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

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 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.

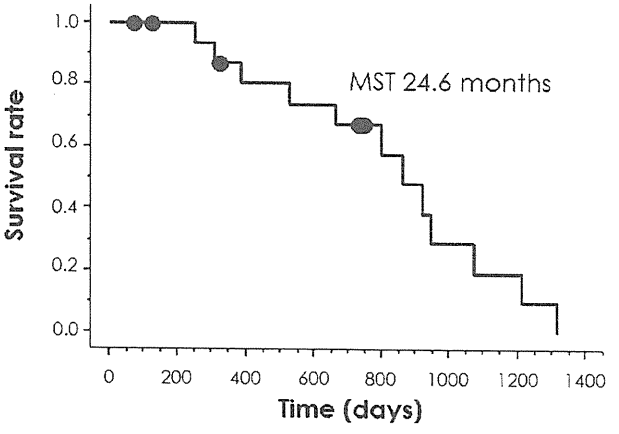

Fig. 2. Overall survival of 18 patients enrolled in this study. MST: Mean survival time. Median follow-up time was 45 months.

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 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.

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ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Neoadjuvant Intraperitoneal and Systemic Chemotherapy for Gastric Cancer Patients with Peritoneal Dissemination

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ABSTRACT

Background. The present study was designed to assess the feasibility and efficiency of intraperitoneal and intravenous neoadjuvant chemotherapy in gastric cancer patients with peritoneal dissemination.

Methods. The study subjects were 25 treatment-naïve patients with gastric cancer. Patients with positive cytology or with peritoneal carcinomatosis received neoadjuvant intraperitoneal and systemic chemotherapy (NIPS), comprising intraperitoneal (i.p.) mitomycin C (MMC) and cisplatin (CDDP), followed by two cycles of intravenous triplet chemotherapy of docetaxel, 5-fluorouracil (5-FU), and CDDP. Gastrectomy with lymph node dissection was performed after NIPS in patients free of peritoneal deposits, confirmed by staging laparoscopy.

Results. Seventeen patients had measurable lymph node metastases by the RECIST criteria. CT examination showed response to the treatment in ten (59%, 0 complete response, 10 partial response). Of the 25 patients, 14 (56%) showed negative results on peritoneal cytology with no macroscopic peritoneal metastasis, whereas the remaining 11 were cancer cell-positive on peritoneal cytology or macroscopic peritoneal metastasis even after NIPS. The median survival time for all 25 patients was 16.7 months. Prognosis was better in patients who showed negative cytology and disappearance of peritoneal cancer metastases after NIPS than in those with positive cytology or existing peritoneal deposits ($P < 0.0001$). The predominant toxicity was myelosuppression and grade 3–4 leukopenia and neutropenia occurred in 20 (80%) patients, which were manageable. No treatment-related mortality was observed during and after NIPS and surgery.

Conclusions. The results of this prospective phase II study indicated that the newly designed NIPS was highly effective and well tolerated in patients with advanced gastric cancer and peritoneal dissemination.

The prognosis of patients with advanced gastric cancer, especially those with serosa-invading tumors, remains poor even after curative resection, and in these cases, peritoneal dissemination caused by free cancer cells seeded from a primary gastric tumor is the most common type of recurrence.[1,2] Cytological examination of peritoneal lavage at laparotomy is usually performed to predict peritoneal recurrence.[3–5] Most cases with positive cytology on peritoneal lavage develop peritoneal recurrence even in patients without macroscopic peritoneal dissemination.[4,5]

Recently, a multidisciplinary approach, including chemotherapy, radiation, and surgery for advanced gastric cancer, has been developed and its survival benefit has been investigated worldwide.[6,7] Furthermore, several novel chemotherapeutic agents, including the taxans (paclitaxel and docetaxel), irinotecan, oxaliplatin, S-1, and capecitabine, have shown potent effects in gastric cancer.[8–13]

These advances in chemotherapy for gastric cancer encouraged us to introduce neoadjuvant chemotherapy for gastric cancer patients with poor prognosis, such as those with positive peritoneal lavage cytology. In this study, we performed peritoneal lavage cytology under local anesthesia or staging laparoscopy for patients with T3 or T4 gastric tumors diagnosed using multidetector row computed tomography (CT) and three-dimensional imaging before treatment.[14] Patients with positive cytology on peritoneal lavage specimens or with macroscopic peritoneal metastasis were enrolled in the study.

Intraperitoneal (i.p.) chemotherapy with mitomycin C (MMC) and cisplatin (CDDP) was reported to be safe for

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patients with T3 or T4 gastric tumors defined by preoperative staging laparoscopy in our pilot study.[15] In that study, the toxicity of the preoperative i.p. chemotherapy was minimal and no serious postoperative complications were observed. A course of intravenous triplet chemotherapy of docetaxel, 5-fluorouracil (5-FU), and CDDP, which was developed by our group, was given every 4 weeks.[16] The modified triplet regimen had been developed to reduce the severe hematological toxicities commonly encountered in the V325 phase III study used in western countries.[17] We reported that the modified regimen was less toxic and no serious complications were observed during chemotherapy and surgery.[16] After the sequential combination chemotherapy, a second staging laparoscopy was performed to evaluate the therapeutic effect for peritoneal dissemination and to decide on the indication of surgery. The purpose of this prospective study was to investigate the feasibility and efficacy of the newly developed neoadjuvant triplet chemotherapy in the setting for gastric cancer with positive peritoneal lavage cytology and/or macroscopic peritoneal dissemination.

MATERIALS AND METHODS

Patient Selection

The eligibility criteria for entry in this study were as follows: (1) the presence of gastric cancer confirmed by histopathology; (2) presence of positive peritoneal cytology (PPC) or peritoneal deposits confirmed by staging laparoscopy; (3) absence of noncurative factors, such as distant metastasis to liver, lung, or lymph nodes except for the peritoneal dissemination; (4) performance status [Eastern Cooperative Oncology Group (ECOG)] < 2; (5) age younger than 75 years; (6) no prior chemotherapy or surgery for gastric or other cancers; (7) adequate bone marrow function (leukocyte count > 3,000 ml^{-1} and platelet count > 100,000 ml^{-1}), (8) adequate liver function (serum bilirubin level < 1.5 mg dl^{-1} and serum transaminase levels less than twice the upper limit of normal); (9) adequate renal function (serum creatinine level < 1.5 mg dl^{-1}; (10) no other severe medical conditions, such as symptomatic infectious disease, intestinal pneumonia, active hemorrhage/bleeding, or obstructive bowel disease; (11) no current pregnancy or lactation; and (12) provision of written informed consent in accordance with government guidelines of each institution or hospital. This study was approved by the ethics committee of Osaka University Hospital.

Treatment Strategy

Figure 1 shows the treatment strategy followed in this study. A staging laparoscopy or peritoneal lavage cytology was performed under local anesthesia in gastric cancer patients with serosa-invading tumors.[14] Neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) were administered to patients with positive cytology and/or peritoneal metastasis. Staging laparoscopy was performed in all patients after chemotherapy, followed by gastrectomy with lymph node dissection in patients free of macroscopic peritoneal deposits of cancer metastasis. MMC was administered by i.p. infusion at a dose of 20 mg/body at day 1 and CDDP also was administered by i.p. infusion at a dose of 20 mg/body at days 1–5.[15] After a 2-week recovery period, we administered a chemotherapy combination of docetaxel at a dose of 60 mg/m^2 on day 1, 5-FU at a dose of 350 mg/m^2 on days 1–5, and CDDP at a dose of 10 mg/m^2 on days 1–5, every 4 weeks. The intravenous chemotherapy was repeated twice unless disease progression was observed after one cycle. All 22 patients who underwent surgery received adjuvant chemotherapy using 5-FU and cisplatin or 5-FU derivative, S-1.

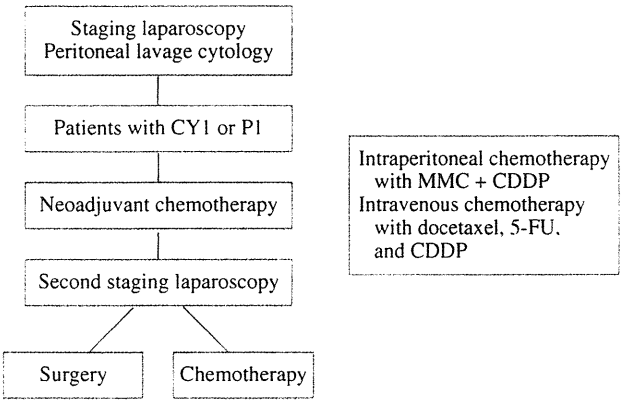

FIG. 1 Flow diagram of the treatment protocol. *CY1* patients with positive peritoneal cytology, *P1* patients positive for macroscopic peritoneal metastasis, *MMC* mitomycin C, *CDDP* cisplatin, *5-FU* 5-fluorouracil

Evaluation of the Disease

Before and after NIPS with i.p. and i.v. infusion of anticancer drugs, conventional examinations, including multidetector row computed tomography and gastric endoscopy were performed to assess the clinical response. A second staging laparoscopy was conducted to evaluate the effect of peritoneal metastasis. The tumor response of measurable metastatic lesions was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria.[18] 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 <50% reduction or <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 >25% increase in more than one region or the appearance of new region. The response of the peritoneal metastasis was evaluated by staging laparoscopy or surgery after NIPS.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 4.0 and recorded.

Statistical Analysis

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

RESULTS

Clinicopathological Characteristics

Between July 2000 and June 2006, a total of 25 patients with gastric cancer with peritoneal dissemination were enrolled in this study. Table 1 shows the clinicopathological characteristics of the enrolled patients treated at the Department of Gastroenterological Surgery, Osaka University Hospital. The patients were 13 men and 12 women with a mean age of 58.9 (range, 31–75) years. Macroscopically, infiltrating-type tumors (type 3 and type 4) accounted for 80% of the cases (20/25). Histopathologically, undifferentiated tumors, including poorly differentiated and signet ring cell carcinoma were dominant (20/25, 80%). Gastrectomy with lymph node dissection was performed in 22 of the 25 patients (88%), who showed no macroscopic peritoneal metastasis at the second staging laparoscopy, whereas surgery was not performed in the remaining 3 patients because of the presence of macroscopic deposits of cancer nests in the abdominal cavity. Eighteen of the 22 patients (82%) underwent total gastrectomy and 14 underwent additional splenectomy. Seventeen of 22 cases (77%) underwent D2 lymphadenectomy and 5 had D2 minus lymph nodes in the region of hilus lienis, which was classified as D1+ alpha.

TABLE 1 Clinicopathological variables of the 25 patients enrolled in the present study

Average age, year (range)	58.9 ± 11.8 (31–75)*
Male/female ratio	13/12
Tumor type	
1	1
2	4
3	8
4	12
Histology	
Diffuse type	20
Differentiated type	5
Distant metastasis except peritoneum	
Present	1 (liver metastasis)
Absent	24
Type of surgery (22 cases)	
Total gastrectomy	18
With splenectomy	14
Without splenectomy	4
Distal gastrectomy	4
Lymph node dissection	
D2	17
D1 + α	5

* Data are mean ± standard deviation

Clinical Response and Toxicity of NIPS

After NIPS, all patients were evaluated for the clinical response and toxicities. Of the 25 patients, 23 (92%) completed the sequence combination chemotherapy, whereas intravenous chemotherapy for the remaining 2 patients was withheld after one cycle due to the appearance of progressive diseases. Seventeen of 25 patients had measurable lymph node metastases by RECIST criteria. As shown in Table 2, the CT scan showed that 10 of 17 (59%) displayed major response (0 CR, 10 PR) to the treatment. Of the 25 patients, 14 (56%) showed negative results on peritoneal cytology and no macroscopic peritoneal metastasis; the remaining 11 patients had positive results on peritoneal cytology or macroscopic peritoneal metastasis after NIPS (Table 2).

Adverse events were graded according to the National Cancer Institute-Common Terminology Criteria for

TABLE 2 Anti-tumor efficacy of neoadjuvant intraperitoneal and systemic chemotherapy (NIPS)

RECIST criteria	*n*	%
Measurable disease	17	
Overall response rate (CR + PR)	10	59
CR	0	0
PR	10	59
SD	6	35
PD	1	6
Nonmeasurable disease	8	
Efficacy for peritoneal disease	25	
CY0 and P0 after NIPS	14	56
CY1 or P1 after NIPS	11	44

TABLE 3 Toxicity profile of neoadjuvant chemotherapy in 25 patients (National Cancer Institute-Common Terminology Criteria for Adverse Events Version 4.0: NCI-CTCAE ver. 4.0)

	Grade 1	Grade 2	Grade 3	Grade 4
Nonhematological				
Fatigue	11 (44)	2 (8)	0 (0)	
Nausea	6 (24)	7 (28)	4 (16)	
Diarrhea	2 (8)	2 (8)	0 (0)	
Alopecia	8 (32)	7 (28)	0 (0)	
Hematological				
Leukopenia	0 (0)	2 (8)	17 (68)	3 (12)
Neutropenia	1 (4)	1 (4)	14 (56)	6 (24)
Anemia	10 (40)	11 (44)	0 (0)	0 (0)
Creatinine	4 (16)	0 (0)	0 (0)	0 (0)
ALT elevation	2 (8)	1 (4)	1 (4)	0 (0)

Data are numbers with percentages in parentheses

TABLE 4 Postoperative complications in 22 patients

Complications	*n*	(%)
Bleeding	0	0
Anastomotic insufficiency	2	9.1
Pancreatic fistula	3	13.6
Wound infection	2	9.1
Intra-abdominal abscess	2	9.1
Intestinal occlusion	0	0
Death resulting from complications	0	0
Any postoperative complication	7	31.8

Adverse Events Version 4.0 (Table 3). In 20 (80%) patients, leukopenia and neutropenia were graded as more than grade 3. Four patients (16%) experienced grade 3 gastrointestinal-related toxicities. However, no chemotherapy-related death was observed. Nineteen patients underwent surgery during a month and three patients did between a month and 6 weeks after recovery of NIPS.

Postoperative Complications

Among 22 patients who underwent surgery, postoperative complications occurred in 7 patients (31.8%, Table 4). Pancreatic fistula was the most frequent complication (three patients). Anastomotic leakage, intra-abdominal abscess, and wound infection occurred in two cases each.

Survival Rates

Figure 2a shows the overall survival time after the introduction of NIPS in all 25 patients enrolled in this study. The median survival time (MST) was 16.7 months. Patients with negative cytology and disappearance of peritoneal cancer metastases ($n = 14$) after NIPS had a significantly better prognosis than those with positive results of cytology or peritoneal deposits (MST 27.1 vs. 9.6 months; $P < 0.0001$; Fig. 2b). Patients who showed major response in metastatic lymph nodes ($n = 10$) also had a significantly better prognosis than those without major response ($n = 7$, $P = 0.0173$; Fig. 2c). Figure 2d shows no significant difference between the prognosis of patients with measurable lymph node metastases and those without measurable disease.

DISCUSSION

In this study, we conducted a prospective phase II study to evaluate the feasibility and efficacy of the neoadjuvant intraperitoneal and systemic chemotherapy, named NIPS, for gastric cancer patients with peritoneal dissemination of cancer cells. After NIPS, 14 (56%) of 25 patients showed negative results on peritoneal cytology, no macroscopic peritoneal metastasis, and had a remarkably better prognosis than those with positive results of cytology or peritoneal deposits (Fig. 2b). Although frequent hematotoxicities were observed in NIPS, they were controllable by specialized oncologists. Furthermore, no chemotherapy-related severe morbidity and mortality were observed. Twenty-two (88%) patients underwent gastrectomy with lymphadenectomy. Total gastrectomy (82%) with D2 lymphadenectomy (77%) was the main surgical approach. Postoperative complications were observed in 32%, which is comparable with previous reports of surgery after neoadjuvant chemotherapy, and no surgery-related mortality was observed.[19,20] These results indicate that NIPS is feasible and effective for gastric cancer patients with peritoneal dissemination.

Multicenter phase III trials have been conducted in gastric cancer and the effects of postoperative adjuvant chemoradiotherapy and perioperative chemotherapy have been demonstrated.[6,7] Furthermore, adjuvant chemotherapy with S-1, an oral fluoropyrimidine (Taiho Pharmaceutical), has an affirmative effect on locally advanced gastric cancer.[21] Although the effect of neoadjuvant chemotherapy on gastric cancer has been studied in several phase III trials, definite conclusions have not been made because of insufficient statistical power and high rate of surgical complications.[19,22,23] However, preoperative chemotherapy may have some advantages, such as the delivery of antitumor agents may be more efficient if administered before surgical disruption of the vasculature, tumor down-staging may increase the rate of complete surgical resection, and preoperative chemotherapy can be used to evaluate chemosensitivity of drugs.

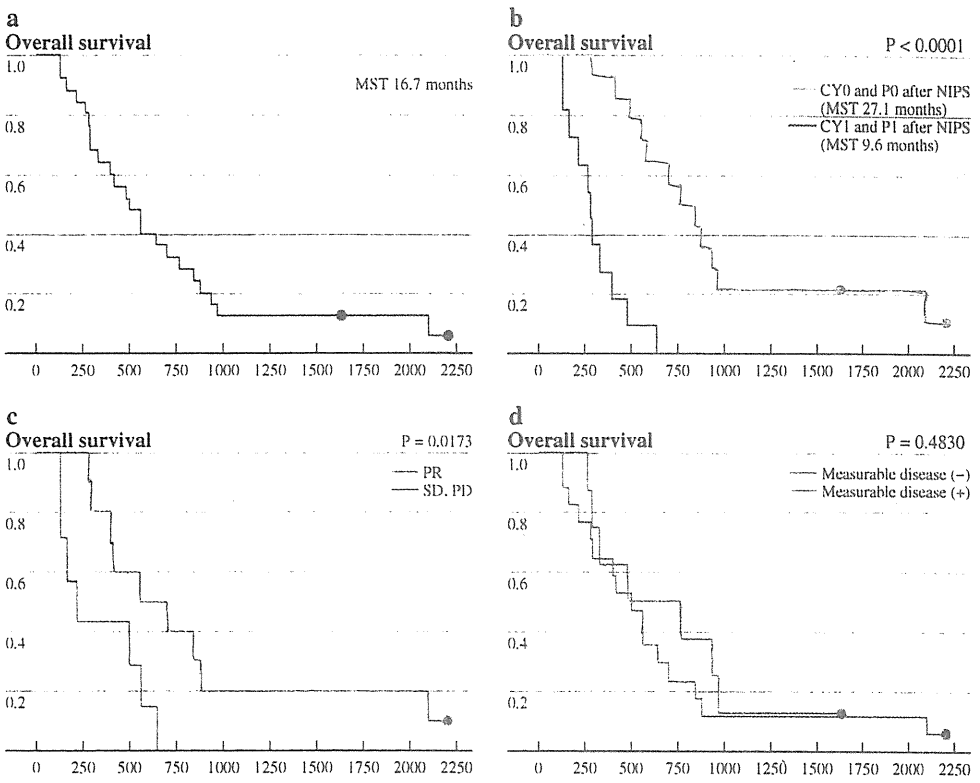

FIG. 2 **a** Overall survival of 25 patients enrolled in this study. *MST* mean survival time. **b** Overall survival according to the effect of NIPS on peritoneal disease. *CY0* negative peritoneal cytology, *CY1* positive peritoneal cytology, *P0* no macroscopic peritoneal metastasis, *P1* presence of macroscopic peritoneal metastasis. **c** Overall survival according to the clinical response evaluated by the RECIST. *PR* partial response, *SD* stable disease, *PD* progressive disease. **d** Overall survival according to the presence or absence of measurable lymph node metastases

Because the prognosis of patients with gastric cancer and peritoneal dissemination is very poor, surgery is not the standard therapy except for patients who require palliative surgery for related symptoms, such as bleeding and obstruction. Several recent retrospective studies have analyzed the effects of neoadjuvant chemotherapy in gastric cancer patients with peritoneal seedlings and/or PPC. Badgwell et al. retrospectively analyzed and concluded that the prognosis of gastric cancer patients with PPC without gross peritoneal diseases was almost similar to that of patients with gross peritoneal disease at preoperative staging laparoscopy.[24] They also reported improvement of prognosis in patients with PCC but without gross peritoneal disease after neoadjuvant chemotherapy compared with a palliative approach. Lorenzen et al. assessed peritoneal cytology before and after neoadjuvant chemotherapy (NAC) and its relation to prognosis.[25] They concluded that some patients with PPC show negative peritoneal cytology after NAC and subsequent improvement of prognosis, although almost 25% of the patients with negative cytology became positive after NAC, which might be a risky strategy. Okabe et al. retrospectively analyzed the effect of induction chemotherapy with S-1 plus cisplatin, which is the standard chemotherapy in Japan, for patients with peritoneal dissemination.[20,26] In that study, 19 (46 %) of 41 patients treated with induction chemotherapy showed disappearance of peritoneal dissemination and negativity of peritoneal cytology and had a curative operation. Furthermore, the prognosis of patients with R0 resection was significantly better than that of patients who underwent noncurative resection.[20]

To our knowledge, our study is the first prospective phase II study on neoadjuvant chemotherapy for gastric cancer with peritoneal disease. We introduced i.p. administration of antitumor drugs combined with systemic chemotherapy. The i.p. chemotherapy was selected to enhance antitumor activity against peritoneal metastasis by maintaining a high concentration of the drug in the peritoneal cavity during a long period of time, and its clinical effects have been verified by a number of convincing clinical trials in ovarian cancer.[27,28] Recent studies also have examined the effects of i.p. administration of taxans, such as paclitaxel and docetaxel, in patients with gastric cancer and peritoneal dissemination because long-term high concentrations of taxans in the peritoneal cavity could be achieved.[29,30] Further studies are needed to define the most suitable regimen for NIPS. Furthermore, the utility of NAC and i.p. chemotherapy for gastric cancer with peritoneal dissemination should be examined by phase III randomized clinical trial.

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REGIV[Q1] 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.

Method: s 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.

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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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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™, 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).

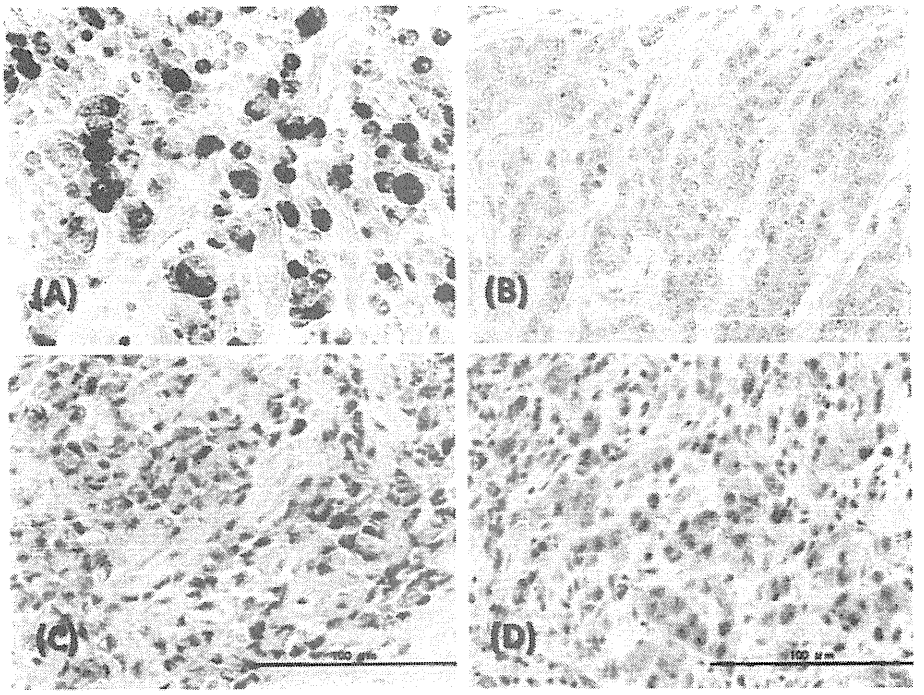

Fig. 1. Immunohistochemistry for REGIV protein in gastric cancer tissues. A: Representative positive staining for REGIV in primary tumor. B: Representative negative staining for REGIV in primary tumor. C: Representative positive staining in endoscopically biopsied specimen from primary tumor. D: Representative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

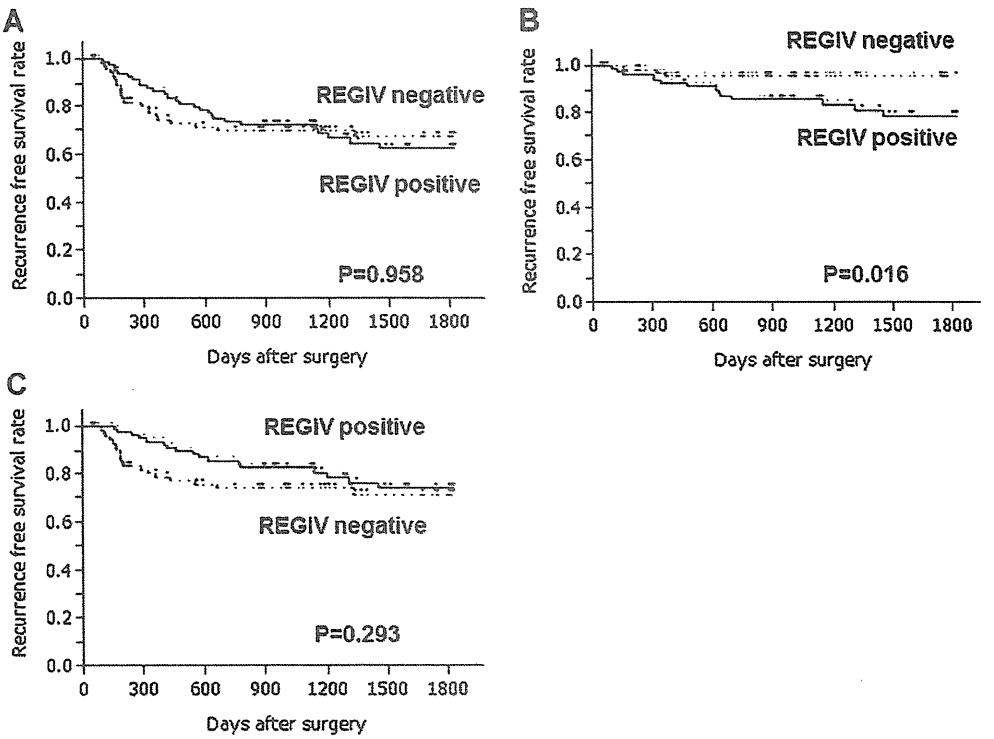

Fig. 2. Survival analysis according to REGIV expression in primary gastric cancer. A: Overall survival. B: Peritoneal recurrence-free survival. C: Recurrence-free survival outside of peritoneal sites.

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.

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

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

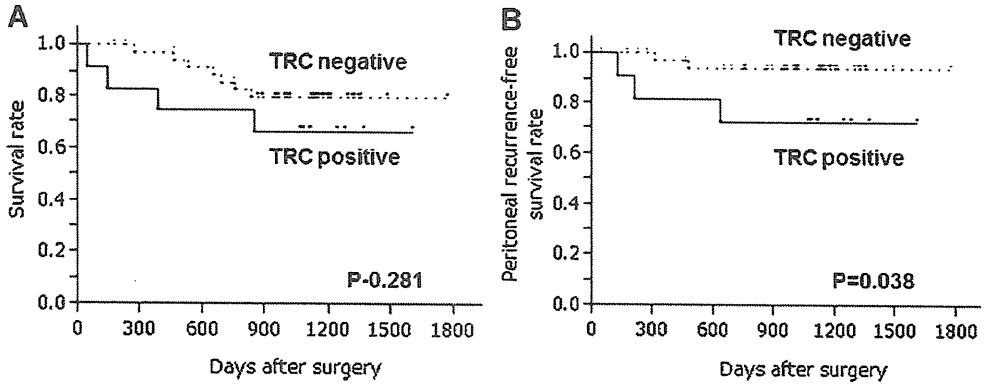

Fig. 3. Survival analysis according to the TRC diagnosis from peritoneal lavage specimens. A: Peritoneal recurrence-free survival. B: Overall survival.

stable and more accurate with respect to quantification. Another advantage is that this method amplifies RNA directly, avoiding the need for reverse transcription to convert RNA to cDNA prior to amplification. These advantages may allow the establishment of more reliable and practical genetic diagnosis of cancer micrometastasis. We reported previously on TRC using carcinoembryonic antigen (CEA) as a biomarker marker for the early detection of peritoneal recurrence after gastric cancer surgery [23]. However, CEA is not a cancer-specific marker and some regions in gastric tumors show no expression of CEA. Additional markers will therefore improve the sensitivity and specificity of our TRC method for predicting peritoneal recurrence following gastric cancer treatment. Our analyses in this study implicated TRC for REGIV as a potential molecular diagnostic method for predicting peritoneal dissemination in advanced gastric cancer in a simple and rapid manner.

In conclusion, we identified REGIV overexpression in peritoneal dissemination of advanced gastric cancer and that the detection of REGIV mRNA in peritoneal lavage fluid by TRC could be a predictor of peritoneal recurrence after curative gastrectomy. Overexpression of REGIV could become a predictor of peritoneal recurrence, although further studies will be needed in a larger population.

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腹膜播種を伴う胃癌に対する外科治療

Surgical treatment for gastric cancer with peritoneal dissemination

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●要旨●従来，胃癌の腹膜播種に対する有効な治療は存在せず，さらに，その診断や効果判定の困難さからほとんど臨床試験は行われてこなかった。胃癌腹膜播種に対し，完全切除をめざして腹膜切除，多臓器合併切除を行った例もあるが，胃癌の場合は再発が必須であること，手術侵襲が大きいことより一般化はされなかった。近年，S-1(TS-1)，タキサン系薬剤など腹膜播種に有効な薬剤の登場，腹腔鏡検査の積極的導入により，腹膜播種を伴う胃癌に対して，術前化学療法，手術，術後化学療法などを組み合わせて，長期生存をめざす試みがなされるようになってきた。この現状を，文献的考察，われわれの試みを含めて報告する。

key words：胃癌，腹膜播種，外科治療，集学的治療，腹腔内化学療法

はじめに

漿膜浸潤胃癌の治療戦略においてもっとも重要な点は，腹膜播種の診断・治療である。開腹時に腹膜播種がみつかった場合は，出血あるいは狭窄症状を回避する目的で胃切除を行うことが多いが，術前に腹膜播種が画像診断あるいは腹腔鏡検査で診断された場合は，手術は一般的ではなく化学療法が行われる。過去に，腹膜播種を伴う進行胃癌に対し，積極的に腹膜切除を含めた根治切除を行う試みがなされたが，再発が必須であること，手術侵襲が大きいことより標準治療とはならなかった。

近年，胃癌に対する新規抗癌剤が登場し，腹膜播種にも有効であることが証明され，さらに化学療法の導入により腹膜播種の消失を確認し根治切除を行うことで予後改善を認めた報告も散見される。本稿では，腹膜播種に対する外科的切除を含めた治療について文献的考察とわれわれの取り組みについて報告する。

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胃癌における腹膜播種

日本胃癌学会の全国集計の結果をみても，胃癌切除後の死因の第1は腹膜播種再発であり，その頻度は深達度が深くなるにつれて増加する[1]。われわれの施設において根治切除を施行した752例では，術後再発形式として最多であったのは腹膜播種であり，全再発例の34％を占めた。その頻度は，MP以浅ではほとんど認められないのに対し，SS，SE，SIでは35％，47％，60％と深達度が進むにつれて増加した[2]。腹膜播種を伴う胃癌に対する姑息的切除の予後に関する効果は，これまでは否定的であり，出血，狭窄などの患者の生活の質の改善を目的とすること以外は，予後改善効果はないと考えられてきた[3]。われわれの施設でも，2001年以前のデータによると，開腹手術後に腹膜播種あるいは細胞診陽性が判明した場合のmedian survival time（MST）は221日と予後不良であった。よって，腹膜播種の有無を治療前に診断し，非根治切除を回避することが重要である。しかし，CT，MRIなどの画像診断が進歩した昨今においても，びまん性に広がり小結節が散在する胃癌の腹膜播種を非観血的に診断することは困難であり，大網結節，腹水貯留，腸間膜肥厚，水腎症，腸閉塞などの間接的所見から推

察するのが現状である。近年，高度進行胃癌の治療方針決定のために，腹腔鏡検査を導入する施設が増えてきており，腹腔鏡検査により画像診断では指摘できない病変を8～40%の頻度で検出できたという報告がなされている[4]。また，腹腔鏡下に腹腔洗浄液を採取し細胞診を行うことで，肉眼ではわからない腹腔内微小癌細胞を検出することも可能であり，治療法の選択に役立つと考えられている。

腹膜播種を伴う胃癌に対する全身化学療法

これまで，腹膜播種を伴う胃癌に対する臨床試験は，その治療効果判定が困難なことや腹膜播種に有効なレジメンが存在しなかったことよりほとんど行われてこなかった。Japanese Clinical Oncology Group（JCOG）が，胃癌腹膜播種237例を対象に5-FU＋leucovolin＋methotrexate（MTX）による全身化学療法のPhase Ⅱ試験を施行した結果，MSTは10.6カ月と不良であった。しかしその後，胃癌に対し有効な抗癌剤が登場し，大規模第Ⅲ相臨床試験が施行された結果，現在のわが国における胃癌に対する標準化学療法は，2010年に改訂された『胃癌治療ガイドライン』にも記載されているようにS-1（TS-1）＋CDDP併用療法である[5]。この試験において，腹膜播種があるがtarget lesionがない症例に対してS-1＋CDDP療法が比較的良好な結果を示していること，ほかにもS-1＋CDDP療法は腹膜播種に奏効するという報告もあり，有望なレジメンと考えられている[6,7]。また，本年1月の2011 Gastrointestinal Cancers Symposiumで報告されたSTART試験の結果は，切除不能進行再発胃癌に対するS-1＋docetaxel併用療法は，S-1に対しoverall survivalでは優越性を示すことはできなかった。しかし，腹膜播種を伴うがtarget lesionを有しない症例に限ると，有意に予後を改善しており，そのMSTは524日と良好であり，有望なレジメンとなり得ると考える。

胃癌腹膜播種に対する腹腔内化学療法

腹膜播種性胃癌に対し，抗癌剤を腹腔内に投与してその腹腔内濃度を高めることを目的に，古くから腹腔内化学療法が行われてきた。

JCOG臨床試験では，胃癌術後にCDDP腹腔内投与を用いた第Ⅲ相試験も行われたが，その有用性を示すことはできなかった[8]。しかしXuらは，胃癌根治切除後の腹腔内化学療法に関する11のランダム化試験のメタアナリシスを行い，腹腔内化学療法の生存率に対する有用性を報告している[9]。腹腔内投与により，5-FU，docetaxel，doxorubicin，gemcitabine，paclitaxelなどの薬剤は，腹腔内AUCが血液内AUCの100倍以上となることが実証されており，腹腔内病変に限局した症例に対しては，副作用を軽減しつつ効果が期待できる方法であることは間違いない。

Ishigamiらは，paclitaxelを経静脈的と経腹腔内の両方に分割し，さらにS-1を投与するレジメンを開発し，腹膜播種を伴う胃癌あるいは腹膜播種再発に対し治療を行い良好な治療成績をあげている（1年生存率78%，MST 20.3カ月）。さらに，腹水の消失，減少を62%の症例に認め，副作用も認容できるものであったと報告している[10]。また今野らは，腹膜播種を伴う胃癌に対して，paclitaxelの腹腔内投与を行い，その後S-1とpaclitaxelによる全身化学療法を行った。結果は，MST 475日，1年生存率63.4%と良好であったと報告している[11]。さらに，多施設臨床試験として，胃癌腹膜播種研究会が行っているdocetaxelの腹腔内投与と，S-1の併用療法がある。docetaxelは2週間おきに腹腔鏡検査時に留置した腹腔ポートより投与し，S-1は2週間投薬，2週間休薬を繰り返し行うレジメンで，現在進行中である[12]。

腹膜切除を伴う外科切除

腹膜播種を伴う胃癌に対し，腹膜切除（peritonectomy）＋腹腔内温熱化学療法を行ったという報告がある[13～15]。34～49例の患者に対しこの方法を行い，MSTは8～11カ月，5年生存率は6～16%であった。手術により肉眼的に全切除できた症例の予後は良好で，MSTは19.2～21.3カ月，5年生存率は27～29.4%であった。ただし，完全切除率は50%前後であり，また手術関連死亡が2～7.1%あり，一般化は困難な治療法である。

腹膜播種に対する外科治療を含めた集学的治療

ユニークな研究として，Kuramotoらは，CY1，P0症例を手術単独群，手術＋CDDP 100mg腹腔内投

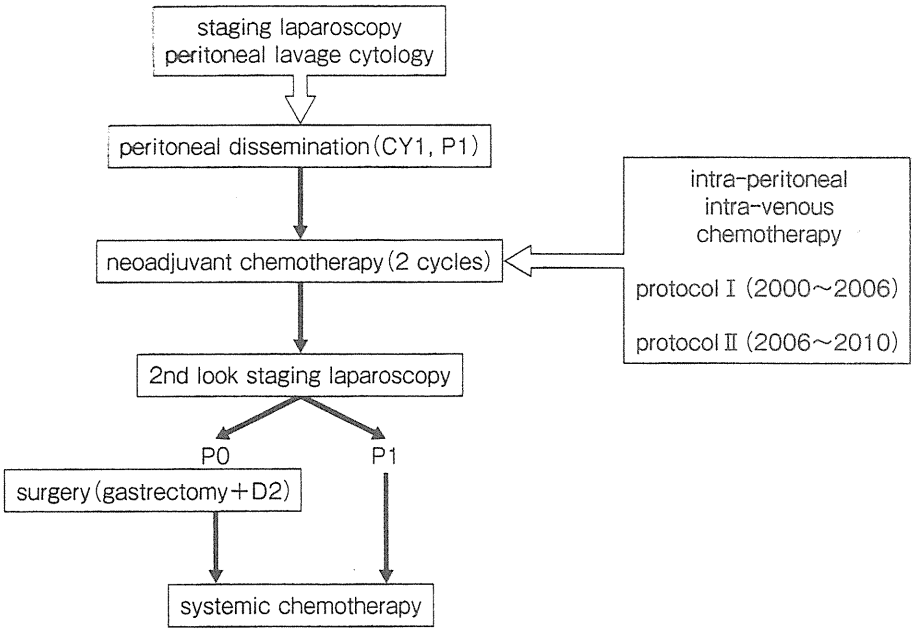


図1　treatment strategy for gastric cancer with peritoneal dissemination

与（i.p.）群，および手術＋腹腔内大量洗浄＋CDDP i.p.群の3群にランダムに振り分け，経過観察結果を報告している[16]。腹腔内大量洗浄（extensive intraoperative peritoneal lavage；EIPL）とは，手術終了後に腹腔内に1,000mlの生理食塩液を入れてよく洗浄し，回収することにより（約900mlが回収できる），腹腔内癌細胞は10倍に希釈される。これを10回繰り返すことで，計算上は1,010個の癌細胞が100個になり，ほぼ消失する。この時点でCDDP i.p.を行う方法である。結果は，surgery＋EIPL＋i.p.群が有意に予後良好であり，5年生存率は43.8％と良好であった（surgery＋i.p.群4.6％，surgery群0％）。EIPL＋i.p.で治癒する症例があるということで興味深い報告であるが，症例数が少なく追試が必要である。

近年の新規抗癌剤の出現により，S-1，docetaxel，taxolなどの腹膜播種に奏効する薬剤が登場し，従来は手術適応ではなかった腹膜播種を伴う進行胃癌が，腹膜播種の消失により切除可能となり，術後長期生存する症例も散見するようになってきた。よって，腹膜播種を伴う切除可能な進行胃癌に対しては新たな治療戦略が提案されつつある（図1）。まず，治療前に腹腔鏡検査を行い，P1あるいはCY1を確認した症例が対象となる。これらの症例に対し，術前化学療法（neoadjuvant chemotherapy；NAC）を施行する。この術前化学療法という言葉の定義には議論があり，もともと手術適応ではない症例であるので導入化学療法（induction chemotherapy）のほうが適切かもしれない。化学療法施行後，再び腹腔鏡検査を施行し，腹膜病変が消失している場合は手術を行う。術後は，補助化学療法を継続するという治療戦略である。これまで，海外を含めいくつかの報告がある。Badgwellらは，retrospectiveな解析を行い，39例のCY1P0であった症例を解析し，そのMSTは13カ月であり，P1を伴う胃癌のMSTは11カ月と差はなかったと報告している[17]。そして，全身化学療法を術前に施行した症例（25例）はNACなしの症例と比較し，有意に予後良好であった（$p=0.005$）と述べている。Lorenzenらは，切除可能胃癌に対し5-FU＋leucovolin＋CDDPによるNACを施行した61症例をretrospectiveに解析した[18]。NAC前後でCY1がCY0になった症例は，予後が改善したと報告しているが，逆にNACのあとでCY0がCY1となり根治手術のタイミングを逃すリスクがあることも報告している。Okabeらは，わが国における現時点での標準的化学療法S-1＋CDDP療法を施行した腹膜播種を伴う胃癌41例についてretrospectiveに解析し報告している[19]。41例中32例（78％）に化学療法後手術を施行し，22例（54％）においてP0，CY0となりR0手術が可能となり，さらにその予後は改善した（MST 40.3カ月）。

腹膜播種に対する治療効果をさらに向上させるために，全身化学療法と腹腔内化学療法を併用し術前投与

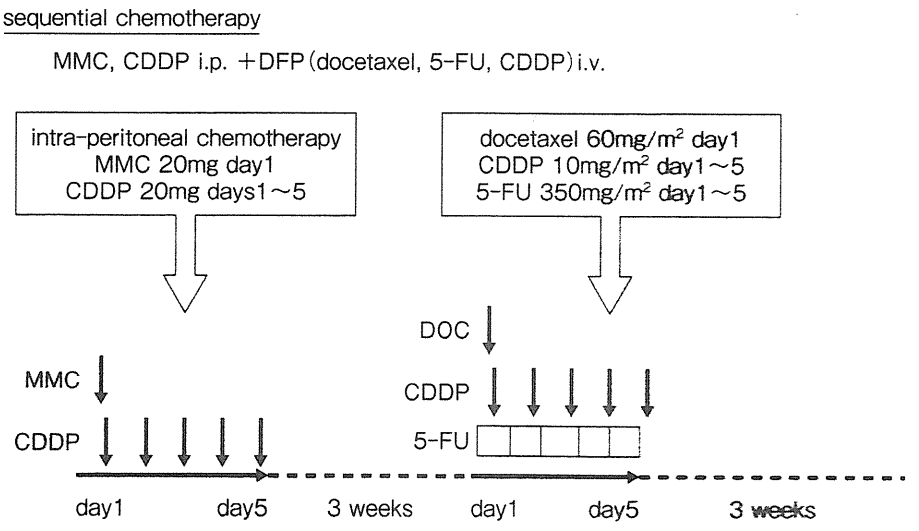


図２　treatment protocol Ⅰ（2000～2006）

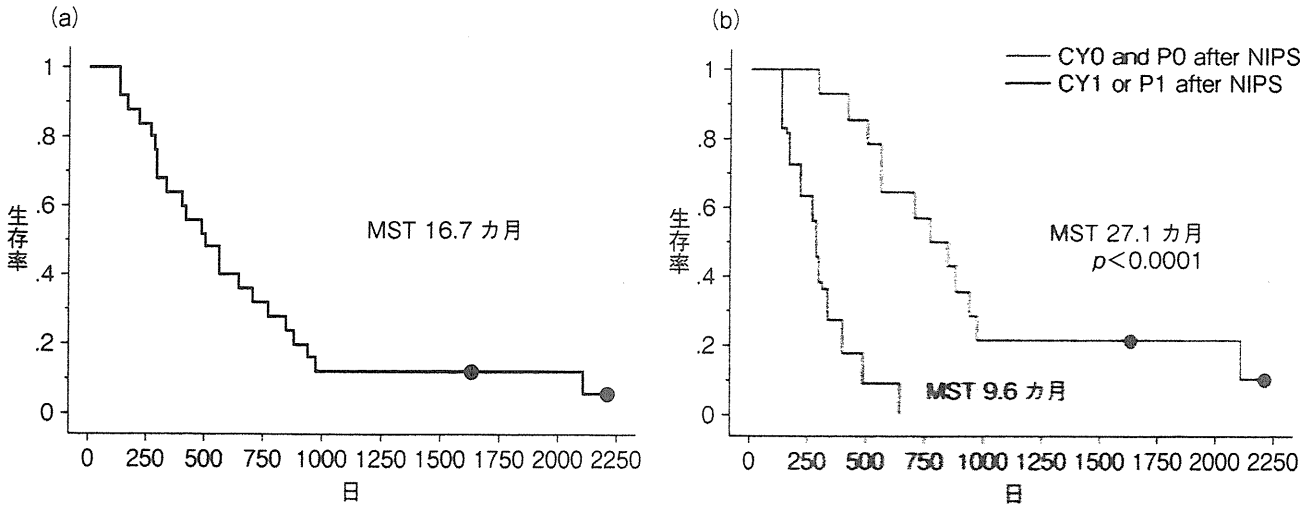


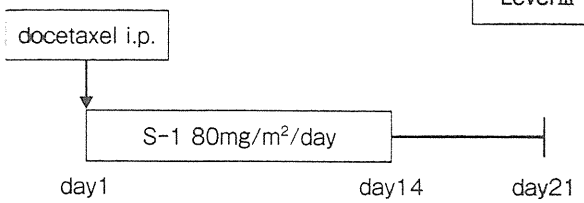
図３　overall survival in protocol Ⅰ

する試みもある。Yonemura らは，neoadjuvant intraperitoneal and systemic chemotherapy（NIPS）という名前を提唱している[20]。彼らは，79症例の腹膜播種を伴う胃癌患者（44症例が初発，35症例が再発患者）に対して腹腔ポートを留置し，docetaxel と CDDP を週に３回投与，１回休薬する腹腔内化学療法に，S-1 による全身化学療法を併用した。結果は，41例（52％）に腹膜播種に対する効果を認め，手術を施行して根治切除が32例（41％）で可能であったと報告している[20]。この根治切除可能であった症例の予後は良好であった（MST 21カ月）と報告している。

われわれの施設では，2000年より術前画像診断で切除可能であるが，漿膜浸潤陽性で腹膜播種が疑われる症例に対し，腹腔鏡検査あるいは腹腔洗浄液を採取して腹膜播種を確認した症例に対し，NIPS を導入する prospective study を行ってきたので報告する。図1．図２は試験の概略であるが，腹膜播種性病変 P1 あるいは CY1を確認した後，まず MMC 20mg を day１に，CDDP 20mg を day１から５日間，1,000ml の生理食塩液とともに腹腔内に投与した。この腹腔内化学療法の認容性については確認ずみである[21]。これに引き続き，docetaxel＋5-FU＋CDDP ３剤併用全身化学療法を２クール施行した。この治療の術前化学療法としての安全性，有効性も確認ずみである[22]。2000～2006年の間に25例が登録され，NIPS を施行後明らかなＰ病変を認めなかった22例に手術（全摘18例，幽門側切除４例）を施行した。臨床効果を示すと，RECIST 評価病変ありの17例での解析では，10例

Phase I study defined recommended dose as 60mg/m²

	docetaxel
Level Ⅰ	40mg/m²
Level Ⅱ	50mg/m²
Level Ⅲ	60mg/m²



2 cycles were given before staging laparoscopy

図4　treatment protocol Ⅱ（2006～）

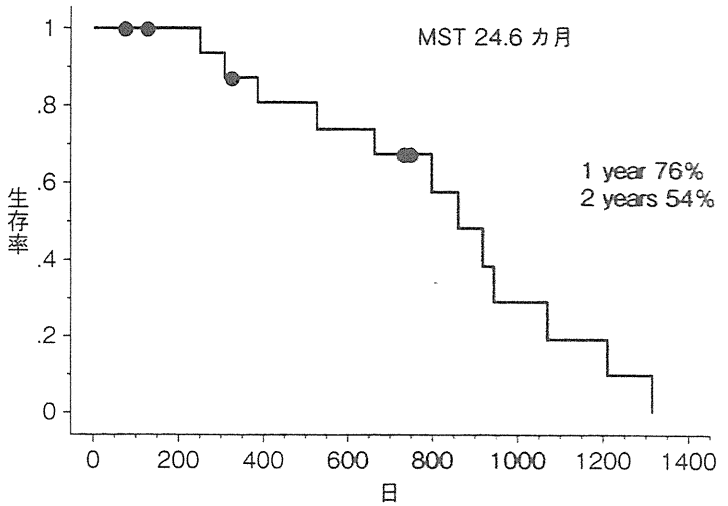


図5　overall survival in protocol Ⅱ

（59%）の症例でPRを認めた。腹膜病変の消失は，14例（56%）に認めた。NIPSのtoxicityは，白血球減少，好中球減少を高頻度に認めたが，休薬，減量で対応可能であり，治療中止は認めなかった。術後合併症であるが，膵液瘻3例，縫合不全2例など，32%の症例に認めたが，NAC後の術後合併症としては平均的なものであり，治療関連死亡は認めなかった。図3は生存率を示しているが，全25例のMSTは16.7カ月であり，NIPSにより腹腔内病変が消失した症例では，MST 27.1カ月と有意に予後が延長した。2006年以降は，図4に示すようにdocetaxelをday 1に腹腔内投与を行い，これとS-1を併用する新たなNIPSを開始した。Phase Iの結果では，重篤な副作用はなく安全性に優れたレジメンであった。これまで18例を登録し，そのうち16例（88.9%）にNIPS後手術（全摘13例，幽門側切除3例）を施行し，術後合併症は2例に腹腔内膿瘍，1例に術後出血を認め，合併症率は18.8%で，治療関連死は認めていない。14例（77.8%）の症例で，NIPS後，腹膜病変の消失を認めた。腹膜病変の消失を認めなかった4例は，骨盤腔を越える腹水貯留例，CTでも確認できるP結節を有する症例であり，術前画像診断にて骨盤腔を越える腹水がなく，腹膜結節を認めない症例に限ると，全例（14例）で腹膜病変は消失した。現時点で観察期間はまだ短いが，全18例の生存率は1年76%，2年54%，MST 24.6カ月と良好であり（図5），安全性，有効性に優れたレジメンであることが示唆される。

おわりに

以上述べてきたように，新規抗癌剤の登場とともに，腹膜播種を伴う胃癌の治療も，大きく変わろうとして

いるのが現状である。腹腔鏡検査にて腹膜播種を治療前に診断し，まず，化学療法を導入する。腹膜病変が著効すれば長期生存，あるいは治癒を期待し，外科的治療による原発巣切除の適応が出てくるものと考える。今後，化学療法のレジメン，腹腔内化学療法の必要性，適応症例（大量腹水，水腎症などは外科治療適応にはならない）の決定など，臨床試験にて検証していく必要があると考える。今後の展開に期待したい。

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