from 162 patients who underwent gastrectomy at the Department of Gastroenterological Surgery, Osaka University Hospital between 2000 and 2008. All tumors were confirmed as gastric adenocarcinoma by histopathological examination. The patients comprised 115 males and 47 females, aged 34–92 years (median, 66 years). Table I lists the characteristics of patients registered in this study. The pathological features were classified based on the 13th edition of the Japanese Classification of Gastric Cancer [18]. Sixteen peritoneal disseminated tumors were obtained from patients by surgery or staging laparoscopy and the corresponding 15 primary tumor specimens were also obtained from patients by surgery or upper gastrointestinal endoscopy. Twenty specimens biopsied during upper gastrointestinal endoscopy and three surgically resected tumor specimens were also obtained from patients treated with the combination chemotherapy of S-1, 5-FU derivative, and cisplatin [17]. The expression of REGIV mRNA by TRC in peritoneal lavage specimens of 95 patients was examined to test for correlation between TRC and cytology. Of those sampled, 50 patients who received no neoadjuvant chemotherapy and whose peritoneal lavage cytology was diagnosed as negative were assessed for further survival analyses.

Evaluation of Clinical Response to Chemotherapy

Before and after chemotherapy with S-1 plus cisplatin, conventional examinations including multidetector row computed tomography and gastric endoscopy were performed to assess the clinical response. The tumor response of measurable metastatic lesions was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) [19]. A complete response (CR) was defined as the disappearance of all evidence of cancer for more than 4 weeks. A partial response (PR) was defined as more than 50% reduction in the sum of the products of the perpendicular diameters of all lesions without any evidence of new regions or progression on any lesions. Stable disease (SD) was defined as less than a 50% reduction or less than a 25% increase in the sum of the products of the perpendicular diameters of all lesions, without any evidence of new lesions. Progressive disease (PD) was defined as a more than 25% increase in more than one region or the appearance of new regions.

Immunohistochemical Analysis

REGIV protein expression was evaluated by immunohistochemical (IHC) analysis of 4-μm thick sections from 10% formalin-fixed and paraffin-embedded blocks. For IHC staining, tissue slides were deparaffinized in xylene, and then rehydrated through a graded ethanol series. For antigen retrieval, slides were autoclaved in 10 mM citrate buffer (pH 6.0) at 121°C for 10 min. Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide in methanol for 20 min, and then nonspecific binding was blocked in 10% normal serum for 20 min. The sections were then incubated overnight at 4°C in a moist chamber with anti-REGIV antibody (dilution 1:50; R&D Systems, Minneapolis, MN). The sites of antibody binding were visualized with the ABC peroxidase detection system (Vector Laboratories, Burlingame, CA). Finally, the sections were incubated in 3,3′-diaminobenzidine tetrahydrochloride with 0.05% H_2O_2 for 3 min and counterstained with 0.1% hematoxylin. The percentage of cancer cells stained with the antibody was evaluated. The presence of REGIV protein was judged as positive if more than 10% of the total observed cancer cells were positively stained; any less was judged as negative.

RNA Extraction

Total cellular RNA was extracted from cell pellets of peritoneal lavage fluid samples and cancer cell lines using TRIZOL reagent according to the manufacturer's protocol. In brief, the cell source mixture was minced using disposable homogenizers (IEDA^{TM}, Tokyo, Japan), mixed with 0.2 ml chloroform, and then centrifuged at 12,000*g* for 15 min. The supernatant was transferred to a fresh tube and mixed with 0.5 ml 100% isopropyl alcohol. After incubation for 10 min at room temperature, RNA was precipitated by centrifugation, washed with 75% ethanol, and then diluted with diethyl pyrocarbonate (DEPC)-treated water.

TABLE I. Relationship Between REGIV Expression and Various Clinicopathological Characteristics in Patients With Gastric Cancer (n = 162)

		REGIV		
	n	Negative	Positive	*P*-value
Age <70/≥70	99/63	45/32	54/31	0.507
Gender (M/F)	115/47	55/22	60/25	0.906
Histological type				
Differentiated	77	47	30	0.001
Undifferentiated	85	30	55	
pT T1/T2/T3/T4	27/82/48/5	19/34/20/4	8/48/28/1	0.022
pN N0/N1/N2/N3	72/55/33/2	37/26/12/2	35/29/21/0	0.232
pStage I/II/III/IV	61/41/51/9	34/18/19/6	27/23/32/3	0.148
Cytology (negative/positive)	157/5	75/2	82/3	0.497
Lymph node recurrence (negative/positive)	152/10	73/4	79/6	0.623
Liver recurrence (negative/positive)	146/16	64/13	79/6	0.052
Peritoneal recurrence (negative/positive)	144/18	74/3	71/14	0.009

pStageI includes pStageIA and pStageIB.
pStageIII includes pStageIIIA and pStageIIIB according to the 13th edition of the Japanese Classification of Gastric Cancer.

Sequences of Primers and Probes for TRC

Synthetic oligonucleotide sequences of a pair of primers, a scissors probe for TRC amplification, and an intercalation-activating fluorescence (INAF) probe for detection of REGIV mRNA are listed in Table II. Numbers in parentheses indicate the corresponding position of the target genome sequences (Gene Bank Accession NM_032044.2). Sequences of the promoter primers indicated in italics are the T7 RNA polymerase-binding sequences. The primers, a scissors probe, and the INAF probe were designed to bind to the secondary-structure-free sites of REGIV mRNA. The INAF probe is a DNA oligonucleotide linked with an intercalating fluorescence dye, oxazole yellow. The 3′-OH end of the scissors probe and INAF probe was capped with an amino group and glycolic acid, respectively, to avoid undesired enzymatic elongation by the Avian Myeloblastosis Virus (AMV) reverse transcriptase reaction. Synthetic oligonucleotides of primers and the scissors probe were provided by Sawady Technology (Tokyo, Japan). Synthesis of the INAF probe for REGIV amplicons was performed as described previously [20].

TRC Reaction

The TRC reaction was conducted as described previously [20]. In brief, 20 μl of the TRC buffer was added to 5 μl of the RNA extract in a thin-wall PCR tube, followed by the addition of 5 μl of enzyme mix. The tube containing the mixture was closed and set in a dedicated instrument, the "TRC monitor," to measure the fluorescence intensity of the reaction mixture incubated at 44°C (excitation wavelength 470 nm, emission wavelength 520 nm).

Real-Time Monitoring of TRC Reaction

The "TRC monitor" was constructed on a round incubator block and rotating fluorescence scanning unit [20]. The temperature of the incubator block was controlled at optimal TRC conditions (44°C) and 32 thin-wall PCR tubes were installed and set in a circle. These were assembled into 1 U to enable synchronous scanning of the fluorescence while irradiating the tube. The LED turns like a beacon to irradiate the excitation light of 470 nm into a tube from outside. The fluorescence (520 nm) is then transferred from the bottom of the tube to a photomultiplier through a light guide.

TABLE II. Synthetic Oligonucleotide Sequences of a Pair of Primers, a Scissors Probe for Amplification, and an INAF Probe for Detection of REGIV mRNA in the TRC Reaction

Scissors probe (68–93)
26 base antisense
5-TATATCTTCTTGCCTCAGGAATTAAT-3
Forward primer (83–106)
45 base sense
5-*CTAATACGACTCACTATAGGG*AAGAAGATATAAAAGCTCCAGAAA-3
Reverse primer (168–194)
27 base antisense
5-GGGTTCTCCTTGATCTGCAAATCTGTT-3
INAF probe (147–166)
20 base antisense
5-GGCAACCAAGACTCTAAGGG-3

INAF, intercalation activating fluorescence; TRC, transcription-reverse transcription concerted reaction.

Numbers in parentheses indicate the corresponding position of the target genome sequences. The sequence indicated by the italicized letters of the promoter primers is the T7 RNA polymerase-binding sequence.

Statistical Analysis

Statistical analysis was performed with JMP® software (JMP version 8.0.2, SAS Institute, Cary, NC). The associations of REGIV expression with the patients' clinicopathological features were assessed by the chi-squared test. Disease-free survival (DFS) and overall survival (OS) were assessed using the Kaplan–Meier method and compared by the log-rank test. Multivariate survival analysis was performed on all parameters that were found to be significant by univariate analysis using the Cox proportional hazard model. P-values <0.05 were considered significant.

RESULTS

REGIV Protein Expression in Gastric Cancer Tissues

The expression of REGIV was investigated in 162 cases of gastric adenocarcinoma by IHC. Of these, 85 cases (52.5%) were considered positive for REGIV, which was detected mainly in the cytoplasm of tumor cells (Fig. 1A). The remaining 77 cases (47.5%) showed negative staining (Fig. 1B). The positive cells for REGIV were detected in various areas of the formed tumor including the surface, central, and deepest areas of the gastric wall.

Correlations Between REGIV Expression and Clinicopathological Parameters

Table II shows the correlations between REGIV overexpression detected by IHC and various clinicopathological parameters for the 162 patients with gastric cancer. The proportion of REGIV-positive cases was significantly higher with diffuse type histology, advanced pathological T stage, and frequent peritoneal recurrence, and REGIV-positive cases tended to harbor infrequent liver metastasis ($P = 0.052$). Other parameters listed in Table II (age, gender, pathological N stage, pathological S stage, and lymph node metastasis) showed no significant correlation with REGIV expression. However, REGIV overexpression did not correlate with recurrence-free survival, but was significantly associated with poorer peritoneal recurrence-free survival and tended to be associated with better recurrence-free survival at sites other than the peritoneum (Fig. 2A–C).

Prognostic Significance of REGIV Expression for Peritoneal Recurrence

Univariate analysis by Cox's proportional hazard model identified several clinicopathological parameters as significant predictors of prognosis (Table III), namely pathological T stage, pathological N stage, and REGIV expression (HR = 8.773, HR = 4.440, and HR = 4.113, respectively; Table III). However histological type was not a significant prognostic factor (HR = 2.253). Multivariate analysis that included all the above significant parameters identified pathological T stage and REGIV expression as significant independent prognostic predictors (HR = 6.359 and HR = 3.362, respectively; Table III).

Expression of REGIV in Peritoneal Metastatic Tumors

Subsequent IHC analysis of REGIV expression in 16 peritoneal tumors metastasized from gastric cancer revealed 15 (93.8%) with overexpressed REGIV (Fig. 1C). Furthermore, 14 out of 15 corresponding primary tumors that overexpressed REGIV protein in peritoneal metastasis showed overexpression of REGIV (Fig. 1D).

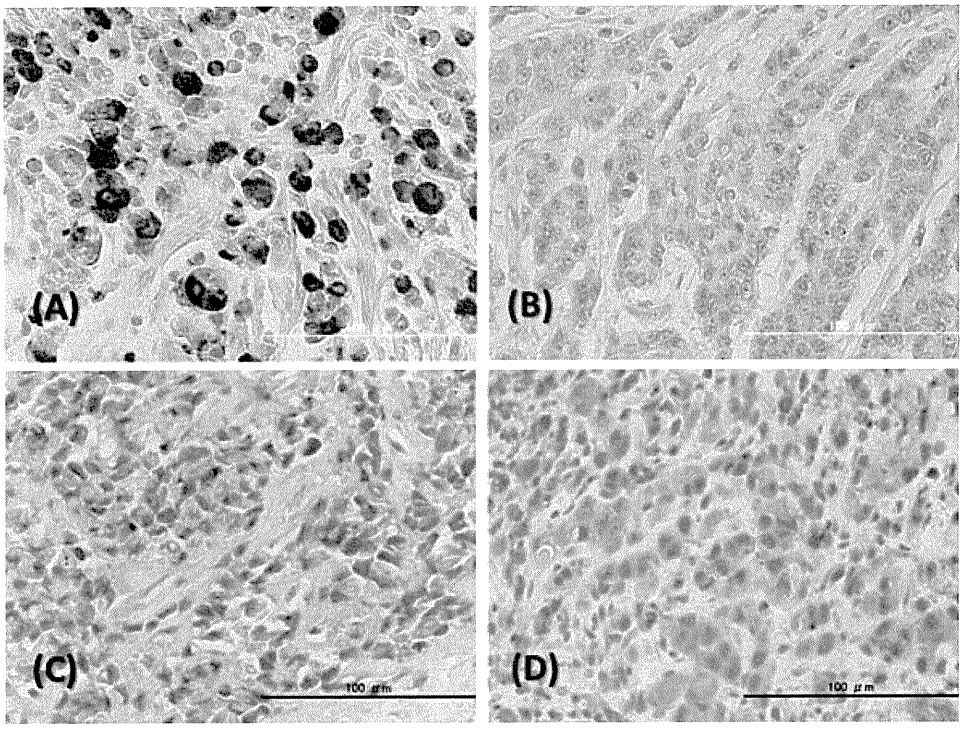

Fig. 1. Immunohistochemistry for REGIV protein in gastric cancer tissues. **A**: Representative positive staining for REGIV in primary tumor. **B**: Representative negative staining for REGIV in primary tumor. **C**: Representative positive staining in endoscopically biopsied specimen from primary tumor. **D**: Representative positive staining in peritoneal metastatic tumor.

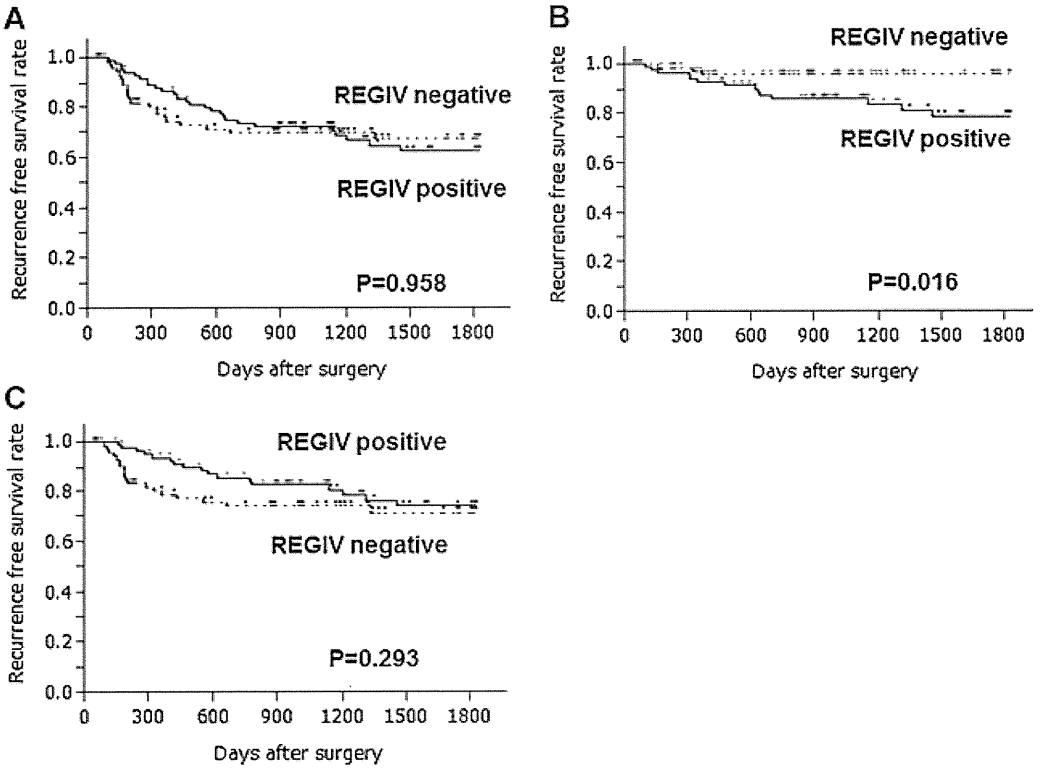

Fig. 2. Survival analysis according to REGIV expression in primary gastric cancer. **A**: Overall survival. **B**: Peritoneal recurrence-free survival. **C**: Recurrence-free survival outside of peritoneal sites.

TABLE III. Result of Univariate and Multivariate Survival Analyses of Peritoneal Recurrence-Free Survival by Cox's Proportional Hazard Model (n = 162)

	n	HR	95% CI	*P*-value
Univariate survival analysis				
Age (≥70/<70)	63/99	0.613	0.172–1.741	0.378
Gender (female/male)	47/115	0.675	0.190–1.909	0.477
Histological type (undifferentiated/differentiated)	85/77	2.253	0.836–7.082	0.111
pT(3–4/1–2)	53/109	8.773	3.096–31.205	<0.001
pN(1–3/0)	90/72	4.440	1.445–19.286	0.008
Cytology (positive/negative)	5/157	3.478	0.191–17.509	0.303
RegIV expression (positive/negative)	85/77	4.113	1.342–17.842	0.011
Multivariate survival analysis				
pT(3–4/1–2)	53/109	6.359	2.157–23.404	<0.001
N(1–3/0)	90/72	2.226	0.687–10.012	0.195
RegIV expression (positive/negative)	85/77	3.362	1.089–14.641	0.034

HR, hazard ratio; 95% CI, 95% confidence interval.

Correlations Between REGIV Protein Expression and Efficacy of Chemotherapy With S-1 Plus Cisplatin

Twenty preoperative specimens were biopsied by upper gastrointestinal endoscopy and 3 were surgically resected from patients subjected to combination chemotherapy of S-1 plus cisplatin [17]. There was no significant correlation between REGIV expression in these specimens and the effect of chemotherapy (CR + PR vs. SD + PD) in these cases.

TRC Analysis of Peritoneal Lavage Samples for REGIV mRNA

Finally, we examined the expression of REGIV mRNA by TRC in peritoneal lavage specimens of 95 patients to test for correlation between TRC and cytology. Of those sampled, 50 patients who received no neoadjuvant chemotherapy and whose peritoneal lavage cytology was diagnosed as negative were assessed for survival analyses. Table IV shows the correlative results, with 24 (96.0%) out of 25 cytology-positive specimens and 12 (17.1%) out of 70 cytology-negative specimens showing a positive TRC diagnosis. Figure 3 shows the comparative OS statistics for patients with gastric cancer after curative resections according to the TRC diagnosis for REGIV from peritoneal lavage specimens. Peritoneal recurrence-free survival in patients with positive TRC was significantly worse than in patients with negative TRC, although OS was not significantly different between the groups.

DISCUSSION

The present study indicated overexpression of REGIV protein in 52.5% of gastric cancers examined and identified an association between this expression and diffuse-type histopathology, tumor progression (advanced pT status), and frequent peritoneal recurrence. Furthermore, the REGIV overexpresssion was significantly associated with poorer peritoneal recurrence-free survival, although with no other type of recurrence-free survival in gastric cancer patients. The clinical significance of REGIV overexpression in gastric cancer is controversial. Oue et al. [7] reported REGIV overexpression in about 30% of gastric adenocarcinomas, in a significant association with poorly differentiated gastric cancer, although they found no associations with T status, N status, or pathological stage. In another study of 63 gastric cancer tumors, Yamagishi et al. [21] observed REGIV overexpression in 49% of cases, but found no relationship with any clinicopathological features including histology, lymph node metastasis, and clinical stage. In the study overexpression of REGI alpha, one of REG family, but not REGIV was an independent prognostic factor.

Mitani et al. [15] reported that REGIV expression correlated significantly with resistance to combination chemotherapy with 5-FU and cisplatin. However, in our study, there was no significant correlation between REGIV expression and the effect of combination chemotherapy with a 5-FU derivative, S-1, and cisplatin.

The present study showed for the first time that REGIV overexpression was common in peritoneal metastatic tumors obtained during surgery or through staging laparoscopy (15/16, 94%), although REGIV protein was expressed in only 52.5% of primary tumors. These results suggested that REGIV overexpression could provide a biomarker for peritoneal dissemination in gastric cancer. Kuniyasu et al. [16] demonstrated that REGIV-transfected gastric cancer cell lines showed increased levels of BCL-2, BCL-XL, survivin, phosphorylated AKT, and phosphorylated EGFR, while peritoneal dissemination mouse models inoculated with REGIV-transfected gastric cancer cells showed increased number and size of peritoneal tumors and lower survival rates compared to untransfected controls. These authors also examined REGIV protein in peritoneal lavage samples obtained from gastric cancer surgery by immunoblot assay and showed that a REGIV-positive peritoneal lavage might be a good marker for peritoneal dissemination. In addition, REGIV mRNA expression assessed by quantitative RT-PCR was shown to be a sensitive predictive marker for peritoneal dissemination in gastric cancer [22]. However, RT-PCR procedures are complicated and time-consuming, thus further refinements are required for the clinical application of molecular diagnostic techniques for REGIV expression.

We reported previously a novel method of quantitative genetic diagnosis using the TRC reaction system for detection of cancer micrometastasis and prediction of cancer recurrence in patients with gastric cancer [23]. The method amplifies and measures a cancer-specific mRNA in a single tube at constant temperature (no thermal cycling) and with only three steps: denaturing, annealing, and extension for PCR. The single temperature reaction is likely to be more

TABLE IV. Relationship Between TRC and Cytology for Peritoneal Lavage Specimens in Patients With Gastric Cancer (n = 95)

	TRC		
	Negative	Positive	Total
Cytology			
Negative	58	12 (17.1%)	70
Positive	1	24 (96.0%)	25
Total	59	36	95