operations. The numbers of TNM stage were as follows: four stage 0, 52 stage I, 103 stage II (IIA, 83; IIB, 20), 95 stage III (IIIA, 6; IIIB, 60; IIIC, 29), and 44 stage IV. The postoperative adjuvant chemotherapy was performed on 75-year-old or less patient of stage III; its frequency was 31.4% of histological curative operations. The regimen of the postoperative adjuvant chemotherapy was taking of oral fluoropyrimidines (tegafur/uracil or 5′-deoxy-5-fluorouridine) for 1 year. The follow-up method is as follows after the operation. The patients of stages 0 and I have gone through the outpatient examination once a year and the tumor marker measurement until 5 years. When abnormality was seen, the computed tomography and/or the colonofiberscopy were performed. The patients of stage II were examined by computed tomography and the tumor marker measurement every 6 months for 2 years and were examined once a year until 5 years afterwards. The patients of stage III were examined by computed tomography and the tumor marker measurement every 4 months for 3 years and were examined once a year until 5 years afterwards. Eighty-six patients (28.9%) died while following up. Deaths due to the colorectal cancer occurred in 63 patients, deaths from other malignancies occurred in 5 patients, and deaths by another sickness occurred in 18 patients. The median follow-up period for surviving patients was 86 (16–141) months.

Cytopathology was determined before the colorectal cancer was excised. The technique of peritoneal lavage cytology was performed with the same method as in the gastric cancer in that the peritoneal lavage cytology was assumed as the prognostic factor [18–21]. The intraperitoneal lavage was performed with 200 ml saline added to 500 U of heparin immediately after the laparotomy. The collected samples were centrifuged at 1,500 rpm for 5 min, and the specimens were stained with Papanicolaou and May Giemsa. A pathology specialist assessed the specimens, and those graded as class IIIb or above of the Papanicolau classification (Table 1), which is used by a lot of pathologists in Japan, were judged to be lavage Cy-positive [lavage Cy (+)] [22]. The cytology results were examined with respect to the following factors based on the *7th edition of the Japanese General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus* [17]: clinical pathological factors, the peritoneal metastasis rate, the peritoneal recurrence rate of the peritoneal metastasis-negative cases, and the overall survival rate.

A diagnosis of postoperative peritoneum metastasis was proven by the positivity of cytology at the peritonitis carcinomatosa or intraabdominal specimen histologically. The histological specimen was excised at reoperation for recurrence, secondary colon cancer, and bowel obstruction.

The data were analyzed using the chi-square test and the Mann–Whitney *U* test. The overall survival rates were calculated by the Kaplan–Meier method and were compared using the log-rank test. A value of $P<0.05$ was considered to be statistically significant for these tests. The multivariate analysis of the pathological factors with respect to the overall survival rate was performed using the Cox's proportional hazards model.

Results

Lavage Cy (+) rate in respect to gender, age, and primary lesion localization

The lavage Cy (+) rate among all of the cases was 6.0% (18/298). The lavage Cy (+) rates for males and females were 3.6% (6/168) and 9.2% (12/130), respectively. The average ages of the lavage Cy (+) and lavage Cy (−) groups were 66.8±8.1 and 64.5±10.2 years, respectively. These values were not significantly different. The lavage Cy (+) rates according to the localization of the primary lesion were 9.1% (1/11) in the cecum and vermiform appendix, 6.3% (2/32) in the ascending colon, 7.7% (2/26) in the transverse colon, 16.7% (2/12) in the descending colon, 5.2% (5/97) in the sigmoid colon, 6.1% (5/82) in the upper rectum, and 2.6% (1/32) in the lower rectum. There were no statistically significant differences among these values (Table 2).

Lavage Cy (+) rate in cases with peritoneal metastasis at excision

In total, 15 cases (5.0%) had peritoneal metastasis at the time of excision of the primary lesion. The lavage Cy (+) rates in the cases with and without peritoneal metastasis were 46.7% (7/15) and 3.9% (11/283), respectively. This difference was statistically significant at the $P<0.05$ level. According to the 7th edition of the *Japanese General Rules for Clinical and Pathological Studies on Colorectal Cancer*, a small amount

Table 1 Papanicolaou classification

Class1	Absence of atypical or abnormal cells
Class2	Atypical cytology but no evidence of malignancy
Class3	Cytology suggestive of, but not conclusive for malignancy
Class3a	Probably benign atypia
Class3b	Malignancy suspected
Class4	Cytology strongly suggestive of malignancy
Class5	Cytology conclusive for malignancy

Table 2 Patients characteristics and peritoneal lavage cytology

	Cytology (+)	Cytology (−)	*P* value
Number of patients in respective cytology grade	18 (6.0%)	280	
	Class3b: 5 (1.7%)	Class1: 92 (30.9%)	
	Class4: 1 (0.3%)	Class2: 189 (63.4%)	
	Class5: 12 (4.0%)	Class3a: 0 (0%)	
Age (years, mean±SD)	67.3±9.2	64.4±10.3	0.2443
Gender			
Men	6 (3.6%)	162	0.0508
Women	12 (9.2%)	118	
Site of lesion			
Cecum	1 (9.1%)	10	0.7296
Ascending colon	2 (6.3%)	30	
Transverse colon	2 (7.7%)	24	
Descending colon	2 (16.7%)	10	
Sigmoid colon	5 (5.2%)	92	
Upper rectum	5 (6.1%)	77	
Lower rectum	1 (2.6%)	37	
Curability			
R0	4 (1.6%)	247	<0.0001
R1	3 (15.8%)	16	
R2	11 (39.3%)	17	

R0: histological curative operation

R1: Macroscopic curative but histological cancer remnant operation

R2: non-curative operation

of peritoneal metastasis near the primary lesion is classified in P1, a small amount of peritoneal metastasis that exists remotely from the primary lesion is classified in P2, and a large amount of peritoneal metastasis is classified in P3. The lavage Cy (+) rates according to the level of peritoneal metastasis were 0% (0/3) for P1, 42.9% (3/7) for P2, and 80% (4/5) for P3. Hence, the higher the level of peritoneal metastasis, the higher the lavage Cy (+) rate (Table 3).

Lavage Cy (+) rate according to clinical pathological factors in cases without peritoneal metastasis at excision

In total, 283 cases (95.0%) showed no peritoneal metastasis at the time of excision.

Histological type The lavage Cy (+) rate was 3.7% (10/270) in cases with well or moderately differentiated adenocarcinoma and 8.3% (1/12) in cases with poorly differentiated adenocarcinoma and mucinous carcinoma. There were no significant differences in histological type.

Table 3 Peritoneal metastases and peritoneal lavage cytology

	Cytology (+)	Cytology (−)	*P* value
P (−)	11 (3.9%)	272	<0.0001
P (+)	7 (46.7%)	8	
P1	0 (0.0%)	3	<0.0001
P2	3 (42.9%)	4	
P3	4 (80.0%)	1	

P: peritoneal metastasis

P1: small peritoneal metastasis in the vicinity

P2: small peritoneal metastasis in the distance

P1: multiple peritoneal metastases in the distance

Invasion depth The lavage Cy (+) rates were 1.5% (1/65) in cases classified as T2 or shallower, 2.8% (4/142) in T3 cases, 6.5% (4/62) in cases T4 cases, and 14.2% (4/14) in cases classified as T4 with additional organ infiltration. Thus, it appeared that the greater the depth of invasion, the higher the lavage Cy (+) rate. However, this trend was not statistically significant.

Lymph node metastasis The lavage Cy (+) rate was 1.9% (3/159) in cases classified as N0, 5.3% (4/76) in N1 cases, 7.4% (2/27) in N2 cases, 9.1% (1/11) in the main feeding arterial lymph node metastasis cases, and 14.3% (1/7) in the paraaortic lymph nodes. Thus, it appeared that the greater the level of lymph node metastasis, the higher the Cy (+) rate. However, this trend was not statistically significant.

Lymphovascular invasion The lavage Cy (+) rate was 5.1% (10/196) in cases with lymphatic-positive invasion and 1.2% (1/84) in cases without invasion. Thus, it appeared that the greater the level of lymphatic invasion, the higher the lavage Cy (+) rate. However, this trend was not statistically significant.

The lavage Cy (+) rate was 5.9% (8/135) in cases with vascular invasion and 2.1% (3/145) in cases without invasion. This difference was not statistically significant.

Table 4 Clinicopathological factors and peritoneal lavage cytology in patients excluding peritoneal metastases

	Cytology (+)	Cytology (−)	*P* value
Histology			
Well & Mod	10 (3.7%)	260	0.0543
Por & Muc	1 (8.3%)	11	
Depth			
Tis/T1/T2	1 (1.5%)	64	0.0881
T3	4 (2.8%)	138	
T4	4 (6.5%)	58	
T4 (additional organ infiltration)	2 (16.7%)	12	
Lymph node metastasis			
N0	3 (1.9%)	159	0.2067
N1	4 (5.3%)	72	
N2	2 (7.4%)	25	
Main feeding arterial lymph node	1 (9.1%)	10	
Paraaortic lymph node	1 (14.3%)	6	
Lymphovascular invasion			
ly(−)	1 (1.1%)	86	0.1816
ly(+)	10 (5.1%)	186	
v(−)	3 (2.0%)	145	0.1245
v(+)	8 (5.9%)	127	
Hepatic metastasis			
H (−)	8 (3.0%)	255	0.0047
H (+)	3 (15.0%)	17	

Well well-differentiated adenocarcinoma, *Mod* moderately differentiated adenocarcinoma, *Por* poorly differentiated adenocarcinoma, *Muc* mucinous adenocarcinoma, *ly* lymphatic invasion, *v* vascular invasion

Hepatic metastasis The lavage Cy (+) rate was 15.0% (3/20) in cases with hepatic metastasis and 3.0% (8/263) in cases without. This difference was statistically significant. The lavage Cy (+) rate was high in the multiple hepatic metastases (Table 4).

Peritoneal recurrence rate

The peritoneal recurrence rate was examined in 283 cases that did not have peritoneal metastasis. The peritoneal relapse rate among all of the cases was 2.5% (7/283). Five cases among seven were diagnosed by excising the peritoneum metastasis specimen at reoperation, which were for two bowel obstructions, one hepatic metastasis, one secondary colon cancer, and one local recurrence. Two cases were proven by positivity of cytology at the peritonitis carcinomatosa. The lavage Cy (+) rate was 9.1% (1/11) in the lavage Cy (+) group and 2.2% (6/272) in lavage Cy (−) group. This difference was not statistically significant (Table 5). The peritoneal recurrence rate in the 264 cases that underwent excision, the radical cure, was 2.3% (6/264); there was no peritoneal recurrence in the lavage Cy (−) group (0/7).

Clinical course of lavage Cy (+) cases

The clinical courses of 11 cases that were lavage Cy (+) without peritoneal metastasis at the time of excision were examined. Four of these cases experienced cancer death, and all of the cases had hepatic metastases. The operations were non-curative in three cases, one of whom already had multiple metastases in the peritoneum and liver and died 2 months after the operation (Table 6).

Overall survival rate

The overall survival rate in the 264 cases that underwent excision, the radical cure, was examined. The 5-year overall survival rates in the seven lavage Cy (+) cases and the 257 lavage Cy (−) cases were 83.3% and 71.4%, respectively, and these values were not significantly different (Fig. 1). The independent factors that contributed to the survival rate were the histological type and the depth of invasion. The peritoneal lavage cytology was not selected by the multivariate analysis as prognostic factor (Table 7).

Table 5 Peritoneal recurrence and peritoneal lavage cytology

	Peritoneal recurrence (+)	Peritoneal recurrence (−)	*P* value
Lavage cytology (+)	1 (9.1%)	10	0.2446
Lavage cytology (−)	6 (2.2%)	266	

Table 6 Clinical courses of patients with positive peritoneal lavage cytology

Age	Gender	Curability	Cytology	H	Site	Histology	T	N	Stage	ly	v	Prognosis	P rec.	Cause of death
67	F	R0	4	0	Low. Rec	w	T3	0	2	0	+	55 months, Alive	(−)	
62	F	R0	3b	0	Low. Rec	w	T3	1	3B	+	0	42 months, Alive	(−)	
64	F	R1	5	0	Upp. Rec	w	T3	1	3B	+	0	31 months, Alive	(−)	
55	M	R0	3b	0	S	w	T2	0	1	+	0	24 months, Alive	(−)	
63	M	R1	5	3	Low. Rec& S	w	T4	1	4	+	+	11 months, Alive	(−)	
74	F	R2	3b	0	Low. Rec	m	T4	4	4	+	+	10 months, Alive	(−)	
61	F	R0	5	0	A	m	T4	2	3C	+	+	9 months, Alive	(−)	
68	F	R2	3b	3	S	m	T3	1	4	+	+	26 months, Death	(−)	Hepatic metastasis
86	F	R2	5	0	Upp. Rec	w	T4	2	3C	+	+	12 months, Death	(−)	Hepatic metastasis
70	F	R1	5	0	Upp. Rec	muc	T4	0	3B	+	+	9 months, Death	(−)	Hepatic and lung metastasis
65	M	R2	5	3	D	w	T4	2	4	+	+	2 months, Death	(+)	Hepatic and peritoneal metastasis

Low. Rec lower rectum, *Upp. Rec* upper rectum, *S* sigmoid colon, *A* ascending colon, *D* descending colon, *w* well-differentiated adenocarcinoma, *m* moderately differentiated adenocarcinoma, *muc* mucinous carcinoma, *P rec.* peritoneal recurrence

Discussion

There is currently no effective treatment for peritoneal metastasis of colorectal cancer. It is often diagnosed at a terminal stage and treated by allopathy. However, cases often show unexpected longevity compared with gastric or pancreatic cancer. Operations on intestinal obstructions might present an opportunity for diagnosis. It is therefore of great importance to predict peritoneal recurrence at an early stage and to adopt a strategy that will impede its progress.

The lavage Cy status is widely used as a predictive factor of peritoneal metastasis in cases of gastric cancer. Many reports have assumed that this is an independent prognostic regulatory factor in multivariate analyses [19, 21]. In many medical facilities, changes to operative procedures and intraperitoneal chemotherapy are made depending on the cytological findings [18–21]. Lavage cytology plays a prominent role in treatment policy decisions for gastric cancer. However, some reports have evaluated the efficacy of peritoneal lavage cytology as a prospective factor of peritoneal metastasis or local recurrence in colorectal cancer cases [8–12]. On the other hand, there were reports that the peritoneal lavage cytology had no detectable impact on survival and peritoneal recurrence [13–16]. The utility of peritoneal lavage cytology has remained controversial until now. The difference in the rate of peritoneal metastasis formation in gastric and colorectal cancers is thought to be due to differences in their biological properties [16, 24]. The rate has been reported as about 20% [9, 10, 12, 13, 15] for colorectal cancer compared with about 40% for T4 gastric cancer [16, 19, 20, 23], while our current study gave a value of 10.5%. It is assumed that cancer cells separate easily from tumors that are exposed on the serosa and also form peritoneal metastases comparatively easily in smaller cancer cell nests in gastric cancer. By contrast, it is thought that peritoneal metastases are not easily formed, even if cancer cells separate to the intraperitoneal cavity, in cancers of the large intestine. The factors regulating peritoneal metastasis seem to involve abnormalities of the characteristics related to hematogenic metastasis. These are associated with all stages in the processes of establishing metastases, including the separation from the original lesion, blood vessel infiltration by the cancer cells, bonding

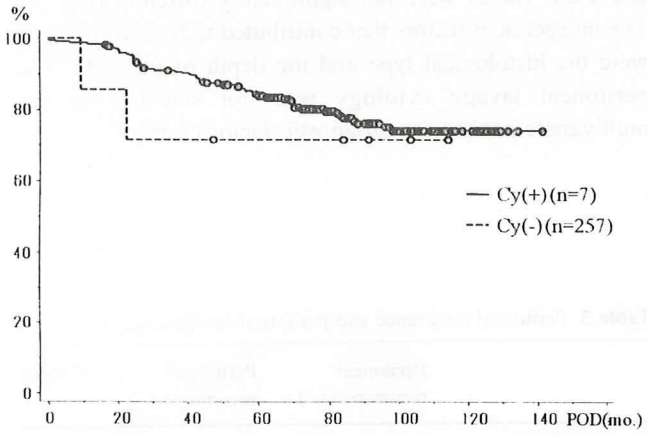

Fig. 1 Cumulative survival rates in patients underwent curative operation

Table 7 Multivariate analysis using the Cox's proportional hazards model between survival and risk of clinicopathological factors

Variable	Hazard ratio	95% Confidential interval	χ^2	*P* value
Peritoneal lavage cytology (+/−)	1.615	0.390–6.678	0.438	0.5082
Histology (por&muc/well&mod)	3.121	1.392–6.997	7.635	0.0057
Depth of invasion (T4/Tis-3)	2.668	1.519–4.688	11.650	0.0006
Lymph node metastasis (+/−)	1.252	0.712–2.202	0.609	0.4350
Lymphatic invasion (+/−)	1.340	0.645–2.786	0.615	0.4328
Vascular invasion (+/−)	1.766	0.960–3.249	3.348	0.0673

of the cancer cells to the endothelium of the target organ, permeation of the blood vessels from the outside, and proliferation within the target organs. Patients with high levels of the sialyl Lewis-X antigen have a high rate of hepatic metastasis [25]. A previous report suggested that a high level of part of the sugar chain was also associated with peritoneal metastasis [26]. We await further clarification of the mechanism of peritoneal metastasis of colorectal cancer by molecular biology techniques in the future.

There can be a problem with the accuracy of the assessment of cytology results. In the current study, the lavage Cy (+) rate in all cases is 6.0%, which differs greatly from the values of 20–25% given in some reports [9, 10]. The data of this study can be trusted because the lavage Cy (+) rate had a high T factor. However, in one T2, three T3, and one lower rectal cancer case, there was a suggestion of false positives. When the details of these cases were examined, the T2 and lower rectal cancer cases were found to have lymphatic invasion. Of the three T3 cases, one had multiple hepatic metastases and one had lymphatic invasion; however, the remaining case had no lymphatic invasion and its histology revealed well-differentiated adenocarcinoma. In the four cases described above, there was a possibility that the cancer cells had originated from the lymph duct or the liver metastasis nest. However, there was a possibility of false positive results in the case with T3 and negative lymphatic invasion. Regarding the other background factors, the more advanced the lymph node metastasis, the higher the lavage Cy (+) rate. This was similar to the finding of a previous report on gastric cancer [27]. It suggested the possibility that the cancer cells are released not only by dropout from the source of origin but also from the metastatic lymph node and the lymph duct as routes of peritoneal metastasis. A previous report showed that lavage Cy (+) rate was according to the histological type by univariate analysis [14]. However, there was no difference between mucinous carcinoma/poorly differentiated adenocarcinoma and well/moderately differentiated adenocarcinoma in this report. This finding was attributed to the fact that the number of cases of mucinous carcinoma/poorly differentiated adenocarcinoma was small and five of the eight cases had lower rectal cancer of mucinous carcinoma. The lavage Cy (+) rate was significantly high for liver metastasis. The lavage Cy positivity was seen in multiple hepatic metastases, but not in single hepatic metastasis. This suggests the possibility that the cancer cell dropout from the hepatic metastasis nest caused the peritoneal metastasis.

A high tendency towards peritoneal recurrence was seen in the lavage Cy (+) cases, although this was not statistically significant. The lavage Cy status was not a useful predictive factor of peritoneal recurrence. This might have been due to a problem with the method used to gather the original specimens. Hase et al. [28] reported that when cytology was performed before and after excision, the peritoneal recurrence rate was 50% for both positivities, whereas it was 16.7% for the positivity before excision alone. One problem with cytology after excision is the possibility of the outflow of cancer cells from the cut lymph duct [29], meaning that the operative procedure might influence the results.

This study is limited in the diagnosis of the peritoneal recurrence. The diagnosis of the peritoneal recurrence was limited to the one that histology proof was done in this study. Four cases of seven in this study presented symptoms of the peritoneal metastases of the ileus and the peritonitis carcinomatosa, etc. The metastasis in the liver and the local recurrence are suggestive of peritoneal recurrence based on the image. However, the peritoneal recurrence was discovered by chance in the case of the secondary cancer. It is suggested that an actual peritoneum metastasis rate is higher because the symptom of the ileus, etc. is not presented in a minute metastasis.

This also suggests the possibility that cytology might be limited by microscopy. Lavage Cy (+) status was not a prognostic factor in curable cases in this study. This suggests that lavage cytology might not be a risk factor for recurrences such as peritoneal, liver, and lung metastases. Previous studies have reported similar findings [16, 30]. Several reports have suggested that immunohistological techniques for the detection of intraperitoneal free cancer cells might be more accurate and useful than cytology. It has also been reported in recent years that a supplementary diagnosis to cytology can be achieved accurately and promptly by the reverse transcription–polymerase chain reaction method [31–34]. Owing to the

rapid advances, gene diagnosis looks likely to be ready for practical applications in the near future.

It is difficult for small metastases to be distinguished in computed tomography and magnetic resonance images. It has been reported that sensitivity and accuracy have been improved in recent years by new diagnostic techniques such as the application of 8-fluoro-deoxy-glucose positron emission tomography [35–37]. It will be necessary to monitor the advances in the accuracy of imaging diagnostics in the future. Moreover, there will be a need to reexamine these methods, particularly with respect to small peritoneal metastases and prospects of recurrence, in light of the expected improvements in diagnostic imaging. In conclusion, intraperitoneal lavage cytology before the resection of colorectal cancer was not a useful predictor of peritoneal recurrence.

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Original Paper

Surgical Strategy for Local Recurrence after Resection of Rectal Cancer

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ABSTRACT

Background/Aims: To evaluate surgery for local recurrence after rectal cancer resection.

Methodology: In total, 76 patients with local recurrence after rectal cancer resection were enrolled between 1978 and 1998. Of these, 61 underwent curative resection. Outcomes were assessed according to treatment. Recurrence was classified as visceral or parietal based on preoperative computed tomography or magnetic resonance imaging.

Results: The 5-year survival rates were 17.8%, 25.9%, and 36.9% for patients who underwent total pelvic exenteration, abdomino-perineal resection, and local resection, respectively. Of the 61 patients who underwent curative resection, 18 (29.5%) showed visceral recurrence and 43 (70.5%) showed parietal recurrence. Among patients with visceral recurrence, 9 (50%) underwent total pelvic exenteration, 6 (33.3%) underwent abdomino-perineal resection, and 3 (16.7%) underwent local resection. Among patients with parietal recurrence, 27 (62.8%) underwent total pelvic exenteration, 4 (9.3%) underwent abdomino-perineal resection, and 12 (27.9%) underwent local resection. Mucinous adenocarcinomas were most common among patients with parietal recurrence. Overall 5-year survival rates were 64.9% and 14.0% for patients with visceral and parietal recurrence, respectively.

Conclusions: Curative resection was effective in rectal cancer patients with visceral recurrence. Novel systemic chemical radiotherapy should be considered for patients with parietal recurrence.

KEY WORDS:
Local recurrence; Rectal cancer; Re-excision; Recurrence pattern

ABBREVIATIONS:
Total Pelvic Exenteration (PE); Abdomino-Perineal Resection (AMP); Local Resection (LE); Continuous Hyperthermic Pelvic Peritoneal Perfusion (CHPPP)

INTRODUCTION

Local recurrence of rectal cancer occurs in 10 to 30 percent of patients who have undergone potentially curative resection (1-4). Surgeries such as pelvic exenteration (PE) and abdominosacral resection, radiation, chemotherapy, cryosurgery, and hyperthermia have been reported as effective in these cases. However, so far no consensus has been reached on the optimal therapeutic method for local recurrences. Previously, we reported on the outcome of total PE for locally recurrent rectal cancer. The 5-year survival rate was 31.6 percent in patients undergoing curative resection and 0 percent in patients undergoing palliative surgery (5). While the overall 5-year survival rate in our previous study was only 14.1 percent, the postoperative morbidity rate was 77.8 percent, and in-hospital death occurred in 13.3 percent of the patients. New approaches, such as FOLFOX (in which fluorinated pyramidine is combined with a platinum-containing drug) and molecule-targeting therapeutic drugs, have reportedly demonstrated excellent results with recurrent colorectal cancers that cannot be excised, and great advances have been made in drug therapies in recent years (6-9). However, the long-term outcomes of these novel techniques have so far remained uncertain. In the current study, the long-term outcomes of total PE, abdomino-perineal resection (AMP), and local resection (LE) in rectal cancer patients with local recurrence were analyzed 10 or more years after surgery.

METHODOLOGY

In total, 76 rectal cancer patients with local recurrence, all of whom had no distant metastases at the time of preoperative diagnosis, underwent surgery at Yokohama City University Hospital, Japan, between 1978 and 1998. Of these, 61 patients were treated by curative resection **(Table 1)**. The remaining 15 patients underwent non-curative resection, which was diagnosed intraoperatively: 9 of these patients had a positive margin of resection, 3 patients had liver metastasis, and 3 patients had peritoneal metastasis. The data from the 61 patients who were treated by curative resection were analyzed as described below.

Initially, the overall 5-year survival rates were compared according to the surgical procedure, the presence of adjuvant therapy, the histological type, and the recurrence pattern. A multivariate analysis

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TABLE 1 Demographics and Characteristics of the Locally Recurrent Rectal Cancer Patients who underwent Curative Resection

Age		59.0±11.3
Gender	Male : Female	39:22
Mode of recurrence	Visceral	18
	Parietal	43
Operative procedure	Pelvic exenteration (PE)	36
	Rectal amputation (AMP)	10
	Local excision (LE)	15
Adjuvant therapy	Preoperative radiation	7
	Continuous hyperthermic pelvic peritoneal perfusion	18
Histology of primary tumor	Well-differentiated adenocarcinoma	24
	Moderately-differentiated adenocarcinoma	27
	Poorly-differentiated adenocarcinoma	1
	Mucinous carcinoma	9

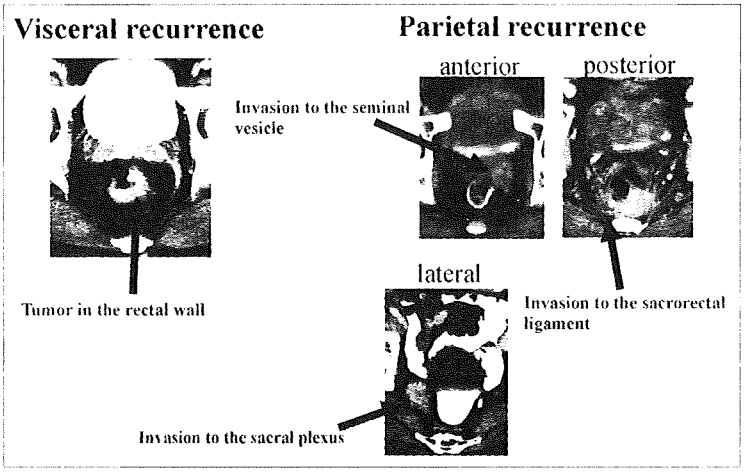

FIGURE 1 Patterns of local recurrence. The panel on the left shows the visceral pattern with the view centered on the rectum. The panel on the right shows the parietal pattern from three views (anterior, posterior, and lateral).

of the risk factors for survival was performed. The surgical procedure was classified as PE (defined as total PE with urinary diversion and rectal amputation), AMP (defined as resection of the perineum including the anus), or LE (defined as resection of the perineum not including the anus). Anterior resection and the Hartmann's procedure were included within LE. The adjuvant therapies were preoperative radiotherapy and intraoperative continuous hyperthermic peritoneal perfusion in the pelvis (CHPPP); the latter was performed routinely for local recurrent rectal cancer from 1992 to 1999. The mean dose of preoperative radiotherapy was 48.6Gy (with a minimum dose of 40Gy and a maximum dose of 50Gy). The CHPPP regimen included the pelvic reflux of 200mg cisplatin, 20mg mitomycin C, and 1,000mg 5-FU with warm water at 42.5°C after resection of the primary lesion. The histological types were compared between the differentiation levels. The recurrence pattern was classified as either visceral, in which the tumor mass formation including the rectal wall was attributed to recurrence at the site of anastomosis and/or implantation at the colorectal wall, or parietal, in which the tumor mass formation including the parietal side of the pelvic cavity was attributed to a positive circumferential radial margin at the site of resection of the primary lesion, implantation at the pelvic wall, and/or lymph-node recurrence **(Figure 1)**. Of the 61 patients, 18 (29.5%) showed visceral recurrence and 43 (70.5%) showed parietal recurrence. Among the patients with visceral recurrence, 16 had anastomotic recurrence and 2 had implantation at the rectum. Among the patients with parietal recurrence, 22 had a positive circumferential margin, 14 had implantation at the pelvic wall, and 7 had lymph-node recurrence.

Among the patients with visceral recurrence and parietal recurrence, PE was employed in 9 (50%) and 27 (62.8%), AMP was employed in 6 (33.3%) and 4 (9.3%), and LE was employed in 3 (16.7%) and 12 (27.9%), respectively. There were no significant differences in the frequencies of these operative procedures.

We also compared the histological findings between the different recurrence patterns (visceral *vs.* parietal), and compared the local re-recurrence frequency between the different operative procedures.

The data were analyzed by the chi-square test and the unpaired *t*-test. The overall 5-year survival rates were compared by the Kaplan-Meier method. A multivariate analysis of the factors that affected the survival rate was performed using the Cox proportional hazard model. Differences were considered statistically significant at the $P<0.05$ level.

RESULTS

Comparisons of 5-year Survival Rate

The overall 5-year survival rate for all of the patients was 26.0 percent **(Figure 2)**.

TABLE 2 Multivariate Analysis of the Prognostic Factors influenced on the Overall Survival by the Cox-proportion Hazard Model

	Coefficient	95% Confidence interval		Exp (coefficient)	*p* value
Parietal/visceral	1.326	1.565	9.062	3.766	0.0031
Op: PE/Others	0.769	1.111	4.195	2.158	0.0232
Histology: Well/Others	-0.297	0.389	1.420	0.743	0.3684
Preop radiation/none	-0.496	0.207	1.788	0.609	0.3669
CHPPP/none	-0.009	0.516	1.905	0.991	0.9793

PE: Total pelvic exenteration; Well: Well-differentiated adenocarcinoma; CHPPP: Continuous hyperthermic pelvic peritoneal perfusion.

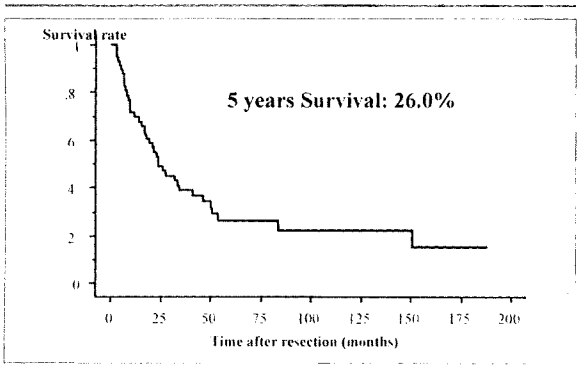

FIGURE 2 Cumulative overall survival curve for all patients. The 5-year survival rate was 26.0 percent.

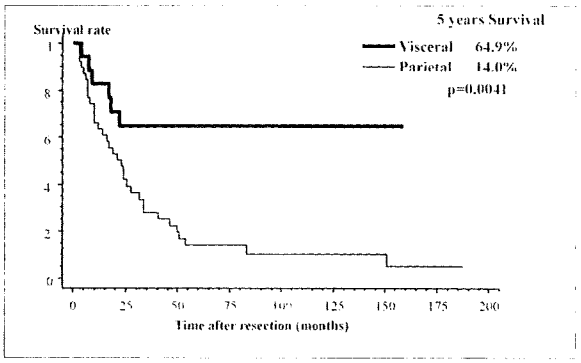

FIGURE 3 Cumulative survival curve according to the patterns of local recurrence. The 5-year survival rate was 64.9 percent for the visceral pattern and 14.0 percent for the parietal pattern. This difference was statistically significant (P=0.0041).

Surgical Procedures

PE, AMP, and LE were performed in 36, 10, and 15 patients, respectively; the curative resection rates for these patients were 80 percent, 83.3 percent, and 78.9 percent, respectively. The 5-year survival rates for the patients treated by PE, AMP, and LE were 17.8 percent, 25.9 percent, and 36.9 percent, respectively. There was a statistically significant difference in the 5-year survival rates for patients treated by PE and AMP (P=0.0236).

Adjuvant Therapy

Preoperative radiotherapy was administered to 7 patients (11.5%). The 5-year survival rates for the patients who did and did not receive preoperative radiotherapy were 40.0 percent and 25.3 percent, respectively; this difference was not statistically significant. In total, 18 patients (29.5%) were treated by the CHPPP regimen. The 5-year survival rates for the patients who did and did not receive CHPPP were 17.5 percent and 31.0 percent, respectively; this difference was not statistically significant.

Histological Type

Overall, 24 patients had well-differentiated adenocarcinoma, 27 patients had moderately differentiated adenocarcinoma, 1 patient had poorly differentiated carcinoma, and 9 patients had mucinous carcinoma. The 5-year survival rates for the patients with well-differentiated adenocarcinoma, moderately differentiated adenocarcinoma, poorly differentiated adenocarcinoma, and mucinous carcinoma were 32.6 percent, 17.6 percent, 100 percent, and 18.2 percent, respectively; these differences were not statistically significant.

Recurrence Patterns

The 5-year survival rates for the patients with visceral and parietal recurrence were 64.9 percent and 14.0 percent, respectively (P=0.0041; **Figure 3**). Within the group with visceral recurrence, the 5-year survival rate was 44.4 percent for patients treated by PE and 87.5 percent for those treated by all methods except PE. Patients treated by PE tended to have a poor prognosis (P=0.0871). Within the group with parietal recurrence, the 5-year survival rate was 7.4 percent for patients treated by PE and 23.8 percent for those treated by all methods except PE. Again, those treated by PE tended to have a poor prognosis (P=0.0568; **Figure 4**).

Multivariate Analysis

Parietal recurrence and PE were both identified as factors that had a significant effect on prognosis **(Table 2)**.

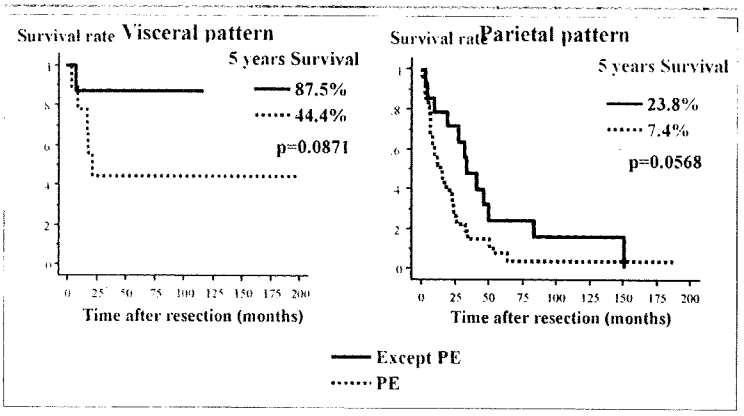

FIGURE 4 Cumulative survival curve according to the surgical procedures for each pattern of local recurrence. A statistically significant difference was found between the group treated by PE and the group treated by all methods except PE (P=0.0871) for the visceral pattern, but not for the parietal pattern (P=0.0568).

TABLE 3 Pathological Findings according to Local Recurrence Pattern

	Visceral (n=18)	Parietal (n=43)	*P* value
Rec. tumor diameter	5.7±3.4cm	5.2±2.2cm	0.5898
Primary tumor diameter	4.7±1.8cm	5.0±2.5cm	0.6181
Histology			0.0087
Well	12	12	(Well/Mod *vs.* Poor/ Muc)
Mod	6	22	
Poor	1	0	
Muc	0	9	

Well: Well-differentiated adenocarcinoma; Mod: Moderately-differentiated adenocarcinoma; Poor: Poorly-differentiated adenocarcinoma; Muc: Mucinous carcinoma.

Comparison of Histological Findings by Recurrence Pattern

There were no differences in tumor diameter between the groups with the two recurrence patterns; however, many cases showed less-differentiated histology within the group with parietal recurrence (P=0.0087; **Table 3**).

Comparison of Local Re-Recurrence Frequency by Recurrence Pattern and Operative Procedure

Local re-recurrence occurred in 31 patients (50.8%), 4 (22.2%) of whom showed visceral recurrence and 27 (62.8%) of whom showed parietal recurrence (P=0.0051). Local re-recurrence was observed in 22 (61.1%), 3 (30.0%), and 6 (40.0%) of the patients treated by PE, AMP, and LE, respectively; these differences were not statistically significant (P=0.1379).

DISCUSSION

Surgical resection for local recurrence of rectal cancer has been reported to have variable outcomes. Estes *et al.* reported a 5-year survival rate of 49 percent in patients undergoing PE for local recurrences. Wanebo *et al.* (10) reported a relatively favorable 4-year survival rate of 33 percent for patients undergoing PE. However, Wiggers *et al.* (11) reported a 5-year survival rate of 0 to 5 percent, and a median survival period of 3.5 to 13 months. Ike *et al.* (5) from our department reported on the outcomes of PE according to the extent of radical resection, and found that the 5-year survival rate of patients undergoing cur-A surgery (in which the tumor was excised with no histological residue) was 31.6 percent; these investigators recommended that PE for local recurrences should be conducted only in patients for whom the long-term outcome could be estimated, because the complication rate could be as high as 77.8 percent. By contrast, Sakai *et al.* (12) reported no cases of 5-year survival with any treatment other than surgery (that is, chemotherapy, radiation chemotherapy, or hyperthermoradiation chemotherapy). At present, surgical resection appears to be the only treatment modality that can achieve long-term survival. The findings of these papers indicated that the patients had a poor surgical outcome because the recurrence was too advanced to achieve curable resection even by PE.

Our current study revealed a significant difference in the long-term outcome between patients who underwent PE and AMP; however, there were no significant differences in the frequency of local re-recurrence among the patients undergoing the three surgical procedures investigated. These results could not be analyzed by a simple comparison, because the background characteristics of the patients were different. There were no differences in the curative resection rates among the different surgical procedures.

The recurrence patterns were classified as visceral or parietal for the purposes of our analysis. Yamada *et al.* (13) reported that the extent of pelvic infiltration was correlated with the prognosis. The causes of local recurrence are thought to include circumferential radial margin shortage, cancer cell scatter, pelvic side wall lymph-node recurrence, and anastomotic cancer cell implantation. However, if the local recurrence has reached a certain size before diagnosis, it can be difficult to determine the exact cause. We examined whether the recurrence occurred at the center of the rectum or the wall of the pelvis. Our findings highlighted a difference in the treatment results. We identified a significant difference in the long-term outcome depending on the recurrence pattern: the 5-year survival rate in patients with visceral recurrence was 64.9 percent, while that in patients with parietal recurrence was 14.0 percent. We therefore believe that the local recurrence type should have a significant influence on the treatment method that is selected. The clinicopathological characteristics of the two groups differed only in the frequencies of mucinous carcinoma and local re-recurrence, which were higher in patients with parietal recurrence.

The efficacy of preoperative radiotherapy as an adjuvant therapy remains unclear. We were unable to reach a definitive conclusion based on the results of the present study alone, because only 7 patients underwent preoperative irradiation and their background characteristics differed considerably from those of the patients who did not undergo preoperative irradiation. Further studies with larger numbers of patients are needed to clarify this point.

In our hospital, CHPPP was used to prevent local recurrences in 120 patients with T3 or T4 rectal cancer between October 1992 and February 1999. Among 111 patients treated with cur-A and cur-B (the latter being ablative surgery in which there is a histological residue although is not visible to the naked eye), 8 (7.2%) developed local recurrences; this frequency was not significantly different from that observed in 131 patients (8 patients; 6.1%) who did not undergo CHPPP during the same period. As a consequence, this approach was not used after March 1999.

The efficacy of chemotherapy as an adjuvant therapy or major treatment could not be assessed in the current study, because the subjects had been treated prior to 1998, at a time when there were no effective evidence-based regimens. More recent reports have demonstrated the remarkable efficacy of chemotherapy for the treatment of colorectal cancer, and the appearance of new regimens, such as FOLFOX and FOLFIRI (in which irinotecan is used in combination with fluorinated pyrimidine), has markedly increased the median survival rates of cases of advanced colorectal cancer with recurrence (6-8). Combination therapy with molecule-targeting drugs is also expected to improve the outcomes in the future (9). Thus, a treatment 'generation gap' seems to have arisen over a period of just a few years. If the combined use of new regimens with molecule-targeting therapeutic drugs or multimodality oncotherapy

including radiation yields a superior outcome to that of surgical resection for resectable local recurrences of rectal cancer, we predict that the need for re-operation will decline in the future.

CONCLUSIONS

Curative resection appears to be effective for cases of rectal cancer with visceral recurrence. By contrast, in patients with parietal recurrence, which is associated with a high frequency of poorly differentiated adenocarcinoma including mucinous carcinoma, resection alone is often inadequate for local control; multimodality oncotherapy, including systemic chemical radiotherapy, might be needed for the treatment of these patients.

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ORIGINAL ARTICLE

Lymphatic vessel invasion detected by monoclonal antibody D2-40 as a predictor of lymph node metastasis in T1 colorectal cancer

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Abstract
Objective When selecting patients who are at high risk for lymph node metastasis, the detection of lymphatic vessel invasion (LVI) is important. We investigated LVI detected by D2-40 staining as a predictor of lymph node metastasis in T1 colorectal cancer.
Materials and methods Clinicopathological factors including LVI were investigated in 136 patients who underwent colectomy with lymph node dissection for T1 colorectal cancer. We used immunostaining with monoclonal antibody D2-40 to detect LVI.
Results Lymph node metastases were found in 18 patients (13.2%), and LVI were detected in 45 (33%); lymph node metastasis was more frequently observed in LVI-positive groups (13/45 vs 5/91, $p<0.001$). Both univariate and multivariate analyses revealed that LVI detected by D2-40 and a poorly differentiated histology at the invasion front were independent risk factors of lymph node metastasis.
Conclusion LVI detected by D2-40 is important for the prediction of lymph node metastasis.

Keywords Lymphatic vessel invasion · D2-40 · Lymph node metastasis · T1 colorectal cancer

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Introduction

With advances in endoscopic techniques and instruments, a local excision with endoscopic submucosal dissection or an endoscopic mucosal resection has become a safe and effective treatment for high-grade dysplasia and early colorectal cancer [1–3]. For T1 colorectal cancer patients with unfavorable histological features, however, additional bowel resections are required because of the risk of lymph node metastasis, even if their tumors are completely removed by local excision. The rate of lymph node metastasis among T1 colorectal cancer cases is approximately 10%, a great many of which can potentially be cured by local excision [4–7]. To avoid over-treatment, patient selection through histopathological estimations of locally excised specimens is important. The pathologic features of the primary tumor provide useful information about the risk of lymph node metastasis and could be used to select patients for additional bowel resection.

Several studies have reported that lymphatic vessel invasion (LVI) is one of the histological risk factors for nodal metastasis of T1 colorectal cancer [5, 8–14]. However, it is difficult to detect LVI with conventional hematoxylin and eosin (HE) staining. Controversies over the detection of LVI arise mainly from the difficulty in visualizing the lymphatic vessel wall [14]. A monoclonal antibody, D2-40, is available to identify the lymphatic vessels, especially when distinguishing them from blood vessels [15, 16]. We introduced D2-40 staining for the diagnosis of LVI as the routine staining of colorectal cancer via the examination of one block of tissue at the maximum invasive depth.

In this study, we investigated the presence of LVI detected by D2-40 staining and whether LVI could still be a risk factor for nodal metastasis in T1 colorectal cancer.

Materials and methods

From September 2002 to December 2007, there were 218 consecutive patients with T1 colorectal cancer treated with colectomy and regional lymph node resections at the Shizuoka Cancer Center. Eighty-two patients were excluded from this analysis because of multiple colorectal cancer (12), familial adenomatous polyposis (one), and LVI and blood vessel invasion (BVI) diagnosed without D2-40 immunostaining or Elastica van Gieson stain (69). The total of 136 patients with T1 colorectal cancer included in this study was comprised of 82 males and 54 females with a mean age of 65 (29–91)years. All clinical data for patients, including tumor location and type of treatment, were collected from our clinical database. One hundred patients underwent laparotomy with lymph node dissection, while 36 were treated with local excision followed by laparotomy. No patients received chemotherapy or radiotherapy preoperatively.

The resected materials were fixed with 10% formalin, mounted in paraffin, thinly sliced, and stained with HE. Regional lymph nodes resected by surgery were routinely examined by one section through the largest cut surface of each node. All cases were assessed as to macroscopic type, size (maximum diameter), histological grade (described below), number of lymph nodes harvested, depth of submucosal invasion, poorly differentiated histology at the invasion front, LVI, BVI, and nodal status. Patients grouped by histological grade were classified as favorable (well and moderately differentiated adenocarcinoma) or unfavorable (mucinous carcinoma and poorly differentiated adenocarcinoma). As for pedunculated lesions, the depth of submucosal invasion was measured as the vertical distance from the baseline (Haggitt's level 2 line) to the invasion front of the tumor [17]. For the non-pedunculated type, the depth was measured as the vertical distance from the deepest muscularis mucosae to the invasion front [12]. If the deepest portion of the tumor was located below the baseline for the pedunculated type or if the depth of submucosal invasion was greater than 1,000 μm for the non-pedunculated type, the cases were classified as deep submucosal invasion, with other cases classified as superficial submucosal invasion. Poorly differentiated histology at the invasion front was defined as the presence of small nests of cancer cells at that site. Pathological parameters of histological grade, the depth of submucosal invasion, and histology at the invasion front of the tumor were examined on HE-stained sections. For the examination of BVI and LVI, one paraffin block of maximum invasive depth was chosen from each case. We used Elastica van Gieson stain for the detection of BVI and immunostaining with monoclonal antibody D2-40 to detect LVI. Immunohistochemistry of D2-40 was performed using the EnVision+ system (DAKO Cytomation, Glostrup, Denmark). After deparaffinization and rehydration of a 5-μm-thick section preparation, the section was treated with 0.3% H_2O_2 in 100% methanol for 20 min for a blockade of intrinsic peroxidase activity, followed by rinsing in running water for 5 min and antigen retrieval by incubation at 121°C for 10 min in 0.01-M citrate buffer. After cooling down and immersing in Tris–HCl buffer, the sections were preincubated with normal horse serum (Invitrogen, 1:100) for 1 h. The sections were then reacted with anti D2-40 monoclonal antibody (DAKO Cytomation, 1:100) for 30 min and were rinsed with Tris–HCl buffer; they were then reacted for 30 min with goat anti-mouse immunoglobulins conjugated to a peroxidase-labeled dextran polymer (EnVision+, DAKO Cytomation). After washing three times with wash buffer, the section products were developed by immersing each one in a 3.3'-diaminobenzidine tetrahydrochloride solution. Nuclei were lightly counterstained with hematoxylin. LVI was considered positive when at least one atypical cell recognized microscopically as a cancer cell was visible inside the D2-40 stained lymphatic lumen (Fig. 1). Two experienced pathologists without knowledge of patient outcomes reviewed all histologic parameters.

The associations between nodal status and clinicopathological parameters were evaluated univariately using a chi-squared test and Fisher's exact test. For quantitative parameters (tumor size, depth of submucosal invasion, and number of lymph nodes harvested), Student's *t* test was used. Multivariate regression analysis was used to assess the hazard ratio for the prediction of nodal metastasis. A p value less than 0.05 was considered to indicate statistical significance. Statistical analysis was performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA).

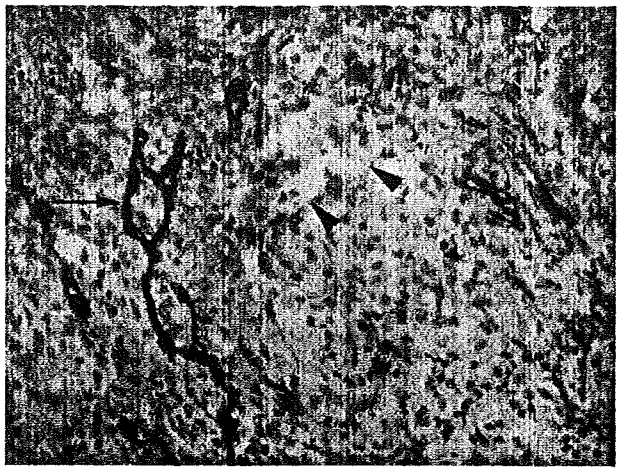

Fig. 1 Immunohistochemically stained section for D2-40 shows linear positivity of a lymphatic vessel by which an atypical cell cluster is surrounded (*arrow*). On the other hand, another atypical cell cluster surrounded by acellular space in the right hand is not surrounded by D2-40 positivity (*arrowhead*)

Results

Tumors were present in the colon (n=98) and rectum (n=38). Macroscopic tumor types were depressed (n=26), flat (n=2), pedunculated (n=13), and sessile (n=95). Tumor size ranged from 0.4 to 7.0 cm (mean, 1.7 cm). The number of lymph nodes harvested varied from four to 59 (mean, 20.5). Nodal metastasis was identified in 18 of 136 patients (13.2%), while the number of metastasis-positive lymph nodes ranged from one to four.

Associations between nodal status and clinicopathological parameters are summarized in Table 1. Forty-five patients were diagnosed as LVI-positive with D2-40 immunostaining. In LVI-positive patients, lymph node metastasis was observed more frequently than in LVI-negative patients (13/45 vs 5/91, p<0.001). Of 18 patients with nodal involvement, five (28%) were not stained with D2-40. No significant difference was found between those with and without nodal involvement as to gender, tumor location, macroscopic type, initial treatment, tumor size, number of lymph nodes harvested, tumor grade, and BVI. Fourteen cases (10.3%) were classified as superficial submucosal invasion, and only one patient classified showed lymph node metastasis; in that case, head invasion and LVI were documented. In 18 cases positive for lymph node metastasis, with the exception of one case of the pedunculated type with head invasion, the depth was 1,500μm or more. Nodal involvement was observed in 21.6% of patients with poorly differentiated histology at the invasion front, which was significantly higher than for lesions without this feature (8.2%, p=0.036).

To investigate the risk factor of lymph node metastasis, multivariate regression analyses were conducted to include patients' gender, tumor location, macroscopic type, histological grade, depth of invasion (deep or superficial submucosal invasion), status of LVI, and BVI (Table 1). The presence of a poorly differentiated histology at the invasion front and LVI constituted a significant risk factor for lymph node metastasis. When LVI is considered a predictive factor of

Table 1 Relationship between clinicopathological factors and lymph node metastasis

		Lymph node metastases		Univariate analysis	Multivariate analysis		
		Negative	Positive	p value	Odds ratio	95% CI	p value
Gender	Female	43	11	0.069[a]	NS		
	Male	75	7				
Macroscopic type	Depressed	25	1	0.408[b]	NS		
	Flat	2	0				
	Pedunculated	11	2				
	Sessile	80	15				
Initial treatment	Local excision	30	6	0.478[a]	NS		
	Surgery	88	12				
Site	Colon	86	12	0.584[a]	NS		
	Rectum	32	6				
Size (mean, cm)		1.98 (0.5–7.0)	1.82 (0.4–3.5)	0.5			
Number of lymph nodes harvested (mean)		19.6 (4–59)	24.3 (7–43)	0.07			
Histological grade	Unfavorable	8	0	0.255[a]	NS		
	Favorable	110	18				
Submucosal invasion	Superficial	13	1	0.478[a]	NS		
	Deep	105	17				
Depth of submucosal invasion (mean, mm)		3.05 (0–10.0)	2.54 (0–6.0)	0.31			
Blood vessel invasion	Negative	87	15	0.381[a]	NS		
	Positive	31	3				
Lymphatic vessel invasion	Negative	86	5	0.001[a]	7.12	2.27–22.2	0.001
	Positive	32	13				
Poorly differentiated histology at the invasion front	Negative	78	7	0.036[a]	3.16	1.05–9.52	0.04
	Positive	40	11				

CI confidence intervals

[a] Fisher's exact test

[b] Chi-squared test

Table 2 Relationship between pathological factors and lymphatic vessel invasion

		Lymphatic vessel invasion		*p* value
		Negative	Positive	
Histological grade	Unfavorable	3	5	
	Favorable	88	40	0.115[b]
Submucosal invasion	Superficial	12	2	
	Deep	79	43	0.142[b]
Blood vessel invasion	Negative	68	34	
	Positive	23	11	0.916[a]
Poorly differentiated histology at the invasive front	Negative	63	22	
	Positive	28	23	0.021[a]

[a] Chi-squared test

[b] Fisher's exact test

lymph node metastasis, sensitivity, specificity, positive predictive value, and negative predictive value are 72.2%, 72.9%, 28.9%, and 94.5%, respectively.

The relation between LVI and other pathological parameters was investigated (Table 2). LVI was observed more frequently in patients with poorly differentiated histology at the invasion front than in those without this feature. Other pathological parameters did not correlate with the incidence of LVI.

Discussion

In this study, we have shown that LVI, detected using immunostaining with monoclonal antibody D2-40, is a risk factor predictive of lymph node metastasis in patients with T1 colorectal cancer. Several studies have suggested that such histological factors as LVI, the depth or the level of submucosal invasion, and budding were also risk factors (Table 3) [5, 7, 8, 10, 11, 17].

Concerning the level of submucosal invasion, Haggitt's classification is generally used. However, according to that classification, a flat and depressed-type tumor per se constitutes a risk factor for lymph node metastasis. Kudo classified the level of invasion of such flat and depressed-type tumors into three categories by dividing the submucosal layer [18]; however, with this classification, it is impossible to evaluate locally excised specimens that do not possess muscularis propria.

The depth of the submucosal layer is evaluated by measuring the distance from the muscularis mucosae to the invasive margin of cancer. Several studies have suggested that the depth of submucosal invasion is a risk factor of lymph node metastasis [8, 10, 12]. However, our study showed that the depth of submucosal invasion is not a significant risk factor. There are two reasons accounting for this result. First, cases in which a cancer invaded no deeper than the superficial layer of the submucosa were excluded by endoscopic examination; this indicates that the remaining cancers had invaded deeper than the superficial layer of submucosa in most cases. Second, the biological character of a cancer such as lymphangiogenesis may have been related to lymph node metastasis in cases in which the cancer had invaded to a certain level of the submucosal layer [19].

There have been several reports suggesting that budding is a risk factor for lymph node metastasis [8, 9, 20, 21]. Budding refers to the emergence of microscopic clusters of undifferentiated cancer cells ahead of the invasive margin

Table 3 Risk factors of lymph node metastasis in T1 colorectal cancer

Author	Material	Number	LNM (%)	Risk Factors
Haggitt RC, et al. 1985 [17]	Sessile and pedunculated colorectal cancer	64	4 (6.3%)	Rectal location, level 4 invasion
Nivatvongs S, et al. 1991 [7]	Sessile and pedunculated colorectal cancer	151	13 (8.6%)	Level 4 invasion
Tanaka S, et al. 1995 [5]	Colorectal cancer	177	21 (12%)	Submucosal invasion >400 μ, lymphatic vessel invasion, tumor configuration, tumor grade at invasion front
Nascimbeni R, et al. 2002 [11]	Sessile colorectal cancer	353	46 (13%)	Lymphatic vessel invasion, sm3 depth of invasion, lower third of rectum
Sakuragi M, et al. 2003 [10]	Colorectal cancer	289	23 (8%)	Lymphatic vessel invasion, submucosal invasion >2,000 μ
Wang HS, et al. 2005 [8]	Colorectal cancer	159	16 (10.1%)	Histological grade, lymphatic vessel invasion, inflammation around cancer, budding
Present study	Colorectal cancer	136	18 (13.2%)	Lymphatic vessel invasion, poorly differentiated histology at invasion front

LNM lymph node metastasis

of the tumor [22]. Poorly differentiated histology at the invasion front in our study resembles budding and is thus regarded as a risk factor for lymph node metastasis. However, budding may also be a factor related to LVI. Morodomi et al. have suggested that by making serial sections of specimens, budding and LVI are revealed as associated [23]. Moreover, some reports suggest that the most important factor regarding the presence of budding is LVI [24, 25]; our study supports those findings. Those reports suggest that the cluster of cancer cells formerly diagnosed as budding using HE stain might be re-diagnosed as LVI if lymphatic vessels are rendered visible by monoclonal immunostain D2-40.

The immunostaining with monoclonal antibody D2-40 has been proven to specifically stain lymphatic endothelial cells [15], making it an essential tool for accurately diagnosing LVI. Several studies have reported that D2-40 immunostaining occasionally changes the diagnosis indicated by HE sections. Walgenbach-Bruenagel et al. have investigated the changes in LVI detection rates using D2-40 immunostaining [26]. LVI is diagnosed 22% more frequently than those cases examined only with HE staining; Saad et al. have reported that the detection of LVI increased by using D2-40 instead of HE staining (53% vs 34%) [19].

Many studies have suggested that LVI is a risk factor for lymph node metastasis, and it will remain problematic as long as diagnoses continue to differ occasionally among pathologists [14]. The factors related to those differences are the differentiation of lymphatic vessels from blood vessels and the tissue space between cancer nests and the surrounding stroma. It has been speculated that D2-40 immunostaining clarifies the structure of lymphatic vessels thus reducing the diagnostic differences among pathologists.

In this study, two thirds of the LVI-positive patients had no lymph node metastasis. However, we assume that there would be substantial occult lymph node metastasis that cannot be detected by a conventional histological examination based on HE staining. Yasuda et al. have reported that single or small clusters of tumor cells were found by immunohistochemistry in 13% of patients who were diagnosed as node-negative by a conventional histological examination [27]. Meanwhile, of the 18 patients with nodal involvement, five (28%) were not positively stained with D2-40. This fact could be accounted for by our procedure, in which we used D2-40 immunostaining only with specimens that involved the maximum invasive depth. Staining more specimens would enable us to detect LVI in more cases thus reducing the proportion of cases with nodal involvement among LVI-negative patients [28].

A combination of LVI and other pathological features would be useful for the management of T1 colorectal cancer treated by local excision. However, if LVI is not identified by D2-40, it would be possible to avoid radical surgery after sufficient explanation to patients about the risk of lymph node metastasis. For example, in patients with lower rectal cancer or with a high risk factor that would make them apt to suffer from intra- or postoperative complications, additional radical surgery would compromise their quality of life.

This study demonstrated that LVI diagnosed by the use of immunostaining with monoclonal antibody D2-40 is important in predicting lymph node metastasis of T1 colorectal cancer. Such a method would provide important information about the risk of lymph node metastasis and may prove useful in evaluating the necessity of an additional resection after local excision in T1 colorectal cancer cases.

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ORIGINAL CONTRIBUTION

Long-Term Prognostic Value of Conventional Peritoneal Lavage Cytology in Patients Undergoing Curative Colorectal Cancer Resection

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PURPOSE: Free malignant cells in the peritoneal cavity might play a role in the metastasis process. However, this phenomenon needs further elucidation. The aims of this study were to investigate the frequency of free cancer cells detected on cytologic examination of lavage fluid after peritoneal washing in patients undergoing curative surgery for colorectal cancer, to explore risk factors for exfoliation of cancer cells into the peritoneal cavity, and to evaluate the influence peritoneal lavage cytology as a prognostic tool.

METHODS: Peritoneal lavage was performed in 697 patients undergoing curative resection of colorectal cancer. Before the manipulation of the tumor, 100 mL of physiologic saline solution was administered into the abdominal cavity and the fluid was collected for cytologic examination. Specimens were classified as positive if at least one cancer cell was detected.

RESULTS: The mean follow-up period was 90.5 months. Overall, 15 (2.2%) of the 697 patients had positive results. Four characteristics were identified as risk factors for exfoliation of cancer cells into the peritoneal cavity: 1) depth of invasion, 2) regional lymph nodes, 3) lymphatic invasion, and 4) venous invasion. In univariate analyses of all 697 patients and the subgroup of 374 patients with pT3 or T4 tumors, patients with positive cytology findings had significantly worse disease-free and cancer-specific survival than patients with negative cytology findings ($P < 0.001$). On multivariate analysis, peritoneal cytology remained an independent predictor of cancer-specific survival in all patients and in patients with pT3 or pT4 tumors. Only peritoneal cytology was a significant prognostic factor for peritoneal recurrence ($P < 0.0001$).

CONCLUSION: Conventional peritoneal cytology is a useful prognostic tool in patients undergoing curative surgery for colorectal cancer and may be helpful in making decisions whether to select intraperitoneal or systemic chemotherapy.

KEY WORDS: Colorectal cancer; Peritoneal cytology; Prognostic factor; Peritoneal recurrence.

The prognosis of colorectal cancer depends on local tumor growth, lymph node involvement, and presence of peritoneal or distant metastasis.[1] The complete removal of the tumor is the most effective treatment for carcinoma of the colon and rectum. However, some metastases are inevitable after curative resections, and it is well known that the most common mechanism of metastases is lymphatic spread to the regional lymph nodes and hematogenous spread *via* the portal vein. Local recurrence may be the result of inadequate local excision or unresected lymphatic permeation.[2]

As noted by Hara *et al.*,[3] peritoneal metastasis consists of 2 steps: first, cancer cells shed from the serosal surface of the primary tumor are transported into the peritoneal cavity; second, free malignant cells in the peritoneum attach themselves preferentially to sites such as the omentum and mesenterium,[4] subsequently growing and disseminating throughout the peritoneal cavity. Such cells may be present within the peritoneal cavity in patients with colorectal cancer before surgery, indicating early peritoneal seeding. The detection of these cells intraoperatively

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through peritoneal lavage cytology may lead to the identification of patients who are more likely to develop peritoneal recurrence.[5,6] The prognostic value of peritoneal washing cytology is well established in gynecologic malignancies, and it has been formally integrated into current staging systems for ovarian and endometrial carcinoma.[7,8] Recently, it has been reported that peritoneal or pleural cytology is useful for predicting the prognosis of gastric, pancreatic, esophageal, and lung cancer.[9–12] Positive findings on cytologic examination of lavage fluid can also contribute to the identification of patients who need frequent postoperative follow-up and possibly adjuvant chemotherapy.[13–16] Conventional cytology,[14,17–23] immunocytochemistry,[16,24] and reverse transcriptase-polymerase chain reaction (RT-PCR).[3,25,26] have been used to analyze the incidence and prognostic significance of free malignant cells in the peritoneal cavity at the time of surgery in patients with colorectal cancer. Some studies have shown no relationship between peritoneal cytology results and prognosis,[3,16,19,20,23] whereas other investigators have reported a significant association.[18,21,22,24–26] A recent meta-analysis found the detection of intraperitoneal free cancer cells in patients with colorectal cancer to be associated with increased recurrence and poor prognosis.[27] However, the impact on survival has not yet been fully established in colorectal cancer.

The aims of this study were to investigate the frequency free cancer cells detected on cytologic examination of lavage fluid after peritoneal washing in patients undergoing curative surgery for colorectal cancer, to explore risk factors for exfoliation of cancer cells into the peritoneal cavity, and to evaluate the influence of positive findings on peritoneal lavage cytology as a prognostic tool for patients with colorectal cancer.

PATIENTS AND METHODS

Patients

From January 1985 through December 1997, intraoperative peritoneal lavage with cytologic evaluation was performed in 697 patients who underwent a curative resection for colorectal cancer. Only patients who had no clinically evident metastatic disease or peritoneal dissemination and who underwent planned surgery with curative intent were studied. R0 resection was performed in all cases. To exclude the presence of distant metastases, the cancer was preoperatively staged in all patients with abdominal plus pelvic CT and chest CT. Tumors were classified according to the UICC pTNM system.[28]

Procedures

Peritoneal lavage was performed just once, immediately after the making of a midline abdominal incision and before manipulation of the tumor. Intraoperatively, 100 mL of physiologic saline solution (37°C) was instilled into the Douglas cavity with the patient in a supine position. After gentle stirring, these fluids were collected. The collected lavage fluid was immediately heparinized and centrifuged at 2,000 rpm for 3 minutes, and the sediment was smeared on 4 glass slides. The slides were stained by the Giemsa and Papanicolaou methods and evaluated by cytologists who were blinded to the clinical information. A slide was classified as positive if at least one cancer cell was detected. A suspicion of malignancy was classified as negative.

Patient Follow-Up

Follow-up to monitor for recurrence of disease after surgery included the following procedures and tests: a physical examination, serum tumor marker, hepatic imaging (ultrasound, CT, or both), abdominal plus pelvic CT, and chest x-ray or CT every 4 to 6 months for the first 3 years and every 6 months for the next 2 years; colonoscopy every one to 2 years.

Peritoneal recurrence was defined as radiologic or histocytologic evidence of cancer recurrence in the abdominal cavity. Liver metastasis, intra-abdominal lymph node metastasis, and local recurrence, defined as radiologic or histocytologic evidence of cancer recurrence at or in the region of the primary tumor bed, were excluded.

Statistical Analysis

Statistical analysis was performed using the StatView version 5.0 software package (Abacus Concepts, Berkeley, CA). Associations between the clinicopathologic parameters were assessed using the chi-squared test or the Fisher exact test for discrete variables. Student *t* test was performed for continuous variables. Disease-free survival curves and cancer-specific survival curves were estimated using the Kaplan–Meier technique and were compared by means of the log-rank test. For cancer-specific survival, only cancer-related deaths were considered; data on patients who had died from other causes or who were still alive at the end of the study were censored. Basically, we used the last visit date for calculating survival times, but for some patients, we confirmed life or death by phone or letter. A Cox proportional hazards model was used to assess risk (expressed as hazard ratio, HR) under simultaneous contributions from several covariates. *P* values of <0.05 were considered statistically significant.

RESULTS

Relationship Between Peritoneal Cytology Results and Clinicopathologic Factors

The mean follow-up period was 90.5 months (range, 6.0 to 211.6 months). Overall, 15 (2.2%) of the 697 patients were positive for free cancer cells in the peritoneal lavage fluid. Table 1 shows the patients' clinical and pathologic characteristics. Comparison of patients who had positive results