Table 1. Baseline Characteristics of the Patients.

Characteristic	S-1 (N=529)	Surgery Only (N=530)	P Value*
Sex — no. (%)			0.98
Male	367 (69.4)	369 (69.6)	
Female	162 (30.6)	161 (30.4)	
Age			0.86
<60 yr — no. (%)	199 (37.6)	195 (36.8)	
60–69 yr — no. (%)	193 (36.5)	215 (40.6)	
70–80 yr — no. (%)	137 (25.9)	120 (22.6)	
Median — yr	63	63	
Range — yr	27–80	33–80	
Tumor stage — no. (%)			0.81
T1	1 (0.2)	0	
T2	289 (54.6)	286 (54.0)	
T3	225 (42.5)	232 (43.8)	
T4	14 (2.6)	12 (2.3)	
Nodal stage, Japanese classification — no. (%)†			0.72
N0	51 (9.6)	64 (12.1)	
N1	296 (56.0)	281 (53.0)	
N2	182 (34.4)	185 (34.9)	
N3	0	0	
No. of lymph-node metastases — no. (%)			0.37
0	51 (9.6)	64 (12.1)	
1–6	331 (62.6)	325 (61.3)	
7–15	117 (22.1)	113 (21.3)	
≥16	30 (5.7)	28 (5.3)	

area of less than 1.25 m^2 received 80 mg daily; those with a body-surface area of 1.25 m^2 or more but less than 1.5 m^2 received 100 mg daily; and those with a body-surface area of 1.5 m^2 or more received 120 mg daily. This 6-week cycle was repeated during the first year after surgery. If patients had hematologic toxic effects of grade 3 or grade 4 (highest possible grade) or nonhematologic toxic effects of grade 2, grade 3, or grade 4, their daily dose was reduced, from 120 mg to 100 mg, 100 mg to 80 mg, or 80 mg to 50 mg. The surgery-only group received no anticancer treatment after surgery, unless there was a confirmed relapse.

Patients in both groups were to be followed up for 5 years postoperatively. Adverse events were assessed according to the Common Toxicity Criteria of the National Cancer Institute (version 2.0).

FOLLOW-UP

Patients in the S-1 group underwent hematologic tests and assessments of clinical symptoms every 2 weeks. Patients in the surgery-only group underwent similar examinations at least every 3 months. Evaluation for adverse events was performed every 3 months for 1 year after surgery.

The presence of a relapse was determined by means of imaging studies, including ultrasonography, computed tomography (CT), gastrointestinal radiography series, and endoscopy. Patients underwent at least one type of imaging study, usually CT, at 6-month intervals during the first 2 years after surgery and at 1-year intervals thereafter until year 5 after surgery. Case-report forms, which included the results of these tests and evaluations and the survival status of patients, were submitted 1 year, 1.5 years, 2 years, 3 years, 4 years,

Table 1. (Continued.)

Characteristic	S-1 (N=529)	Surgery Only (N=530)	P Value*
Cancer stage, Japanese classification — no. (%)‡			0.78
II	236 (44.6)	238 (44.9)	
IIIA	202 (38.2)	207 (39.1)	
IIIB	90 (17.0)	85 (16.0)	
IV	1 (0.2)	0	
Cancer stage, TNM classification — no. (%)			0.37
IB	1 (0.2)	0	
II	264 (49.9)	282 (53.2)	
IIIA	170 (32.1)	157 (29.6)	
IIIB	54 (10.2)	56 (10.6)	
IV	40 (7.6)	35 (6.6)	
Type of lymph-node dissection — no. (%)			0.69
D1	0	1 (0.2)	
D2	501 (94.7)	497 (93.8)	
D3	28 (5.3)	32 (6.0)	
Type of gastrectomy — no. (%)			0.26
Total	220 (41.6)	201 (37.9)	
Distal	301 (56.9)	316 (59.6)	
Proximal	4 (0.8)	11 (2.1)	
Other	4 (0.8)	2 (0.4)	

* P values for sex and type of gastrectomy were calculated with the use of the chi-square test. P values for age, tumor stage, nodal stage, number of lymph-node metastases, cancer stage (Japanese and tumor–node–metastasis [TNM] classifications), and type of lymph-node dissection were calculated with the use of the Wilcoxon test.

† Nodal stages according to the Japanese classification were defined as follows: N0, no evidence of lymph-node metastasis; N1, metastasis to group 1 lymph nodes; N2, metastasis to group 2 lymph nodes; and N3, metastasis to group 3 lymph nodes. Groups 1, 2, and 3 are regional lymph-node classifications defined according to the location of the primary tumor and based on the results of studies of lymphatic flow at various tumor sites and the observed survival associated with metastasis at each nodal station (i.e., position in relation to primary node).

‡ Cancer stages according to the Japanese classification were defined as follows: stage IA, T1N0; stage IB, T1N1 or T2N0; stage II, T1N2, T2N1, or T3N0; stage IIIA, T2N2, T3N1, or T4N0; stage IIIB, T3N2 or T4N1; and stage IV, T4N2, any T stage with N3, or distant metastasis.

and 5 years after surgery. Patients, their physicians, endoscopists, and radiologists were aware of the group assignment after surgery, and no placebo was used. However, relapses and other events were evaluated by members of the steering committee, who were unaware of the group assignments.

STATISTICAL ANALYSIS

The results of a previous study conducted in Japan[20] served as the basis for determining the required numbers of patients.[21] The 5-year overall survival rate in the surgery-only group was assumed to be 70%. We calculated that a total enrollment of 1000 patients was needed for a hazard ratio for death of 0.70 in the S-1 group as compared with the surgery-only group, with the use of the log-rank test, a two-sided alpha of 5%, and a statistical power of 80%, assuming 3 years of recruitment and an additional 5 years of follow-up.

Efficacy was to be evaluated in two interim analyses performed by an independent data and safety monitoring committee 1 year and 3 years after the completion of enrollment. Significance was evaluated with the use of the method of Lan and DeMets[22] and the O'Brien–Fleming boundary. Person-years were used to estimate information fractions for use in interim analyses. When calculating information fractions, we assumed that patients who had not completed the study before the interim analysis were continuously observed until the final analysis.

Data for all randomly assigned patients, whether eligible or not, were included in efficacy analyses. Data for eligible patients were also analyzed to evaluate the robustness of the results. Overall survival was defined as the period between randomization and death. All deaths, including those from other diseases, were considered to be events. Relapse-free survival was defined as the period between randomization and the occurrence of an event — relapse or death — whichever came first. Data for patients who had not had an event were censored as of the date of the final observation.

The median time from surgery to randomization was 28 days (range, 7 to 42) in the S-1 group and 28 days (range, 6 to 42) in the surgery-only group. Because the number of days from surgery to randomization varied among patients, we also calculated the overall survival from the date of surgery. In the first interim analysis, overall survival was also measured from the date of surgery. The Kaplan–Meier method was used to estimate the cumulative survival. The primary confirmatory analysis was performed with the use of the stratified log-rank test, with the cancer stage — which was used in the random assignment of patients at enrollment — as a stratification factor. The Cox proportional-hazards model was used to calculate the hazard ratios. All P values calculated in the subgroup analysis were two-sided and were not adjusted for multiple testing. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

CHARACTERISTICS OF PATIENTS

We enrolled and randomly assigned 1059 patients — 529 to the S-1 group and 530 to the surgery-only group — at 109 centers between October 2001 and December 2004. After randomization, 25 patients (14 in the S-1 group and 11 in the surgery-only group) were found to be ineligible. The reasons for ineligibility were as follows: the absence of cytologic examination of the peritoneal fluid (nine patients), cancers other than gastric cancer (five), previous treatment for gastric cancer (four), laboratory test values at enrollment that did not meet the protocol requirements (four), limited (D1) surgery (one), stage IV cancer (one), and T1 cancer (one). The main analyses were based on data from all randomly assigned patients, including those who were ineligible. The two groups were well balanced with regard to baseline clinical characteristics, surgical procedures, and pathological findings (Table 1).

INTERIM ANALYSIS

The first interim analysis was based on data derived from case-report forms submitted by December 2005, 1 year after enrollment of the last patient. This analysis (median follow-up, 2.0 years) was conducted by the independent data and safety monitoring committee in June 2006. In this interim analysis, both overall survival and relapse-free survival differed between the two groups, both for all randomly assigned patients (overall survival, $P=0.002$; relapse-free survival, $P<0.001$) and for all eligible patients (overall survival, $P<0.001$; relapse-free survival, $P<0.001$). The significance level of the differences was close to the predetermined threshold for the interim analysis, $P=0.001$. Given these results, the data and safety monitoring committee recommended discontinuation of the trial and publication of the results based on updated data (from follow-up surveys as of June 30, 2006).

ADVERSE EVENTS AND TREATMENT COMPLIANCE

Data on 517 patients in the S-1 group and 526 in the surgery-only group were analyzed for adverse events. Data from the remaining 12 patients in the S-1 group, who did not receive S-1, and from the remaining 4 patients in the surgery-only group, who requested that their treatment assignment be changed after randomization, were not included in the safety analysis. Adverse events of grade 1, 2, 3, or 4 (defined according to the Common Toxicity Criteria of the National Cancer Institute, version 2.0) — including leukopenia, anemia, thrombocytopenia, elevated total serum bilirubin levels, and nonhematologic toxic effects — were more frequent in the S-1 group than in the surgery-only group. The adverse events of grade 3 or 4 that were more frequent in the S-1 group were anorexia, nausea, diarrhea, leukopenia, anemia, elevated total serum bilirubin level, stomatitis, and rash (Table 2).

Among the 517 patients in the safety population who received S-1, treatment was continued for at least 3 months in 452 patients (87.4%), at least 6 months in 403 patients (77.9%), at least 9 months in 366 patients (70.8%), and 12 months in 340 patients (65.8%). The reasons for withdrawal of treatment included refusal of the patient to continue treatment because of adverse events

Table 2. Adverse Events, According to Treatment Group.*

Event	S-1 (N=517)					Surgery Only (N=526)				
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3 or 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3 or 4
	no. of patients				%	*no. of patients*				%
Leukopenia	157	144	6	0	1.2	93	32	2	0	0.4
Anemia	293	167	6	0	1.2	311	64	3	1	0.8
Thrombocytopenia	123	10	1	0	0.2	32	2	2	0	0.4
Elevated AST level	193	30	9	0	1.7	177	30	17	1	3.4
Elevated ALT level	192	26	6	0	1.2	182	27	16	1	3.2
Elevated total serum bilirubin level	155	75	7	1	1.5	40	13	5	1	1.1
Elevated creatinine level	25	2	0	0	0.0	24	2	1	1	0.4
Stomatitis	139	26	1	0	0.2	16	2	0	0	0.0
Anorexia	213	72	30	1	6.0	63	9	8	3	2.1
Nausea	146	37	19	—	3.7	40	7	6	—	1.1
Vomiting	88	23	6	0	1.2	42	6	7	3	1.9
Diarrhea	227	66	16	0	3.1	85	11	1	0	0.2
Rash	111	52	5	0	1.0	6	4	2	0	0.4
Pigmentation	204	37	—	—	—	2	0	—	—	—
Fatigue	242	60	3	0	0.6	88	4	3	0	0.6

* Grades of adverse events were defined according to the Common Toxicity Criteria of the National Cancer Institute (version 2.0). AST denotes aspartate aminotransferase, and ALT alanine aminotransferase; dashes indicate not available.

or other factors (71 patients), the decision of the investigators to terminate treatment because of adverse events or complications (72), the detection of metastasis or relapse (25), the presence of cancers other than gastric cancer (2), post-enrollment ineligibility (5), and transfer to another hospital (2). The dose of S-1 was decreased in 219 of the 517 patients (42.4%) who received S-1. Of the 340 patients who received treatment for 12 months, the dose was decreased in 158 patients (46.5%).

OVERALL SURVIVAL AND RELAPSE-FREE SURVIVAL

On the basis of follow-up data updated on June 30, 2006, the median time from randomization to follow-up was 2.9 years in both the S-1 group and the surgery-only group. Seven patients in the S-1 group and six patients in the surgery-only group were lost to follow-up. A total of 102 patients died in the S-1 group, and 140 patients died in the surgery-only group. The causes of death in the S-1 and surgery-only groups were as follows: relapse (in 96 and 124 patients, respectively), other cancer (1 and 2), a cause other than cancer (4 and 7), and unknown causes (1 and 7). The number of patients who had recurrent metastasis was 133 in the S-1 group and 188 in the surgery-only group.

The hazard ratio for death in the S-1 group, as compared with the surgery-only group, was 0.68 (95% confidence interval [CI], 0.52 to 0.87; P=0.003). The 3-year overall survival rate was 80.1% in the S-1 group (95% CI, 76.1 to 84.0) and 70.1% in the surgery-only group (95% CI, 65.5 to 74.6) (Fig. 1A). The hazard ratio for relapse in the S-1 group, as compared with the surgery-only group, was 0.62 (95% CI, 0.50 to 0.77; P<0.001). The rate of relapse-free survival at 3 years was 72.2% in the S-1 group (95% CI, 67.9 to 76.4) and 59.6% in the surgery-only group (95% CI, 54.9 to 64.3) (Fig. 1B). Among eligible patients, the hazard ratio for death in the S-1 group, as compared with the surgery-only group, was 0.66 (95% CI, 0.51 to 0.86; P=0.002). The results for all randomly assigned patients were similar.

SITE OF FIRST RELAPSE

Common sites of first relapse were the peritoneum, hematogenous sites, and lymph nodes (Table 3). Local relapse occurred in 7 patients in the

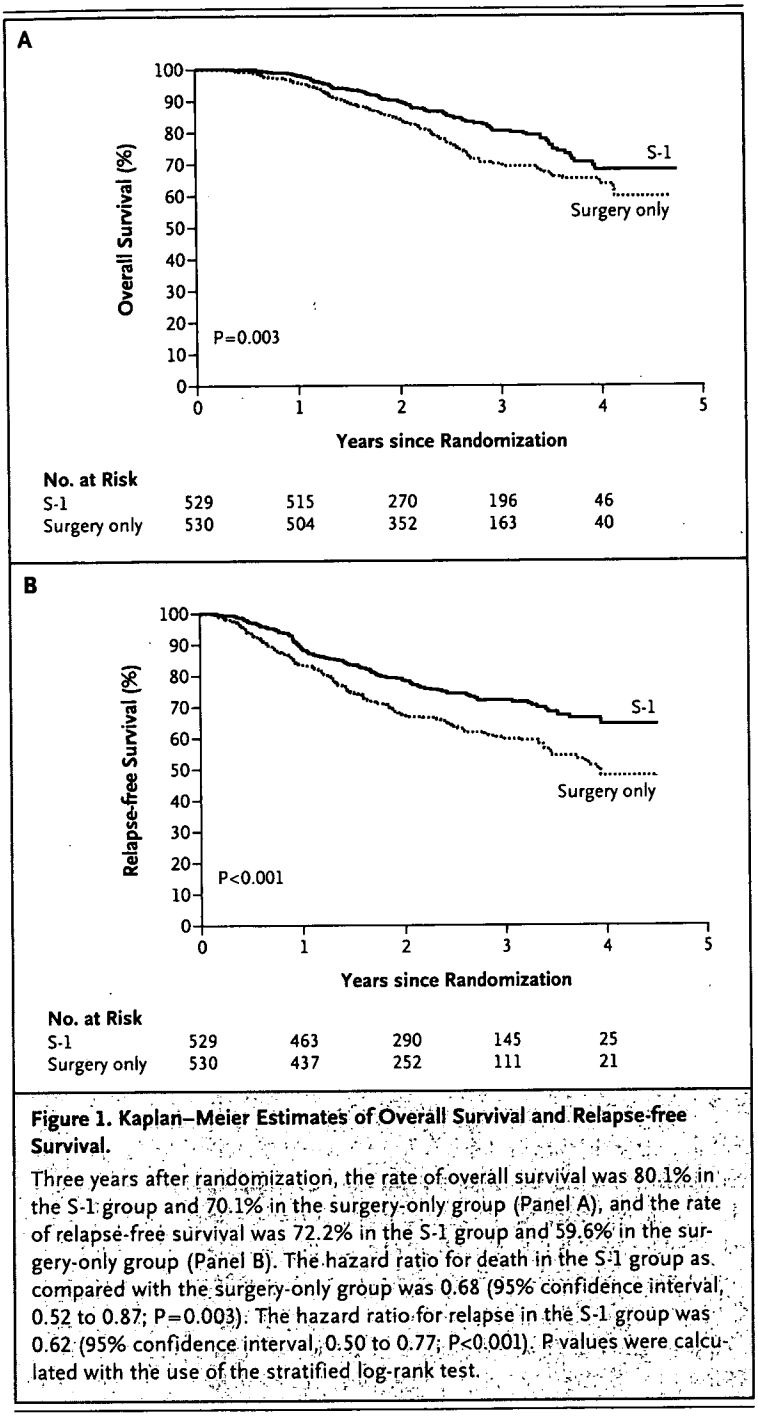

Figure 1. Kaplan–Meier Estimates of Overall Survival and Relapse-free Survival.

Three years after randomization, the rate of overall survival was 80.1% in the S-1 group and 70.1% in the surgery-only group (Panel A), and the rate of relapse-free survival was 72.2% in the S-1 group and 59.6% in the surgery-only group (Panel B). The hazard ratio for death in the S-1 group as compared with the surgery-only group was 0.68 (95% confidence interval, 0.52 to 0.87; P=0.003). The hazard ratio for relapse in the S-1 group was 0.62 (95% confidence interval, 0.50 to 0.77; P<0.001). P values were calculated with the use of the stratified log-rank test.

S-1 group (1.3%) and in 15 patients in the surgery-only group (2.8%). Postoperative treatment with S-1 reduced the frequencies of peritoneal and lymph-node relapses. In the surgery-only group, 84 patients (15.8%) had peritoneal relapse, and 46 patients (8.7%) had lymph-node relapse. In the S-1 group, 59 patients (11.2%) had peritoneal relapse, and 27 (5.1%) had lymph-node relapse.

SUBGROUP ANALYSIS

The overall survival of eligible patients was analyzed according to sex, age, cancer stage (Japanese classification and tumor–node–metastasis [TNM] classification), tumor stage, nodal stage (Japanese classification[18] and TNM classification), and histologic type (Fig. 2). A total of 25 ineligible patients (14 in the S-1 group and 11 in the surgery-only group), including those who had stage IV disease, were excluded. There was no significant interaction between the treatment group and any of the variables studied.

DISCUSSION

Few large-scale trials (those with >500 patients) have compared postoperative adjuvant therapy with surgery alone among patients with gastric cancer. Such large trials have been performed by Nakajima et al. (the JCOG [Japan Clinical Oncology Group] 8801 study),[20] Macdonald et al. (the INT-0116 study),[7] and Cunningham et al. (the MAGIC trial).[8] The JCOG 8801 study in Japan failed to demonstrate therapeutic benefits of adjuvant chemotherapy. The authors suggested that surgery probably resulted in a cure only in patients with T1 tumors, who accounted for nearly one third of all patients in the study, possibly masking differences in overall survival. The INT-0116 study, performed in the United States, showed that adjuvant chemoradiotherapy prolonged overall survival and relapse-free survival. Most patients in the INT-0116 study underwent either D0 or D1 surgery, with only 10% undergoing D2 surgery. The characteristics of patients in the INT-0116 study differed from those in the JCOG 8801 study and those in our trial. An analysis of benefit according to the type of lymph-node dissection showed no effect of adjuvant chemoradiotherapy among patients who underwent D2 surgery. In the MAGIC trial, conducted mainly in the United Kingdom, perioperative and postoperative chemotherapy with epirubicin, cisplatin, and infused fluorouracil significantly prolonged overall survival and relapse-free survival. In that study, D2 surgery was not performed as a standard procedure.

In addition to the JCOG 8801 study, the JCOG

9206-1,[23] JCOG 9206-2,[24] and National Surgical Adjuvant Study Group for Gastric Cancer (N-SAS-GC)[25] studies have evaluated postoperative adjuvant chemotherapy after D2 surgery in Japan. These studies involved only about 200 patients each. Although the results of the N-SAS-GC study showed that adjuvant chemotherapy with uracil–tegafur (an oral fluoropyrimidine prodrug) was effective, confirmatory studies were needed. Because of the high incidence of gastric cancer in Japan, an effective regimen for adjuvant chemotherapy is particularly important there. Our decision to perform this phase 3 trial of S-1 in patients who underwent curative resection was based on the results of previous clinical trials showing that S-1 alone is effective for the treatment of advanced gastric cancer and may therefore be useful as adjuvant chemotherapy.

More than 100 centers located throughout Japan participated in our trial. To ensure a uniform level of surgical quality, the participating centers were selected from among hospitals performing at least 100 operations annually for gastric cancer. All of our patients, except one who received D1 surgery and was therefore ineligible, underwent surgery that was at least D2. The rate of local relapse in the surgery-only group was 2.8% (15 of 530 patients), indicating that surgery alone was satisfactory in terms of local control. In all, 29% of patients underwent splenectomy, whereas 4% underwent pancreatectomy.

After a median follow-up of 2.9 years, the rate of overall survival was higher in the S-1 group than in the surgery-only group. This outcome is consistent with the results of a previous trials conducted in Japan.[3,25] Our results are also consistent with those of a meta-analysis showing that the hazard ratio for death among patients who received S-1, as compared with those who did not, ranged from 0.70 to 0.82.[1-6] In addition, the effectiveness of postoperative adjuvant chemotherapy with S-1 for gastric cancer is consistent with the high response rates among patients with advanced gastric cancer.[14,15] Adverse events of grade 3 or grade 4 occurred in less than 5% of patients in the S-1 group, except for anorexia (which occurred in 6% of patients), and compliance with S-1 treatment was good. We therefore believe that S-1 is useful as adjuvant chemotherapy after curative surgery in patients with gastric cancer.

Table 3. Site of First Relapse, According to Treatment Group.*

Site	S-1 (N=529)	Surgery Only (N=530)	Hazard Ratio for Relapse in the S1 Group (95% CI)	P Value
	no. of patients (%)			
Total no. of relapses	133 (25.1)	188 (35.5)		
Local	7 (1.3)	15 (2.8)	0.42 (0.16–1.00)	0.05
Lymph nodes	27 (5.1)	46 (8.7)	0.54 (0.33–0.87)	0.01
Peritoneum	59 (11.2)	84 (15.8)	0.64 (0.46–0.89)	0.009
Hematogenous	54 (10.2)	60 (11.3)	0.84 (0.58–1.21)	0.35

* Some patients had a first relapse at more than one site.

Our results were obtained at a median follow-up of 2.9 years after randomization (median follow-up after surgery, 3.0 years). The survival rate 3 years after surgery was 80.5% in the S-1 group and 70.1% in the surgery-only group. These results were similar to those obtained when survival rates were measured from the date of randomization, with a hazard ratio for death in the S-1 group, as compared with the surgery-only group, of 0.68 (P=0.002). The results may change marginally at the 5-year follow-up. However, the number of events in the surgery-only group has already reached nearly 80% of that initially predicted for 5 years. At the first interim analysis, the predicted probability that overall survival in the S-1 group would be significantly better than that in the surgery-only group at final analysis was estimated to be 99.3%.[26]

Although it has sometimes been suggested that there may be differences in certain aspects of gastric cancer in Japan and the West, there have been no significant differences identified between Japan and the United Kingdom with regard to possible genetic influences or between Japan and European countries in the distribution of important prognostic factors.[27-29] Moreover, Italian investigators have reported that pancreas-preserving D2 dissection performed at centers with experience in this procedure can provide a survival benefit.[30] If adequate D2 dissection were performed, we believe that treatment outcomes in Western countries would be similar to those in Japan. We acknowledge that the results of our trial may not be valid in countries where D2 surgery is not considered the standard operation.

Baseline Characteristic	S-1 No. of deaths/total	Surgery Only no. of patients	P Value for Interaction
Sex			0.59
Male	70/358	101/362	
Female	27/157	36/157	
Age			0.42
<60 yr	27/192	46/191	
60–69 yr	36/193	54/211	
70–80 yr	34/130	37/117	
Cancer stage (Japanese classification)			0.86
II	24/232	38/233	
IIIA	43/194	63/203	
IIIB	30/89	36/83	
Cancer stage (TNM classification)			0.69
II	26/260	49/278	
IIIA	38/165	48/153	
IIIB	19/53	23/53	
IV	14/37	17/35	
Tumor stage			0.55
T2	43/283	60/282	
T3	52/219	72/225	
T4	2/13	5/12	
Nodal stage (Japanese classification)			0.35
N0	3/49	11/63	
N1	44/290	62/273	
N2	50/176	64/183	
Nodal stage (TNM classification)			0.23
N0	3/49	11/63	
N1	44/323	70/319	
N2	38/115	42/109	
N3	12/28	14/28	
Histologic type			0.53
Differentiated	44/214	55/209	
Undifferentiated	53/301	80/307	
Total	97/515	137/519	

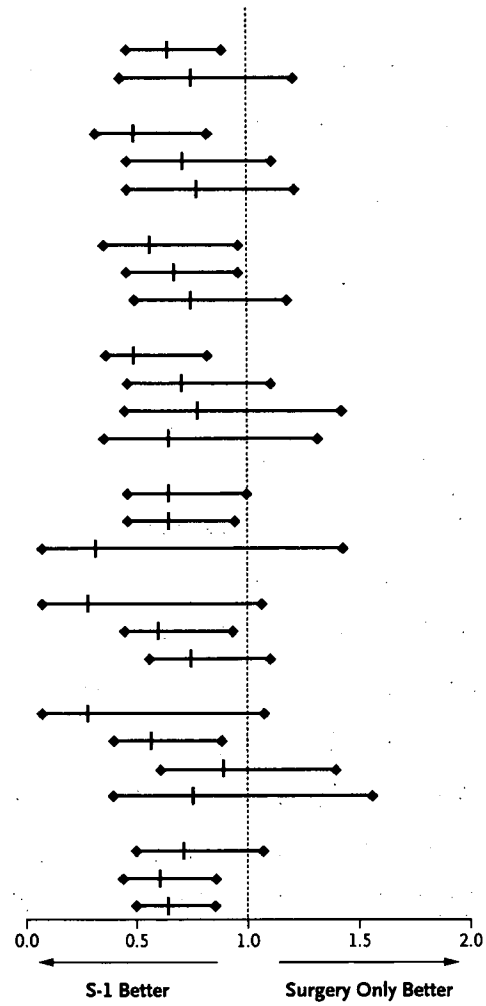

Figure 2. Hazard Ratios for Death and P Values for the Interaction of Treatment Group and Baseline Characteristic among Eligible Patients.

In the surgery-only group, the cancers in three patients could not be classified as differentiated or undifferentiated; one of these patients is still alive.

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APPENDIX

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Gastrointestinal Oncology

Detection of Minimal Gastric Cancer Cells in Peritoneal Washings by Focused Microarray Analysis with Multiple Markers: Clinical Implications

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Background: Peritoneal cytology is an important prognostic factor of gastric cancer. However, peritoneal cytology requires great skill, which may explain its low prevalence. A reverse transcriptase–polymerase chain reaction–based assay with multiple marker genes or immunocytochemistry was assessed as an alternative method of gathering the same kind of data as cytology.

Methods: Peritoneal washings from 179 patients with gastric cancer were analyzed by multiplex reverse transcriptase–polymerase chain reaction with 10 marker genes and subsequent hybridization to a customized oligo-nucleotide array. Results with this assay were either validated as a prognostic factor or confirmed by demonstrating the presence of cancer cells by immunocytochemical cytology.

Results: Only 1 (2.2%) of 44 disease-free cases was shown to be positive by the microarray assay, whereas 13 (93%) of 14 conventional cytology–positive cases were found to be positive. This assay further detected approximately one-third of cytology-negative patients either with peritoneal recurrence (7 of 20, 35%) or with non-peritoneal recurrence (6 of 22, 27%). A high concordance between the microarray assay and immunocytochemical cytology with five antibodies against CK20, FABP1, MUC2, TFF1, and MASPIN was confirmed. The clinical outcome of the microarray assay–positive cases was poor, as was that of the cytology-positive cases.

Conclusions: Our assay, though time-consuming and requiring special equipment, demonstrated a specificity and sensitivity equal to or better than cytology in our institutes. The minimal

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free peritoneal cancer cells detected by the microarray assay may provide the same clinical information as larger amounts of cancer cells for patients with gastric cancer. An anti-MASPIN antibody may be helpful in peritoneal cytology of gastric cancer.

Key Words: Gastric cancer—Peritoneal cytology—RT-PCR—Microarray—MASPIN.

Gastric cancer is one of the most frequently encountered malignancies.[1] Although it can often be cured through surgical resection, leaving no evident residual disease, recurrences are experienced after a potentially curative surgical operation, with peritoneal metastasis as the most common form.[2] Therefore, peritoneal cytology as an assessment of the risk for peritoneal recurrence is often performed for advanced disease, and it is recognized as one of the most important prognostic factors for gastric cancer.[3] Beginning in 1999, the Japanese Gastric Cancer Association began including a description of the intraoperative peritoneal wash cytology as "CY status" in its documentation system, and any surgical procedure labeled as CY1 (cytology positive) is considered noncurative.[4] In addition, usage of laparoscopic disease evaluation and peritoneal cytology before surgical resection is being advocated for patients with advanced gastric cancer.[5] Neoadjuvant therapies may be indicated as a potentially better treatment option for cases with T3 or T4 disease (tumor penetrates serosa or invades adjacent structures) or positive peritoneal cytology proven by a laparoscopic staging system.[6] Thus, peritoneal cytology is establishing an increasing clinical implication in the management of advanced gastric cancer.

However, because conventional peritoneal cytology provides only a limited sensitivity, short-time peritoneal recurrences or even synchronous peritoneal metastases are not rare in cytology negative cases. Specimens containing few atypical cells or containing indeterminate cells mimicking reactive mesothelium are often defined as negative, perhaps because the job requires great skill by trained cytologists (or pathologists). With these problems, the peritoneal cytology in gastric cancer operation is not prevailing in spite of its importance. Trained cytologists are always used in large specialist hospitals, such as those of a university or cancer center, but are often unavailable at other general hospitals. Our goals were twofold: first, to discover a molecular biological method that could be used in a clinical laboratory agency as a substitute for skilled cytology; and second, to ensure that the procedure has uniform accuracy, regardless of the site where the procedure is performed.

To improve the sensitivity of peritoneal cytology, reverse transcriptase–polymerase chain reaction (RT-PCR)-based methods or immunocytochemical cytology have been introduced.[7–9] However, these challenges experienced problems resulting from the use of a single marker gene or only a few marker genes to detect cancer cells even though the cancer cells show expressional variations from cell to cell and from case to case. For example, *CEA* has been reported to be lacking in specificity and *CK20* in sensitivity.[10,11] We previously identified 11 genes as potentially useful markers: *CK20, FABP1, MUC2, TFF1, TFF2, MASPIN, GW112, PRSS4, MDK, SOX9*, and *CDX1*. Among them, five markers, *CK20, FABP1, MUC2, TFF1*, and *TFF2*, showed highly specific results in nested RT-PCR for peritoneal cytology of gastric cancer.[12] Considering the advantage in the use of multiple markers,[13] we here report a sensitive microarray assay that uses multiple marker genes for detecting minimal cancer cells in peritoneal washings of patients with gastric cancer. Concurrently, we also validated the usefulness of our previously identified five marker genes (*CK20, FABP1, MUC2, TFF1*, and *MASPIN*) in immunocytochemical peritoneal cytology.

METHODS

Clinical Materials

A total of 179 peritoneal wash samples from patients with gastric cancer who gave written informed consent were collected at the National Cancer Center (NCC, 98 cases), the Aichi Cancer Center (ACC, 47 cases), and the University of Tokyo Hospital (34 cases). The NCC samples were those used in our previous studies,[12] and the ACC samples were representative, and thus not consecutive, cases (18 stage Ia cases, 12 recurrence-free cases, and 17 relapsed cases) described in another previous study.[7] Each set of samples derived from these three institutes was used for three analyses: the capability of predicting recurrence, the impact on clinical outcome, and concordance with findings of immunostaining of the assay. Our study design was approved in the institutional review board of each institute. The method for the RNA extraction is detailed in our previous reports.[7,10]

TABLE 1. *Sequences of primers and probes*

Gene	Forward and reverse primers	Probe
TFF1	[a]CCTTTGGAGCAGAGAGGAGGCAAT [b]TCAGAGCAGTCAATCTGTGTTGTGAGC	TTCGACGACACCGTTCGTGGGGTCCCCTGGT GCTTCTATCCTAATACCATCGAC
TFF2	ATAACAGGACGAACTGCGGCTTCC AGCTGATAAGGCGAAGTTTCTTTCTTGG	TTGAAGTGCCCTGGTGCTTCTTCCCGAACTCT GTGGAAGACTGCCATTACTAAGAGAGGC
FABP1	TCATGAAGGCAATCGGTCTG CAATGTCACCCAATGTCATGG	CATTCTGCACGATTTCCGACACCCCCTTGATA TCCTTCCCCTTCTGGATGAGCTCTTCCG
CK20	ACACGGTGAACTATGGGAGCGATCT CTTCCAGAAGGCGGCGGTAAGTAG	GTACGAMCCMCGCCCCGAGGGCTGGTCGC GACTACAGTGCATATTACAGAC
MUC2	CCGGGGAGTGCTGTAAGAAG GCTCTCGATGTGGGTGTAGG	CTTGAAGTCCCCGGGCTTCAGGATGACGTGC TGGTTGTCGGGCCGTTTGATGATA
CEA	AACTTCTCCTGGTCTCTCAGCT GCAAATGCTTTAAGGAAGAAG	GGTTGGGGTTGCTCTGATATAGGAGCCCTGG TGTAGTTTCTTCATTTCAGGAAGACTGAC
TACSTD1	TGCTGGGGTCAGAAGAACAG TTGAGTTCCCTATGCATCTCA	GCAGGGTCTAAAAGCTGGTGTTATTGCTGTTA TTGTGGTTGTGGTGATAGCAGTTGTTGC
MASPIN	TCCGGGGTAGTTGGCAGAAATACAG TGCATGTCAAGGAAGAGATGGGAGA	GTAATTTGTAAAGTTGGGTGGATAAGCTATCC CTGTTGCCGGTTCATGGATTACTTCTCT
PRSS4	CTGGGCACAGTTGCTGTCCC GGCCACCAGAGTCACGCTGG	GATGCTCCGGTGCTGACCCAGGCTGAGTGTA AAGCCTCCTACCCTGGAAAGATTACCAAC
GW112	CAGAAGCCCCAGTAAGCTGTTTAGGA GCACTTTGTCACTGCCATCAGATTTT	AACCAGAGTTACTAACCAATTCCACCCCCCAC CAACCCCCTTCTACTGCCTACTTTAAAA
β-Actin	TCATCACCATTGGCAATGAG CACTGTGTTGGCGTACAGGT	CCTGTGGCATCCACGAAACTACCTTCAACTCC ATCATGAAGTGTGACGTGGACATCCGCA

[a]Forward primer.
[b]Reverse primer.

Marker Gene Set

After identifying 11 potentially useful marker genes (*CK20, FABP1, MUC2, TFF1, TFF2, MASPIN, GW112, PRSS4, MDK, SOX9*, and *CDX1*) in our previous study,[12] we further screened additional thousands of genes for marker genes with a new version of microarray GeneChip U133A (Affimetrix, Santa Clara, CA) by use of the same gene screening procedures.[12] We first selected four marker candidates, *PRSS2, PRSS8, TACSTD1*, and *BCAS1*., However, 6 (*MDK, SOX9, CDX1, PRSS2, PRSS8*, and *BCAS1*) of the 15 genes showed clear false-positive signals in representative clinical samples with an earlier version of a focused microarray containing the 15 genes and a most popular marker *CEA*. Thus, these six genes were excluded. Finally, we selected 10 markers, *CK20, FABP1, MUC2, TFF1, TFF2, MASPIN, GW112, PRSS4, TACSTD1*, and *CEA*.

Manufacturing Microarrays for Detecting Minimal Gastric Cancer Cells in Peritoneal Washings

Focused microarray is constructed by fixing 50–60 mer of oligonucleotide probes on a slide glass by Bubble Jet technology.[14] The microarray contains a single spot for each sequence of 10 marker genes and *β-actin* as a control gene. Each probe sequence used for the microarray is listed in Table 1.

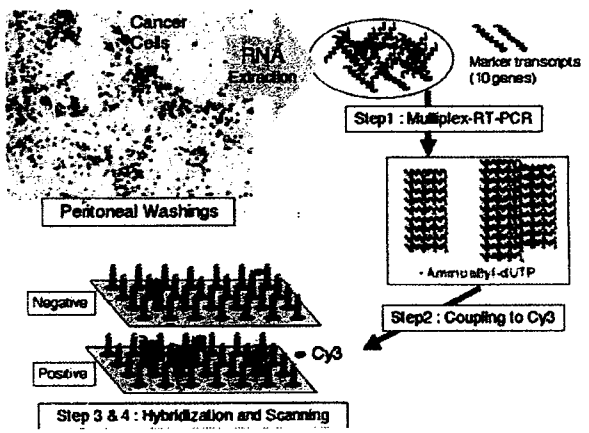

FIG. 1. Schematic flow diagram of MiniChip assay. Microscopic photo depicts one peritoneal wash cytology slide stained by anti-MASPIN antibody. Atypical cells are rare (arrows). Marker transcripts among extracted total RNAs were amplified and labeled with amino allyl–dUTP by multiplex reverse transcriptase–polymerase chain reaction (step 1) and stained by coupling to Cy3 dye (step 2), followed by hybridization to focused microarray (step 3) and fluorescence intensity scanning (step 4).

Marker Gene Detection by Focused Microarray

As shown in a schematic flow diagram (Fig. 1), the MiniChip assay consists of four steps: (1) amino allyl–dUTP labeling by multiplex RT-PCR; (2) coupling the labeled RT-PCR products to Cy3 dye; (3) hybridization Cy3-labeled cDNA to microarray; and (4) fluorescence scanning. Representative microarray

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.

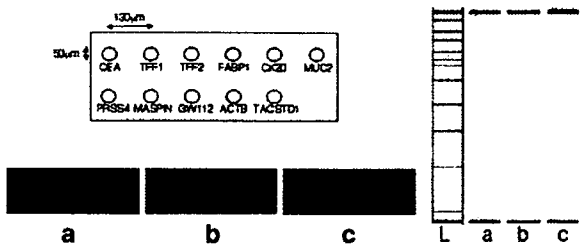

FIG. 2. Image of focused microarrays. and distribution of MiniChip assay results. Positions of probes for marker genes (left, top). Images of microarrays (left, bottom) and electrophoreses (right), respectively, show results obtained by MiniChip assay and capillary electrophoresis system from three representative 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

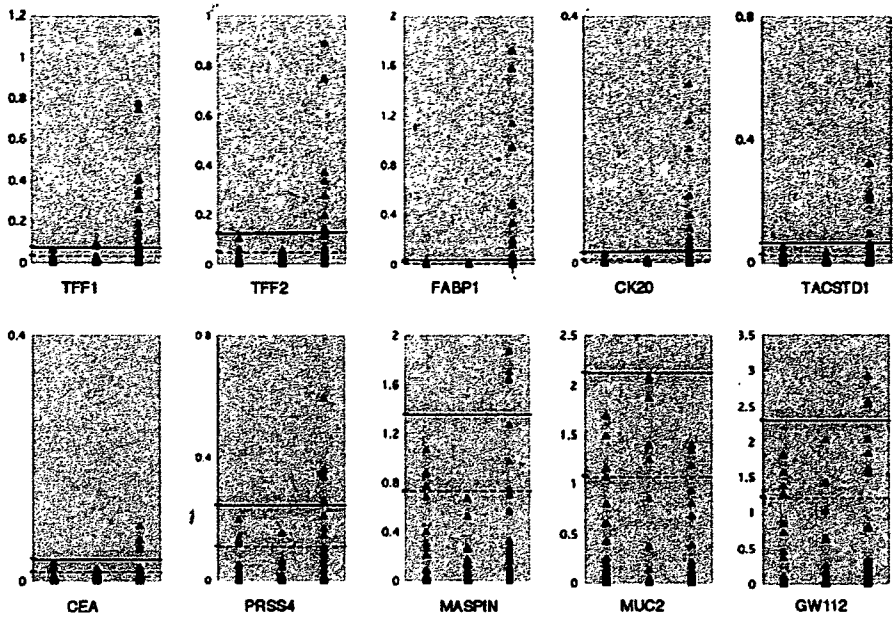

FIG. 3. Distribution of MiniChip assay results. Contrast among three groups of cases is clear in microarray images, whereas electrophoresis system detects polymerase chain reaction products only in cytology-positive cases. Plots on left, middle, and right rows are fluorescence ratios of stage Ia, disease-free, 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, $P < .001$).

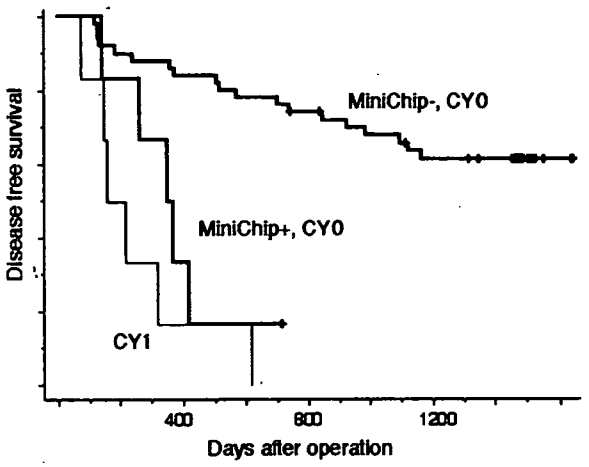

FIG. 4. MiniChip assay results and conventional cytology and relationship to disease-free survival. Cases defined as cancer positive by MiniChip assay but negative by conventional cytology (MiniChip positive, CY0) showed disease-free curve similar to that of cytology positive cases (CY1) and had significantly 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

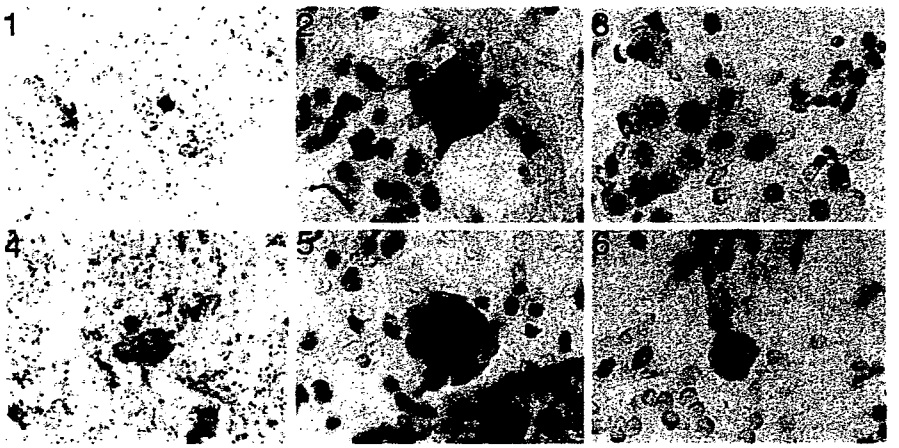

FIG. 5. Atypical cells found in conventional cytology–negative but MiniChip assay–positive cases by immunocytochemistry. Stained slides by anti-MASPIN and anti-CK20 antibodies in two cases. (1) Case 9, MASPIN (x 40). (2) Case 9, MASPIN (x 200). (3) Case 9 CK20 (x 200). (4) Case 13, MASPIN (x 40). (5)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