data of cytology-positive and -negative cases are shown in Fig. 2. This assay was able to detect fluorescence signals in cases where RT-PCR products could not be visualized even by a highly sensitive capillary electrophoresis system such as the Agilent Bioanalyzer 2100 (Fig. 2).

Multiplex RT-PCR and Coupling of Cy3 Dye

From .5 to 1 μg of total RNA prepared from 20–50 mL of peritoneal washings, reverse transcription was performed with Superscript II (Invitrogen, Carlsbad, CA) with random hexamer in a total volume of 20 μL according to the manufacturer's protocol. Multiplex RT-PCR was performed in two tubes at different PCR cycles: 30 cycles for relatively cancer-specific genes, *CK20, FABP1, MUC2, TFF1, TFF2*, and *CEA*, and 25 cycles for *MASPIN, GW112, PRSS4*, and *TACSTD1* together with *β-actin*. PCR primer sequences are listed in Table 1. Ten microliters of the PCR solution in each tube consisted of 1 μL of template cDNA, primers (2.5 pmol each), 50 μM of amino allyl-dUTP, 1 μL of AccuPrime 10 × buffer 1 (2 mM of dNTP, 15 mM of $MgCl_2$) and .25 μL of AccuPrime Taq polymerase (Invitrogen). A thermal cycler was set with initial heating at 94°C for 2 minutes, followed by an amplification cycle heated at 94°C for 15 seconds, 60°C for 45 seconds, and 72°C for 3 minutes. The two PCR solutions were mixed and purified with QIAquick PCR purification Kit (Qiagen, Tokyo, Japan). Purified cDNAs were dried by evaporation and dissolved in 9 μL of .2 M carbonate buffer (pH 9.0). Two microliters of Cy3 monoreactive dye (Amersham Biosciences, Piscataway, NJ) was added to the cDNA solution, followed by incubation at room temperature for 1 hour. After two cycles of this process, cDNA was purified by QiaQuick purification kit.

Hybridization to Focused Array and Fluorescence Scanning

The entire Cy3-labeled cDNA solution (50 μL) was mixed in 120 μL of a hybridization cocktail (6 × buffer containing 900 mM of NaCl, 60 mM of NaH_2PO_4, and H_2O, and 6 mM of EDTA, pH 7.4/10% formamide/.05% sodium dodecyl sulfate). By a hybridization apparatus, HybStation (Genomic Solutions, Ann Arbor, MI), an array was preheated to 65°C for 3 minutes, filled with the hybridization cocktail, then incubated at 92°C for 2 minutes and then at 55°C for 4 hours. Subsequently, the array was washed with 2 × standard saline citrate (SSC), .1% sodium dodecyl sulfate at 25°C and then 2 × SSC at 20°C, and rinsed with .1 × SSC, in accordance with the steps laid out in a conventional manual, and finally dried in a spin drier. The array was scanned by an apparatus for DNA microarrays (Genepix 4000B; Axon Instruments, Union City, CA), and the fluorescence intensity from each probe spot was obtained after subtracting the background level. Fluorescence intensity from a *β-actin* probe was used as an internal control so that the ratio of fluorescence intensity to that of *β-actin* could be analyzed.

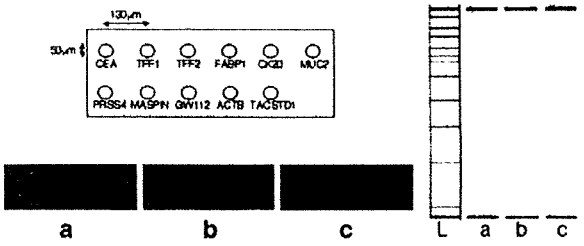

FIG. 2. Image of focused microarrays. and distribution of MiniChip assay results. Positions of probes for marker genes (left, top). Images of microarrays (left, bottom) and electrophoreses (right), respectively, show results obtained by MiniChip assay and capillary electrophoresis system from three representative cases. (a) Stage Ia case. (b) Cytology-negative case with peritoneal recurrence. (c) Cytology-positive case. L, marker ladder.

Establishing Diagnostic Criteria for MiniChip Assay

A putative cutoff value for each gene was established from the fluorescence ratio of 39 samples derived from stage Ia cases (cancer confined to the submucosa without nodal involvement) as negative control cases because cases of this stage seldom develop peritoneal metastasis.[15] Two cutoff values were attempted, one at a value corresponding to the maximum value plus standard deviation (MAX SD) and the other at the average value plus twice standard deviation (AVG 2SD) of the 39 stage Ia cases. On the basis of the fluorescence ratios between each marker gene and *β-actin* under these two cutoff values, we defined in this study any case with two or more positive markers as a MiniChip assay–positive case because we ascertained that tumor-negative samples may well be weakly positive by a single marker, whereas a tumor-negative sample with two or more positive markers would be rare.

Analyses of Clinical Outcome

The clinical outcome of the NCC and ACC patients was investigated to evaluate the sensitivity and specificity for the disease recurrence of the MiniChip

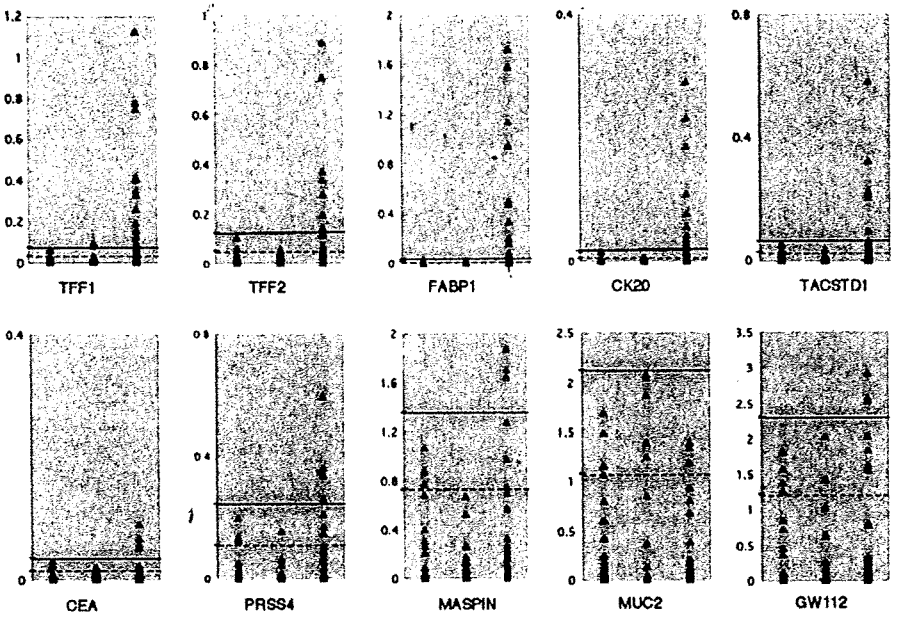

FIG. 3. Distribution of MiniChip assay results. Contrast among three groups of cases is clear in microarray images, whereas electrophoresis system detects polymerase chain reaction products only in cytology-positive cases. Plots on left, middle, and right rows are fluorescence ratios of stage Ia, disease-free, and metastatic cases, respectively. Dashed line indicates level of average value plus twice standard deviation (AVG 2SD) of stage Ia cases; solid line indicates maximum value plus standard deviation (MAX SD). Upper five genes, *TFF1, TFF2, FABP1, CK20,* and *TACSTD1*, showed more specific results than other genes.

assay. Patients of the two institutes were followed up in a same manner with clinical imagings and measurement of carcinoembryonic antigen (CEA) and cancer antigen (CA) 19-9 every 3 to 6 months.[7,10] Cases with at least 2 years (700 days) of follow-up and without a recent history of other malignancies were eligible for the analyses of clinical outcome. For NCC patients, the disease-free survival was analyzed by Kaplan-Meier curve with a diagnosis of disease recurrence as an end point to compare the impact of the MiniChip assay and its clinical outcome with that of conventional cytology. ACC cases were selected representative cases and thus were not used for the survival analyses. Samples from the University of Tokyo Hospital lacked adequate clinical follow-up time and thus were used only for the immunocytochemistry below.

Immunocytochemistry

To evaluate the usefulness of our previously identified five marker genes (*CK20, FABP1, MUC2, TFF1*, and *MASPIN*) in immunocytochemical peritoneal cytology, we used anti-MASPIN (BD Pharmingen, San Diego, CA), anti-CK20 (Dako, Kyoto, Japan), anti-TFF1 (Dako), anti-MUC2 (Novo-Castra, New Castle, UK), and anti-FABP1 protein (Abçam, Cambridge, UK) as primary antibodies (all are mouse monoclonal IgG) and a Histofine Simple Stain Max PO (M) (Nichirei, Tokyo, Japan) as peroxidase-conjugated secondary antibody. By use of four gastric cell lines, the mRNA levels of the five genes of which have been previously examined,[12] the quality of all primary antibody products was verified and an optimal dilution ratio of each antibody was established.

RESULTS

Application of the Focused Microarray Analysis to Detect Minimal Gastric Cancer Cells in Peritoneal Washings and Its Clinical Impact

In our previous report[12] and in this study, we selected the 10 genes as tumor cell–specific genes. In fact, 6 genes (*CK20, FABP1, MUC2, TFF1, TFF1*, and *CEA*) of the 10 have never been detected in early cancers (tumor cell–negative cases) despite nested RT-PCR with outer and inner primer sets, and the other 4 genes (*MASPIN, GW112, PRSS4*, and *TACSTD1*) have also shown no or quite weak bands in the early cancers with high 30 PCR cycles (12 for *MASPIN, GW112*, and *PRSS4*, and data not shown

TABLE 2. *Number of cases defined as cancer positive by MiniChip assay and conventional cytology*

	5 genes (AVG 2SD)				10 genes (MAX SD)		CY
	NCC (n)		ACC (n)		NCC (n)	ACC (n)	NCC (n)
Disease recurrence	n		n				
Peritoneal	14	8	11	4	8	2	5
Others[a]	16	3	6	3	4	1	1
None[b]	32	1[c]	12	0	0	0	0
	62		29				

[a] Recurrences without detectable peritoneal metastasis.
[b] Disease-free more than 2 years (700 days).
[c] Disease-free for up to 713 days.

TABLE 3. *MiniChip assay and immunostaining*

		Immunostaining[a]					Operative findings	
MiniChip assay	Case	MASPIN	CK20	MUC2	TFF1	FABP1	CY[b]	P[c]
Positive	1	< 5	Many	Many	NS	< 20	1	1
	2	< 5	< 20	< 20	< 5	< 5	1	1
	3	< 5	2 cluster	0	< 5	NS	1	0
	4	< 5	< 5	< 5 + 1 cluster	0	0	1	0
	5	Many	0	–	1 cluster	0	1	0
	6	< 5	0	0	0	0	1	0
	7	0	0	–	0	–	1	0
	8	Many	< 5	0	0	0	0[d]	1
	9	Many*	Many*	< 5	1 cluster	< 5	0	1
	10	Many	< 5	0	< 5	NS	0	1
	11	0	0	–	–	–	0	0
	12	Many	< 5	–	< 5 + NS	< 5 + NS	0	0
	13	1 cluster*	Many + NS*	0	0	0	0	0
	14	< 5	0	0	0	0	0	0
Negative	15	Many	Many	< 5	< 5	0	1	0
	16	0	–	–	–	–	0	0
	17	0	0	–	0	NS	0	0
	18	0	0	–	0	0	0	0
	19	0	NS	0	NS	–	0	0
	20	–	0	–	–	0	0	0
	21	0	0	–	0	0	0	0
	22	0	0	0	0	0	0	0
	23	0	NS	0	0	NS	0	0
	24	0	0	0	0	0	0	0
	25	< 5	0	–	0	0	0	0
	26	0	0	0	0	0	0	0
	27	0	0	–	0	–	0	0
	28	0	0	0	0	0	0	0
	29	0	0	0	0	0	0	0
	30	0	Many	0	0	< 5 + 1 cluster	0	0
	31	< 20	< 5	1 cluster + 1	0	0	0	0
	32	0	0	0	0	NS	0	0
	33	0	0	–	–	–	X	0
	34	0	0	0	0	0	X	0

[a] Numbers of stained atypical cells in one whole cytology slide. Many indicates > 20; NS, nonspecific staining of noncancerous background cells; and –, no slide available.
[b] Results of the conventional cytology. 0 and 1 indicate negative and positive, respectively. An X indicates cases without cytology reports.
[c] Peritoneal metastasis status confirmed by operative findings. 1 indicates positive for peritoneal metastatic nodules.
[d] Defined as class III by conventional cytology.
* Photomicrographs are shown in Fig. 4.

in *TACSTD1*). Therefore, the MiniChip assay belongs in a negative or positive assay. However, it is required for determining the cutoff values.

The distribution of fluorescence ratios between each marker gene and *β-actin* in 39 stage Ia–early cancer cases, 44 disease-free cases, and 65 metastatic cases are plotted in Fig. 3. High-level signals of five genes, *TFF1, TFF2, CK20, FABP1*, and *TACSTD1*, were found to be obviously more specific to the above metastatic cases. Therefore, as described in detail in Methods, we attempted two methods for the diagnosis: in one, all 10 genes were used in the diagnosis with a cutoff set at MAX SD, and in the other, 5 genes with a cutoff set at AVG 2SD were used.

After excluding the 39 early cancer cases used in the establishment of the cutoff values and 18 cases with synchronous metastases, 62 cases (30 patients experienced relapse) at the NCC and 29 cases (17 patients experienced relapse) at the ACC were eligible as a validation set for predicting recurrence.

The number of the two kinds of MiniChip assay–positive cases at both the NCC and the ACC and the conventional cytology–positive cases in the 62 NCC cases and recurrence status are shown in Table 2. Comparing the two cutoff sets, the cutoff set at AVG 2SD for five genes showed better results in the diagnosis than that with a cutoff set at MAX SD for 10 genes. Therefore, we focused on the results with a cutoff set at AVG 2SD for five genes.

Only 1 case (2.2%) of 44 disease-free cases (1 of 32 at the NCC and 0 of 12 at the ACC) was shown to be positive by the MiniChip assay, whereas 13 (93%) of 14 conventional cytology–positive cases (6 of 6 at the NCC and 7 of 8 at the University of Tokyo Hospital; Tables 2 and 3) were found to be positive. These results demonstrate that this assay has low false-positive and false-negative findings. In 14 NCC cases with peritoneal recurrence, the MiniChip assay detected more than did the conventional cytology (eight cases vs. five), and accordingly, three of nine cytology-negative patients with peritoneal recurrence were detected by the MiniChip assay. For the 29 ACC cases, which included only cytology-negative patients, the MiniChip assay detected 4 of 11 cases with peritoneal recurrence. The MiniChip assay detected approximately one-third (7 of 20, 35%) of cases found to be falsely negative by conventional cytology at both institutes. Interestingly, the MiniChip assay also detected 3 of 16 NCC cases and 3 of 6 ACC cases with nonperitoneal recurrence (6 of 22, 27%), in accordance with our previous finding.[12]

As shown in Fig. 4, the Kaplan-Meier curve demonstrates the impact of the MiniChip assay on the disease status of patients with gastric cancer treated with potentially curative surgery. The clinical outcome of the MiniChip assay–positive patients (MiniChip positive, CY0) as well as conventional cytology–positive patients (CY1) was found to be quite poor compared with the MiniChip assay–negative patients with negative conventional cytology (MiniChip negative, CY0) (log rank test, $P < .001$).

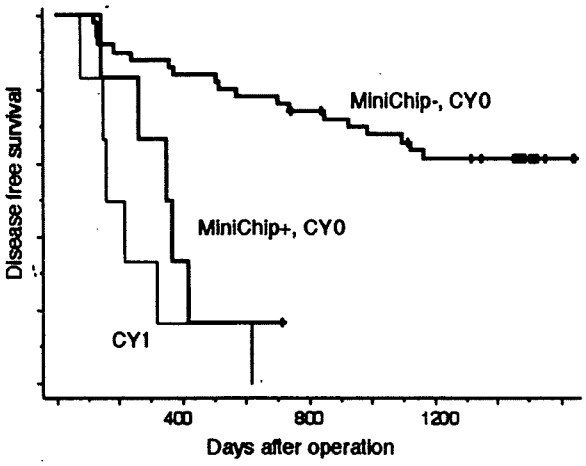

FIG. 4. MiniChip assay results and conventional cytology and relationship to disease-free survival. Cases defined as cancer positive by MiniChip assay but negative by conventional cytology (MiniChip positive, CY0) showed disease-free curve similar to that of cytology positive cases (CY1) and had significantly worse prognosis compared with both negative cases (MiniChip negative, CY0) (log rank test, $P < .001$).

High Concordance Between the MiniChip Assay and Immunocytochemical Cytology

To evaluate the usefulness of the five marker genes (*CK20, FABP1, MUC2, TFF1*, and *MASPIN*) in immunocytochemical cytology, we performed immunostaining with available antibodies for these genes' products in 34 cases at the University of Tokyo Hospital. The results of the MiniChip assay, immunostaining, and conventional cytology accompanied by peritoneal metastatic status as an operative finding are summarized in Table 3. Of the 34 cases, 14 were found to be positive by MiniChip assay. These 14 cases included seven of eight cases found to be positive by conventional cytology. Of the 14 cases, anti-MASPIN, anti-CK20, aniti-TFF1, anti-MUC2, and anti-FABP1 detected 12 (86%), 9 (64%), 6 (43%), 4 (29%), and 4 (29%) cases, respectively. Two cases (patients 7 and 11) were not detected with any of the antibodies used. One case (patient 15), whose results were negative by MiniChip assay but positive by

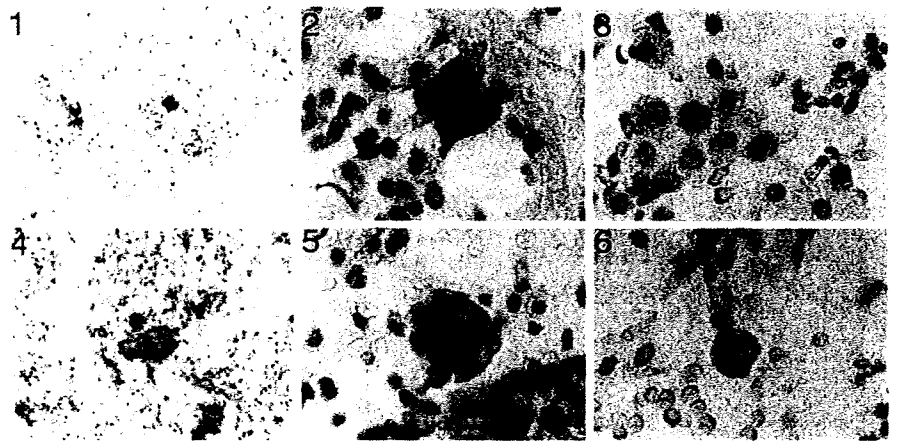

FIG. 5. Atypical cells found in conventional cytology–negative but MiniChip assay–positive cases by immunocytochemistry. Stained slides by anti-MASPIN and anti-CK20 antibodies in two cases. (1) Case 9, MASPIN (x 40). (2) Case 9, MASPIN (x 200). (3) Case 9 CK20 (x 200). (4) Case 13, MASPIN (x 40). (5) Case 13, MASPIN (x 200). (6) Case 13, CK20 (x 200). Immunocytochemistry clearly demonstrated atypical cells in conventional cytology–negative slides and confirmed positive findings of MiniChip assay.

conventional cytology, was detected with four antibodies except anti-FABP1. All of the five cases with peritoneal metastasis confirmed as an operative finding were detected by both the MiniChip assay and immunostaining. These results suggest that immunocytochemical cytology with the five antibodies as well as the MiniChip assay could contribute to the prediction of cancer recurrence. Among the five antibodies, anti-MASPIN was highly specific to atypical cells and rarely stained noncancerous cells. Representative immunostaining results are shown in Fig. 5.

DISCUSSION

Although the role of peritoneal cytology is established as an important tool in the management of gastric cancer, it is nevertheless a job that requires great skill by trained cytologists, which may account for its low prevalence in clinical practice. However, cytologists should be able to find support from the MiniChip assay and/or immunocytochemical analysis, in particular with anti-MASPIN antibody, demonstrated here to have an improved and stable sensitivity for a minimal amount of cancer cells. Present results of immunocytochemistry with anti-MASPIN antibody encourage its application in the detection of micrometastases or isolated tumor cells in the lymph nodes for which anti-CK20 was frequently used in previous studies.[11,16]

In the MiniChip assay, five genes, *TFF1*, *TFF2*, *FABP1*, *CK20*, and *TACSTD1*, showed highly specific results; however, *CEA* and *MUC2* were unexpectedly less contributing, despite our previous findings.[7,12] This is possibly because of inefficient or nonspecific amplification by multiplex RT-PCR. For evaluating the sensitivity of the MiniChip assay, a fraction (estimated at 3.8×10^3 cells) of gastric cancer cell line HSC60 was serially diluted ($1:4^n$) by a peritoneal wash sample from one early gastric cancer patient and was analyzed by the MiniChip assay. Reproducible positive results with the MiniChip assay were observed from samples diluted as much as 1:16, suggesting that the threshold for the detection of the assay was approximately 200 cells per 1.5 mL of peritoneal washings (data not shown). Although the sensitivity of RT-PCR–based methods is expected to be higher than that of immunocytochemistry,[17] both of the two methods, the MiniChip assay and immunocytochemistry, detected twice as many positive cases as did conventional cytology. Therefore, further optimization of the primers or probes, including those for additional markers recently identified,[18,19] is required to improve the results.

As shown in Fig. 4, the disease-free survival curve of the MiniChip assay–positive patients was almost identical to that of the conventional cytology–positive patients. If this observation is consistent with a larger cohort of patients, rare free peritoneal tumor cells, beyond the sensitivity limit of conventional cytology, detected by the MiniChip assay should be interpreted as indicating a poor prognosis with recurrence likely to occur shortly—as short a time as that in cases with positive conventional cytology. However, because the benefits of adjuvant therapies

in some solid tumors were reported to relate to the amount of remnant tumor burden after potentially curative surgery,[20,21] patients with a MiniChip assay–positive result might show a better overall survival after adjuvant therapies compared with those with a positive conventional cytology result.

Our present data suggest an improved sensitivity and reliability of the MiniChip assay and immunocytochemical cytology by anti-MASPIN compared with conventional cytology. Additional information about the status of remnant tumor burden known through these assays might be helpful for future clinical trials to identify a cluster of patients who would most benefit from adjuvant therapies.

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Original article

Role of staging laparoscopy with peritoneal lavage cytology in the treatment of locally advanced gastric cancer

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Abstract

Background. **More accurate preoperative staging is necessary to determine the treatment strategy for locally advanced gastric cancer. Laparoscopy has been suggested as an appropriate staging modality. The aim of this study was to clarify the role of staging laparoscopy in patients with locally advanced gastric cancer.**

Methods. **One hundred patients with primary gastric adenocarcinoma underwent laparoscopy with peritoneal lavage cytology. The disease stages determined were compared with those obtained by conventional methods.**

Results. **The disease stages were corrected after laparoscopy for 47 of the 100 patients (47%), with downstaging in 3 (3.0%) and upstaging in 44 (44%). Peritoneal deposits were found in 7 patients with peritoneal dissemination diagnosed by conventional examination. An unsuspected peritoneal deposit was found in 21 of 93 patients (22.6%), and unsuspected free cancer cells without deposits were found in 27 of 93 patients (29.0%). Gastrectomy after staging laparoscopy was performed in 39 patients. Laparoscopy showed no peritoneal deposits in any of these patients. Free cancer cells were found in 9 patients (23.1%), but 4 of these had peritoneal deposits at operation. R0 resection was performed in 34 of the 39 patients (87.2%). Neoadjuvant chemotherapy after staging laparoscopy was performed in 35 patients. All 35 patients underwent gastrectomy, which resulted in 27 R0 and 8 R2 resections. Of 18 patients with positive cytology at laparoscopy, 11 had no free cancer cells at operation. Neoadjuvant chemotherapy induced downstaging of the disease in 11 of the 18 patients with positive cytology (61.1%). Of 26 patients with massive peritoneal deposits, 4 underwent palliative resection because of pyloric stenosis. Twenty-two patients (22.0%) were able to avoid unnecessary laparotomy because of the staging laparoscopy.**

Conclusion. **Staging laparoscopy with peritoneal lavage cytology is a safe, effective tool in patients with locally advanced gastric cancer, especially in patients receiving neoadjuvant chemotherapy.**

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Key words Gastric cancer · Staging laparoscopy · Peritoneal lavage cytology

Introduction

Selection of the appropriate treatment for patients with gastric cancer requires accurate tumor staging. Conventional imaging techniques often understage the extent of the intraabdominal spread of advanced gastric cancer, which results in a high rate of unnecessary exploratory laparotomy [1]. The clinical staging can be improved by laparoscopy, since this may identify intraabdominal tumor deposits on peritoneal surfaces, which are not detectable by preoperative noninvasive imaging. Patients with peritoneal seeding found at laparoscopy may be spared an exploratory laparotomy, and they are currently the only ones to benefit from diagnostic laparoscopy.

Peritoneal carcinomatosis is the most frequent pattern of metastasis and recurrence in patients with gastric cancer [2]. Presumably, disseminated lesions originate from free cancer cells exfoliated from the cancer-invaded serosa. To detect these free cells, several Japanese institutions have performed washing cytology [3]. Recently the prognostic value of positive cytology findings was confirmed also in the West [4]. But the role of cytology during laparoscopy in advanced gastric cancer is controversial. In previous reports, cytology during laparoscopy provided no additional information compared to laparoscopy findings alone [5,6].

Neoadjuvant chemotherapy is expected to lead to downstaging of the primary tumors that are thought to be unresectable and thus permit higher curability with subsequent surgery. Several studies showed that preoperative chemotherapy induced downstaging of the disease and resulted in a higher curative resection rate for surgically staged unresectable cancer [7–9]. In patients

with advanced gastric cancer, accurate staging is necessary to decide on preoperative chemotherapy. Recently, the absence of peritoneal deposits at laparoscopy was included in the eligibility criteria for randomized controlled trials of neoadjuvant chemotherapy.

In the present study we examined the role of laparoscopy with peritoneal lavage cytology in accurate preoperative staging, in order to choose the appropriate treatment modalities for patients with locally advanced gastric cancer.

Patients and methods

Between January 1999 and June 2005, staging laparoscopy was performed in 100 patients with clinical T3 or T4 advanced gastric adenocarcinoma, at the Department of Surgery, Niigata Cancer Center Hospital. The patients were newly diagnosed and had had no prior treatment. The eligibility for staging laparoscopy included macroscopic type 4 or type 2 and type 3 with positive metastasis in regional lymph nodes. The diagnostic assessments included barium meal, endoscopy, abdominal ultrasonography, and computed tomography (CT) as appropriate to identify metastases or local infiltration. The disease stage was reported according to the criteria in the second English edition of the *Japanese classification of gastric carcinoma* [10]. The absence or presence of residual tumor after the operation was determined by the R classification, based on International Union Against Cancer (UICC) criteria [11]. Patients who had positive lavage cytology but no macroscopic peritoneal metastasis were regarded as having had an R0 resection. Written informed consent to participate in the study was obtained from all patients. The patients were followed up at our hospital until December 2005, with follow-up durations of 17 to 2202 days (median, 505 days). Any deaths after staging laparoscopy, including deaths from other causes, were included in the survival analysis.

Laparoscopy was performed under general anesthesia as an independent procedure, or immediately before surgery. The patient was positioned as for an open upper abdominal procedure, and the operating table was repositioned according to the intraabdominal region to be inspected. A small (2-cm) laparotomy incision was made, into which was inserted a 12-mm disposable trocar for the flexible laparoscope superior to the umbilicus. The abdomen was insufflated with carbon dioxide until a pressure of 10–12 mmHg was reached. A 3-mm access needle was then inserted in the right upper quadrant, under visual control, for washing cytology. All four quadrants of the peritoneal cavity were thoroughly inspected for evidence of malignant deposits, but biopsy of suspect metastases was not performed. Peritoneal lavage fluid was taken from the Douglas pouch and/or left subphrenic space.

At the beginning of the study, immediate laparotomy was performed in asymptomatic patients without evidence of peritoneal deposits (P0) after staging laparoscopy. Gastrectomy was performed for those patients who were diagnosed at laparoscopy as having a few peritoneal deposits graded as P1 according to the first English edition of the *Japanese classification of gastric carcinoma* [12]. Starting in 2001, patients eligible for staging laparoscopy underwent neoadjuvant chemotherapy, with a combination of cisplatin along with S-1 (oral fluoropyrimidine agent), given orally, or 5-fluorouracil (Fu), CPT-11 (irinotecan), or paclitaxel given intravenously, with the aim of downstaging the disease after the staging laparoscopy. Patients with extensive peritoneal dissemination, graded as P2-P3 by the *Japanese classification of gastric carcinoma* [12], were referred for systemic chemotherapy unless symptomatic disease (obstruction and bleeding) required palliative gastrectomy.

After the neoadjuvant chemotherapy was completed, conventional examinations were routinely carried out to assess the clinical response. The treatment response was categorized using the response assessment of chemotherapy and radiotherapy for gastric carcinoma [10]. A complete response (CR) was defined as 100% regression of the disease. A partial response (PR) was defined as regression of more than 50% of the tumor and metastatic lymph nodes, as confirmed by barium meal, endoscopy, and CT scans. Progressive disease (PD) was defined as an increase in the tumor mass or metastatic nodes (or both) or the appearance of new lesion(s). Patients not in these groups were considered to have stable disease (no change; NC).

The χ^2 test, Fisher's exact probability test, and the Mann-Whitney *U*-test were used to evaluate differences in clinicopathologic features. Survival was estimated using the Kaplan-Meier method, and statistical differences were analyzed using the log-rank test. A *P* value of less than 0.05 was considered significant.

Results

Clinicopathologic characteristics of patients

Staging laparoscopy was performed in 100 patients with T3 or T4 advanced gastric cancer. Laparoscopy was uneventful in all patients, and there were no procedure-related complications. The patients' clinicopathologic characteristics are shown in Table 1. There were 65 men and 35 women, with a median age of 62 years (range, 28–83 years). In 80% of the patients, the tumors were macroscopic type 3 or 4. Histologically, undifferentiated

tumors (poorly differentiated adenocarcinoma, signet-ring cell carcinoma, and mucinous adenocarcinoma) were predominant (73%). On conventional staging, the tumors were clinical stage II in 42 patients, stage IIIA in 31, stage IIIB in 12, and stage IV in 15.

Comparison of conventional and laparoscopic staging

As shown in Table 2, the disease stages were corrected after laparoscopy for 47 of the 100 patients (47%), with downstaging in 3 (3%) and upstaging in 44 (44%). Peritoneal deposits were found in 7 patients with peritoneal dissemination diagnosed by conventional examination. Table 3 presents clinicopathologic factors relevant to unsuspected peritoneal deposits and free cancer cells. An unsuspected peritoneal deposit was found in 21 of 93 patients (22.6%), and unsuspected free cancer cells without deposits were found in 27 of 93 patients (29%). More patients with type 4 tumors than those with type 2 tumors were found to have peritoneal deposits ($P = 0.02$). Patients with type 3 or 4 tumors were more likely to have free cancer cells than those with type 2 tumors ($P = 0.02$ for type 3 and $P = 0.001$ for type 4).

Treatment after staging laparoscopy

Gastrectomy after staging laparoscopy was performed in 39 patients. At laparoscopy, peritoneal deposits were not found in any of the 39 patients, and free cancer cells were found in 9 patients. At operation, 4 of the 9 patients with positive cytology had peritoneal deposits that had not been discovered at laparoscopy. In 34 patients (87.2%), R0 resection was performed, and positive cytology was found in 7 (20.6%) of the 34 patients with R0 resection. Resection for the remaining 5 pa-

Table 1. Clinicopathologic characteristics of 100 patients

Sex, M/F	65/35
Age (years; mean ± SD)	61.0 ± 11.9
Cancer location: (U/M/L)	23/38/39
Macroscopic type: (0/1/2/3/4)	0/0/20/29/51
Histology (differentiated/undifferentiated)	27/73
cT, (cT3/cT4)	97/3
cN, (cN0/cN1/cN2/cN3)	46/33/12/9
cH, (cH0/cH1)	99/1
cP, (cP0/cP1)	93/7
cStage, (IA/IB/II/IIIA/IIIB/IV)	0/0/42/31/12/15

U, Upper third of stomach; M, mid-third of stomach; L, lower third of stomach; cT,clinical tumor; cN, clinical lymph node metastasis; cH, clinical liver metastasis; cP, clinical peritoneal disease; cStage, clinical stage

Table 2. Comparison of conventional and laparoscopic staging

Conventional staging		Laparoscopic staging				
		IB	II	IIIA	IIIB	IV
II	$n = 42$	3	18	0	0	21
IIIA	$n = 31$	0	0	13	0	18
IIIB	$n = 12$	0	0	0	7	5
IV	$n = 15$	0	0	0	0	15
Correction of disease staging		47/100 (47%)				
Downstaging		3/100 (3%)				
Upstaging		44/100 (44%)				

Table 3. Clinicopathologic factors relevant to unsuspected peritoneal metastasis and free cancer cells

Clinicopathologic factors		Unsuspected peritoneal deposits ($n = 21$)	Unsuspected free cancer cells without peritoneal deposits ($n = 27$)
Macroscopic type			
2	$n = 20$	1 (5.0%)	0
3	$n = 29$	4 (13.8%)	7 (24.1%)
4	$n = 51$	16 (31.4%)	20 (39.2%)
Cancer location			
U	$n = 23$	7 (30.4%)	4 (17.4%)
M	$n = 38$	9 (23.7%)	13 (34.2%)
L	$n = 39$	5 (12.8%)	10 (25.6%)
Histology			
Differentiated	$n = 27$	6 (22.2%)	5 (18.5%)
Undifferentiated	$n = 73$	15 (20.5%)	22 (30.1)
Stage before laparoscopy			
II	$n = 42$	8 (19.0%)	13 (31.0%)
IIIA	$n = 31$	7 (22.6%)	12 (38.7%)
IIIB	$n = 12$	4 (33.3%)	1 (8.3%)
IV	$n = 15$	2 (13.3%)	1 (6.7%)

U, Upper third of stomach; M, mid-third of stomach; L, lower third of stomach

tients was R2. Neoadjuvant chemotherapy after staging laparoscopy was performed in 35 patients. At laparoscopy, peritoneal deposits were found in 2 of these patients, and free cancer cells were found in 18. The clinical response (PR in 13, NC in 18, PD in 4), as determined by conventional methods, did not correspond to operative curability. All 35 patients underwent gastrectomy without a second staging laparoscopy, which resulted in 27 with an R0 resection (77.1%) and 8 with an R2 (22.9%). Positive cytology was found in 1 of the 27 patients that received R0 resection (3.7%). Peritoneal deposits were found in all 8 patients who received an R2 resection. Of the 18 patients with positive cytology at staging laparoscopy, 11 had no free cancer cells at operation. Thus, neoadjuvant chemotherapy induced downstaging of the disease in 11 of 18 patients with positive cytology (61.1%). Of the 26 patients with P2-P3 disease, 4 underwent palliative resection because of pyloric stenosis. Palliative chemotherapy was performed in 22 patients with P2–P3 disease. As a result, 22 of the 100 patients (22.0%) were able to avoid unnecessary laparotomy because of the staging laparoscopy. But 4 of the 22 patients who received chemotherapy required palliative gastrectomy at a later stage due to obstruction and bleeding from the tumor.

Patient outcome

The overall 5-year survival rate of the 100 patients was 33.1%. The overall survival of the 74 patients without or with a few peritoneal deposits (P0-P1) was 42.7%, but none of the patients with P2-P3 disease survived for more than 3 years after treatment (Fig. 1). Figure 2 shows the survival curves of the 39 patients who received immediate gastrectomy decided upon according to the washing cytology status at laparoscopy. The 5-year survival rate of patients with positive cytology was significantly worse than that of patients with negative cytology ($P = 0.01$). Of the 9 patients with positive cytology, 6 developed peritoneal carcinomatosis. Of the 27 patients who received curative resection after staging laparoscopy, 10 died of recurrent disease; 4 of these 10 patients had peritoneal carcinomatosis, 3 had hematological recurrence (2 in liver, 1 in bone), 1 had lymph node recurrence, and 2 had combined recurrence (1 in peritoneal dissemination and liver, 1 in lymph node and liver). Of the 28 patients who received neoadjuvant chemotherapy followed by curative resection, 3 died of recurrent disease (2 with recurrence of peritoneal carcinomatosis and 1 with lymph node metastasis at the paraaortic lesion). Four patients that underwent palliative gastrectomy died after a median of 232 days. Of the 22 patients who received palliative chemotherapy, 18 died after a median of 289 days and 4 were alive with disease at 148, 230, 553, and 563 days.

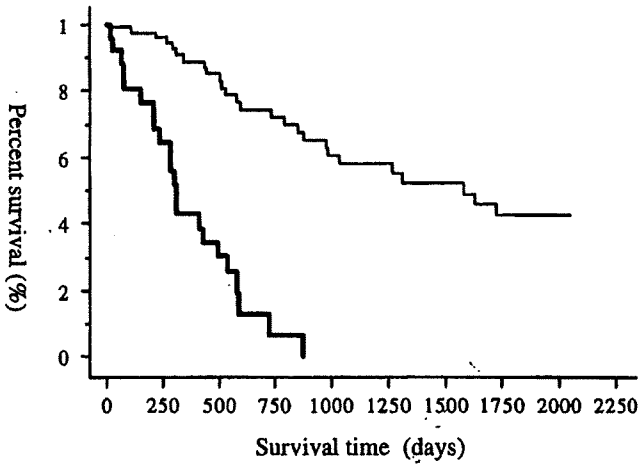

Fig. 1. Survival curves for 100 patients with advanced gastric cancer who received staging laparoscopy. The patients were stratified according to the results of the laparoscopic findings. The difference between the curves was significant ($P < 0.0001$). *Thin curve*, P0-P1, patients (without or with a few peritoneal deposits; $n = 74$); *thick curve*, P2-P3, patients (with extensive peritoneal dissemination; $n = 26$)

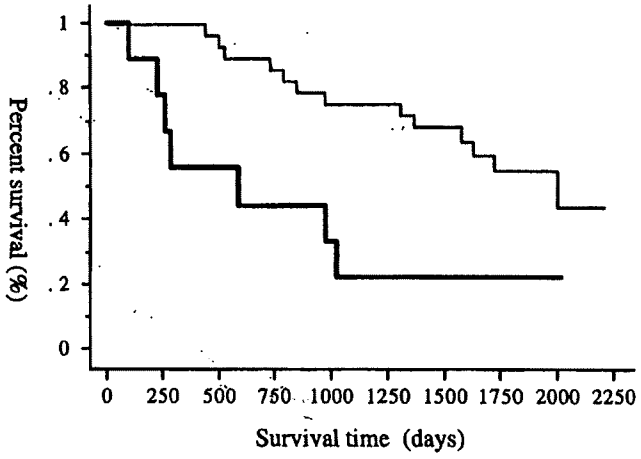

Fig. 2. Survival curves for 39 patients who received immediate gastrectomy according to the status of washing cytology at laparoscopy. The difference between the curves was significant ($P = 0.01$). *Thin curve*, cytology-negative ($n = 30$); *thick curve*, cytology-positive ($n = 9$)

Discussion

Laparoscopy has the potential to fulfill two roles in patients with advanced gastric cancers: (1) avoiding an unnecessary laparotomy in patients with incurable metastatic diseases and (2) staging patients for preoperative treatments.

Peritoneal dissemination is the most common pattern of metastasis in patients with incurable neoplastic diseases [2]. Peritoneal metastasis is usually not detected by conventional techniques, but surgical laparoscopy

offers high accuracy for detecting small intraabdominal metastases [13]. Many laparoscopic studies of patients with gastric cancer have shown that conventional examinations failed to detect peritoneal seeding in about 13% to 37% of patients [13–16]. Among the 100 patients enrolled in the present study, 21 (21%) were found to have unsuspected peritoneal metastasis (a finding which is compatible with the above reports), and these patients were able to avoid unnecessary laparotomy because of the staging laparoscopy.

Early detection of peritoneal dissemination by the cytological examination of peritoneal lavage fluid has been established in Japan [2, 3]. The intraoperative cytological examination of peritoneal lavage fluid is important for predicting survival and peritoneal recurrence in patients with gastric cancer. Recently the prognostic value of positive cytology findings was confirmed also in the West [4]. During staging laparoscopy, cytology of peritoneal lavage fluid is easily performed. But the usefulness of cytology during laparoscopy for gastrointestinal malignancies has been a subject of debate in the literature [5,6]. Van Dijkum et al. [5] maintained that cytology of peritoneal lavage fluid should no longer be performed during the laparoscopic staging of gastrointestinal malignancies, because it offered little benefit. However, in their study, patients with gastric cancers were excluded. Sotiropoulos et al. [6] found that cytology during laparoscopy gave no additional information compared to laparoscopic findings alone. In our study, 27 of 93 patients (29%) were found to have unsuspected positive cytology without malignant deposits. In 39 patients who received immediate gastrectomy, the survival rate of the patients with positive cytology was significantly worse than the survival rate of those with negative cytology. Also, peritoneal deposits that had not been discovered at laparoscopy were found in 4 patients with positive cytology. These findings reveal that conducting cytology of peritoneal lavage fluid at laparoscopy could be beneficial and could make up for the false-negative results of laparoscopy.

Accurate staging by laparoscopy is necessary in patients with advanced gastric cancers, to assess the benefits of preoperative neoadjuvant chemotherapy. In the past decade, neoadjuvant chemotherapy has attracted interest as a promising treatment strategy [7–9]. Although neoadjuvant chemotherapy has not significantly improved the prognosis for patients with potentially resectable gastric cancers, promising results have come from studies dealing with patients who had surgically staged unresectable cancer. Several studies showed that preoperative chemotherapy induced downstaging of the disease and resulted in a higher curative resection rate for surgically staged unresectable cancer. In the present study, 11 of 18 patients with positive cytology at staging laparoscopy revealed no free cancer cells or downstaging at operation. If peritoneal lavage cytology had not been performed at laparoscopy, these patients might have been included in the category of those without negative cytology before treatment. The significance of this change is not clear, but the patients with positive cytology before treatment could have far more advanced diseases relative to those with negative cytology.

The role of a second staging laparoscopy after neoadjuvant chemotherapy is unclear. Conventional imaging examinations have been routinely performed to assess the clinical response to the therapy, but these examinations have not been useful for diagnosing peritoneal metastasis. However, laparoscopy is more invasive and expensive than the conventional examinations. Yano et al. [17] reported that a second staging laparoscopy could accurately assess the response to neoadjuvant chemotherapy to aid in decisions on salvage surgery, especially in patients in whom peritoneal metastasis was the only reason for noncurability. In our study, a second staging laparoscopy was not performed. In 8 of the 35 patients who received neoadjuvant chemotherapy, the resection was R2. Thus, a second staging laparoscopy could be valuable for these patients to aid in decisions on gastrectomy. The role of a second staging laparoscopy in patients after adjuvant chemotherapy should be examined to determine whether the procedure has additional value for these patients.

In conclusion, staging laparoscopy is a safe, effective tool for diagnosing locally advanced gastric cancer. It can increase the curative resection rate and decrease unnecessary laparotomies in patients with advanced gastric cancer by detecting previously unsuspected peritoneal metastasis. Moreover, cytology of peritoneal lavage fluid at laparoscopy should be done in patients receiving neoadjuvant chemotherapy, as this cytology could be beneficial for these patients.

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胃癌治療の新しいエビデンスを求めて－臨床試験の取り組み－
JCOGでの取り組みと現状

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胃癌治療の新しいエビデンスを求めて－臨床試験の取り組み－

JCOGでの取り組みと現状

Japan Clinical Oncology Group (JCOG) - Activity and current status -

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研究者集団，支援機構，監視機構が存在するJCOGは，科学的/倫理的な臨床試験を遂行しEvidenceを創出している．拡大手術のPhase III(9501，9502)によって標準D2手術が確立した．術後化療Phase III(8801，9206-1，9206-2)でのNegative resultsから示唆された情報はACTS-GCの「有効中止」に結実した．さらなるEvidenceを創出すべく，手術，集学的治療の臨床試験に取り組んでいる．

I. JCOGの機構とJCOG胃癌外科グループ

JCOG(Japan Clinical Oncology Group)は各種固形腫瘍に対する標準治療の確立を目的に設立された多施設共同臨床試験グループである．標準治療確立のためのEvidence創出に必須な科学的/倫理的な臨床試験を遂行するために，JCOGは，組織を整備してきた．

臨床試験に必要な組織は，診療と研究を行う臨床研究者集団，データ管理や統計解析を行う研究者支援機構，試験の審査や効果/安全性の評価とデータの品質保証を行う第三者的監視機構である．JCOGにおける臨床研究者集団は13の臓器/治療別研究グループとして存在し，この中に胃がん外科グループが所属する．研究者支援機構はデータセンターが担当する．第三者的監視機構として，意思決定を行う運営委員会のもと，プロトコール審査委員会，効果/安全性評価委員会，施設監査を行う監査委員会などが存在する．研究者集団，支援機構，監視機構が独立して存在することで，科学的で倫理的な臨床試験が遂行できる．

外科の臨床試験では，手術の品質管理が重要となる．当グループは，3回/年の全体会議を行い，研究者が手術ビデオを供覧し手技について議論する．さらに，個々の臨床試験では，手術時間，出血量，合併症，郭清リンパ節個数などの客観的指標が取り入れられている．原資料は第三者的監視機構によりcheckされる．

これまでに取り組んできた試験と現在進行中/計画中の試験につき解説する．

II. 術後補助化学療法の臨床試験(表1)

1. JCOG8801[1]

sT1N+/T2を対象として，手術単独群を対照

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表1 JCOGにおける術後補助化学療法のPhase III臨床試験

試験名 期間	対象	対照治療 (C)	試験治療 (T)	仮説	予定症例 登録症例	結果
8801 88-92	sT1N+ sT2	手術単独	MMC/5-FU UFT	C：5y 70% T：5y 80% (HR=0.67)	508 579	C：5y 82.9% T：5y 85.8% N.S.
9206-1 93-94	sT1N1-2 sT2N0-2	手術単独	MMC/5FU/Ara-C 経口FU	C：5y 70% T：5y 85% (HR=0.5)	220 252	C：5y 86.1% T：5y 91.2% P=0.13
9206-2 93-98	sT3-4CY0	手術単独	CDDPip 5FU/CDDP UFT	C：5y 40% T：5y 55% (HR=0.75)	280 268	C：5y 60.9% T：5y 62.0% N.S.

とし，MF+UFTによる術後化療の優越性を検証する試験である．全生存期間をPrimary endpointとし，化療群でハザード比0.67(5年生存率70%→80%)の生存率改善効果を，片側検定alpha error = 0.05，beta error = 0.2で検証する．必要症例数は508例となった．化療群では，MMC (1.4 mg/m^2，1回/3週間)+5FU(166.7 mg/m^2，2回/3週間)を行った後に，UFT 300 mg連日で18ヵ月間行う．

1988～1992年に579例が登録された．5年生存率(5生)は，単独群82.9%，化療群85.8%と有意差を認めなかった．層別解析では，T1-単独群94.9%，T1-化療群92.0%に対し，T2-単独群76.9%，T2-化療群83.0%であった．

以上より，T1の予後は手術単独で十分に良好であり補助化療は必要ないこと，T2に対し補助化療が有効か今後の試験の対象とすべきことが示された．

2．JCOG9206-1[2]

sT1N1-2/T2N0-2を対象として，手術単独群を対照とし，MFC+経口FUによる術後化療の優越性を検証する試験である．無再発生存期間(RFS)をPrimary endpointとし，化療群でハザード比0.5(5年無再発生存率=5y-RFS：70%→85%)の改善効果を，両側検定alpha error = 0.05，beta error = 0.2で検証する．必要症例数は220例となった．化療群では，MMC 1.33 mg/m^2，FU 166.7 mg/m^2，Ara-C 13.3 mg/m^2，2回/週を術後3週間施行後，経口FU 134 mg/m^{2}を18ヵ月間内服する．

1993年1月～1994年12月に252例が登録された．5y-RFSは，単独群83.7%，化療群88.8%と化療群で良好であったが有意差を認めなかった(p=0.14)．5生も同様に，単独群86.1%，化療群91.2%と化療群で良好であったが有意差を認めなかった(p=0.13)．層別解析では，sT1-2やpN0症例の予後が90%程度ときわめて良好であった．本試験では化療群で良好な成績であったが，ハザード比0.63＞0.50と差を証明できなかった．今から考えれば，あまりにも欲張ったハザード比の見直しを行い，症例数を増加させて，もう少し少ない差でも検出するという議論を行うべきであったが，その当時の研究者達の知識や経験ではこのような改変を科学的な保証を崩さずに行うすべを持っていなかったのが実情である．

3．JCOG9206-2[3]

sT3-4CY0を対象として，手術単独群を対照としCDDPip+5-FU+CDDP(FP)+UFTによる術後化療の優越性を検証する試験である．全生存期間をPrimary endpointとし，化療群でハザード比0.75(5生：40%→55%)の改善効果を両側検定alpha error = 0.05，beta error = 0.2で検証する．必要症例数は280例となった．化療群では，術中にCDDP 70 mg/m^{2}をip投与，術後14日目よりFU 700 mg/m^2/day，CDDP 70 mg/m^2/dayを3日間投与，術後4週目よりUFT 267 mg/m^{2}を

12ヵ月間内服する化学療法を行う．

1993年1月～1998年3月に268例が登録された．5生は，単独群60.9%，化療群62.0%と差を認めなかった(p=0.99)．5y-RFSも同様に，単独群55.6%，化療群57.5%と差を認めなかった(p=1.01)．

以上より，本レジメンはT3-4胃癌に対して有効ではないと結論づけられた．当時としては，進行・再発胃癌に対して有効な薬剤が用いられ，期待されたが，逆にコンプライアンスの低さが問題となった．

4．JCOG9701

肉眼的根治切除可能なsP0CY1/P1-2を対象として，手術単独群を対照とし，CDDPip + 5FUdivによる術後補助化療の優越性を検証するPhase III試験である．1997年から2年間に，10例が登録されたのみで登録中止となった．本試験が失敗した理由として，①予後がきわめて不良な対象に手術単独群を置いたことで主治医のMotivationが上がらなかった，②術前診断での適格症例に苦労して同意を得ても，術中適格となるのは約10～20%にという効率の悪さ，があげられる．臨床試験の成功にはMotivationと効率が重要であることが示唆された．

III．手術の臨床試験(表2)

1．JCOG9501[4)5)]

根治切除可能なsT2-4N0-2CY0を対象として，D2を対照とし，D2+#16郭清(D3)の優越性を検証する試験である．全生存期間をPrimary endpointとし，D3群でハザード比0.84(5生：50%→58%)の改善効果を，両側検定alpha error = 0.05，beta error = 0.25で検証する．必要症例数は520例となった．

1995年6月～2001年4月に合計523例(D2群263例，D3群260例)が登録された．周術期死亡を両群に1例(0.8%)ずつ認めた．合併症は，D2群20.9%，D3群28.1%とD3群で多い傾向にあった(p=0.067)．手術時間，出血量と輸血の有無で両群に有意差を認めた[4)]．Primary endpointである生存期間は，両群でまったく差を認めず，5生はD2群69.2%，D3群70.3%であった(p=0.57，HR=1.03)[5)]．

以上より，D2に比しリスクの高いD3は，標準治療として推奨されないことが示された．

2．JCOG9502[6)]

3 cm以内の食道浸潤T2-4胃癌を対象とし，開腹経裂孔アプローチ(Transhiatal approach，TH群)に対して，左開胸開腹アプローチ(Left thoraco-abdominal approach，LTA群)の優越性を検証するPhase III試験である．TH群では，開腹経裂孔アプローチによる胃全摘，腹部D2+16a2lat郭清，および，経裂孔的に無理なく郭清できる下部食道周囲の縦隔リンパ節郭清を行った．一方，LTA群では，左開胸開腹アプローチによる胃全摘，腹部D2+16a2lat郭清，および下肺静脈までの下縦隔郭清を行った．全生存期間をPrimary endpointとし，LTA群での10.5%の生存率改善効果を片側検定alpha error = 0.05，

表2　JCOGにおける手術のPhase III臨床試験

試験名 期間	対象	対照治療 (C)	試験治療 (T)	仮説	予定症例 登録症例	結果
9501 95-01	sT2ss-4 N0-2CY0	D2	D2 +#16郭清	C：5y 50% T：5y 58% (HR=0.84)	520 523	C：5y 69.2% T：5y 70.3% N.S.
9502 95-03	食道浸潤 3 cmまで sT2-	開腹	左開胸開腹	Cに比し Tで5y：+10.5%	302→250 165	C：5y 52.3% T：5y 37.9% (中間解析)

beta error = 0.2で検証する．必要症例数302例，4年間の登録期間を予定したが，登録が進まず，8年後に改訂された．片側検定 alpha error = 0.1，beta error = 0.2と設定し直し，必要症例数250例で12年間の登録期間を見込み直した．

1995年7月～2003年10月，両群165例が登録された段階の中間解析で，5生はTH群53.4%，LTA群38.9%であった．このまま登録を続けた場合に，LTA群がTH群を有意に上回る可能性はきわめて低い(3.65%)ことが統計学的に明らかとなり，本臨床試験は無効中止となった．Updateされた生存解析では，TH群(n=82)の5生は52.3%，LTA群(n=85)の5生は37.9%であった．TH群に対するLTA群のハザード比は1.36(0.89-2.08，p=0.92)であった．

在院死は，LTA群4%，TH群0%，合併症発生割合はLTA群49%，TH群34%といずれもLTA群で高かった．侵襲が大きくリスクの高いLTAは，5生で10.5%上回ることで有効な治療と判断される試験であったが，結果は，リスクは高いが生存率で設定を上回る可能性がほとんどない治療法であることが明らかとなった．

以上より，3 cm以内の食道浸潤胃癌に対して，左開胸開腹アプローチは推奨されないことが示された．

IV．術前補助化学療法の臨床試験(表3)

1．JCOG0001[7]

P0H0M0だが根治切除を行っても予後不良な#16転移，および根治切除を行うことが困難なBulky massを形成するN2の予後はきわめて不良であり，Historical controlの3生は10%程度しかない．これらを対象とし，CPT-11+CDDPによる術前補助化学療法を行うことで，3生の期待値25%と閾値15%を得られるか，治療関連死亡(TRD)の点推定値が5%を下回るか，を評価するPhase II試験である．60例の登録を予定したが，55例の登録段階で3例のTRDを認めたことから，TRD=5.5%>5%となり登録中止となった．一方，登録55例の3生は27%，3生の95% CI下限は15.2%であった．

以上より，本治療法は，有効であるが，毒性が強くTRDを起こさないような症例選択と管理が重要であると結論づけられた．

2．JCOG0002-DI

根治切除可能な4型胃癌を対象とし，Historical controlに対してS1による術前化学療法の有効性と安全性を評価するPhase II試験である．生存においては歴史的対照群を上回ることはなく，4型胃癌に対するTS-1単剤による術前化学療法は無効であった．最終結果を論文報告予定である．

3．JCOG0210

P0H0M0CY0の4型/巨大3型胃癌を対象とし，Historical controlに対してS1+CDDPによる術前化学療法の有効性と安全性を評価するPhase II試験である．本試験の特徴は，術前化学療法の完遂＋根治切除の達成率をprimary endpointにしたfeasibility studyであるという点である．このendpointは，仮説をクリアーし，その後同じ対象に対して，同じレジメンで手術単独を対照群とした第III相試験を開始した(JCOG0501)．登録と予定観察が終了し，

表3 JCOGにおける術前補助化学療法のPhase II臨床試験

試験名 期間	対象	試験治療 (T)	仮説	予定症例 登録症例	結果
0001 00-03	cN3(#16) Bulky-N2	CPT11+ CDDP	T：3y 15～25% TRD点推定値 ≦5%	60 55(登録中止)	T：3y 27% (95%CI：15～39%) TRD：5.5% (3/55)

Secondary endpointである遠隔成績も近々米国臨床腫瘍学会で報告予定である.

V. 現在, 進行中の臨床試験

1. JCOG0110[8]

根治切除可能な胃上部進行胃癌を対象として, 脾臓摘出を伴うD2胃全摘術(対照群)と脾臓温存D2手術を比較する. 全生存期間をPrimary endpointとし, 対照群の5生が65～70%に対して, 試験治療群で5%以上劣らないことを検証する非劣性デザインのPhase III試験である. 脾臓摘出は, D2胃全摘術における合併症発症の危険因子である. 脾臓を温存することで, 合併症発症は減少すると予測されるが, 郭清が犠牲になることで生存率が犠牲になるのか否か, 世界中から注目されている.

2. JCOG0302

センチネルリンパ節理論を応用することで, 早期胃癌の局所切除を行うことが妥当であるかを評価する妥当性試験である.

3. JCOG0405

JCOG0001の後継試験として計画された. 術前化学療法としてCPT-11+CDDPよりも安全性が高いと考えられるTS-1+CDDPを用いている. P0H0M0CY0のN3転移またはBulky N2転移症例を対象とし, 術前化学療法の有効性と安全性を評価するPhase II試験である.

4. JCOG0501

P0H0M0CY0の4型/巨大3型胃癌を対象とし, 手術単独群に対するS1+CDDPによる術前補助化学療法の優越性を検証するPhase III試験である. 16例を登録した時点で, ACTS-GCの有効中止が報じられ, 本試験はいったん登録を中止し, 現在両群にTS-1による術後補助化学療法を加えるデザインに変更して, 再開を予定している.

VI. 現在, 計画されている臨床試験

腹膜転移や肝転移を伴うStage 4を対象として減量手術の優越性を検証するPhase III, および早期胃癌に対する腹腔鏡手術の忍容性と安全性を評価するPhase II試験が計画されている.

ま と め

以上のように, JCOG胃癌外科グループでは, 科学的/倫理的でSocial value / Scientific valueの高い臨床試験を行うことで, Evidenceを創出してきた. 拡大手術の有効性を検証したPhase III(9501, 9502)によって, 拡大手術は否定されD2標準術式が確立した. 本試験結果は, 本邦の胃癌外科医の高い技術力と優れた治療成績を世界に知らしめる結果となった. 手術の限界が示されたという点でも意義深く, 今後のさらなる治療成績の向上には, 化学療法を取り入れた集学的治療が不可欠となった. また, 現在の標準手術であるD2がOver surgeryであるのか否か, 脾温存D2手術の非劣性試験の結果も世界から注目されている.

術後補助化学療法のPhase III(8801, 9206-1, 9206-2)では有効性を証明することはできなかった. しかしながら, Negative resultsから示唆された情報は, その後のNSAS-GC(T2N+に対する術後UFT補助化学療法のPhase III)へと引き継がれ, ACTS-GC(Stage II/IIIに対する術後S-1補助化学療法のPhase III)での「有効中止」に結実した.

現在, さらなるEvidenceを創出すべく, 手術, 集学的治療の臨床試験に取り組んでいる.

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胃癌に対する術前補助化学療法

Neoadjuvant chemotherapy for advanced gastric cancer

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◎高度のリンパ節転移を有する進行胃癌や大型3型，4型胃癌は，たとえ手術が可能であったとしても予後不良である．これらの胃癌に対する有望な治療戦略として術前補助化学療法が注目されている．術前補助化学療法のメリットは，術後補助化学療法に比較して高いcomplianceが期待でき，切除標本の治療効果を病理組織学的に判定することで薬剤の感受性を確認できる点などである．一方，効果がなかった場合に手術が遅れ，本来可能であった根治術ができなくなることがある点，手術に先行して化学療法を行うことで術後の合併症が増加する可能性などのデメリットが考えられる．現在，日本臨床腫瘍研究グループ(Japan Clinical Oncology Group：JCOG)では，TS-1＋CDDP療法を用いた第Ⅱ相および第Ⅲ相臨床試験が進行中である．

Key word：進行胃癌，術前補助化学療法，TS-1＋CDDP療法，臨床試験

わが国における胃癌の年間新規発生数は10.4万人で悪性腫瘍の第一位を占める[1]．しかし，診断技術の進歩により早期胃癌が相対的に増加したことと進行胃癌の治療成績が向上したことなどの理由により，全体での5年生存率は70%以上に上昇している[2]．一方，高度のリンパ節転移を有する進行胃癌や，診断時には胃壁全体に浸潤していることの多い大型3型，4型胃癌は，たとえ手術が可能であったとしても予後不良であり，これらの胃癌に対する標準的治療は確立されていないのが現状である．これら手術療法のみでは難治性の予後不良群に対して，補助化学療法の開発によって予後の大幅な向上をめざすあらたな治療戦略が必要である．

高度リンパ節転移を有する胃癌

高度リンパ節転移には，第3群リンパ節である大動脈周囲リンパ節の肉眼的な転移と，腹腔動脈を取り巻く2群リンパ節が一塊となって腫瘤を形成するもの(bulky N2)の2つがある．このような病態においては遠隔転移がない症例で，たとえリンパ節郭清範囲を広げた拡大手術を行い，肉眼的に病変を完全切除したとしても予後が不良である．この要因としては，手術範囲より遠隔に微小転移を伴っている場合が多いこと，拡大手術後に高率に発生する合併症により切除直後あるいは術後早期の補助化学療法が行われていない点などが考えられている[3]．いずれの予後も不良で，3年生存率はほぼ5～10%程度である[4,5]．

大型3型，4型胃癌

笹子ら[6]によると，3型胃癌の予後は直径が大きくなるにつれて不良となり，5年生存率は病理組織学的腫瘍径が4～8 cmで37.0%，8～12 cmで20.3%，12 cmを超えると0%であり，4型胃癌のそれぞれ11.2%，14.0%，6.4%と比較すると病理組織学的腫瘍径が10 cmを越える大型3型胃癌ではむしろ4型胃癌よりも予後が不良であった．都立駒込病院における根治切除が施行された直径8 cm以上の3型胃癌と4型胃癌の5年生存率