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A New Formula for Predicting Liver Metastasis in Patients with Colorectal Cancer: Immunohistochemical Analysis of a Large Series of 439 Surgically Resected Cases

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Key Words

Clinicopathological study · Colorectal cancer · Dysadherin · E-cadherin · Liver metastasis · Matrilysin

Abstract

Objective: The purpose of this study was to establish a new formula predicting liver metastasis in patients with colorectal cancer (CRC). ***Methods:*** Nine previously reported predictive markers for liver metastasis and/or prognosis (COX-2, dysadherin, E-cadherin, β-catenin, Ki-67, p53, laminin5-γ2, matrilysin and MUC-1) were immunohistochemically investigated in 439 consecutive patients with CRC. We tried to determine the combination of molecules which best predicted liver metastasis. A formula for predicting liver metastasis was constructed using a training cohort comprising 150 cases, and applied to a validation cohort comprising 190 cases and another comprising 99 cases from an outside hospital. ***Results:*** A combination of dysadherin, E-cadherin and matrilysin was identified to be best for predicting liver metastasis (area under the curve value, 0.807). The predictive formula: 3× dysadherin score [0 for low expression (≤50% of tumor cells positive) or 1 for high expression (>50%)] + 4× E-cadherin score [0 for preserved (>80% of tumor cells positive) or 1 for reduced (≤80%)] + 2× matrilysin score [0 for low expression (≤30% of tumor cells positive) or 1 for high expression (>30%)] was able to discriminate patients with liver metastasis in the training cohort with a sensitivity of 85.7% and a specificity of 58.9%. The discriminative capacity of the formula was validated in the first cohort with a sensitivity of 87.0% and a specificity of 66.5%, and in the second cohort with a sensitivity of 80% and a specificity of 60.0%. ***Conclusions:*** We have established a formula for predicting liver metastasis in patients with CRC, and confirmed that it has a high sensitivity potentially useful for clinical application.

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Introduction

Colorectal cancer (CRC) is the third most common malignant tumor in the world [1]. Its prognosis after curative resection depends exclusively on the development of metachronous metastases, especially liver metastasis [1]. To improve the prognosis of CRC, the most important considerations are the selection of patients at high risk for liver metastasis and subsequently the institution of appropriate adjuvant therapy. Adjuvant therapy in patients with CRC after curative resection has been reported to be useful for improving overall and disease-free survival [2–4]. Resection of liver metastases offers a chance for prolonged survival [5, 6]. Patients with intermediate-stage disease (stage II or III) have a recurrence rate of about 20–50%, including liver and lung metastases, recurrence in lymph nodes and peritoneal dissemination [2, 3, 7]. The remaining 50–80% have no recurrence, and therefore these patients underwent unnecessary adjuvant chemotherapy. To increase the survival benefit from adjuvant chemotherapy and the early detection rate of surgically resectable liver metastasis, the selection of patients at high risk for liver metastasis is essential.

Conventional risk factors for liver metastasis include lymph node metastasis, venous, serosal and lymphatic invasion, tumor dedifferentiation, white streak sign and resection margin [1, 8–14]. The accuracy of diagnosing liver metastasis using these conventional markers has been reported to be between 24 and 98% in terms of sensitivity, and between 34 and 97% in terms of specificity [1, 8–13]. Recently, many molecular markers have been reported to be useful for predicting liver metastasis and thus prognosis in CRC patients [15–19]. Therefore, in the present study, we tried to determine the best combination of the immunohistochemically detectable molecules already reported for predicting liver metastasis, and to establish a new formula for accurate prediction of liver metastasis in CRC patients.

Materials and Methods

Patients and Samples

Four hundred thirty-nine patients with CRC were selected from the lists of patients treated at the National Cancer Center Hospital (Tokyo, Japan) between 1995 and 1998 and the Kitasato University (Kanagawa, Japan) between 2000 and 2002. The patients included 267 (60.8%) men and 172 (39.2%) women, ranging in age from 21 to 93 years (median 62 years). Sample selection was restricted to consecutive cases diagnosed as stage II (44.2%, 194 of 439) or III (55.8%, 245 of 439). All patients had undergone curative resection. None of the patients had received chemotherapy or radiotherapy preoperatively. Follow-up studies were complete in all patients, ranging from 0.1 to 8.3 years (median, 5.5 years). Two patients who were followed up for 0.1 months died of pulmonary embolism 3 and 4 days after surgery, respectively. Recurrence after surgery was diagnosed by ultrasonography, computed tomography and angiography. Tumor location, lymph node, liver and lung metastases, tumor size, and lymphatic and venous invasion were all classified according to the TNM classification [20]. Histologically, tumors were classified according to the International Histological Classification of Tumors of the World Health Organization [21]. Among the study cases, 188 (42.8%) were classified as well-differentiated adenocarcinomas, 231 (52.6%) as moderately differentiated, 11 (2.5%) as poorly differentiated, 6 (1.37%) as mucinous and 2 (0.46%) as signet-ring-cell adenocarcinomas. During the follow-up period, liver metastases were observed in 49 (11.2%) cases, and at the time of writing this has proved fatal in 28 (57.2%) cases.

We divided the 439 patients into three groups. Group I included 150 consecutive patients, 94 men (62.7%) and 56 women (37.3%), ranging in age from 21 to 87 years (median, 63 years), operated on at the National Cancer Center Hospital between January 1, 1995, and July 1, 1996. In group I, 21 patients (14%) developed liver metastases and were used as a training cohort. Group II included 190 consecutive patients, 116 men (61.1%) and 74 women (38.9%), ranging in age from 32 to 93 years (median, 62 years), who were operated on at the National Cancer Center Hospital between July 1, 1996, and January 1, 1998. In group II, 24 patients (12.6%) developed liver metastases; they were used as the first validation cohort. Group III included 99 consecutive patients, 57 men (57.6%) and 42 women (42.4%), ranging in age from 27 to 85 years (median, 62 years), who were operated on at the Kitasato University between January 1, 2000, and January 1, 2003. In group III, 5 patients (5.1%) developed liver metastases; they were used as the second validation cohort.

Search Strategy and Selection Criteria for Antibodies

We selected nine previously reported molecules for immunohistochemical study – β-catenin [22–26], cyclooxygenase-2 (COX-2) [16, 27, 28], dysadherin [18, 29–31], E-cadherin [18, 23, 32], Ki-67 [33, 34], p53 [11, 34–36], matrilysin [37, 38], MUC-1 [19, 33] and laminin5γ2 [17, 39, 40] – as the prognostic significance of the expression of these markers has already been reported in several papers in which multivariate logistic regression analysis was performed, and reliable figures and descriptions of immunostaining were demonstrated (table 1).

Immunohistochemistry

Resected primary colon cancers were cross-sectioned in order to obtain tissue sections according to the general rules for clinical and pathological studies on cancer of the colon, rectum and anus [41]. Representative tissue sections taken at the maximum cross-section, each containing the deepest site of cancer invasion, were subjected to immunohistochemical staining using the avidin-biotin peroxidase complex method [42]. After deparaffinization in xylene and rehydration in ethanol, the sections were heated in citrate buffer (10 mM, pH 6.0) at 120°C for 10 min for antigen retrieval. Endogenous peroxidase was blocked with 0.3% hydrogen peroxidase in methanol for 20 min. The sections were then incubated with anti-dysadherin antibody (M53; 1:500 dilution, established in our laboratory [31]), anti-E-cadherin antibody (HECD-

Table 1. List of antibodies used and working conditions

Antibody	Clone	Dilution	AR	City/location	Source
β-Catenin	14	1:5,000	MW	Lexington/Ky./USA	Transduction
COX-2	160112	1:200	MW	Ann Arbor/Mich./USA	Cayman
Dysadherin	M53	1:4,000	MW	Tokyo/Japan	original
E-cadherin	HECD-1	1:4,000	MW	Tokyo/Japan	original
Ki-67	MIB-1	1:500	MW	Glostrup/Denmark	DAKO
Laminin5γ2	1-97	1:4,000	MW	Tokyo/Japan	original
Matrilysin	141B-2	1:800	MW	Tokyo/Japan	Fine Chemical
MUC-1	Ma695	1:200	MW	Newcastle/UK	Novocastra
p53	DO-7	1:500	MW	Newcastle/UK	Novocastra

AR = Antigen retrieval; MW = microwave.

1; 1:2,000 dilution, established in our laboratory [43]), anti-β-catenin antibody (clone 14; 1:5,000 dilution, Transduction Laboratories, Lexington, Ky., USA), anti-COX-2 antibody (160112; 1:200 dilution, Cayman, Ann Arbor, Mich., USA), anti-laminin5γ2 antibody (1-97; 1:4,000 dilution, established in our laboratory [40]), anti-Ki-67 antibody (MIB-1; 1:500 dilution, Dako, Glostrup, Denmark), anti-matrilysin antibody (141B-2; 1:800 dilution, DFC, Toyama, Japan), anti-MUC-1 antibody (Ma695; 1:200 dilution, Novocastra, Newcastle-upon-Tyne, UK) and anti-p53 antibody (DO7; 1:500 dilution, Novocastra) at 4°C. The sections were washed with phosphate-buffered saline, incubated with biotin-labeled anti-mouse IgG antibody and avidin-biotin complex (ABC kit, Vector Laboratories, Peterborough, UK) and visualized using diaminobenzidine tetrahydrochloride. The sections were counterstained with hematoxylin. As internal positive controls for dysadherin and laminin5γ2 staining, positive staining of endothelial cells present in the primary tumor tissue was used. As an internal positive control for E-cadherin staining, membranous staining of normal epithelial cells adjacent to the tumor specimens was used. As internal positive controls for COX-2, MUC-1, β-catenin, matrilysin, p53 and Ki-67 staining, colon cancer samples known to stain positively for each antibody were used. As a negative control, normal mouse IgG (Vector Laboratories, Burlingame, Calif., USA) was used instead of the primary antibody.

Evaluation of Immunohistochemistry

All the slides were first reviewed by two observers (H.O. and Y.N.) independently without knowledge of the clinical data. All discrepancies were resolved by joint review of the slides in question. After selecting three markers – dysadherin, E-cadherin and matrilysin – from the training cohort, group I, immunohistochemical stainings were scored by a third independent pathologist (Y.F.) to allow validation of the evaluation of the immunohistochemical results.

The percentages of tumor cells positive for p53, Ki-67, β-catenin, COX-2, laminin5γ2, dysadherin, E-cadherin and MUC-1 were evaluated semiquantitatively as the ratio of the number of positive tumor cells relative to the total number of tumor cells. Cutoff indices were fixed according to previous reports as follows. Expression of E-cadherin was defined as preserved when membrane staining of >80% of the tumor cells was observed and reduced when membrane staining ≤80% of the tumor cells was observed [18]. Expression of dysadherin and β-catenin was defined as high when membrane staining >50% of the tumor cells was observed, and as low when membrane staining ≤50% of the cells was observed [18]. Expression of laminin5γ2 was categorized into three groups as: few, <10% of tumor cells positive; moderate, 10–50% of tumor cells positive, and high, >50% of tumor cells positive [17]. Expression of matrilysin was defined as high when >30% of tumor cells were stained at the invasive front, and as low when ≤30% of cells were stained at the invasive front [15, 38]. Expression of COX-2 was defined as positive when cytoplasmic staining of >10% of tumor cells was observed [16]. Expression of MUC-1 [19] and p53 and Ki-67 [34] was defined as positive when >10% of tumor cells were stained.

Statistical Analysis

All the data were tabulated, and statistical tests were performed with SAS version 9.1 (SAS Institute, Cary, N.C., USA). The relationship between clinicopathological findings and the scores of immunohistochemical markers were analyzed by Fisher's exact test for a two-by-two contingency table or by the χ^2 test for other contingency tables.

Selection of the best combination of markers was performed in group I by a stepwise selection procedure in a multivariate logistic regression model. The stepwise procedure was set to a threshold of 0.05 for inclusion and 0.15 for exclusion. Each selected independent liver metastasis factor was given a coefficient suggested by the multivariate logistic regression model, as a parameter estimate. In order to evaluate the goodness of fit for the final model, we applied the Hosmer-Lemeshow test [44] on eight distinct groups, and the Akaike Information Criterion (AIC) test [45] to the combination set of markers. AIC is widely used as a criterion for model selection. The model with the minimum AIC is chosen as the best one, and the AIC is therefore formally biased against overly complex models. The immunohistochemical metastatic score (IMS) was calculated according to the formula composed of selected factors. The scoring formula was applied to patients in groups II and III as well as those in group I. The thresh-

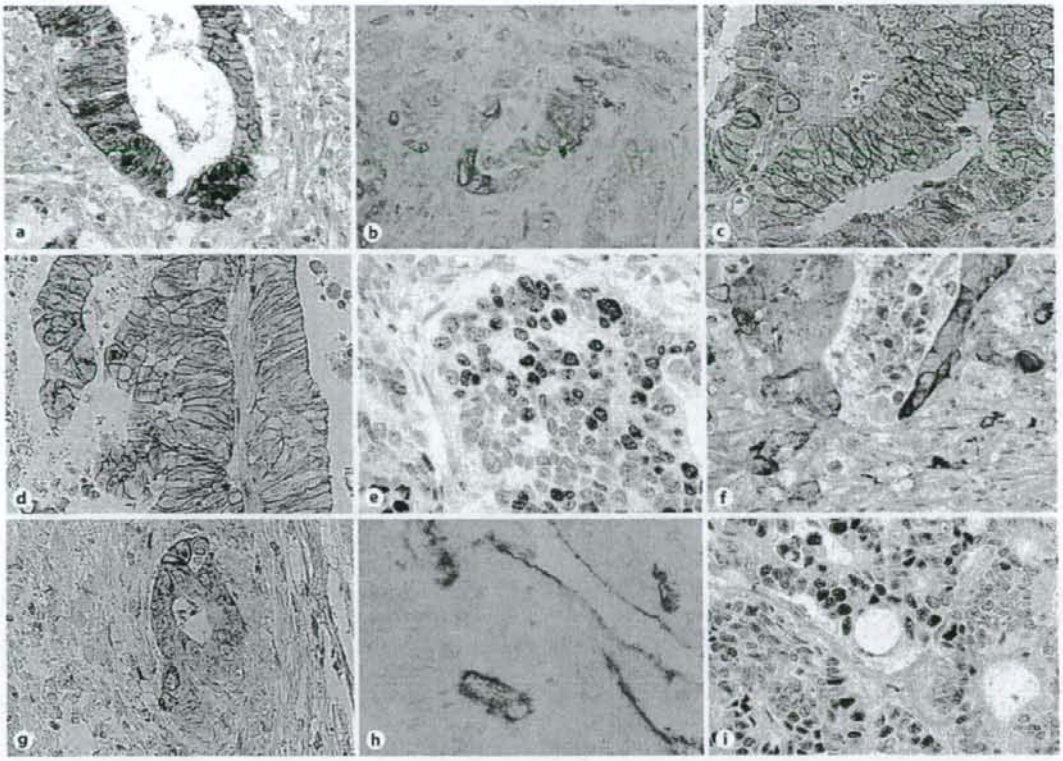

Fig. 1. Immunohistochemical staining pattern of each molecular marker (×400). β-Catenin expression was localized at the cell-cell borders, in the cytoplasm and in the nuclei of cancer cells (**a**). COX-2 expression was observed in the cytoplasm of cancer cells (**b**). Membranous dysadherin (**c**) and E-cadherin (**d**) expression was observed at the cell-cell borders of cancer cells. Ki-67 (**e**) and p53 expression (**i**) was observed in the nuclei of cancer cells. Laminin5γ2 (**f**) and matrilysin expression (**g**) was predominately intracytoplasmic, and preferentially located at the invasive front. MUC-1 (**h**) expression was located at the surface of glandular structures of cancer cells.

old was set at five points. Two theoretical potential groups at risk for liver metastasis were defined as follows: group A, low risk of liver metastasis, total score $0 \leq IMS \leq 4$; group B, high risk of liver metastasis, total score $5 \leq IMS$.

Results

Biomarkers in Primary Colon Cancers with Respect to the Occurrence of Liver Metastasis

The associations between clinicopathological factors and liver metastasis in all samples are shown in table 2. The representative staining pattern of each molecular marker is shown in figure 1. The associations between liver metastasis and immunohistochemical molecular markers in group I are shown in table 3. There was a significant association between liver metastasis and E-cadherin ($p = 0.001$), laminin5γ2 ($p = 0.005$), dysadherin ($p = 0.004$) and matrilysin expression ($p = 0.017$; table 3).

Identification of Candidate Markers in the Training Cohort, Group I, by Stepwise Analysis of the Logistic Regression Model

Although two markers – dysadherin and E-cadherin – were significantly associated with liver metastasis ($p = 0.013$ and 0.004, respectively) by the multivariate re-

Table 2. Association between liver metastasis and clinicopathological factors in all samples

Characteristics	Liver metastasis		p value
	positive (n = 49)	negative (n = 390)	
Age			
<65 years	27	226	
≥65 years	22	164	0.759
Gender			
Female	18	154	
Male	31	236	0.758
Tumor location			
Colon	35	238	
Rectum	14	152	0.210
Maximum tumor diameter			
<4.5 cm	30	191	
≥4.5 cm	19	199	0.129
Pathological tumor status			
T_2	1	31	
T_3	46	345	
T_4	2	14	0.351
Lymph node metastasis			
Absent	11	183	
Present	38	207	0.001
Histological grade			
G_1	18	170	
G_2	30	201	
G_3	1	18	0.474
Lymphatic invasion			
Absent	8	93	
Present	41	297	0.282
Venous invasion			
Absent	11	149	
Present	38	241	0.039

T_2 = Tumor invades the muscularis propia; T_3 = tumor invades through the muscularis propia into the subserosa or peritoneal tissues; T_4 = tumor directly invades other organs or structures and/or perforates the visceral peritoneum; G_1 = well-differentiated adenocarcinoma; G_2 = moderately differentiated adenocarcinoma; G_3 = poorly differentiated adenocarcinoma including signet-ring cell adenocarcinoma and mucinous adenocarcinoma.

Table 3. Association between liver metastasis and immunohistochemical molecular markers

Characteristics	Liver metastasis		p value
	positive (n = 21)	negative (n = 129)	
β-Catenin: membranous			
<70%	3	21	
≥70%	18	108	1.000
β-Catenin: cytoplasmic			
<50%	9	54	
≥50%	12	75	1.000
β-Catenin: nuclear			
<50%	12	88	
≥50%	9	41	0.328
COX-2			
<10%	12	76	
≥10%	9	53	1.000
Dysadherin			
<50%	5	75	
≥50%	16	54	0.004
E-cadherin			
Reduced	4	77	
Preserved	17	52	0.0006
Ki-67			
<30%	11	55	
≥30%	10	74	0.480
Laminin5γ2			
<10%	2	40	
≥10% and <50%	12	67	
≥50%	7	22	0.005
Matrilysin			
<30%	5	69	
≥30%	16	60	0.017
MUC-1			
<10%	12	72	
≥10%	9	57	1.000
p53			
<10%	9	48	
≥10%	12	81	0.801

β-Catenin: membranous/cytoplasmic/nuclear = Membranous/cytoplasmic/nuclear staining of β-catenin.

gression model, three markers – dysadherin, E-cadherin and matrilysin – were selected as candidate markers to establish a formula using a stepwise selection procedure in the multivariate logistic regression model (table 4). This combination set of markers showed an AIC value of 104.9. The receiver-operating characteristic curve of this combination set in the 150 individuals of group I is shown in figure 2. The area under the curve value was 0.807.

We carried out a stepwise method using the minimum value of the AIC as the selecting criterion. In cases where the model included dysadherin and E-cadherin, the AIC was 106.9. On the other hand, when the model included dysadherin, E-cadherin and matrilysin, the AIC was 104.9. As a result, although matrilysin was not significant in the multivariate regression analysis, it was included in the formula. Additionally, we obtained the Hosmer-

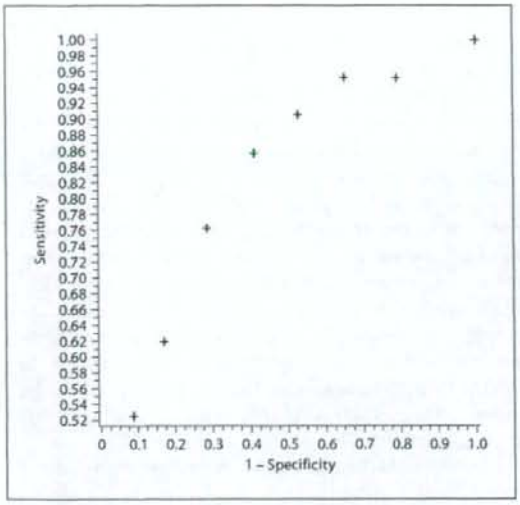

Fig. 2. Receiver-operating characteristic curve of the immunohistochemical metastatic scores for 150 independent patients (group I).

Table 4. Summary of the stepwise selection of the logistic regression model

Variable	Estimate	Standard error	Odds ratio (95% confidence interval)	P value
Intercept	–4.4458	0.787		<0.0001
Dysadherin	1.4216	0.569	4.144 (1.357, 12.66)	0.013
E-cadherin	1.7611	0.603	5.819 (1.782, 19.01)	0.004
Matrilysin	1.0931	0.573	2.984 (0.969, 9.186)	0.056

Table 5. Scoring formula for predicting liver metastasis in CRC patients: IMS

IMS = 3 × dysadherin score + 4 × E-cadherin score + 2 × matrilysin score

Dysadherin score
- 0 for low expression (≤50% of tumor cells positive)
- 1 for high expression (>50% of tumor cells positive)

E-cadherin score
- 0 for preserved (>80% of tumor cells positive)
- 1 for reduced (≤80% of tumor cells positive)

Matrilysin score
- 0 for low expression (≤30% of tumor cells positive)
- 1 for high expression (>30% of tumor cells positive)

IMS = Immunohistochemical metastatic score.

Lemeshow χ^2 with 6 degrees of freedom equal to 2.647 and p = 0.852. It appeared, therefore, that our model fit was acceptable.

Predictive Formula for Liver Metastasis

A formula for predicting liver metastasis was established using the above three markers. The predictive formula: 3× dysadherin score [0 for low expression (≤50% of tumor cells positive) or 1 for high expression (>50% of tumor cells positive)] + 4× E-cadherin score [0 for preserved (>80% of tumor cells positive) or 1 for reduced (≤80% of tumor cells positive)] + 2× matrilysin score [0 for low expression (≤30% of tumor cells positive) or 1 for high expression (>30% of tumor cells positive)] was established (table 5). Total scores calculated using this formula predicted liver metastasis with a sensitivity of 85.7% (18 of 21) and a specificity of 58.9% (76 of 129) in the training cohort (group I).

Confirmation of the Evaluation of Immunohistochemistry by the Third Independent Pathologist

Slides immunostained for dysadherin, E-cadherin and matrilysin were also evaluated by the third independent pathologist, and the expression of these markers was significantly correlated with liver metastasis in the training cohort (group I), confirming the evaluation done by the other two pathologists. Each concordance rate for the dysadherin, E-cadherin and matrilysin expression scores between a third pathologist and the other two pathologists was 72, 70 and 78%, respectively. The concordance rate for the risk of liver metastasis calculated by our new formula between a third pathologist and the other two pathologists was 69%.

Confirmation of the Prediction Formula in the Validation Cohort (Group II)

The discriminating performance of the prediction formula was validated in a blinded manner using an independent validation cohort (group II), consisting of 190 patients. The same calculation showed a predictive accuracy with a sensitivity of 87.0% (20 of 23) and a specificity of 66.5% (111 of 167).

Confirmation of the Prediction Formula in the Second Validation Cohort (Group III) from the Kitasato University

The discriminating performance of the prediction formula was validated in a blinded manner using the second independent validation cohort, group III, consisting

of 99 patients from the Kitasato University Hospital. The same calculation showed a predictive accuracy with a sensitivity of 80% (4 of 5) and a specificity of 60.0% (56 of 94).

Discussion

We used a supervised learning method which requires the use of a training data set of known markers to identify the best combination of immunohistochemical markers for predicting liver metastasis in patients with CRC after curative surgery, and dysadherin, E-cadherin and matrilysin expression was found to be the best combination for this purpose. Patients were divided into two categories – a high-risk group for liver metastasis and a low-risk group for liver metastasis – based on the scores obtained using the formula. The choice of a threshold should primarily depend on the purpose of the overall clinical scheme; some investigators may require a higher sensitivity for clinical applications while sacrificing specificity, whereas others may choose the opposite. In this study, we determined 5 as the threshold, for which the sensitivity was >80%, and can be regarded as sufficient for use as a screening test. Liver metastasis was predicted with an accuracy of 85.7% in terms of sensitivity and 58.9% in terms of specificity using our formula. Pathological risk factors for liver metastasis have been reported to be venous, lymphatic and serosal invasion, tumor dedifferentiation, lymph node metastasis and white streak sign, observed macroscopically at the invasive front of the cut surface of a tumor [1, 8–14]. We used stepwise multivariate analysis to look for the best combination set of markers for predicting liver metastasis, including conventional clinicopathological factors. However, no conventional clinicopathological factors were selected as candidate markers useful for constructing a predictive formula for liver metastasis, indicating that our formula is able to predict liver metastasis more precisely than conventional clinicopathological factors. Additionally, we applied survival analysis to liver metastasis event data. The results obtained were similar to those of logistic regression analysis, and the selected markers were the same as those selected by the Cox regression models (data not shown). We also performed multivariate analysis using the logistic regression model between liver metastasis and immunohistochemical molecular markers for patients with 219 colon cancers and patients with 140 rectal cancers separately. In both cancer groups, all three selected markers – dysadherin, E-cadherin and matrylsin – showed a similar tendency in the stepwise logistic regression model (data not shown). Our formula was validated using independent sets of patients, including 190 from our institution and 99 from another institution. Furthermore, our new predictive formula was validated not only in cases from an outside hospital but also by a third independent pathologist who was instructed to evaluate immunostained slides without prior knowledge of the cases. This predictive formula might be helpful for selecting patients who should undergo adjuvant chemotherapy after curative surgery, or who require close follow-up to detect liver metastasis at a sufficiently early stage for curative resection, and ultimately for avoiding unnecessary adjuvant chemotherapy in patients who are unlikely to develop liver metastasis. In order for our formula to be applied for practical clinical care, however, it must be validated in a large-scale prospective clinical trial.

We examined the differences in immunohistochemical positivity for the three molecular markers between older samples (resected between 1995 and 1996) and relatively new samples (resected between 1997 and 2001) in order to evaluate the suitability of older samples for immunohistochemical study. There were no differences in immunohistochemical positivity for the three molecular markers between the two sample groups (data not shown). Therefore, we consider that even older samples, such as specimens resected over 10 years ago, are reliably applicable for immunohistochemical study for prediction of liver metastasis.

Our study showed that E-cadherin, dysadherin and matrilysin expression was significantly correlated with liver metastasis, confirming the results of previous studies [15, 18–38]. Although multivariate logistic analysis failed to reveal a significant association between laminin5γ2 expression and liver metastasis, the χ^2 test showed that laminin5γ2 was significantly associated with liver metastasis, confirming the results of previous studies [17, 39]. The expression of p53, Ki-67, COX-2, β-catenin or MUC-1 failed to demonstrate any significant association with liver metastasis, even though these markers were selected on the basis of the fact that their prognostic significance had been reported in several previous papers [3, 16, 19, 26, 28, 34]. These discrepancies could be explained on the basis of differences in treatment modalities, scoring system, sample size analyzed, tumor heterogeneity and interobserver variations in evaluating immunostained slides.

A number of previous studies have investigated the usefulness of combining several molecular markers for predicting liver metastasis in CRC patients [46–48]. Na-

gai et al. [11] analyzed 100 patients, comprising 48 with liver metastasis and 52 without evidence of liver metastasis, and established a predictive formula for liver metastasis using a combination of factors such as tumor location, host inflammatory cell reaction, p53 staining, and extent of tumor and venous invasion using multivariate analysis. The predictive value for liver metastasis was 81.3% in terms of sensitivity and 92.3% in terms of specificity [11]. Barozzi et al. [49] investigated five clinicopathological factors and seven molecular markers – TGF-α, IGF-II, MMP-2, VEGF, CD34, c-erb B2 and EGFR – in 101 patients, comprising 49 patients without evidence of metastasis, 27 with synchronous liver metastasis and 25 with metachronous liver metastasis. Using multivariate analysis, they found that TGF-α, IGF-II and MMP-2 were independent predictors of liver metastasis. They reported that if the expression levels of all three of these molecular markers were high, then the probability of liver metastasis was 99.5%, whereas if the expression levels of all three were low, then the probability of liver metastasis was only 0.3% [49]. Although the sensitivity and specificity in these previous reports were high, their sample sizes were rather small in comparison with our present study. Also, before drawing any conclusions about their usefulness, these previous reports need to be validated in patients from an outside hospital and by another independent pathologist to confirm the accuracy of the immunostaining evaluation.

Several recent studies have demonstrated the potential clinical utility of gene expression profiles, including the identification of prognostic subclasses. Eschrich et al. [50] reported that in 78 patients with Dukes B and C stage disease, a 43-gene signature was demonstrated to identify 3-year survival significantly better with a sensitivity of 73% and a specificity of 84%. Wang et al. [51] identified a 23-gene signature that predicted prognosis in 74 patients with Dukes B stage disease with a sensitivity of 72% and a specificity of 83%. Bertucci et al. [52] found a 244-gene signature that separated 22 patients from among a group with all stages of CRC with a significant difference in 5-year survival of 100 vs. 30% ($p = 0.001$). These previous reports suggest that microarray gene expression profiling could be a valuable tool for highly accurate prognostication in CRC patients. At present, however, the cost of cDNA analysis, the complexity of the method and accuracy in the interpretation of DNA microarrays are problems that remain to be solved before this approach can be applied routinely in a standard clinical setting. On the other hand, immunohistochemistry is an already standardized method that can easily be performed in every laboratory. Although application of the specific scoring calculation is less feasible for timely routine diagnostics, our formula based on immunohistochemical results has the advantage of feasibility compared with methods using DNA extracted from tumor tissue.

In conclusion, we have established a formula for predicting liver metastasis in CRC patients and confirmed its high sensitivity potentially for clinical application.

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References

1 Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW: Colorectal cancer. Lancet 2005;365:153–165.

2 Cascinu S, Georgoulias V, Kerr D, Maughan T, Labianca R, Ychou M: Colorectal cancer in the adjuvant setting: perspectives on treatment and the role of prognostic factors. Ann Oncol 2003;14(suppl 2):ii25–ii29.

3 Watanabe T, Wu TT, Catalano PJ, Ueki T, Satriano R, Haller DG, Benson AB 3rd, Hamilton SR: Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med 2001;344:1196–1206.

4 O'Connell MJ, Laurie JA, Kahn M, Fitzgibbons RJ Jr, Erlichman C, Shepherd L, Moertel CG, Kocha WI, Pazdur R, Wieand HS, Rubin J, Vukov AM, Donohue JH, Krook JE, Figueredo A: Prospectively randomized trial of postoperative adjuvant chemotherapy in patients with high-risk colon cancer. J Clin Oncol 1998;16:295–300.

5 Doci R, Gennari L, Bignami P, Montalto F, Morabito A, Bozzetti F: One hundred patients with hepatic metastases from colorectal cancer treated by resection: analysis of prognostic determinants. Br J Surg 1991;78:797–801.

6 Fortner JG: Recurrence of colorectal cancer after hepatic resection. Am J Surg 1988;155: 378–382.
7 Chang GJ, Rodriguez-Bigas MA, Skibber JM, Moyer VA: Lymph node evaluation and survival after curative resection of colon cancer: systematic review. J Natl Cancer Inst 2007; 99:433–441.
8 Galandiuk S, Wieand HS, Moertel CG, Cha SS, Fitzgibbons RJ Jr, Pemberton JH, Wolff BG: Patterns of recurrence after curative resection of carcinoma of the colon and rectum. Surg Gynecol Obstet 1992;174:27–32.
9 Krasna MJ, Flancbaum L, Cody RP, Shneibaum S, Ben Ari G: Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance. Cancer 1988;61:1018–1023.
10 Adachi Y, Inomata M, Kakisako K, Sato K, Shiraishi N, Kitano S: Histopathologic characteristics of colorectal cancer with liver metastasis. Dis Colon Rectum 1999;42:1053–1056.
11 Nagai E, Yao T, Sakamoto M, Akazawa K, Utsunomiya T, Tsuneyoshi M: Risk factors related to liver metastasis in colorectal carcinoma: a multivariate analysis of clinicopathologic and immunohistochemical variables. Jpn J Cancer Res 1994;85:1280–1287.
12 Ouchi K, Sugawara T, Ono H, Fujiya T, Kamiyama Y, Kakugawa Y, Mikuni J, Tateno H: Histologic features and clinical significance of venous invasion in colorectal carcinoma with hepatic metastasis. Cancer 1996;78: 2313–2317.
13 Ono M, Sakamoto M, Ino Y, Moriya Y, Sugihara K, Muto T, Hirohashi S: Cancer cell morphology at the invasive front and expression of cell adhesion-related carbohydrate in the primary lesion of patients with colorectal carcinoma with liver metastasis. Cancer 1996;78:1179–1186.
14 Inomata M, Ochiai A, Sugihara K, Moriya Y, Yamaguchi N, Adachi Y, Kitano S, Hirohashi S: Macroscopic features at the deepest site of tumor penetration predicting liver metastases of colorectal cancer. Jpn J Clin Oncol 1998;28:123–128.
15 Adachi Y, Yamamoto H, Itoh F, Arimura Y, Nishi M, Endo T, Imai K: Clinicopathologic and prognostic significance of matrilysin expression at the invasive front in human colorectal cancers. Int J Cancer 2001;95:290–294.
16 Yamauchi T, Watanabe M, Kubota T, Hasegawa H, Ishii Y, Endo T, Kabeshima Y, Yorozuya K, Yamamoto K, Mukai M, Kitajima M: Cyclooxygenase-2 expression as a new marker for patients with colorectal cancer. Dis Colon Rectum 2002;45:98–103.
17 Aoki S, Nakanishi Y, Akimoto S, Moriya Y, Yoshimura K, Kitajima M, Sakamoto M, Hirohashi S: Prognostic significance of laminin-5 gamma2 chain expression in colorectal carcinoma: immunohistochemical analysis of 103 cases. Dis Colon Rectum 2002;45: 1520–1527.
18 Aoki S, Shimamura T, Shibata T, Nakanishi Y, Moriya Y, Sato Y, Kitajima M, Sakamoto M, Hirohashi S: Prognostic significance of dysadherin expression in advanced colorectal carcinoma. Br J Cancer 2003;88:726–732.
19 Hiraga Y, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G, Shimamoto F, Kohno N: Immunoreactive MUC1 expression at the deepest invasive portion correlates with prognosis of colorectal cancer. Oncology 1998;55:307–319.
20 Sobin LH, Fleming ID: TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer 1997;80:1803–1804.
21 World Health Organization: Classification of Tumors. IARC Press, Lyon, 2000.
22 Hugh TJ, Dillon SA, Taylor BA, Pignatelli M, Poston GJ, Kinsella AR: Cadherin-catenin expression in primary colorectal cancer: a survival analysis. Br J Cancer 1999;80:1046–1051.
23 Nakanishi Y, Ochiai A, Akimoto S, Kato H, Watanabe H, Tachimori Y, Yamamoto S, Hirohashi S: Expression of E-cadherin, alpha-catenin, beta-catenin and plakoglobin in esophageal carcinomas and its prognostic significance: immunohistochemical analysis of 96 lesions. Oncology 1997;54:158–165.
24 Gunther K, Brabletz T, Kraus C, Dworak O, Reymond MA, Jung A, Hohenberger W, Kirchner T, Kockerling F, Ballhausen WG: Predictive value of nuclear beta-catenin expression for the occurrence of distant metastases in rectal cancer. Dis Colon Rectum 1998;41:1256–1261.
25 Maruyama K, Ochiai A, Akimoto S, Nakamura S, Baba S, Moriya Y, Hirohashi S: Cytoplasmic beta-catenin accumulation as a predictor of hematogenous metastasis in human colorectal cancer. Oncology 2000;59: 302–309.
26 Wong SC, Lo ES, Lee KC, Chan JK, Hsiao WL: Prognostic and diagnostic significance of beta-catenin nuclear immunostaining in colorectal cancer. Clin Cancer Res 2004;10: 1401–1408.
27 Fux R, Schwab M, Thon KP, Gleiter CH, Fritz P: Cyclooxygenase-2 expression in human colorectal cancer is unrelated to overall patient survival. Clin Cancer Res 2005;11: 4754–4760.
28 Soumaoro LT, Uetake H, Higuchi T, Takagi Y, Enomoto M, Sugihara K: Cyclooxygenase-2 expression: a significant prognostic indicator for patients with colorectal cancer. Clin Cancer Res 2004;10:8465–8471.
29 Ino Y, Gotoh M, Sakamoto M, Tsukagoshi K, Hirohashi S: Dysadherin, a cancer-associated cell membrane glycoprotein, down-regulates E-cadherin and promotes metastasis. Proc Natl Acad Sci USA 2002;99:365–370.
30 Nakanishi Y, Akimoto S, Sato Y, Kanai Y, Sakamoto M, Hirohashi S: Prognostic significance of dysadherin expression in tongue cancer: immunohistochemical analysis of 91 cases. Appl Immunohistochem Mol Morphol 2004;12:323–328.
31 Shimamura T, Sakamoto M, Ino Y, Sato Y, Shimada K, Kosuge T, Sekihara H, Hirohashi S: Dysadherin overexpression in pancreatic ductal adenocarcinoma reflects tumor aggressiveness: relationship to E-cadherin expression. J Clin Oncol 2003;21:659–667.
32 Hirohashi S: Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. Am J Pathol 1998;153:333–339.
33 Aoki R, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G, Shimamoto F, Kohno N: MUC-1 expression as a predictor of the curative endoscopic treatment of submucosally invasive colorectal carcinoma. Dis Colon Rectum 1998;41:1262–1272.
34 Allegra CJ, Paik S, Colangelo LH, Parr AL, Kirsch I, Kim G, Klein P, Johnston PG, Wolmark N, Wieand HS: Prognostic value of thymidylate synthase, Ki-67, and p53 in patients with Dukes' B and C colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project collaborative study. J Clin Oncol 2003;21:241–250.
35 Crowe PJ, Yang JL, Berney CR, Erskine C, Ham JM, Fisher R, Russell PJ, Genetic markers of survival and liver recurrence after resection of liver metastases from colorectal cancer. World J Surg 2001;25:996–1001.
36 Soong R, Grieu F, Robbins P, Dix B, Chen D, Parsons R, House A, Iacopetta B: p53 alterations are associated with improved prognosis in distal colonic carcinomas. Clin Cancer Res 1997;3:1405–1411.
37 Adachi Y, Yamamoto H, Itoh F, Hinoda Y, Okada Y, Imai K: Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. Gut 1999;45:252–258.
38 Masaki T, Matsuoka H, Sugiyama M, Abe N, Goto A, Sakamoto A, Atomi Y: Matrilysin (MMP-7) as a significant determinant of malignant potential of early invasive colorectal carcinomas. Br J Cancer 2001;84:1317–1321.
39 Lenander C, Habermann JK, Ost A, Nilsson B, Schimmelpenning H, Tryggvason K, Auer G: Laminin-5 gamma 2 chain expression correlates with unfavorable prognosis in colon carcinomas. Anal Cell Pathol 2001;22: 201–209.
40 Ono Y, Nakanishi Y, Ino Y, Niki T, Yamada T, Yoshimura K, Saikawa M, Nakajima T, Hirohashi S: Clinicopathologic significance of laminin-5γ2 chain expression in squamous cell carcinoma of the tongue: immunohistochemical analysis of 67 lesions. Cancer 1999; 85:2315–2321.
41 Japanese Society for Cancer of the Colon and Rectum: General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus, ed 7. Tokyo, Kanehara, 2006.

42 Nakanishi Y, Noguchi M, Matsuno Y, Saikawa M, Mukai K, Shimosato Y, Hirohashi S: p53 expression in multicentric squamous cell carcinoma and surrounding squamous epithelium of the upper aerodigestive tract. immunohistochemical analysis of 95 lesions. Cancer 1995;75:1657–1662.
43 Shimoyama Y, Hirohashi S, Hirano S, Noguchi M, Shimosato Y, Takeichi M, Abe O: Cadherin cell-adhesion molecules in human epithelial tissues and carcinomas. Cancer Res 1989;49:2128–2133.
44 Hosmer DW, Hosmer T, Le Cessie S, Lemeshow S: A comparison of goodness-of-fit tests for the logistic regression model. Stat Med 1997;16:965–980.
45 Akaike H: A new look at the statistical model identification. IEEE Trans Autom Control 1974;19:716–723.
46 Mitomi H, Mori A, Kanazawa H, Nishiyama Y, Ihara A, Otani Y, Sada M, Kobayashi K, Igarashi M: Venous invasion and down-regulation of p21(WAF1/CIP1) are associated with metastasis in colorectal carcinomas. Hepatogastroenterology 2005; 52: 1421–1426.
47 Gunther K, Dworak O, Remke S, Pfluger R, Merkel S, Hohenberger W, Reymond MA: Prediction of distant metastases after curative surgery for rectal cancer. J Surg Res 2002;103:68–78.
48 Maeda K, Kang SM, Ogawa M, Onoda N, Sawada T, Nakata B, Kato Y, Chung YS, Sowa M: Combined analysis of vascular endothelial growth factor and platelet-derived endothelial cell growth factor expression in gastric carcinoma. Int J Cancer 1997;74: 545–550.
49 Barozzi C, Ravaioli M, D'Errico A, Grazi GL, Poggioli G, Cavrini G, Mazziotti A, Grigioni WF: Relevance of biologic markers in colorectal carcinoma: a comparative study of a broad panel. Cancer 2002;94:647–657.
50 Eschrich S, Yang I, Bloom G, Kwong KY, Boulware D, Cantor A, Coppola D, Kruhoffer M, Aaltonen L, Orntoft TF, Quackenbush J, Yeatman TJ: Molecular staging for survival prediction of colorectal cancer patients. J Clin Oncol 2005;23:3526–3535.
51 Wang Y, Jatkoe T, Zhang Y, Mutch MG, Talantov D, Jiang J, McLeod HL, Atkins D: Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. J Clin Oncol 2004;22:1564–1571.
52 Bertucci F, Salas S, Eysteries S, Nasser V, Finetti P, Ginestier C, Charafe-Jauffret E, Loriod B, Bachelart L, Montfort J, Victorero G, Viret F, Ollendorff V, Fert V, Giovaninni M, Delpero JR, Nguyen C, Viens P, Monges G, Birnbaum D, Houlgatte R: Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters. Oncogene 2004;23:1377–1391.

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Clinical Significance of Circulating Tumor Cells in Blood from Patients with Gastrointestinal Cancers

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Background: Circulating tumor cells (CTCs) measured by the CellSearch system in metastatic breast cancer have been reported to correlate with shorter overall survival. The purpose of this study was to clarify the clinicopathologic characteristics of CTCs in gastrointestinal cancers.

Methods: Pre- and postoperative CTCs from 130 gastrointestinal cancer patients and 41 healthy volunteers were measured by this system. Correlation between CTC counts and clinicopathologic variables was examined.

Results: The number of CTCs in metastatic patients ($n = 79$) was larger than in nonmetastatic patients ($n = 35$) and in healthy donors ($n = 41$) ($P < 0.001$). CTC counts were larger in metastatic gastric cancer ($n = 27$) than in nonmetastatic gastric cancer ($n = 14$) ($P = 0.016$). Two or more CTCs was significantly correlated with advanced tumor stage in all gastrointestinal cancers ($P < 0.001$) and in gastric cancer ($P = 0.032$). Two or more CTCs had significant correlation with peritoneal dissemination of gastric or colorectal cancer ($P = 0.007$) and pleural dissemination of esophageal cancer ($P = 0.033$). The survival of patients with ≥2 CTCs was shorter than that of patients with <2 CTCs ($P = 0.005$). The change in CTCs tended to correlate with disease progression and chemotherapeutic effect.

Conclusion: This study suggests that measurement of CTCs in gastrointestinal cancer patients could be useful as a tool for judging tumor stage, predicting the presence of peritoneal or pleural dissemination and patients' survival, and monitoring response to cancer therapy.

There are numerous reports on the isolation and characterization of circulating tumor cells (CTCs) in patients with various cancers.[1–8] The detection of circulating tumor cells in blood requires highly sensitive, specific, and reproducible methods. During the last decade, immunocytochemistry,[9,10] reverse-transcription polymerase chain reaction (RT-PCR) procedures,[11,12] and flow cytometry[13,14] have been used for the detection of these rare circulating tumor cells in blood.

The newly developed CellSearch system (Veridex LLC, Warren, NJ) was designed to detect tumor cells in whole blood.[15] By use of the CellSearch system, it is possible to obtain highly reproducible quantitative results from different laboratories.[15] Previous studies reported that the presence of CTCs assessed by the CellSearch system in metastatic breast cancer was associated with shorter overall survival.[16–18] Assessment of CTCs in metastatic breast cancer was reported to be an earlier, more reproducible indication of disease status than current imaging meth-

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TABLE 1. *Clinicopathologic features of patients with gastrointestinal cancers*

Subjects	Esophageal cancer*	Gastric cancer**	Colorectal cancer**	Total
Total number	38	44	48	130
Histology				
Well	7 (18%)	7 (16%)	7 (15%)	21 (16%)
Moderately	14 (37%)	16 (36%)	37 (77%)	67 (52%)
Poorly	2 (5%)	15 (34%)	3 (6%)	20 (15%)
Others or unknown	15 (39%)	6 (14%)	1 (2%)	22 (17%)
Nonmetastatic	10	14	11	35
Stage I	2 (20%)	8 (57%)	4 (36%)	14 (40%)
Stage II	1 (10%)	1 (7%)	1 (9%)	3 (9%)
Stage III	7 (70%)	5 (36%)	6 (55%)	18 (51%)
Metastatic	23	27	29	79
Liver metastasis	5 (22%)	6 (22%)	21 (72%)	32 (41%)
Lung metastasis	14 (61%)	1 (4%)	7 (24%)	22 (28%)
Bone metastasis	1 (4%)	0 (0%)	2 (7%)	3 (4%)
Pleural dissemination	4 (17%)	1 (4%)	0 (0%)	5 (6%)
Peritoneal dissemination	1 (4%)	21 (78%)	12 (41%)	34 (43%)
Post curative resection (clinically no recurrence)	5	3	8	16

* Squamous cell carcinoma; ** Adenocarcinoma.

ods.[19] Allard et al.[15] reported that CTCs were detected in patients with various types of carcinomas, including gastrointestinal cancers. Cohen et al.[20] conducted a pilot study demonstrating that CTCs can be isolated and enumerated in patients with metastatic colorectal cancer by immunomagnetic separation targeting the epithelial-cell adhesion molecule (EpCAM). They reported that patients with disease progression had greater serial increases in CTC number than nonprogressors.[20] However, the clinical significance of CTCs detected by the CellSearch system in patients with gastrointestinal cancers has not been well documented. Our hypothesis is that CTCs in patients with gastrointestinal cancers are associated with tumor stage, metastatic status, and prognosis. In this study, we evaluated CTCs in 130 gastrointestinal cancer patients using the CellSearch system and clarified the clinicopathologic characteristics of CTCs in gastrointestinal cancers.

PATIENTS AND METHODS

Characteristics of Patients and Healthy Controls

A total of 130 patients (114 preoperative and 16 postoperative) with gastrointestinal cancers were enrolled in this study. Table 1 shows the clinicopathologic features of the cases. Of these, 35 patients (23 men and 12 women) had nonmetastatic cancers which were diagnosed pathologically as American Joint Committee on Cancer (AJCC) stage I to III. They consisted of 10 (28.6%) patients with esophageal cancer, 14 (40.0%) patients with gastric cancer, and 11 (31.4%) patients with colorectal cancer. Their mean age was 68.9 ± 9.6 years. Blood samples from these nonmetastatic cancer patients were collected before surgery.

Additionally, 79 patients (54 men and 25 women) had distant-organ metastatic cancers which were diagnosed either clinically using computed tomography (CT) or pathologically after surgery. They consisted of 23 (29.1%) patients with esophageal cancer, 27 (34.2%) patients with gastric cancer, and 29 (36.7%) patients with colorectal cancer. Their mean age was 63.3 ± 11.9 years. From the patients with metastatic cancers, blood samples were collected before surgery or chemotherapy, or after chemotherapy. The median interval between the latest chemotherapy and CTC measurement was 1.1 months (range 1.0–2.0 months). In this study, the chemotherapy regimen was 5-fluorouracil/cisplatin or docetaxel/nedaplatin for esophageal cancer, and S-1/cisplatin or paclitaxel for gastric cancer. The chemotherapy for colorectal cancer patients consisted of modified FOLFOX-6, FOLFIRI, and irinotecan (CPT-11) plus tegafur-uracil (UFT) and leucovorin (LV).

In addition, 16 patients (12 men and 4 women) who had gastrointestinal cancers were examined after curative resection. They were proved to be free from residual tumor and recurrence by imaging modalities such as CT scan when the blood draw was done. They consisted of five (31.3%) patients with esophageal cancer, three (18.8%) patients with gastric cancer, and eight (50.0%) patients with colorectal cancer. Their mean age was 66.9 ± 10.1 years. The median interval between surgery and blood draw was 1.0 month (range 0.7–97.0 months).

As a control group, 41 healthy volunteers with no known illness and no history of cancer were examined.

All cases were followed prospectively, and the median survival time in metastatic patients was 5.8 months (range 1.0–15.0 months). All cases were enrolled using an institutional review board-approved protocol and the patients provided informed consent.

Isolation and Enumeration of Circulating Tumor Cells by CellSearch

The 7.5 mL of blood was drawn into the CellSave Preservative Tubes (Veridex LLC, Raritan, NJ). Samples were maintained at room temperature and processed within 72 h after collection. All evaluations were performed without knowledge of the clinical status of the patients and the healthy controls at one central laboratory (Veridex, Ibaraki, Japan). The CellSearch system was used for the isolation and enumeration of circulating tumor cells. It consists of a semi-automated system for the preparation of a sample and is used with the CellSearch Epithelial Cell Kit. The procedure enriches the sample for cells expressing EpCAM with antibody-coated magnetic beads, and it labels the nucleus with the fluorescent nucleic acid dye 4, 2-diamidino-2-phenylidole dihydrochloride. Fluorescently labeled monoclonal antibodies specific for leukocytes (CD45-allophycocyan) and epithelial cells (cytokeratin 8, 19, 19-phycoerythin) are used to distinguish epithelial cells from leukocytes. The identification and enumeration of circulating tumor cells were performed with the use of the CellSpotter and CellTracks Analyzer, a semi-automated fluorescence-based microscopy system that permits computer-generated reconstruction of cellular images. CTCs were defined as nucleated cells lacking CD45 and expressing cytokeratin. Criteria used in the CellSearch system to define a tumor cell have been described previously.[15] Results are expressed as the number of cells per 7.5 mL of whole blood. Technical details of the CellSearch and CellSpotter systems, including accuracy, precision, linearity, and reproducibility, were described by Allard et al.[15] It was reported that detection of ≥2 CTCs per 7.5 mL of blood is to be regarded as a positive result after evaluation of CTC counts of 145 healthy donors, 199 patients with nonmalignant diseases, and 964 patients with metastatic carcinomas.[15] Based on these results, we also considered ≥2 CTCs per 7.5 mL of blood to be a positive result.

EpCAM Expression Analysis for Gastrointestinal Cancers

Detection of CTCs by the CellSearch system was based on isolation and enumeration of cells expressing EpCAM with antibody-coated magnetic beads. To evaluate whether the CellSearch system is applicable for gastrointestinal cancers, we investigated EpCAM expression of gastrointestinal cancers. EpCAM mRNA expression of esophageal cancer cell lines (TE1, TE6, TE9), gastric cancer cell lines (MKN45, MKN74, TMK1), and colorectal cancer cell lines (HCT116, HT29, Lovo15) was analyzed. RNA isolation from 1×10^6 cells of each cell line was performed by means of the RNeasy Mini Kit (Qiagen, Westburg b.v., Leusden, The Netherlands), and RT-PCR was performed using the LightCycler (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. The sequence of EpCAM-specific primers and probes used in this study was previously described by Schroder et al.[21] The amplified products were analyzed using polyacrylamide gel electrophoresis followed by silver staining.

Accuracy, Sensitivity, and Linearity of the CellSearch Assay for Detecting Circulating Tumor Cells

Of the nine cell lines described before, the TE1 cell line was used for this analysis. Varying numbers of TE1 cells were spiked into blood, and recovery was measured using the CellSearch system. Predetermined numbers of TE1 cells (0, 1, 4, 12, 25, 50, 100) were spiked into 7.5-mL samples of peripheral blood from three healthy volunteers. Collection of TE1 cells was performed by counting precisely with the use of light microscopy and micropipettes. The collected cells were mixed with 7.5 mL of whole blood, and the CTC count was performed using the CellSearch system 24 h later.

Statistical Analysis

Single regression analysis was performed to assess the linearity of the CellSearch Assay for detecting CTCs. Correlation of CTC counts and tumor stage was analyzed using the Mann–Whitney U test and the Kruskal–Wallis H test. The distributions of patients above and below the cutoff level in the form of metastasis were compared using Fisher's exact test. The relationship between CTC counts and the tumor marker was examined by Spearman's rank correlation coefficient. Survival analysis was done using the

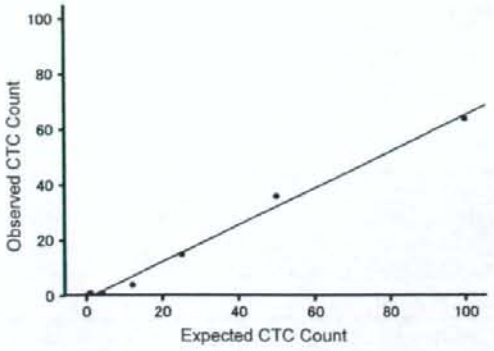

FIG. 1. Recovery of known numbers of spiked esophageal cancer cells from whole blood. Representative result of the number of spiked TE1 cells plotted against the observed number of cells is shown.

product-limit Kaplan–Meier and the log-rank test. Multivariate analysis was performed using a Cox proportional hazard model to test for independent prognostic variables. All statistical calculations were carried out using SPSS for Windows release 15.0J (SPSS Japan Inc., Tokyo, Japan). The required significance was $P < 0.05$.

RESULTS

EpCAM Expression in Gastrointestinal Cancers and Sensitivity of the CellSearch Assay for Detecting Esophageal Cancer Cells

EpCAM mRNA expression of gastrointestinal cancer cell lines (TE1, TE6, TE9, MKN45, MKN74, TMK1, HCT116, HT29, and Lovo15) was detectable in all gastrointestinal cancer cell lines using an RT-PCR assay. Of the nine cell lines, TE1 was used for the analysis of accuracy, sensitivity, and linearity of the CellSearch assay. Representative results of the expected number of TE1 cells spiked into the healthy donor samples (0, 1, 4, 12, 25, 50, and 100) plotted against the actual number of TE1 cells observed in the samples are shown in Fig. 1. Regression analysis of the number of observed tumor cells versus the number of expected tumor cells produced a slope of 0.41 [95% confidence interval (CI), 0.30–0.52], an intercept of 0.2 (95% CI, −4.7–5.1), and a correlation coefficient (R^2) of 0.758. Even the single cell spiked into the samples was detected using this system.

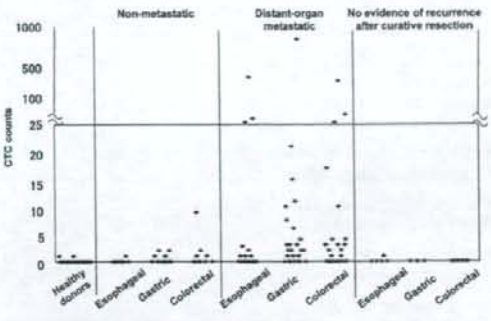

FIG. 2. Scatter plot of CTC counts in healthy donors and patients with gastrointestinal cancers.

Detection of Gastrointestinal Cancer Cells in Patient Blood Samples

Figure 2 shows the scatter plot of CTC counts from healthy donors and patients with gastrointestinal cancers. The numbers of blood specimens tested for CTCs from healthy donors and patients with gastrointestinal cancers are shown in Table 2. The number of CTCs in patients with metastatic gastrointestinal cancers was significantly larger compared with that in healthy donors and that in patients with nonmetastatic gastrointestinal cancers ($P < 0.001$). The number of CTCs was significantly larger in metastatic gastric cancer than in nonmetastatic gastric cancer ($P = 0.016$). In the group of esophageal cancer patients and colorectal cancer patients, CTC counts did not have significant correlation with tumor stage, which was considered to be due to the small number of patients. The number of CTCs in clinically tumor-free patients after curative resection was as low as that in healthy donors.

We considered ≥2 CTCs per 7.5 mL of blood to be a positive result based on the report by Allard et al.[15] In our study, there was no ≥2 CTCs result in the 38 healthy donors. In contrast with the result for healthy donors, ≥2 CTCs were detected in 32 of 79 (40.5%) patients with metastatic gastrointestinal cancers (Table 2). Samples from 4 of 35 (11.4%) patients with nonmetastatic gastrointestinal cancers were found to contain ≥2 CTCs. Two or more CTCs were more frequently found in patients with metastatic gastrointestinal cancers than in healthy donors and in patients with nonmetastatic gastrointestinal cancers ($P < 0.001$). Table 2 also shows the incidence of ≥2 CTCs in various groups. Two or more CTCs was significantly correlated with advanced tumor stage in gastric cancer patients ($P = 0.032$).

TABLE 2. *CTC counts in healthy donors and in patients with gastrointestinal cancers*

Subject	No. of patients	Mean ± SD	Median	*P* value	≥2 CTCs (%)	*P* value
Healthy donors	41	0.0 ± 0.2	0		0	
Nonmetastatic gastrointestinal cancers	35	0.6 ± 1.7	0		11.4	
Metastatic gastrointestinal cancers	79	22.6 ± 108.1	1	<0.001	40.5	<0.001
Nonmetastatic esophageal cancer	10	0.1 ± 0.3	0		0	
Metastatic esophageal cancer	23	17.7 ± 66.0	0	0.121	21.7	0.343
Nonmetastatic gastric cancer	14	0.4 ± 0.7	0		14.3	
Metastatic gastric cancer	27	36.2 ± 168.0	2	0.006	55.6	0.032
Nonmetastatic colorectal cancer	11	1.3 ± 2.8	0		18.2	
Metastatic colorectal cancer	29	13.9 ± 51.6	1	0.225	41.4	0.267
Post curative resection	16	0.1 ± 0.2	0		0	

SD, standard deviation.

TABLE 3. *Correlation of CTCs with metastatic organs in gastrointestinal cancers*

Variable	Number of subjects	≥2 CTCs	*P* value
All subjects with metastatic gastrointestinal cancer	79	32 (40.5%)	
Liver metastasis			
+	32	13 (40.6%)	0.584
−	47	19 (40.4%)	
Lung metastasis			
+	23	6 (26.1%)	0.076
−	56	26 (46.4%)	
Bone metastasis			
+	3	1 (33.3%)	0.642
−	76	31 (40.8%)	
All subjects with metastatic gastric or colorectal cancer	56	27 (48.2%)	
Peritoneal dissemination			
+	33	21 (63.6%)	0.006
−	23	6 (26.1%)	
All subjects with metastatic esophageal cancer	23	5 (21.7%)	
Pleural dissemination			
+	4	3 (75.0%)	0.021
−	19	2 (10.5%)	

The distributions of metastatic organs in patients with metastatic gastrointestinal cancer above the cutoff level are shown in Table 3. Two or more CTCs in patients with metastatic gastric or colorectal cancer had significant correlation with peritoneal dissemination ($P = 0.006$). Two or more CTCs in patients with metastatic esophageal cancer had significant correlation with pleural dissemination ($P = 0.021$). Positive (≥2) CTC counts in those patients were significantly correlated with pleural or peritoneal dissemination even if the cutoff level was 3, 4, 5, and 10 (data not shown). There were no significant correlations between positive CTC counts and liver metastasis, lung metastasis, or bone metastasis.

Serum tumor marker was added in this analysis to be compared with CTC counts. In all patients enrolled in this study, carcinoembryonic antigen (CEA) was measured within 3 weeks from CTC measurement as a routine evaluation. A normal range in the hospital is 0–5.6 ng/mL. High levels of CEA (>5.6 ng/mL) were found in 5 of 23 (21.7%) metastatic esophageal cancer patients, 13 of 27 (48.1%) metastatic gastric cancer patients, and 24 of 29 (82.8%) metastatic colorectal cancer patients. The relationship between CTC counts and CEA level was examined by Spearman's rank correlation coefficient. It revealed that there was a weak positive correlation between these two variables ($\rho = 0.265$, $P = 0.018$).

To investigate whether positive CTC counts were associated with patients' survival, the cumulative survival curves were calculated via the Kaplan–Meier method and compared via the log-rank test. The survival of the CTC-positive group was significantly shorter than that of the CTC-negative group in total patients with metastatic gastrointestinal cancers ($P = 0.005$) (Fig. 3A). Patients with ≥2 CTCs had a significantly worse prognosis compared with the patients with <2 CTC in the group of metastatic esophageal cancer ($P < 0.001$) (Fig. 3B) and gastric cancer ($P = 0.039$) (Fig. 3C). There was no significant correlation between positive CTC counts and survival in the group of metastatic colorectal cancer patients ($P = 0.297$) (Fig. 3D).

A multivariate analysis was done to test whether CTC positivity was an independent prognostic factor in metastatic gastrointestinal cancer patients. Factors enrolled in this analysis were CTC counts, CEA level, and the sites of metastasis, which were considered to be significant characteristics of these patients. The Cox model showed that CTC positivity remained significant (hazard ratio, 3.32: 95% confidence interval, 1.26–8.77; $P = 0.016$) and was independent of the other factors (Table 4).

In 10 of 32 CTC-positive patients with metastatic gastrointestinal cancers, CTC was measured before

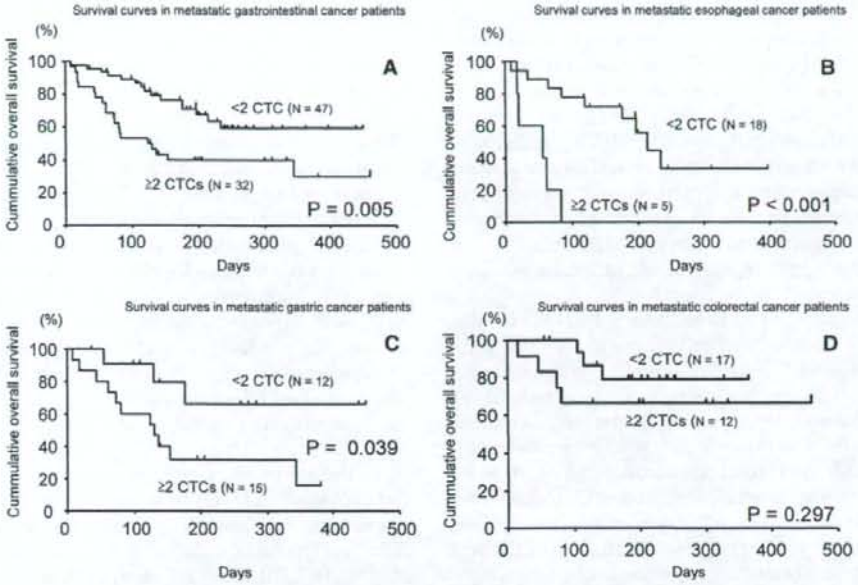

FIG. 3. Overall survival for patients with positive (≥2) CTCs versus negative (<2) CTC: **(A)** metastatic gastrointestinal cancer patients, **(B)** metastatic esophageal cancer patients, **(C)** metastatic gastric cancer patients, and **(D)** metastatic colorectal cancer patients.

TABLE 4. *Multivariate Cox proportional hazards analysis of patients with metastatic gastrointestinal cancers*

Factor	Hazard ratio	95% CI	*P*
CTC counts (≥2/<2)	3.32	1.26–8.77	0.016
Serum CEA (>5.6/≤5.6)	0.82	0.35–1.95	0.656
Liver metastasis (±)	1.05	0.41–2.72	0.922
Lung metastasis (±)	2.38	1.08–5.25	0.031
Bone metastasis (±)	1.35	0.17–10.48	0.776
Pleural or peritoneal dissemination (±)	1.45	0.51–4.13	0.491

initiation of a new line of chemotherapy and more than 3 weeks after initiation of therapy. The response to the new line of chemotherapy was measured with other imaging modalities such as CT scan and evaluated using response evaluation criteria in solid tumors (RECIST) in each case. Four of ten (40.0%) cases had stable disease after chemotherapy, whereas their CTC counts decreased (Fig. 4A). Six of ten (60.0%) cases had progressive disease, whereas all of their CTC counts increased (Fig. 4B). These data suggested that CTC measurement is useful for monitoring response to chemotherapy in the metastatic setting.

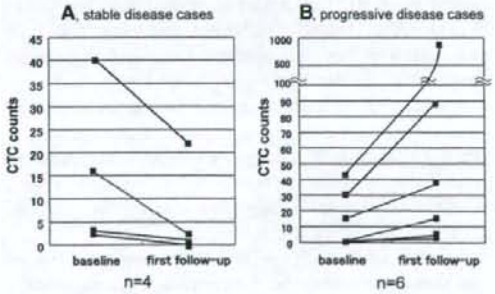

FIG. 4. CTCs measured before initiation of a new line of chemotherapy and more than 3 weeks after initiation of therapy. **(A)** CTCs did not increase in the patients with stable disease. **(B)** CTCs increased in the patients with progressive disease.

DISCUSSION

In this study, we investigated the utility of the CellSearch system for gastrointestinal cancers. We assessed the assay for CTCs in 79 patients with metastatic gastrointestinal cancers, 35 patients with nonmetastatic gastrointestinal cancers, 16 patients after curative resection, and 41 healthy volunteers.

Our preliminary results strongly suggest that the CellSearch system can be used to measure circulating gastrointestinal cancer cells. The number of CTCs in distant-organ metastatic patients was significantly larger than that in healthy donors and that in nonmetastatic patients. Positive CTCs significantly correlated with advanced tumor stage, pleural and/or peritoneal dissemination, and shorter survival. To our knowledge, this is the first report that demonstrates the clinical significance of CTCs assessed by the CellSearch system for patients with esophageal or gastric cancer.

In general, CTCs have been observed in the peripheral blood of cancer patients at very low concentrations of 10^{-7}–10^{-8} of normal peripheral blood cells.[5] Their characterization is of considerable biomedical interest in order to understand how these cells can travel via the blood stream to anatomically distant sites and form metastatic disease. Consequently, an instrument for measuring CTCs could be a valuable diagnostic tool. The number of these cells has been shown to correlate with outcome for cohorts of metastatic breast cancer patients with progressive disease at the time of sampling.[16–18]

The CellSearch system was designed to detect tumor cells in the peripheral blood. The system is based on the enumeration of epithelial cells, which are separated from the blood by EpCAM antibody-coated magnetic beads and identified with the use of fluorescently labeled antibodies against cytokeratin and with a fluorescent nuclear stain and fluorescent cytokeratin antibodies. Criteria used to define a tumor cell have been an issue for immunohistochemical techniques, in which reproducibility across laboratories is poor despite attempts to standardize such criteria. Criteria used in the CellSearch system make it possible to obtain highly reproducible quantitative results from different laboratories.[15]

Identification with the use of fluorescently labeled antibodies enables the CellSearch system to offer lower false-positive rates than RT-PCR and than flow cytometry. Although RT-PCR and flow cytometry are sensitive techniques for rare circulating tumor cell identification, they do not permit visual confirmation of malignant cells, and false-positive results on normal controls have been reported.[22,23]

Allard et al.[15] reported the presence of ≥2 CTCs in 4 of 13 (31%) patients with metastatic gastric cancer and in 99 of 333 (30%) patients with metastatic colorectal cancer. In our study, ≥2 CTCs were detected in 32 of 79 (40.5%) patients with metastatic gastrointestinal cancers, which was compatible with the previous report.[15] By use of the CellSearch system, a higher proportion of positive CTCs was seen in patients with metastatic prostate cancer, ovarian cancer, and breast cancer than in patients with metastatic gastrointestinal cancers.[15] It was suggested that filtration via the portal circulation might be effective in prevention of tumor cell dissemination in gastrointestinal cancer patients.[15]

Our study showed that CTC measurement in gastrointestinal cancer was useful for distinguishing metastatic patients from nonmetastatic ones. This distinction is necessary to judge whether patients are eligible for curative resection or not. Measurement of CTCs can improve palliation by avoiding nontherapeutic laparotomy. In fact, among the patients enrolled in this study, seven patients with preoperative stage I to III were upstaged to stage IV at surgery, whereas four of them (57.1%) were positive for CTCs before surgery.

In this study, there was no significant correlation between positive CTC counts and liver, lung, or bone metastasis. The sample size may be too small for these comparisons to be valid. CTC measurement in this study by the CellSearch system was performed with antibodies specific to epithelial cells (EpCAM, cytokeratins 8, 18, and 19). In fact, some of the metastatic patients were negative for CTC counts in our study. These results suggest that some of the CTCs may not express these epithelial markers and may be undetectable by the CellSearch system.

There has been no report on correlation between CTC counts and peritoneal and/or pleural dissemination in gastrointestinal cancer patients. The mechanism of these clinical entities remains unclear. Sakakura et al.[24] performed a global analysis of the different gene expressions of a gastric cell line established from metastases to the peritoneal cavity with the application of a high-density cDNA microarray method, but the role of the detected genes is still unclear. CTCs may be the key to solving the mechanism of peritoneal and pleural dissemination in the future. Considering that detection of CTCs by the CellSearch system is based on isolation and enumeration of cells expressing EpCAM, it can be hypothesized that EpCAM plays an important role in peritoneal and pleural dissemination. The trifunctional antibody catumaxomab (anti-EpCAM × anti-CD3) is reported to show convincing efficacy in patients with malignant ascites.[25,26] These reports might support the hypothesis.

Due to the inaccuracy of CT and other modalities for the detection of peritoneal and pleural dissemination, it is often difficult to judge patients to be eligible for potentially curative resection by current-

generation CT scanning. Measurement of CTCs may be a useful diagnostic tool to predict peritoneal and/or pleural dissemination.

In the present study, we showed that metastatic gastrointestinal cancer patients with ≥2 CTCs had significantly shorter survival. Multivariate analysis revealed that positive CTCs were an independent prognostic factor. There has been no report on such a powerful marker that provides prognostic information for patients with metastatic gastrointestinal cancers. In this study, detection of ≥2 CTCs was also an important prognostic factor for the subgroups including metastatic esophageal cancer and metastatic gastric cancer patients. However, it was not a significant prognostic factor for the subgroup including metastatic colorectal cancer patients. The result may be due to chemotherapy such as modified FOLFOX-6 and FOLFIRI, which is more effective for metastatic colorectal cancer than for metastatic esophageal or gastric cancer. Chemotherapy may prolong survival of metastatic colorectal cancer patients, even those who are positive for CTCs. Serial CTC monitoring may be useful to select effective chemotherapy for patients with advanced gastrointestinal cancers.

Although tumor markers such as CEA are widely used for the follow-up of patients with gastrointestinal cancers, their lack of sensitivity remains unsolved. Current imaging modalities and tumor markers are sometimes useless for monitoring therapy. They might cause a delay in judging response of therapy. Our results also demonstrated that CTC measurement in gastrointestinal cancers could be more sensitive and useful for monitoring response to chemo- and/or radiotherapy than imaging modalities and tumor markers. Cohen et al.[20] demonstrated that metastatic colorectal cancer patients with disease progression had greater serial increases in CTC number than did nonprogressors, which was similar to our results. They also reported that change in CTC number might help guide therapy by predicting clinical response.[20] In this study, CTC numbers in patients with no evidence of recurrence after curative surgery were as small as those in healthy donors. These results suggest that CTC measurement may be useful for monitoring disease status. We are planning a survey of a much larger population as a multicentric study to confirm the utility of this assay.

In conclusion, this study suggested that measurement of CTCs in patients with advanced gastrointestinal cancers could be useful as a tool for judging tumor stage, predicting the presence of peritoneal or pleural dissemination and patients' survival, and monitoring response to cancer therapy. Although larger multicentric studies are required to verify our results, monitoring CTCs should be considered to select effective multimodal therapy for patients with advanced gastrointestinal cancers.

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REFERENCES

1. Racila E, Euhus D, Weiss AJ, et al. Detection and characterization of carcinoma cells in the blood. *Proc Natl Acad Sci USA* 1998; 95:4589–94.
2. Ghossein RA, Bhattacharya S, Rosai J. Molecular detection of micrometastases and circulating tumor cells in solid tumors. *Clin Cancer Res* 1999; 5:1950–60.
3. Gaforio JJ, Serrano MJ, Sanchez-Rovira P, et al. Detection of breast cancer cells in the peripheral blood is positively correlated with estrogen-receptor status and predicts for poor prognosis. *Int J Cancer* 2003; 107:984–90.
4. Molnar B, Ladanyi A, Tanko L, et al. Circulating tumor cell clusters in the peripheral blood of colorectal cancer patients. *Clin Cancer Res* 2001; 7:4080–5.
5. Witzig TE, Bossy B, Kimlinger T, et al. Detection of circulating cytokeratin-positive cells in the blood of breast cancer patients using immunomagnetic enrichment and digital microscopy. *Clin Cancer Res* 2002; 8:1085–91.
6. Mehes G, Witt A, Kubista E, et al. Circulating breast cancer cells are frequently apoptotic. *Am J Pathol* 2001; 159:17–20.
7. Pierga JY, Bonneton C, Vincent-Salomon A, et al. Clinical significance of immunocytochemical detection of tumor cells using digital microscopy in peripheral blood and bone marrow of breast cancer patients. *Clin Cancer Res* 2004; 10:1392–400.
8. Fehm T, Sagalowsky A, Clifford E, et al. Cytogenetic evidence that circulating epithelial cells in patients with carcinoma are malignant. *Clin Cancer Res* 2002; 8:2073–84.
9. Shariat SF, Roudier MP, Wilcox GE, et al. Comparison of immunohistochemistry with reverse transcription-PCR for the detection of micrometastatic prostate cancer in lymph nodes. *Cancer Res* 2003; 63:4662–70.
10. Pantel K, Schlimok G, Angstwurm M, et al. Methodological analysis of immunocytochemical screening for disseminated epithelial tumor cells in bone marrow. *J Hematother* 1994; 3:165–73.
11. Straub B, Muller M, Krause H, et al. Quantitative real-time RT-PCR for detection of circulating prostate-specific antigen mRNA using sequence-specific oligonucleotide hybridization probes in prostate cancer patients. *Oncology* 2003; 65:12–7.
12. Gao CL, Rawal SK, Sun L, et al. Diagnostic potential of prostate-specific antigen expressing epithelial cells in blood of prostate cancer patients. *Clin Cancer Res* 2003; 9:2545–50.
13. Moreno JG, O'Hara SM, Gross S, et al. Changes in circulating carcinoma cells in patients with metastatic prostate cancer correlate with disease status. *Urology* 2001; 58:386–92.
14. Hamdy FC, Lawry J, Anderson JB, et al. Circulating prostate specific antigen-positive cells correlate with metastatic prostate cancer. *Br J Urol* 1992; 69:392–6.
15. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res* 2004; 10:6897–904.

16. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004; 351:781–91.
17. Cristofanilli M, Hayes DF, Budd GT, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *J Clin Oncol* 2005; 23:1420–30.
18. Hayes DF, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin Cancer Res* 2006; 12:4218–24.
19. Budd GT, Cristofanilli M, Ellis MJ, et al. Circulating tumor cells versus imaging—predicting overall survival in metastatic breast cancer. *Clin Cancer Res* 2006; 12:6403–9.
20. Cohen SJ, Alpaugh RK, Gross S, et al. Isolation and characterization of circulating tumor cells in patients with metastatic colorectal cancer. *Clin Colorectal Cancer* 2006; 6:125–32.
21. Schroder CP, Ruiters MH, de Jong S, et al. Detection of micrometastatic breast cancer by means of real time quantitative RT-PCR and immunostaining in perioperative blood samples and sentinel nodes. *Int J Cancer* 2003; 106:611–8.
22. de Cremoux P, Extra JM, Denis MG, et al. Detection of MUC1-expressing mammary carcinoma cells in the peripheral blood of breast cancer patients by real-time polymerase chain reaction. *Clin Cancer Res* 2000; 6:3117–22.
23. De Luca A, Pignata S, Casamassimi A, et al. Detection of circulating tumor cells in carcinoma patients by a novel epidermal growth factor receptor reverse transcription-PCR assay. *Clin Cancer Res* 2000; 6:1439–44.
24. Sakakura C, Hagiwara A, Nakanishi M, et al. Differential gene expression profiles of gastric cancer cells established from primary tumour and malignant ascites. *Br J Cancer* 2002; 87:1153–61.
25. Heiss MM, Strohlein MA, Jager M, et al. Immunotherapy of malignant ascites with trifunctional antibodies. *Int J Cancer* 2005; 117:435–43.
26. Burges A, Wimberger P, Kumper C, et al. Effective relief of malignant ascites in patients with advanced ovarian cancer by a trifunctional anti-EpCAM × anti-CD3 antibody: a phase I/II study. *Clin Cancer Res* 2007; 13:3899–905.

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Results of a multicenter study of 1,057 cases of rectal cancer treated by laparoscopic surgery

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Abstract
Background The aim of this study was to clarify the feasibility of laparoscopic surgery for rectal cancer retrospectively in 28 centers throughout Japan.
Methods Between May 1994 and February 2006, 1,057 selected patients with rectal cancer underwent laparoscopic surgery. All the data regarding the patient details, and operative and postoperative outcome were collected retrospectively.
Results Mean follow-up was 30 months. Procedures included anterior resection in 938, abdominoperineal resection in 107, Hartmann's procedure in 10, and others in two patients. Conversion to open procedures occurred in 77 patients (7.3%). Postoperative surgical complications developed in 235 patients (22.2%), including anastomotic leakage in 84 (9.1%). Median length of postoperative hospital stay was 15 days (7–271 days). Patients with upper rectal cancer had shorter hospital stay than those with lower rectal cancer (14 versus 18 days, $p < 0.01$). Tumor–node–metastases (TNM) stage included 83(7.9%) stage 0, 495 (46.8%) stage I, 197 (18.6%) stage II, 230 (21.8%) stage III, and 52 (4.9%) stage IV. Recurrence was developed in 67 patients (6.6%) of the 1,011 curatively treated patients. Local recurrence occurred in 11 patients (1.0%). There was no port-site metastasis. Of the 1,011 curatively treated patients, the 3-year disease-free survival rate was 100% in stage 0, 94.6% in stage I, 82.1% in stage II, and 79.7% in stage III.
Conclusions Laparoscopic surgery is feasible and safe in selected patients with rectal cancer, with favorable short-term and mid-term outcome.

Keywords Rectal cancer · Laparoscopic surgery · Short-term outcome · Multicenter study

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The role of laparoscopic surgery has gained acceptance in the treatment of benign diseases, but it remains controversial in the treatment of malignancies, because of concerns about adequacy of lymphadenectomy, the extent of resection, early findings of port-site metastasis, and the lack of long-term results [23]. There are some retrospective and prospective comparative studies reporting on the feasibility and favorable outcome of laparoscopic surgery for colorectal cancer including earlier return of bowel motility [6, 9, 17, 27], less postoperative pain [6, 27], and shorter hospital stay [6, 8, 9, 27]. Recently, results of large