Table 4 RT-PCR studies in patients with histologically node-negative gastric cancer diagnosed by hematoxylin–eosin staining

Years	Study	No. of patients	No. of total LNs	Depth of invasion	Method	Markers	No. of patients with micrometastases (%)
2001	Okada et al. [37]	24	335	Tl–T4a	RT-PCR	CEA, CK20, MAGE3	10 (41.7)
2002	Matsumoto et al. [38]	50	312	T1–T4	RT-PCR	CEA	14 (28.0)
2005	Arigami et al. [39]	80	1,862	T1–T3	RT-PCR	CEA	25 (31.3)
2006	Sonoda et al. [40]	33	310	Tl	RT-PCR	MUC2, TFF1	11 (33.3)
2007	Wu et al. [41]	10	–	–	RT-PCR	CK20	2 (20.0)

with pN0 gastric cancer [39]. LNM was identified in 9 of 80 patients (11.3 %) and in 34 of 1,862 nodes (1.8 %) by IHC, whereas RT-PCR assay demonstrated LNM in 25 patients (31.3 %) and 66 nodes (3.5 %). Of those 66 nodes, 33 were detected only by RT-PCR. The detection rate of LNM was generally higher by RT-PCR than by IHC due to the high sensitivity of RT-PCR. These reports did not examine the relationship between LNM and prognosis, so further investigation will be necessary in the future.

Lymph node micrometastasis in colorectal cancer

Table 5 summarizes findings for LNM determined by RT-PCR in patients with colorectal cancer [42–55]. According to 14 reports, the number of patients and average number of lymph nodes ranged from 30 to 395 and from 5.3 to 21.3, respectively. Almost all reports dealt with relatively early-stage cancer, such as stage II or Dukes A–B. CK antibody was commonly used for detection of LNM, as for esophageal and gastric cancer. LNM was examined using multiple sections in many reports. LNM was defined as newly found metastasis in patients showing pN0 status on routine HE staining in 9 of 16 reports. In the others, LNM was defined according to the size of metastasis. The incidence of LNM ranged from 5.1 to 70.9 % and the detection rate was >30 % in half of the reports (7/14). Detection rates were >30 % for 33.3 % (4/12) of reports on esophageal cancer and 25.0 % (4/16) of reports on gastric cancer. The incidence of LNM was thus higher in colorectal cancer than in esophageal and gastric cancer. In terms of prognostic impact, a significant correlation was found in only 3 of 13 reports (23.1 %). Positive rates for a prognostic impact of LNM were high in both esophageal and gastric cancer, at 58.3 % (7/12) and 64.3 % (9/14), respectively, compared with colorectal cancer. Rahbari et al. [56] conducted a systematic review with meta-analyses of studies that evaluated the prognostic significance of molecular tumor-cell detection in regional lymph nodes. Meta-analysis revealed that molecular tumor-cell detection in regional lymph nodes was associated with poor overall survival, disease-specific survival, and disease-free survival. Subgroup analyses showed the prognostic significance of molecular tumor-cell detection independent of the applied detection method, molecular target, or number of retrieved lymph nodes. They concluded that molecular detection of occult disease in regional lymph nodes is associated with an increased risk of disease recurrence and poor survival in patients with node-negative colorectal cancer. In node-negative patients, LNM is thought to represent a crucial prognostic factor, since it indicates metastatic potential.

Four studies have examined LNM detected by RT-PCR in colorectal cancer (Table 6) [44, 57–59]. The numbers of patients and numbers of examined nodes were relatively small. Like esophageal and gastric cancer, CEA and/or CK were used as markers. The detection rate of LNM was high, at >50 % in three of the four reports. In esophageal and gastric cancer, no reports showed detection rates over 50 %. As with IHC, a high positive rate of LNM with RT-PCR was seen for colorectal cancer. The difference may be due to organ specificity. Interestingly, all authors found a significant correlation between LNM and prognosis. In comparison, a significant association was found in only 23 % of studies using IHC, differing markedly from the RT-PCR method. As the meta-analysis by Rahbari et al. [56] included results from both IHC and RT-PCR, LNM might be a prognostic factor in colorectal cancer. Comparing prognostic significance of LNM between IHC and RT-PCR in the same cases thus seems warranted.

Clinical utility and future perspectives for lymph node micrometastasis

The presence of LNM means that the process of metastasis from the primary tumor has already started. According to the results of this review, a high incidence of LNM ≥10 % is present in patients with pN0 GI cancer. Whether all tiny tumor cells implant and grow in lymph nodes remains unclear, but the potential presence of LNM should be kept in mind. In our study, LNM already showed proliferative activity even in ITC [36]. If LNM is present in patients diagnosed as pN0, we think that such patients should be

Table 5 Immunohistochemical studies in patients with histologically node-negative colorectal cancer diagnosed by hematoxylin–eosin staining

Year	Study	No. of patients	Average no. of LNs	Tumor stage	Method	Antibody	No. of sections for IHC	Definition of micrometastasis	No. of patients with micrometastases (%)	5-year survival (positive vs. negative)	*P*	Prognostic significance
2001	Yasuda et al. [42]	30	21.3	Dukes B	IHC	CK (CAM5.2)	Multiple	pN0 by HE staining	21 (70.0)	–	–	–
2002	Noura et al. [43]	55	12.0	T1–T3	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	27 (49.1)	–	0.817	No
2002	Noura et al. [44]	64	5.5	Stage II	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	35 (54.7)	90.8 vs. 85.1 %	n.s.	No
2003	Palma et al. [45]	38	10.3	Dukes B	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	6 (15.8)	–	0.804	No
2003	Bukholm et al. [46]	156	5.5	Stage II	IHC	CK (CAM5.2)	Multiple	≤0.2 mm	59 (37.8)	–	0.029	Yes
2005	Perez et al. [47]	56	9.6	Stage II (post-CRT)	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	4 (7.1)	–	n.s.	No
2006	García-Sáenz et al. [48]	105	6.3	Stage II	IHC	CK (AE1/AE3)	Multiple	pN0 by HE staining	26 (24.8)	–	0.759	No
2006	Messerini et al. [49]	395	20.9	Stage IIA	IHC	CK (CK20; clone K 20.8)	Multiple	>0.2 mm and < 2 mm	39 (9.9)	64.1 vs. 78.1 %	0.046	No
2008	Davies et al. [50]	105	5.3	Dukes A–B	IHC	CK (AE1/AE3, MNF 116)	–	pN0 by HE staining	49 (46.7)	–	0.54	No
2008	Bosch Roig et al. [51]	39	9.8	Stage II	IHC	CK (AE1/AE3)	Multiple	>0.2 and <2 mm	2 (5.1)	–	<0.0001	Yes
2008	Park et al. [52]	160	17.8	Stage I–II	IHC	CK (CK20; clone K 20.8)	Multiple	pN0 by HE staining	8 (5.0)	91.7 vs. 93.1 %	0.59	No
2010	Uribarrena-Amezaga et al. [53]	85	10.8	Dukes A-B	IHC	CK (AE1/AE3)	–	pN0 by HE staining	31 (36.5)	–	0.2916	No
2011	Oh et al. [54]	124	19.2	Stage II	IHC	CK (AE1/AE3)	Single	<2 mm	33 (26.6)	96.3 vs. 97.6 %	0.75	No
2011	Faerden et al. [55]	126	–	Stage I–II	IHC	CK (CAM5.2)	Multiple	≤2 mm	39 (31.0)	75.0 vs. 93.0 %	0.012	Yes

Table 6 RT-PCR studies in patients with histologically node-negative colorectal cancer diagnosed by hematoxylin–eosin staining

Years	Study	No. of patients	No. of total LNs	Tumor stage	Method	Markers	No. of patients with micrometastases (%)	5-year survival (positive vs. negative)	*P*	Prognostic significance
1998	Futamura et al. [57]	13	202	Stage I-III	RT-PCR	CEA, CK20	13 (100)	–	–	–
1998	Liefers et al. [58]	26	192	Stage II	RT-PCR	CEA	14 (53.8)	50.0 vs. 91.0 %	0.02	Yes
2002	Noura et al. [44]	64	350	Stage II	RT-PCR	CEA	19 (29.7)	78.2 vs. 95.3 %	0.015	Yes
2002	Rosenberg et al. [59]	85	25 (median)	Stage I–II	RT-PCR	CK20	44 (51.8)	70.6 vs. 95.9 %	0.001	Yes

categorized as pN1. Examination of LNM is thus useful for accurate staging, particularly in pN0 patients. Since prognosis differs significantly between patients with and without LNM according to several reports, adjuvant therapy seems to be necessary for patients with LNM. Prospective randomized controlled studies should be conducted to examine the effectiveness of adjuvant therapies in patients with LNM.

Recently, rapid examination using IHC and RT-PCR has been developed to detect LNM even during surgery. Particularly when performing less-invasive surgeries, intraoperative diagnosis of lymph node metastasis, including LNM, is essential. For example, we applied intraoperative diagnosis of LNM to esophageal cancer surgery in which supraclavicular lymphadenectomy was omitted if negative results were obtained for LNM at the recurrent nerve and cervical paraesophageal nodes [60]. In recent years, sentinel node navigation surgery (SNNS) has been clinically introduced for breast cancer and malignant melanoma [61, 62]. SNNS has also been trialed for GI cancer. We investigated LNM in all dissected lymph nodes, including the sentinel node (SN), as SN mapping using IHC and RT-PCR, yielding good results in patients with esophageal and gastric cancer classified as clinical T1 and N0 [63, 64]. We thus think that SNNS is applicable to clinical T1 and N0 patients based on intraoperative identification of LNM. In fact, if intraoperative histological and molecular examinations demonstrate no metastasis in any SNs identified from cT1 and cN0 patients, treatment using thoracoscopic and laparoscopic approaches with SN dissection may be feasible. On the other hand, standard surgery with standard lymph node dissection is currently necessary in patients with SN metastasis verified by intraoperative diagnostic tools. Furthermore, in the future, endoscopic submucosal dissection (ESD) with thoracoscopic and laparoscopic SN dissection might serve as an ultimate organ-preserving surgery to avoid lymph node recurrence in selected patients with extended indications for ESD. SNNS will add to the development of minimally invasive surgeries with individualized lymphadenectomy and good postoperative quality of life.

In conclusion, LNM needs to be recognized as the first step on the path to lymphatic metastasis. Minimally invasive surgery can be safely performed in clinical situations with accurate diagnosis of LNM. New treatment strategies applying the diagnosis of LNM are to be expected for each type of cancer.

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Conflict of interest The authors declare that they have no conflict of interest.

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Original Article

Clinical Significance of Circulating Tumor Cells in Peripheral Blood From Patients With Gastric Cancer

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BACKGROUND: The authors hypothesized that circulating tumor cells (CTCs) in patients with gastric cancer are associated with prognosis and disease recurrence. In this study, they evaluated CTCs in gastric cancer and clarified the clinical impact of CTCs. **METHODS:** In total, 265 consecutive patients with gastric cancer were enrolled. Fourteen patients were excluded from the analysis, including 12 patients who another cancer and 2 patients who refused the treatment. The remaining 251 patients were divided into 2 groups: 148 patients who underwent gastrectomy (the resection group) and 103 patients who did not undergo gastrectomy (the nonresectable group). Peripheral blood samples were collected before gastrectomy or chemotherapy. A proprietary test for capturing, identifying, and counting CTCs in blood was used for the isolation and enumeration of CTCs. **RESULTS:** CTCs were detected in 16 patients (10.8%) from the resection group and in 62 patients (60.2%) from the nonresectable group. The overall survival rate for the entire cohort was significantly lower in patients with CTCs than in those without CTCs ($P<.0001$). In the resection group, relapse-free and overall survival in patients with CTCs was significantly lower than in patients without CTCs ($P<.0001$). It was noteworthy that the expression of CTCs was an independent factor for determining the overall survival of patients with gastric cancer in multivariate analysis ($P=.024$). In the nonresectable group, the overall survival rate was significantly lower in patients with CTCs than in those without CTCs ($P=.0044$). **CONCLUSIONS:** The evaluation of CTCs in peripheral blood may be a useful tool for predicting tumor progression, prognosis, and the effect of chemotherapy in patients with gastric cancer. ***Cancer* 2013;119:3984-91.** *© 2013 American Cancer Society.*

KEYWORDS: *circulating tumor cells, gastric cancer, prognosis, peritoneal dissemination, hematogenous recurrence.*

INTRODUCTION

The presence of circulating tumor cells (CTCs) has been evaluated in blood from patients with gastrointestinal cancers.[1-4] The early detection of CTCs has the possibility of providing useful information before the start of treatment, including surgery and/or systemic chemotherapy. Some patients develop recurrent disease after surgery, even after undergoing complete resection of their primary tumor. Currently, the prognosis for patients with gastric cancer has been improved by the development of new anticancer drugs However, if the presence of CTCs is confirmed before surgery, then the use of neoadjuvant chemotherapy may be indicated, and this may have an impact on the timing of surgical intervention. Furthermore, the presence of CTCs in patients with distant metastasis would be a useful parameter for evaluating the effect of chemotherapy. Various methods for detecting rare CTCs have been attempted using a molecular biologic approach, such as reverse transcriptase-polymerase chain reaction (RT-PCR) analysis and flow cytometry in gastric cancer.[4-7] Although CTCs have been evaluated in blood from patients with gastric cancer, the clinical significance of CTCs remain unclear. Several authors have reported that the detection of CTCs using RT-PCR in gastric cancer is useful for predicting prognosis.[8-11] The detection of CTCs in blood requires high sensitivity and reproducibility.

The CellSearch system (Veridex LLC, Warren, NJ) was developed to identify CTCs in blood, and its utility has been reported in patients with breast cancer and prostate cancer.[12,13] The presence of CTCs is correlated with shorter overall survival in patients with metastatic disease. However, there have been few reports regarding the evaluation of CTCs in patients with gastric cancer using the CellSearch system. We hypothesized that CTCs in patients with gastric cancers are

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associated with prognosis and the recurrence. In this study, we evaluated CTCs in patients with gastric cancer and explored the clinical impact of CTCs using the CellSearch system.

MATERIALS AND METHODS

Gastric Cancer Cell Line

To prepare for an examination of the CellSearch system, we used the KATO III gastric cancer cell line for the analysis. KATO III cells were cultured in RPMI 1640 (Nissui Pharmaceutical Company, Ltd., Tokyo, Japan) supplemented with 10% fetal calf serum (Mitsubishi Kasei, Tokyo, Japan), 100 U/mL penicillin, and 100 U/mL streptomycin. Cancer cells were grown at 37°C in a humidified atmosphere containing 5% CO_2, as previously described.

Clinical Study Design

Patients with gastric cancer who received treatment at 2 medical centers (Kagoshima University Hospital and Jiaikai Imamura Hospital, Kagoshima, Japan) were analyzed using prospectively collected data. Informed consent was obtained from all patients in accordance with the ethical standards of the Committee on Human Experimentation of Kagoshima University Hospital and Jiaikai Imamura Hospital. We evaluated the usefulness of measuring CTC levels with regard to the overall survival of patients with gastric cancer. In total, 265 consecutive patients with gastric cancer were enrolled between February 2005 and December 2012 at 2 medical centers. Two hundred twenty-eight patients from Kagoshima University Hospital and 37 patients from Jiaikai Imamura Hospital were registered on the study. Fourteen patients were excluded from the analysis, including 12 patients who had another cancer, such as esophageal, colorectal, or prostate cancer, and 2 patients who refused the treatment for gastric cancer. The patients were divided into 2 groups; those who underwent gastrectomy (the resection group; N = 148) and those who did not undergo gastrectomy (the nonresectable group; N = 103) (Fig. 1). Patients in the resection group underwent gastrectomy with standard lymphadenectomy. Patients who had received any preoperative radiotherapy or chemotherapy were excluded from this study. Peripheral blood samples were collected before gastrectomy. Clinical stage was assigned according to the TNM classification.[14]

Patients in the nonresectable group did not undergo surgery because of the presence of distant metastasis or recurrence. Peripheral blood was collected before the beginning of chemotherapy in these patients. In the current study, various chemotherapy regimens were used and mainly included the oral fluoropyrimidine S-1, such as S-1 alone, S-1 plus cisplatin, S-1 plus paclitaxel, and so on.

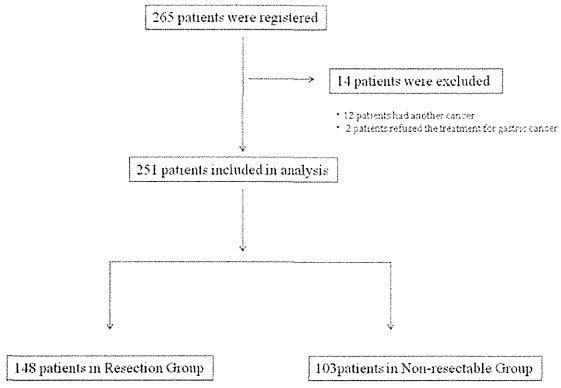

Figure 1. In total, 265 consecutive patients with gastric cancer were enrolled in the study. Fourteen patients were excluded from the analysis: 12 patients had another cancer, and 2 patients refused the treatment for gastric cancer. There were 148 patients with gastric cancer in the resection group and 103 patients who did not undergo gastrectomy in the nonresectable group.

All patients in the resection group were followed after discharge by physical examinations, routine blood tests, serum tumor marker tests (carcinoembryonic antigen [CEA] and cancer antigen 19-9 [CA 19-9]), and computed tomography scans every 3 to 6 months. Follow-up data after discharge were obtained for all patients, and the median follow-up was 31.6 months (range, 4-72 months). In the nonresectable group, patients were evaluated for chemotherapy every 2 to 3 months until death.

Isolation and Enumeration of Circulating Tumor Cells

Ten-milliliter blood samples were drawn into CellSave Preservative Tubes (Veridex, LLC). The samples were maintained at room temperature and processed within 72 hours after collection. All evaluations were performed by technical assistants without knowledge of the clinical status of the patients. The CellSearch system was used to isolate and enumerate CTCs using 7.5 mL of the 10-mL samples. CellSearch is a semiautomated system for the preparation of a sample and is used with the CellSearch Epithelial Cell Kit. The procedure enriches the sample for cells that express epithelial cell adhesion molecule (EpCAM) with antibody-coated magnetic beads, and it labels the nucleus with the fluorescent nucleic acid dye 4,2-diamidino-2-phenylidole dihydrochloride (DAPI).

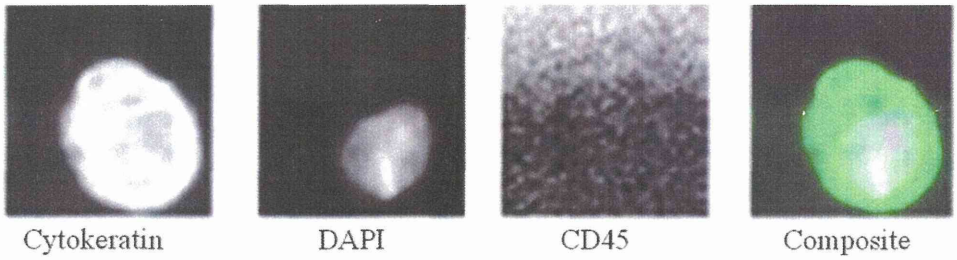

Figure 2. Circulating tumor cells were defined as nucleated cells that lacked allophycocyan (CD45) and expressed cytokeratin. DAPI indicates 4,2-diamidino-2-phenylidole dihydrochloride.

Fluorescently labeled monoclonal antibodies specific for leukocytes (CD45 allophycocyan) and epithelial cells (cytokeratin 8, cytokeratin 19, and 19-phycoerythrin) are used to distinguish epithelial cells from leukocytes. We identified and enumerated CTCs using the Celltracks analyzer II (Veridex, LLC), a semiautomated, fluorescence-based microscopy system that permits the computer-generated reconstruction of cellular images. CTCs were defined as nucleated cells that lacked CD45 and expressed cytokeratin (Fig. 2). Criteria used in the CellSearch system to define a tumor cell have been described previously. The results are expressed as the number of cells per 7.5 mL of whole blood.

Peripheral blood samples for use as a control group were obtained from 15 healthy volunteers who consented to participate. No volunteers had any illness or past history of cancer.

A spiking study was conducted to investigate the detectable limit of the CellSearch system. Therefore, the sensitivity and linearity of the CellSerach system was assessed by spiking a series of 10-fold serial dilutions of KATO III cells (10^2, 50, 10^1, 5, 10^0, and 0 cells) into whole blood from a normal healthy volunteer who did not have any cancer. This in vitro experiment was repeated 3 times for each series.

Statistical Analysis

The chi-square test and the Fisher exact test were used to compare the status of CTCs with categorical clinicopathologic factors. The Kaplan-Meier method was used for survival analysis, and the differences in survival were examined using the log-rank test. Prognostic factors were assessed in univariate and multivariate analyses using Cox proportional hazards regression models. All statistical calculations were performed using SAS statistical software (SAS Institute, Inc., Cary, NC). A P value $< .05$ was considered statistically significant.

RESULTS

Patient Characteristics

The 170 men and 81 women in the cohort ranged in age from 28 to 87 years (mean age, 64.4 years). Sixty-four percent of all patients remained alive at the time of this analysis.

In the resection group, 82 patients underwent distal gastrectomy, 13 patients underwent proximal gastrectomy, and 53 patients underwent total gastrectomy. The final pathologic findings indicated that all patients with disease greater than stage II had oral S-1 recommended as adjuvant chemotherapy for 1 year after surgery. Seventy-four patients (88.1%) were able to tolerate S-1; however, 10 patients (11.9%) were not able to tolerate S-1 because of anorexia and leucopenia. Twenty-six patients (17.6%) in the resection group had developed recurrent disease at the time of this analysis. These patients relapsed an average of 14.9 months after surgery.

In the nonresectable group, 72 patients had primary tumors of the stomach and distant metastasis, and 31 patients had recurrent distant metastasis after gastrectomy. Sixty-one patients had peritoneal dissemination, and 24 patients had para-aortic lymph node or Virchow lymph node swelling. Hematogenous distant metastases were identified in 24 patients. All patients in the nonresectable group received treatment with chemotherapy. The chemotherapy for gastric cancer consisted of S-1 plus cisplatin in 51 patients and S-1 plus paclitaxel in 52 patients.

CTCs were not identified in any samples from the healthy volunteers. In this study, the presence of ≥ 0 CTCs per 7.5 mL of blood was considered a positive result.

Circulating Tumor Cells and Clinical Correlation

Seventy-eight of 251 patients had CTCs detected. CTCs were detected in 16 patients (11.3%) from the resection

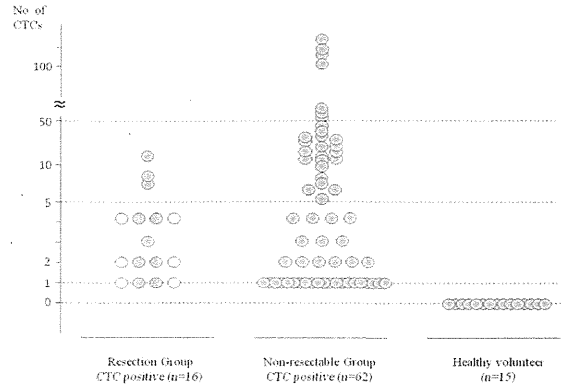

Figure 3. Circulating tumor cells (CTCs) were detected in 16 patients (10.8%) from the resection group and in 62 patients (60.2%) from the nonresectable group. The average number of CTCs was 3.5 in the resection group and 109.3 in the nonresectable group. CTCs were not observed in any samples from healthy volunteers.

group and in 62 patients (60.2%) from the nonresectable group. There was a significant difference in the positive rate between the 2 groups ($P < .0001$). Among those who had CTCs detected, the average count was 3.5 CTCs in patients from the resection group and 109.3 CTCs in patients from the nonresectable group (Fig. 3). The overall survival rate for all patients was significantly lower among those who had CTCs detected than among those who did not ($P < .0001$) (Fig. 4A).

In the resection group, CTCs were detected in 1 patient (1.6%) with a T1 tumor, in 2 patients (11.1%) with T2 tumors, in 6 patients (16.2%) with T3 tumors, and in 7 patients (23.3%) with T4 tumors. Clinicopathologic findings from the resection group are provided in Table 1. CTCs in patients who underwent gastrectomy were significantly correlated with the depth of tumor invasion, lymph node metastasis, distant metastasis, disease stage, vessel invasion, and lymphatic invasion. Although serum tumor markers like CEA and CA 19-9 were added to our analysis to be compared with CTCs, there was no significant correlation between CTCs and serum tumor markers.

Among 132 patients without CTCs, 14 patients (10.6%) had a recurrence after surgery. Eight patients had peritoneal dissemination, and 3 patients had hematogenous recurrences. Conversely, 12 of 16 patients (75%) with CTCs had a recurrence after surgery. The patients who had CTCs detected had a significantly higher relapse rate compared with patients who did not have CTCs detected ($P < .0001$). Two patients without recurrence on diagnostic imaging had transient elevation of serum CEA.

TABLE 1. Characteristics of Patients in the Resection Group

Variable	CTCs: No. of Patients (%)		
	Positive, n = 16	Negative, n = 132	*P*
Sex			
Men	11 (68.8)	88 (66.7)	.867
Women	5 (31.2)	44 (33.3)	
Age, y			
<70	8 (50)	83 (62.9)	.317
>70	8 (50)	49 (37.1)	
Tumor classification			
T1	1 (6.3)	62 (47)	.009
T2	2 (12.5)	16 (12.1)	
T3	6 (37.5)	31 (23.5)	
T4	7 (43.8)	23 (17.4)	
Lymph node classification			
N0	2 (12.5)	80 (60.6)	< .0001
N1	0 (0)	19 (14.4)	
N2	1 (6.3)	17 (12.9)	
N3	13 (81.3)	16 (12.1)	
Distant metastasis			
Yes	3 (18.8)	5 (3.8)	.012
No	13 (81.2)	127 (96.2)	
Stage			
I	1 (6.3)	63 (47.7)	.0002
II	1 (6.3)	25 (18.9)	
III	11 (68.8)	39 (29.5)	
IV	3 (18.8)	5 (3.8)	
Lymphatic invasion			
0	1 (6.3)	71 (53.8)	.0003
1	15 (93.7)	61 (46.2)	
Vessel invasion			
0	3 (18.8)	73 (55.3)	.006
1	13 (81.2)	59 (44.7)	
Histologic type			
Differentiated	3 (18.8)	39 (29.5)	0.365
Undifferentiated	13 (81.2)	93 (70.5)	—

Abbreviations: CTCs, circulating tumor cells.

Peritoneal dissemination was the most common pattern of recurrence, and 5 patients had hematogenous recurrences (Table 2). There were no significant differences in the recurrence pattern between patients with and without CTCs. However, all patients who had CTCs detected, at the least, had either peritoneal dissemination or hematogenous distant metastases. The sensitivity and specificity for predicting recurrences were 46.2% and 96.7%, respectively.

When we analyzed relapse-free survival according to whether patients were positive for CTCs, relapse-free survival in patients who were positive for CTCs was significantly lower than in those who were negative ($P < .0001$) (Fig. 4B). Furthermore, the 5-year survival rate also was significantly lower in patients with CTCs than in those without CTCs ($P < .0001$) (Fig. 4C). Multivariate analysis demonstrated that the presence of CTCs was an

independent prognostic factor (Table 3). Factors that we included in this analysis were CTCs, tumor classification, lymph node classification, lymphatic invasion, and vessel invasion, all of which were considered to be significant characteristics in these patients. It is noteworthy that positive expression of CTCs in peripheral blood was identified as an independent factor for overall survival in patients with gastric cancer (hazard ratio, 1.73; 95% confidence interval, 1.08-2.77; $P = .024$).

All patients of the nonresectable group received chemotherapy. There was no significant correlation between the presence of CTCs and nonresectable factors (Table 4). In these 103 patients, the presence of CTCs was correlated with a lower survival rate ($P = .0044$) (Fig. 4D). The median survival was 248 days in patients with CTCs and 582 days in patients without CTCs.

TABLE 2. Recurrence Pattern of 16 Patients With Circulating Tumor Cells in the Resection Group

Postgastrectomy	No. of Patients (%)
Recurrence pattern	12 (75)
Peritoneal dissemination	9 (56.3)
Liver metastasis	2 (12.5)
Bone metastasis	2 (12.5)
Adrenal gland metastasis	1 (6.3)
Lymph node metastasis	1 (6.3)
No recurrence	4 (25)

Sensitivity of the CellSearch System With Cell Line

KATO III cells were used for the analysis of sensitivity and linearity of the CellSearch system. Representative results from the expected number of KATO III cells spiked into healthy donor samples plotted against the actual number of KATO III cells observed in the samples are illustrated in Figure 5. Regression analysis of the

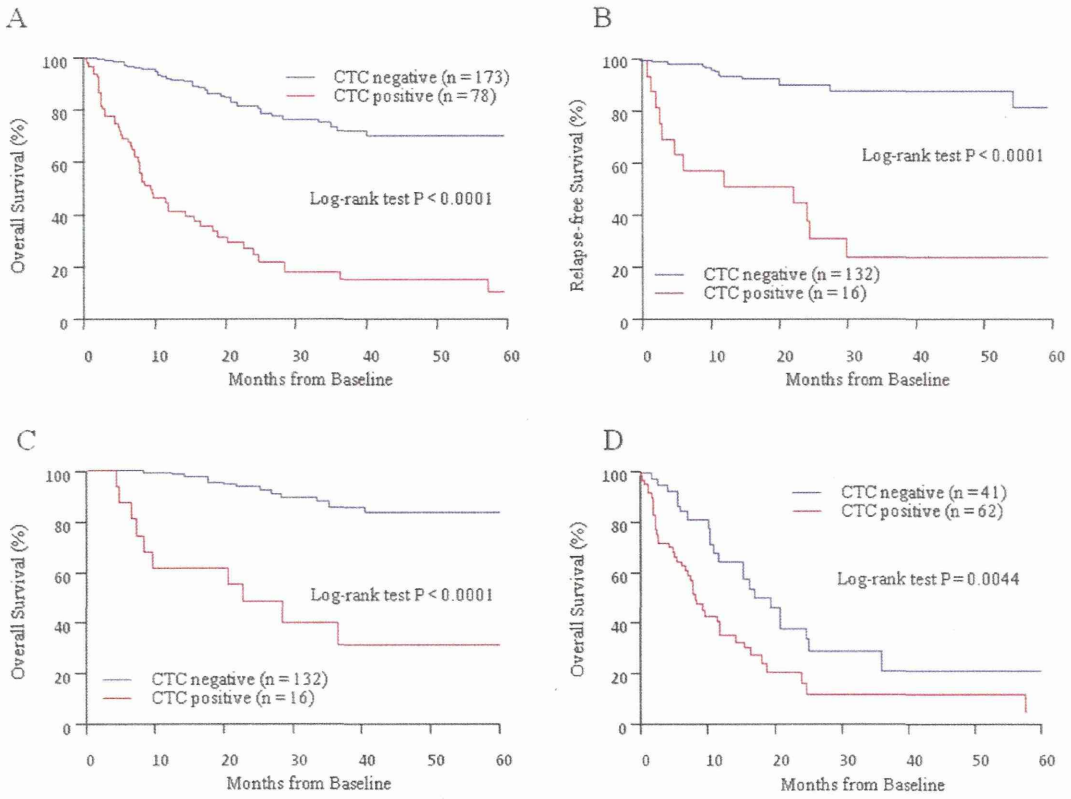

Figure 4. (A) Circulating tumor cells (CTCs) were detected in 78 of 251 patients. The 5-year survival rate was significantly lower in patients with CTCs than in those without CTCs ($P < .0001$). (B) In the resection group, the relapse-free survival rate was significantly lower in patients with CTCs than in those without CTCs ($P < .0001$). (C) The overall survival rate also was significantly lower in patients with CTCs than in those without CTCs ($P < .0001$). (D) In the nonresectable group, the overall survival rate was significantly lower in patients with CTCs than in those without CTCs ($P = .0044$). The median survival was 248 days in patients with CTCs and 582 days in patients without CTCs.