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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, laminin5γ2, 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-

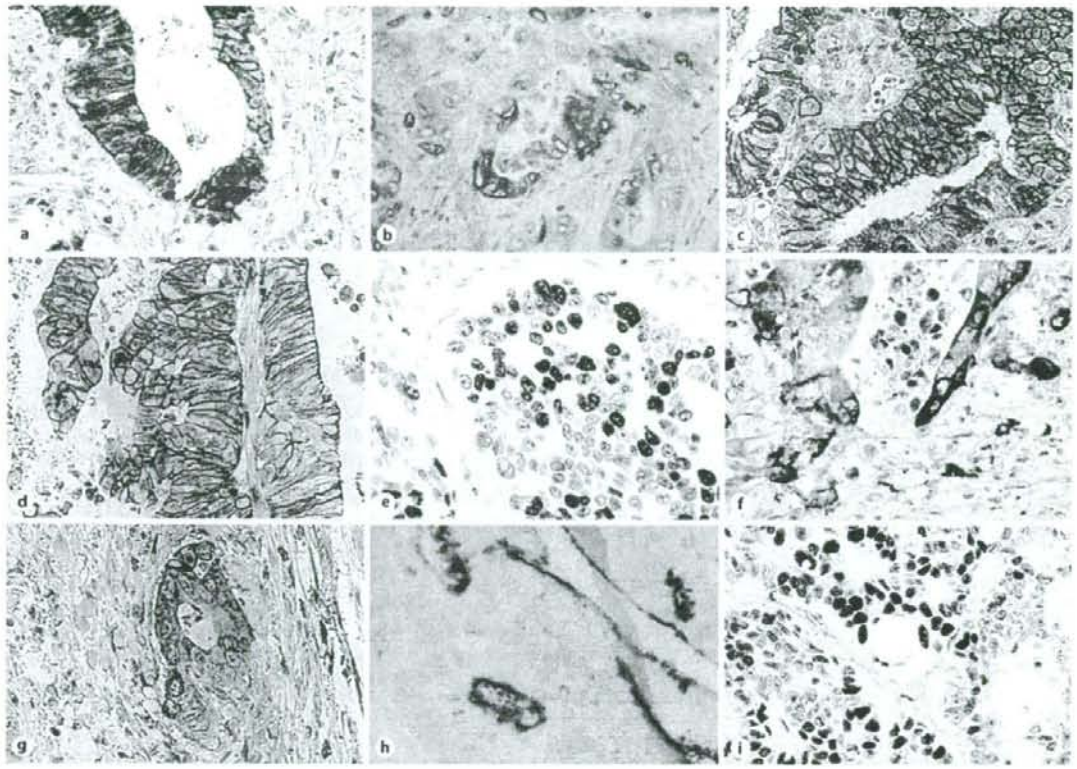

Fig. 1. Immunohistochemical staining pattern of each molecular marker (×400). β-Catenin expression was localized at the cell-cell borders, in the cytoplasm and in the nuclei of cancer cells (**a**). COX-2 expression was observed in the cytoplasm of cancer cells (**b**). Membranous dysadherin (**c**) 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-

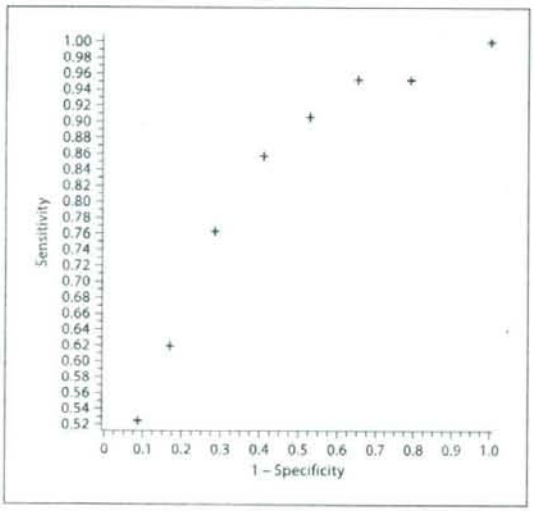

Fig. 2. Receiver-operating characteristic curve of the immunohistochemical metastatic scores for 150 independent patients (group I).

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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Intersphincteric Resection for Very Low Rectal Adenocarcinoma: Univariate and Multivariate Analyses of Risk Factors for Recurrence

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Background: The aim of this study was to analyze the risk factors for local and distant recurrence after intersphincteric resection (ISR) for very low rectal adenocarcinoma.

Methods: One hundred twenty consecutive patients with T1–T3 rectal cancers located 1–5 (median 3) cm from the anal verge underwent ISR. Univariate and multivariate analyses of prospectively recorded clinicopathologic parameters were performed.

Results: Fifty patients had disease categorized as stage I, 21 as stage II, 46 as stage III, and 3 as stage IV on the basis of International Union Against Cancer tumor, node, metastasis staging system. Median follow-up time was 3.5 years. The 3-year rates of local and distant recurrence were 6% and 13%, respectively. Univariate analysis of the risk factors for local recurrence revealed pathologic T, pathologic stage, focal dedifferentiation, microscopic resection margins, and preoperative serum CA 19-9 level to be statistically significant. Multivariate analysis showed resection margin, focal dedifferentiation, and serum CA 19-9 level to be independently significant. Univariate analysis of the risk factors for distant recurrence indicated tumor location, combined resection, tumor annularity, pathologic N, lateral pelvic lymph node metastasis, pathologic stage, histologic grade, lymphovascular invasion, perineural invasion, and adjuvant chemotherapy to be significant. Multivariate analysis identified pathologic N, histologic grade, and tumor location to be independently significant.

Conclusion: Profiles of risk factors for local and distant recurrences after ISR are different. With local recurrence, the resection margin, focal dedifferentiation, and serum CA 19-9 level are important. For distant recurrence, the lymph node status, histologic grade, and tumor location need to be taken into account.

Key Words: Rectal cancer—Surgery—Intersphincteric resection—Local recurrence—Recurrence—Prognostic factor.

Standard surgery for patients with massively invasive rectal adenocarcinoma located within 5 cm from the anal verge is abdominoperineal resection.[1] To avoid permanent colostomy for such patients, modern intersphincteric resection (ISR) was developed in the 1980s and became well established in the 1990s.[2–4] ISR is currently defined as a procedure obtaining sufficient margins by removing part or whole of the internal sphincter and restoring bowel continuity for rectal cancers involving or next to the

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anal canal. ISR is usually performed in combination with total mesorectal excision. Cautious performance of this operation has been reported to allow satisfactory results with regard to both defecatory function and oncologic outcome.[4–7]

In our previous study, we showed that ISR with meticulous dissection and irrigation after closure of the distal stump does not increase risk of local or distant recurrence.[8] ISR without radiotherapy is usually sufficient for patients with T1–T2 tumors. However, with T3 tumors, neoadjuvant therapy should be considered if resection margins are estimated to be insufficient.[8]

Appropriate selection of patients who are at high risk for recurrence is essential to reduce unnecessary toxicity and costs of neoadjuvant and adjuvant therapy for those at low risk. Selection criteria are thus needed to stratify patients for neoadjuvant and adjuvant therapy and also for clinical trials. At present, however, there have been few studies addressing risk factors for recurrence after ISR. The aim of this study was thus to conduct retrospective exploratory analysis of the risk factors for local and distant recurrences after ISR for very low rectal adenocarcinoma.

PATIENTS AND METHODS

Between October 1993 and February 2007, 122 patients with massively invasive rectal adenocarcinomas located within 5 cm from the anal verge underwent ISR at the National Cancer Center Hospital, Tokyo. Selection criteria for ISR were as follows: (1) sufficient medical fitness; (2) normal sphincter function; (3) distance between the tumor and the anorectal junction (upper edge of the surgical anal canal) of <2 cm; (4) no involvement of the external sphincter; and (5) no signs of disseminated disease. The patients were assessed with chest and abdominal computed tomography (CT), digital anorectal examination, and radiological studies, including endorectal ultrasound, thin-section helical CT, or high-resolution magnetic resonance imaging (MRI). Approval by the institutional review board was not required for the observational study. All patients gave informed consent for usage of their data for analysis.

Univariate and multivariate analyses of 26 prospectively recorded clinicopathologic variables were conducted for the 120 consecutive patients who did not receive neoadjuvant radiotherapy. Data from the remaining two given radiotherapy were excluded from the present analysis.

Surgical Procedures

The surgical procedures were described previously[8] and are basically similar to those originally documented by Schiessel et al.[4,7] The intersphincteric plane between the puborectalis and the internal sphincter was dissected cautiously as caudal as possible under direct vision by electrocautery. If the lower edge of the tumor was reached, the anal canal was closed just below the tumor, and then washed with povidone iodine followed by saline. A self-holding retractor was applied to the anal canal, the internal sphincter was circumferentially incised and the intersphincteric plane was dissected. A resection margin of at least 1 cm was always attempted. After removal of the rectum, the pelvic cavity and anal canal were washed, and then a coloanal anastomosis was made.

Histopathologic Examination

Results of histopathologic examination were prospectively documented in the pathology report form. Evaluated variables included gross tumor morphology, pathologic depth of transmural invasion (pT), pathologic regional lymph node metastasis (pN), pathologic lateral pelvic lymph node metastasis, pathologic distant metastasis (pM), pathologic stage, histopathologic grade, lymphovascular invasion, perineural invasion, mucin production, focal dedifferentiation, and circumferential and distal resection margins. pT, pN, pM, pathologic stage, and histopathologic grade were classified according to the International Union Against Cancer tumor, node, metastasis (TNM) classification.[9] Perineural invasion was defined as the presence of cancer cells inside the perineurium.[10] Focal dedifferentiation was defined as the presence of polygonal, noncolumnar cancer cells which had a single or a solitary trabecular form with indistinct polarity and had a diffusely infiltrative pattern at the invasive front.[11,12]

Follow-up

All of the patients were followed up with a median follow-up time of 3.5 (range, .9–11.7) years for those who remained alive, and 93 patients (78%) could be followed up for more than 2 years. Patients with stage I tumors were examined by chest and abdominopelvic CT, as well as carcinoembryonic antigen measurement every year for at least 5 years. Patients with stage II tumors were examined every 6 months for 2 years, then yearly for at least 3 years. Patients with

stage III tumors were examined every 4 months for 2 years, then every 6 months for at least 3 years.

Statistical Analysis

The starting point for the recurrence-free interval was the day of surgery, and data on patients who were alive or free of recurrence were censored at the last follow-up. Local recurrence was defined as confined to the pelvis and distant recurrence as present outside of the pelvis.

Survival curves were estimated by the Kaplan-Meier method, and differences in survival were evaluated with the log rank test. Multivariate analysis was performed by Cox regression model with the forward stepwise method (likelihood ratio). All statistical analyses were conducted by SPSS for Windows, version 11.0J (SPSS-Japan, Tokyo, Japan). *P* values were two sided and were considered to be statistically significant at <.05.

RESULTS

Clinicopathologic Findings

Findings for 26 clinicopathologic variables related to patient and tumor characteristics, treatment, and pathology are summarized in Table 1. There were 92 male and 28 female patients with a median age of 57 (range, 26–75) years. The median distance from the anal verge to the tumor was 3 (range, 1–5) cm.

A total of 103 patients underwent partial resection of the internal sphincter and 17 underwent complete resection. A small part of the external sphincter was resected in six patients to obtain sufficient surgical margins. All patients underwent total mesorectal excision. In addition to total mesorectal excision, 46 patients received extended lateral pelvic lymph node dissection. The median number of lymph nodes removed at surgery was 29 (range, 4–88), and 108 patients (90%) underwent dissection of 12 or more nodes. Combined resection of adjacent organs was performed for 12 patients. Two patients with a solitary liver metastasis and one with a solitary lung metastasis underwent complete resection of their metastases. A total of 108 patients had a protective stoma, which was closed 3 months after ISR. Postoperatively, 26 patients with stage III disease and 1 with curatively resected liver metastasis received adjuvant chemotherapy with 5-fluorouracil plus leucovorin, or uracil-tegafur plus leucovorin on the basis of the results from the National Surgical Adjuvant Breast and Bowel Project Protocol C-06,[13] or oral uracil-tegafur on the basis of the results of the National Surgical Adjuvant Study of Colorectal Cancer 01 randomized trial.[14]

The median tumor size was 3.7 (range, 1–12) cm. Data on TNM classifications and histopathologic findings are provided in Table 1. Resection margins were macroscopically negative in all patients, but microscopically positive in four. One patient had both circumferential and distal positive margins, and the other three had a circumferential positive margin. Excluding these four patients, the median distal margin was 1.2 (range, .3–4) cm.

Of 39 patients (33%) who experienced complications, 30 were treated conservatively and 9 received surgery. Of 15 patients (13%) with anastomotic leakage, 6 underwent emergency operations. One patient who had anastomotic leakage and sepsis died on the third postoperative day (30-day mortality rate = .8%).

Survival and Recurrence

At the last follow-up in February 2008, 112 patients were alive and 8 were dead. Causes of death included rectal cancer (n = 4 patients), other cancers (n = 2), anastomotic leakage (n = 1), and cerebral contusion (n = 1). The estimated overall 3- and 5-year survival rates were 95% and 91%, respectively, including one hospital death.

A total of 20 patients (17%) experienced recurrence. Estimated 3- and 5-year cumulative rates for overall recurrence were 17% and 23%, respectively (Fig. 1). Sites of the first recurrence included the pelvis in six patients, pelvis and lung in one, inguinal lymph nodes in two, inguinal lymph nodes and lung in one, lung in four, lung and liver in one, and liver in five. The incidences of overall recurrence for stage I, II, III, and IV disease were 5%, 22%, 27%, and 50%, respectively.

In total, eight patients (6.7%) developed local recurrence, with estimated 3- and 5-year cumulative rates of 6% and 10%, respectively (Fig. 1). Detailed sites of local failure included the internal iliac or obturator nodes in three patients, circumferential resection margin in two, anastomosis in one, seminal vesicle in one, and sacrum in one. The incidences of local failure for stage I, II, III, and IV disease were 2%, 19%, 7%, and 0%, and for pathological T1, T2, and T3 tumors were 4%, 2%, and 12%, respectively.

Estimated 3- and 5-year cumulative rates for distant recurrence, found in 15 patients (13%), were 13% and 18%, respectively (Fig. 1). The incidences

TABLE 1. *Univariate analyses of 26 clinicopathologic variables related to patient and tumor characteristics, treatment, and pathology*

Characteristic	No. of patients	3-Year cumulative local recurrence rate		3-Year cumulative distant recurrence rate	
		%	*P*	%	*P*
Sex					
Male	92	6	.87	16	.38
Female	28	7		4	
Age					
<60 y	71	10	.098	16	.57
≥60 y	49	0		9	
Distance of tumor from the anal verge					
<2.5 cm	21	0	.91	28	.034
≥2.5 cm	99	7		11	
Internal sphincter resection					
Partial	103	7	.27	11	.14
Complete	17	0		27	
Combined resection					
No	108	7	.33	11	.021
Yes	12	0		33	
Extended lateral pelvic lymph node dissection					
No	74	5	.58	10	.25
Yes	46	8		17	
Tumor size					
<3.5 cm	50	5	.86	9	.95
≥3.5 cm	70	7		15	
Tumor annularity					
<3/4	101	7	.35	10	.034
≥3/4	16	0		44	
Unknown	3				
Gross tumor contour					
Clear	117	6	.69	12	.17
Diffuse	2	0		50	
Unknown	1				
Gross anal margin					
<1 cm	28	4	.49	18	.72
≥1 cm	92	7		12	
Pathological depth of transmural invasion (pT)					
1	25	0	.027	9	.055
2	46	3		5	
3	49	13		23	
Pathological lymph node metastasis (pN)					
0	72	5	.24	5	<.0001
1	30	4		17	
2	18	0		50	
Histopathologic lateral pelvic lymph node metastasis					
Negative	112	6	.27	10	<.0001
Positive	8	13		53	
Pathological distant metastasis (pM)					
0	117	6	.73	12	.14
1	3	0		50	
Pathological UICC TNM stage					
I	50	0	.012	5	.0058
II	21	17		5	
III	46	8		25	
VI	3	0		50	
Histopathologic grade					
Well differentiated	59	5	.47	11	.029
Moderately differentiated	53	7		12	
Poorly differentiated	8	13		38	
Lymphovascular invasion					
Negative	48	3	.22	2	.0077
Positive	71	8		22	
Unknown	1				

TABLE 1. *continued*

Characteristic	No. of patients	3-Year cumulative local recurrence rate		3-Year cumulative distant recurrence rate	
		%	*P*	%	*P*
Perineural invasion					
Negative	99	6	.80	11	.022
Positive	13	8		40	
Unknown	8				
Mucin production					
Negative	108	5	.26	13	.80
Positive	12	17		18	
Focal dedifferentiation					
Negative	55	0	.018	7	.13
Positive	58	13		21	
Unknown	7				
Microscopic resection margins					
Negative	116	4	<.0001	14	.50
Positive	4	50		0	
Transfusion					
No	112	6	.47	12	.21
Yes	8	0		25	
Anastomotic leak					
No	105	7	.31	14	.55
Yes	15	0		8	
Adjuvant chemotherapy					
No	93	5	.47	8	.0008
Yes	27	8		38	
Preoperative serum CEA level					
<5 ng/mL	95	5	.73	14	.44
≥5 ng/mL	25	8		11	
Preoperative serum CA 19-9 level					
<37 U/mL	113	5	<.0001	13	.17
≥37 U/mL	7	29		25	

UICC, International Union Against Cancer; TNM, tumor, node, metastasis system; CEA, carcinoembryonic antigen.

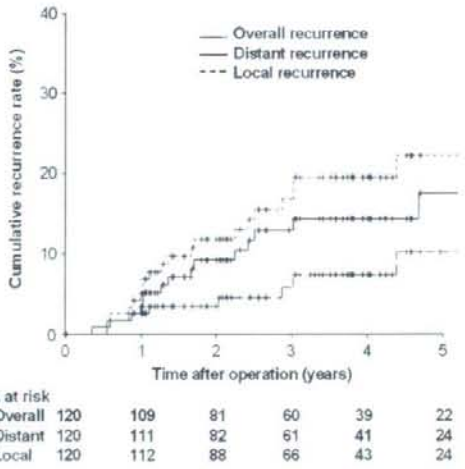


FIG. 1. Cumulative rates for overall recurrence, distant recurrence, and local recurrence among 120 patients undergoing intersphincteric resection.

of distant recurrence for stage I, II, III, and IV disease were 6%, 5%, 22%, and 33%, respectively.

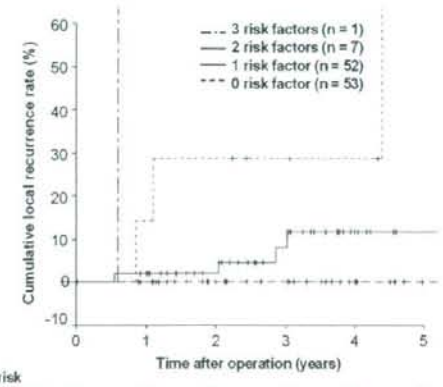
Univariate Analysis

Cumulative local recurrence was statistically significantly associated with pT, pathologic stage, focal dedifferentiation, microscopic resection margins, and the preoperative serum CA 19-9 level (Table 1).

Cumulative distant recurrence was statistically significantly associated with distance of the tumor from the anal verge, combined resection, tumor annularity, pN, lateral pelvic lymph node metastasis, pathologic stage, histopathologic grade, lymphovascular invasion, perineural invasion, and adjuvant chemotherapy (Table 1).

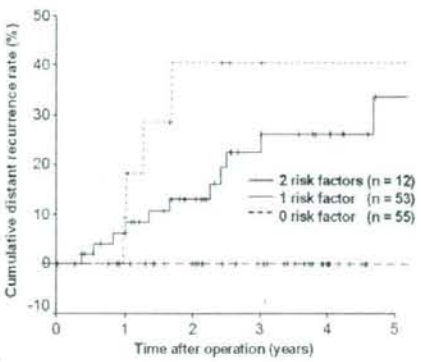
Multivariate Analysis

Multivariate analysis was performed for those factors associating statistically significantly with cumu-



No. at risk						
0 risk factor	53	50	39	32	23	14
1 risk factor	52	48	37	25	12	5
2 risk factors	7	5	4	2	1	
3 risk factors	1					

FIG. 2. Cumulative rates for local recurrence among 120 patients undergoing intersphincteric resection, according to the number of risk factors for local recurrence.



No. at risk						
0 risk factor	55	52	46	38	25	15
1 risk factor	53	44	32	21	15	8
2 risk factors	12	10	5	3	2	2

FIG. 3. Cumulative rates for distant recurrence among 120 patients undergoing intersphincteric resection, according to the number of risk factors for distant recurrence.

lative local recurrence and distant recurrence on univariate analysis. The multivariate analysis of four variables, excluding pathological stage, revealed that a positive microscopic resection margin (hazard ratio, 19 [95% confidence interval, 3.3–111], $P = .001$), positive focal dedifferentiation (22 [1.6–333]; $P = .021$), and preoperative serum CA 19-9 level of >37 U/mL (5.6 [1.1–29]; $P = .04$) were independently associated with an high local recurrence rate. The 3-year cumulative local recurrence rates for patients with zero, one, two, and three positive risk factors were estimated to be 0%, 8%, 29%, and 100%, respectively (Fig. 2).

Multivariate analysis for distant recurrence with seven variables, excluding pathological stage and lateral pelvic lymph node metastasis, revealed that pN1 or pN2 (hazard ratio, 13 [95% confidence interval, 3.0–53]; $P < .001$), poorly differentiated histology (6.4 [1.4–29]; $P = .015$), and distance of tumor from the anal verge less than 2.5 cm (3.7 [1.2–12]; $P = .026$) were independently associated with a poorer prognosis. The 3-year cumulative distant recurrence rates for patients with zero, one, and two positive risk factors were estimated to be 0%, 23%, and 40%, respectively (Fig. 3).

DISCUSSION

In the present study, we found by univariate analysis that pT, pathologic stage, focal dedifferentiation, microscopic resection margins, and preoperative serum CA 19-9 level were statistically significantly associated with local recurrence after ISR. On multivariate analysis, resection margins, focal dedifferentiation, and serum CA 19-9 level were independently associated with local recurrence. We could stratify high-risk patients by using these three factors. With two or more factors, the 3-year local recurrence rate was estimated to be 29% or higher. Therefore, when two or more risk factors identified here become apparent, neoadjuvant chemoradiotherapy should be provided to widen resection margins, or abdominoperineal resection should be substituted for ISR.

Preoperative radiotherapy or chemoradiotherapy are generally applied as standard neoadjuvant therapy for T3 and/or lymph node positive rectal cancers in general, in addition to total mesorectal excision. This is based on evidence that radiotherapy better reduces local recurrence and prolongs disease-free and overall survival when compared with surgery alone,[15,16] and that preoperative chemoradiotherapy is more effective for local control, less toxic, and more favorable for sphincter preservation than postoperative therapy.[17]

However, neoadjuvant and adjuvant therapy strategies to prevent local and distant recurrence after ISR have yet to be established. There is great variation in application of radiotherapy as a neoadjuvant against local recurrence, with reported rates ranging from 0% to 88%. Schiessel et al.[7] never provided radiation, even for T3 tumors, like us, whereas Rullier et al.[18] irradiate

all T3 tumors and some T1 and T2 lesions. The other authors provided radiotherapy to 25% to 75% of patients.[5,6,19,20] Although there is some dependence on selected patient population, tumor stage, and staging accuracy, usage also seems to reflect surgeons' preferences.

Radiotherapy for rectal cancer in general is well known to have the potential to cause damage to anorectal[21,22] and sexual[23,24] functions. In cases with very low rectal cancer necessitating ISR, if radiation is provided, damage to the sphincter is inevitable and may be catastrophic for some patients whose sphincters are also damaged by surgery. Chamlou et al.[20] reported that functional results after ISR are statistically significantly altered by preoperative radiotherapy. Ten (42%) of 24 patients who received preoperative radiotherapy of 45 Gy were incontinent as compared with 7 (15%) of 46 without radiotherapy ($P = .02$).[20] Therefore, more selective usage of radiotherapy in case with ISR seems to be preferable than in the general rectal cancer population.

Because preoperative radiotherapy is known to be more effective and less toxic,[17] it is preferable to stratify patients preoperatively. For this purpose, high-resolution MRI is accurate at estimating the resection margin status. In a prospective study of 408 rectal cancer patients, the MERCURY Study Group found that high-resolution MRI predicted a clear circumferential resection margin with an overall accuracy rate of 88%.[25]

Focal dedifferentiation,[11,12] also known as "tumor budding,"[26] can be accurately estimated by excisional or transanal submucosal biopsy[26] before ISR. The transanal submucosal biopsy allows specimens to be removed from the boundary zone between the tumor and the normal mucosa, including the submucosal tissue with exploratory excisional forceps. On the basis of this technique, the submucosal invasive frontal region can be predicted with up to 86% accuracy.[26]

Although the exact relation between CA 19-9 and local recurrence is unclear, CA 19-9 is easily assessable. Thus, preoperative performance of high-resolution MRI, excisional/submucosal biopsy, and serum CA 19-9 measurement is recommended for every candidate for ISR and warrants further investigation and validation.

In our previous study, we observed the 3-year cumulative local recurrence rate of 0% in patients with T1–T2 tumors and thus stated that meticulous performance of ISR allows local control without radiotherapy for such lesions.[8] In the present investigation, however, we found two cases (2.8%) with local recurrence. One patient with a pT1 tumor and a microscopic positive margin developed anastomotic recurrence 4.4 years after ISR. Another with a pT2 tumor had liver metastasis 10 months after ISR and underwent partial hepatectomy. This patient subsequently developed lung, liver, arm, and brain metastases, and also experienced pelvic recurrence in a seminal vesicle 2 years after ISR. Because the former case had a disease-positive surgical margin and the latter developed pelvic recurrence after fatal disseminated diseases, these two cases should be regarded as exceptional. Therefore, we still consider ISR without radiotherapy to be sufficient treatment for T1–T2 tumors in general. However, if patients have two or more of the risk factors we identified in the present study, neoadjuvant therapy should be considered.

Of the other studies on ISR, only three mentioned risk factors for local recurrence. Schiessel et al.[7] reported that only Dukes' stage and the T stage had an impact on local failure. Chamlou et al.[20] described overall survival to be statistically significantly influenced by pathologic TNM stage and pT on univariate analysis, but found no impact of the investigated factors on local recurrence. Analyzing data of 134 patients with rectal cancer located 2 to 11 (median 6.5) cm from the anal verge, undergoing not only ISR, but also low anterior resection or coloanal anastomosis, Paty et al.[27] found that mesenteric implants, a positive microscopic resection margin, a T3 tumor, perineural invasion, blood vessel invasion, and poorly differentiated histology were statistically significantly associated with pelvic recurrence on univariate analysis. In line with the previous reports, pT and pathologic staging were identified as prognostic factors in the present study as well. In addition, these variables are regarded as category I prognostic factors.[28] Therefore, they should be included with the above-mentioned list for further validation.

To our knowledge, risk factors for distant as opposed to local recurrence have hitherto not been studied separately and sufficiently. We here found that by univariate analysis, cumulative distant recurrence was statistically significantly associated with distance of tumor from the anal verge, combined resection, tumor annularity, pN, lateral pelvic lymph node metastasis, pathologic stage, histopathologic grade, lymphovascular invasion, perineural invasion, and adjuvant chemotherapy. By multivariate analysis, pathologic lymph node involvement, histologic grade, and tumor location were independently associated with distant recurrence. For patients with one or more positive risk factors, the 3-year cumulative distant recurrence rate was estimated to be 23% or

higher. Because adjuvant chemotherapy for stage II and III disease is believed to reduce distant recurrence by 30% to 40%, we recommend adjuvant chemotherapy for patients with one or more risk factors, in line with other authors' recommendations.[7,18,19]

As we showed, the risk factor profiles for distant and local recurrence differ, as do the prophylactic treatments. Therefore, local and distant recurrence should be analyzed separately. This is now feasible in the era of modern imaging technologies, with the availability of high-resolution MRI, multidetector row CT, and positron emission tomography-CT, which enable accurate diagnosis and differentiation of local and distant recurrence.

In conclusion, this retrospective exploratory study suggests that profiles of risk factors for local and distant recurrence after ISR may differ greatly. With local recurrence, the resection margin, focal dedifferentiation, and serum CA 19-9 level seem to be important. For distant recurrence, the lymph node status, histologic grade, and tumor location need to be taken into account. By using these factors, we may be able to stratify patients for neoadjuvant and adjuvant therapy and also for future clinical trials. These results warrant further investigation and validation with larger data sets or in future prospective trials according to the scoring system we have outlined.

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切除不能肝転移を有する大腸癌症例に対しFOLFOX療法施行後，切除可能となった2例

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