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Original Research Article

Factors Affecting Positive Peritoneal Lavage Cytology in Patients with Stage II and III Colorectal Cancer with R0 Resection: A Multi-institutional, Prospective Study

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Abstract

Objectives: This study aimed to explore the risk factors associated with cancer cell exfoliation in Stage II and III colorectal cancer (CRC).

Methods: This multicenter, prospective, observational study targeted 1,698 patients with cStage II and III CRC who underwent R0 resection between 2013 and 2017. Clinicopathological variables were analyzed for correlations with positive peritoneal lavage cytology (PLC).

Results: The positive PLC rate was 2.7% (46/1,694 cases) at laparotomy and 1.6% (25/1,590 cases) after tumor resection. Logistic regression analyses identified that undifferentiated histologies diagnosed by preoperative biopsy specimen, cT4, and pN+ were independent factors that affected the positive PLC at laparotomy. The positive PLC rate at laparotomy was 4.5% (33/736 cases) among the patients with undifferentiated histology and/or cT4. Logistic regression analyses revealed that the presence of ascites and undifferentiated histology by biopsy independently affected positive PLC after tumor resection.

Conclusions: The undifferentiated histology and/or T4 indicated by preoperative diagnosis were identified as factors affecting PLC at laparotomy. Furthermore, ascites and preoperative histological type were identified as factors affecting positive PLC after tumor resection. As factors affecting positive PLC, these preoperative findings were found to be equivalent to pathological findings.

Keywords

colorectal cancer, peritoneal lavage cytology, peritoneal metastasis, R0 resection

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Introduction

Colorectal cancer (CRC) is the third most common cause of death in Japan, and it has an increasing prevalence. Complete tumor removal is the most effective treatment for CRC. However, some recurrences are inevitable after curative resections, even in cases of apparently localized tumors, and the liver, lung, and peritoneum are the most common recurrence sites[1]. Despite recent advances in knowledge regarding various clinical, biological, and pathological features as-

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sociated with CRC prognosis, the depth of tumor invasion and lymph node involvement have been regarded as the most important factors affecting recurrence and prognosis. These factors are used in Dukes staging and the tumor-node-metastasis (TNM) classification system[2,3].

The prognostic values of peritoneal lavage cytology (PLC) and ascites cytology have been well established for gynecologic cancers[4,5]. Peritoneal and pleural lavage cytologies are also useful prognostic indicators for gastric, pancreatic, esophageal, and lung cancers[6-9] and contribute to patient selection for close follow-up and/or intensive chemotherapy[10-13]. On the other hand, although positive PLC has been reported to be associated with increased recurrence risk and poor prognosis[14-17], the true prognostic value of PLC for CRC has not yet been established[3,18]. In previous reports, the positive PLC rates in patients with CRC who had R0 resections were considerably low[14,15,19,20].

The identification of factors associated with cancer cell exfoliation into the peritoneal cavity must be able to clarify selection criterion for PLC. Although some previous reports examined such factors[17-21], most of them included a small number of patients, especially patients who underwent R0 resection. Furthermore, many reported factors associated with cancer cell exfoliation into the peritoneal cavity were assessed after surgery, such as pathological findings of the resected specimen.

This study aimed to explore the factors associated with cancer cell exfoliation into the peritoneal cavity using preoperative factors.

Methods

Study design

This multicenter, prospective, observational study targeted patients with CRC and was conducted by the Japanese Society for Cancer of the Colon and Rectum (JSCCR). Patients were enrolled at 21 institutions in Japan from September 2013 to December 2017. The eligibility criteria were age ≥20 years and histologically confirmed colorectal adenocarcinoma diagnosed as Stage II or III before surgery. To diagnose the CRC stage before surgery, all patients underwent barium enema, colonoscopy, abdominal ultrasound, and abdominal and chest computed tomography, regardless of the cancer site. Pelvic magnetic resonance imaging was performed for low rectal cancer. Patients with synchronous or metachronous (within 5 years) malignancies, other than carcinoma, *in situ*, were excluded.

Written informed consent was obtained from all patients before enrollment. The study protocol was approved by the ethics committee of the JSCCR and the local institutional review board.

Procedures

PLC was performed twice for each case: once immediately after the abdomen was opened and before the exploration and manipulation of the tumor (at laparotomy) and once after anastomosis or tumor resection (after tumor resection). PLC just after anastomosis or tumor resection was performed before peritoneal lavage. Any free fluid present in the abdominal recesses was removed for examination (ascites and peritoneal cytology). In the absence of obvious peritoneal fluid, 50 ml of physiologic saline solution was instilled into the vesicorectal/vesicouterine pouch, 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 four glass slides and fixed with cytospray. The slides were stained using the Giemsa and Papanicolaou methods and evaluated by cytologists who were blinded to the clinical information. The smears were classified according to the Papanicolaou classification, and Class III, IV, and V were classified as positive. When both PLC and peritoneal cytology of ascites were performed, the result was classified as positive if either test was positive.

Parameters

The parameters assessed in this study were as follows: age, gender, comorbidity, neoadjuvant therapy, Eastern Cooperative Oncology Group Performance Status, location of the primary tumor, size of the primary tumor, number of tumors, presence or absence of ascites, preoperative serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), laparoscopic surgery, instrument anastomosis, lymph node dissection, additional resection, volume of bleeding, operative time, complications, histological type, depth of tumor invasion, lymph node metastases, lymphatic invasion, and venous invasion.

Definitions and diagnosis

Clinical and pathological data were recorded according to the JSCCR classification system[18]. The depth of tumor invasion and lymph node metastases were classified according to the eighth edition of the TNM classification system[4]. The cutoff values for CEA and CA19-9 were 5.0 ng/mL and 37.0 U/mL, respectively. For the analyses of factors affecting positive cytology, the cutoff values for body mass index (BMI) were determined using receiver operating characteristic curve analyses.

Statistical analysis

The differences between continuous and categorical variables were identified using the Mann-Whitney *U* test and the chi-squared test or Fisher's exact test, respectively. The factors affecting positive PLC were analyzed using binomial

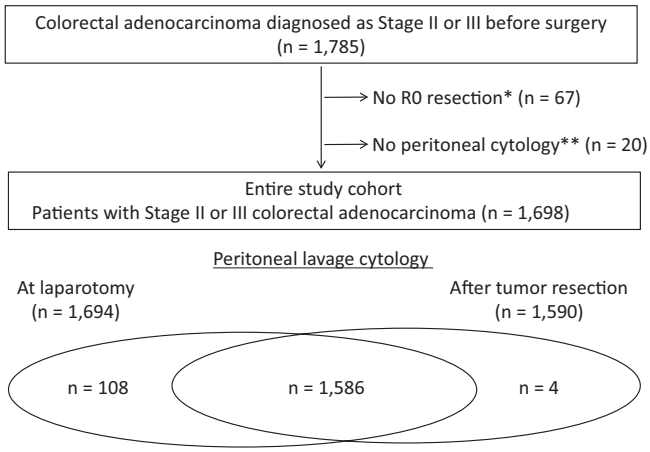

Figure 1. Flow diagram for the inclusion of patients in this study. *: R0 resection according to the JSCCR classification system **: Peritoneal lavage cytology was not performed either at laparotomy or after tumor resection.

logistic regression analyses, and the identified factors were compared using the chi-squared test or Fisher's exact test, at the $p < 0.01$ level, to determine factors independently associated with positive PLC. Multivariate analyses were performed for both preoperative and pathological findings.

All data were expressed as the total number of patients and proportion of the population (%). The p-values < 0.05 were considered significant. All analyses were performed using the JMP 11 statistical software (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

Figure 1 shows the flow diagram used to determine patient inclusion in this study. A total of 1,785 patients with clinical Stage II and III CRC were enrolled from 21 institutions in Japan. A total of 67 patients who were not curable with surgical treatment according to the JSCCR classification system and 20 patients who had not undergone PLC at laparotomy or after tumor resection were excluded. Thus, the study population comprised 1,698 patients with CRC. PLC was performed in 1,694 patients at laparotomy and 1,590 patients after tumor resection.

The positive PLC rate was 2.7% (46/1,694 cases) in the patients who underwent PLC at laparotomy. The positive PLC rate was 3.0% (46/1,512 cases) in patients with pStage II and III CRC. The positive PLC rate after tumor resection was 1.6% (25/1,590 cases). Among these, the PLC at laparotomy were positive in 44% (11/25 cases). The positive PLC rate was 0.9% (14/1,544 cases) in patients with negative PLC at laparotomy and positive PLC after tumor resection.

Cutoff value of BMI

The cutoff values at laparotomy determined using receiver operating characteristic curve analyses were as follows. The area under the curve was 0.550. When the cutoff value for BMI was defined as 24, the sensitivity and specificity of BMI for predicting positive peritoneal cytology were 82.6% and 32.0%, respectively.

The cutoff values after tumor resection determined using receiver operating characteristic curve analyses were as follows. The area under the curve was 0.678. When the cutoff value for BMI was defined as 21, the sensitivity and specificity of BMI for predicting positive peritoneal cytology were 62.5% and 63.8%, respectively.

Concordance rate between pre- and postoperative findings for histology and T and N classification

Of the 1,603 patients with preoperative diagnosis of differentiated histology, 96.0% were identical with postoperative histological diagnosis, and of the 82 patients with preoperative diagnosis of undifferentiated histology, 69.5% coincided with the postoperative findings.

Of the 981 patients with cT1/T2/T3, 94.3% were pT1/T2/TT3, and of the 329 patients with cT4, 46.4% were pT4. Of the 732 patients with cN0, 69.0% were pN0, and of the 955 patients with cN+, 56.8% were pN+.

The concordance rates between the pre- and postoperative findings for histology and T and N classification were 94.7%, 74.2%, and 62.1%, respectively.

Factors affecting positive peritoneal cytology at laparotomy

Positive PLC at laparotomy was significantly associated with histology diagnosed before surgery ($p = 0.003$), cT ($p < 0.001$), pT ($p < 0.001$), and pN ($p < 0.001$), as determined by univariate analyses (Table 1).

When using the cT, logistic regression analyses revealed that preoperative histology, cT, and pN independently affected positive PLC at laparotomy (Table 2). When using the pT, logistic regression analyses revealed that preoperative histology (hazard ratio: 2.70, p-value < 0.04), pT (hazard ratio: 3.20, p-value < 0.001), and pN (hazard ratio: 2.70, p-value = 0.01) independently affected positive PLC at laparotomy.

Factors affecting positive peritoneal cytology after tumor resection

Positive PLC after tumor resection was significantly associated with preoperative histology ($p = 0.005$), ascites ($p = 0.004$), pT ($p = 0.004$), and pN ($p = 0.004$) according to the univariate analyses (Table 3). Logistic regression analyses revealed that, among these factors, ascites and preoperative histology independently affected positive PLC after tumor resection (Table 3).

Table 1. Univariate Analyses of Factors Affecting Positive Peritoneal Cytology at Laparotomy for Colorectal Cancer, Which Was Curability A with Surgical Treatment According to the JSCCR Classification System.

Variable		Category	Number	Performed case	Positive case (%)		Negative case	Univariate analysis p-value
Preoperative factor	Gender	Male	999	999	20	2.0%	979	0.03
		Female	698	694	26	3.7%	668	
		Unknown	1	1	0	0.0%	1	
	Age (year old)	<70	864	860	26	3.0%	834	0.43
		70≤	831	831	20	2.4%	811	
		Unknown	3	3	0	0.0%	3	
	Comorbidities	+	1086	1083	26	2.4%	1057	0.38
		-	610	609	19	3.1%	590	
		Unknown	2	2	1	50.0%	1	
	Neoadjuvant therapy	+	54	54	1	1.9%	53	0.69
		-	1644	1640	45	2.7%	1595	
	Performance status	0, 1	1482	1478	40	2.7%	1438	0.99
		2, 3, 4	148	148	4	2.7%	144	
		Unknown	68	68	2	2.9%	66	
	Tumor location	Colon	1268	1265	38	3.0%	1228	0.21
		Rectum	430	429	8	1.9%	421	
	Histological type	Differenciated type	1529	1526	35	2.3%	1491	0.003
		Undifferenciated type	77	76	6	7.9%	70	
		Unknown	92	92	5	5.4%	87	
	Depth of invasion	T1, T2, T3	988	984	13	1.3%	971	<0.001
		T4a, T4b	710	710	33	4.6%	677	
		Unknown	0	0	0	0.0%	0	
	LN metastases	-	736	734	12	1.6%	722	0.02
		+	959	957	34	3.6%	923	
		Unknown	3	3	0	0.0%	3	
	Number of tumor	Solitary	1614	1610	42	2.6%	1568	0.19
		Multiple	79	79	4	5.1%	75	
		Unknown	5	5	0	0.0%	5	
	Size of tumor (mm)	<60	1183	1179	24	2.0%	963	0.04
		60≤	505	505	22	4.4%	483	
		Unknown	10	10	0	0.0%	10	
	Ascites	+	94	94	4	4.3%	90	0.34
		-	1604	1600	42	2.6%	1558	
	Serum CEA level	Low	1055	1051	21	2.0%	1030	0.04
		High	597	597	22	3.7%	575	
		Unknown	46	46	3	6.5%	43	
	Serum CA19-9 level	Low	1456	1452	35	2.4%	1417	0.12
		High	185	185	8	4.3%	177	
		Unknown	57	57	3	5.3%	54	
Operative factor	Laparoscopic surgey	+	667	663	13	2.0%	650	0.13
		-	1031	1031	33	3.2%	998	
Histological factor	Histological type	Differenciated type	1567	1564	40	2.6%	1524	0.12
		Undifferenciated type	122	121	6	5.0%	115	
		Unknown	9	9	0	0.0%	9	
	Depth of invasion	T1, T2, T3	1305	1301	21	1.6%	1280	<0.001
		T4a, T4b	385	385	25	6.5%	360	
		Unknown	8	8	0	0.0%	8	
	LN metastasis	+	772	771	33	4.3%	738	<0.001
		-	922	919	13	1.4%	906	
		Unknown	4	4	0	0.0%	4	
	Lymphatic invasion	0	619	618	10	1.6%	608	0.04
		1, 2, 3	1070	1067	35	3.3%	1032	
		Unknown	9	9	1	11.1%	8	
	Venous invasion	0	494	493	9	1.8%	484	0.16
		1, 2, 3	1192	1189	36	3.0%	1153	
		Unknown	12	12	1	8.3%	11	

JSCCR: Japanese Society for Cancer of the Colon and Rectum LN: lymph node

Table 2. Multivariate Regression Analysis for Factors Affecting Positive Peritoneal Cytology at Laparotomy for Colorectal Cancer, Which Was Curability A with Surgical Treatment According to the JSCCR Classification System.

Variables selected	Hazard ratio	95% confidence interval	*p*-value
Histological type (before surgery)	3.02	1.20–7.58	0.020
Depth of invasion (before surgery)	2.67	1.36–5.24	0.004
Lymph node metastasis (histological)	3.10	1.54–6.27	0.002

JSCCR: Japanese Society for Cancer of the Colon and Rectum

Among the patients with negative PLC at laparotomy, positive PLC after tumor resection was significantly associated with ascites ($p < 0.001$) and preoperative histology ($p = 0.002$) according to the univariate analyses (Table 4). In the logistic regression analyses, among these two factors, both were identified as independent factors affecting positive PLC after tumor resection (Table 4).

Positive PLC rates according to factors affecting positive peritoneal cytology

Table 5 shows the positive PLC rates, according to the factors affecting positive peritoneal cytology results at laparotomy. At least one of the identified factors that could be assessed before surgery (preoperative histology, cT4) were identified in 736 patients (43.4%) of the 1,694 patients who had PLC at laparotomy, and the positive PLC rate was 4.5% (33/1,694 cases). Furthermore, at least one of the identified factors including pN+ was identified in 1,131 patients (66.8%), and the positive PLC was 3.8% (43 cases). At least one of the identified factors (preoperative histology, pT4, and pN) was identified in 949 patients (56.0%), and the positive PLC rate was 4.4% (42/949 cases). Among the 13 patients who had positive PLC at laparotomy, despite having no factors affecting positive PLC at laparotomy, which were possible to know before surgery, four patients were Stage IIIa, six were Stage IIIb, and three were Stage group II based on both pre- and postoperative information.

Among the 1,590 patients who had PLC after tumor resection, ascites and/or preoperative undifferentiated histology, which were factors identified to affect positive PLC after tumor resection, were identified in 8.4% (133/1,590 cases), and the positive PLC rate among these was 5.3% (7/133 cases). Among the 1,544 patients who had negative PLC at laparotomy and had PLC after tumor resection, ascites and/or preoperative undifferentiated histology were identified in 8.0% (124/1,544 cases), and the positive PLC rate among these was 4.8% (6/124 cases).

Discussion

The major spreading routes of CRC are hematogenous, lymphogenous, and peritoneal dissemination[22]. Although peritoneal metastasis is one of the most common sites of recurrence following liver and lung metastasis, it is less frequent and considered to be less prognostically important than other routes[23]. Diagnosing peritoneal metastasis can be difficult because of the multiplicity and microscopic sizes of the implants, although peritoneal metastasis can result in intractable ascites, intestinal obstructions, and further tumor proliferation, and peritoneal metastasis must often be diagnosed by surgery[24]. Peritoneal recurrence is often diagnosed at terminal stages, and most patients with peritoneal metastasis have a poor prognosis[24]. Therefore, identifying the well-defined risk factors that can predict peritoneal recurrence during early stages may facilitate the treatment of high-risk patients using strategies such as more intensive postoperative surveillance and adjuvant chemotherapy that may improve prognoses.

The presence of free cancer cells in the abdominal cavity is thought to represent a precursor state for peritoneal dissemination. In CRC, positive lavage cytology has been associated with poor oncological outcomes[14-17], although its significance on the prognosis has not been fully established[3,18]. The reported positive rate of PLC ranged from 2.2% to 20.0%[14,15,17,19,20,25], and this large range may be due to the differences in patient populations, lavage methods, or criteria used for assessment. In many of these reports, the numbers of enrolled cases were small and likely insufficient to reach definitive conclusions. Furthermore, T2 cancer, which rarely progresses to positive peritoneal cytology, and Stage IV CRC, including peritoneal metastasis cases that are highly likely to progress to positive peritoneal cytology, were included in many of these studies. In addition, most reports included PLC at laparotomy; however, none of these reports included PLC after tumor resection, although cancer cells might exfoliate into the peritoneal cavity during the surgical procedure[26,27]. The present study assessed the incidence of free cancer cells and the factors affecting the exfoliation of cancer cells into the peritoneal cavity, by performing peritoneal cytology before and after curative resections in patients with CRC diagnosed as Stage II and III.

In our series, peritoneal cytology at laparotomy was positive in 2.7% of all patients and 3.0% of patients with patho-

Table 3. Univariate and Multivariate Analyses of Factors Affecting Positive Peritoneal Lavage Cytology after Tumor Resection for All Colorectal Cancer, Which Was Curability A with Surgical Treatment According to the JSCCR Classification System.

	Variable	Category	Number	Performed case	Positive case		Negative case	Univariate analysis	Multivariate analysis		
					Number	%	Number	p-value	HR	95% CI	p-value
Preoperative factor	Gender	Male	999	937	15	1.6%	922	0.91			
		Female	698	652	10	1.5%	642				
		Unknown	1	1	0	0.0%	1				
	Age (year old)	<70	864	819	13	1.6%	806	0.97			
		70≤	831	768	12	1.6%	756				
		Unknown	3	3	0	0.0%	3				
	Comorbidities	+	1086	1017	14	1.4%	1003	0.56			
		-	640	571	10	1.8%	561				
		Unknown	2	2	1	50.0%	1				
	Neoadjuvant therapy	+	54	51	0	0.0%	51	0.36			
		-	1644	1539	25	1.6%	1514				
	Performance status	0, 1	1482	1384	20	1.4%	1364	0.055			
		2, 3, 4	148	138	5	3.6%	133				
		Unknown	68	68	0	0.0%	68				
	Tumor location	Colon	1268	1176	21	1.8%	1155	0.240			
		Rectum	430	414	4	1.0%	410				
	Histological type	Differenciated type	1529	1441	20	1.4%	1421	0.005	3.39	1.08–10.61	0.04
		Undifferenciated type	77	70	4	5.7%	66				
		Unknown	92	79	1	1.3%	78				
	Depth of invasion	T1, T2, T3	988	957	13	1.4%	944	0.40			
		T4a, T4b	710	633	12	1.9%	621				
		Unknown	0	0	0	0.0%	0				
	LN metastases	-	736	704	9	1.3%	695				
		+	959	957	16	1.7%	941	0.40			
		Unknown	3	3	0	0.0%	3				
	Number of tumor	Solitary	1613	1512	23	1.5%	1489	0.40			
		Multiple	79	72	2	2.8%	70				
		Unknown	5	5	0	0.0%	5				
	Size of tumor (mm)	<60	1183	1124	13	1.2%	1111	0.03			
		60≤	505	457	12	2.6%	445				
		Unknown	10	9	0	0.0%	9				
	Ascites	+	94	68	4	5.9%	64	0.004	3.86	1.25–11.95	0.02
		-	1604	1522	21	1.4%	1501				
	Serum CEA level	Low	1055	1010	15	1.5%	995	0.99			
		High	597	539	8	1.5%	531				
		Unknown	46	41	2	4.9%	39				
	Serum CA19-9 level	Low	1456	1366	21	1.5%	1345	0.70			
		High	185	173	2	1.2%	171				
		Unknown	57	51	2	3.9%	49				

logical Stage II and III. The positive peritoneal cytology rates at laparotomy have been reported as 3.1% in T3 and T4 CRC with curative surgery by Nishikawa et al.[14], as 3.6% in Stage II and III CRC by Noura et al.[19], and as 4.6% in T3 and T4 CRC by Fujii et al.[20]. The positive PLC rate in Stage II and III CRC, for which the clinical utility of peritoneal cytology at laparotomy would be expected, was assumed to be approximately 3%-4.6%. The previously reported factors that were significantly correlated with positive PLC at laparotomy are as follows: peritoneal dissemination, liver metastasis, ascites, curability, histological type, depth of invasion (pT), lymph node metastasis (pN), lymphatic invasion, and venous invasion[14,17,19-21]. However, the factors that have been identified, including

Table 3. Univariate and Multivariate Analyses of Factors Affecting Positive Peritoneal Lavage Cytology after Tumor Resection for All Colorectal Cancer, Which Was Curability A with Surgical Treatment According to the JSCCR Classification System (continued).

	Variable	Category	Number	Performed case	Positive case		Negative case	Univariate analysis	Multivariate analysis		
					Number	%	Number	p-value	HR	95% CI	p-value
Operative factor	Laparoscopic surgey	+	667	647	12	1.9%	635	0.45			
		-	1031	943	13	1.4%	930				
	Instrument anastomosis	+	1149	1062	18	1.7%	1044	0.60			
		-	539	519	7	1.3%	512				
		Unknown	10	9	0	0.0%	9				
	LN dissection	D0, D1	17	11	1	9.1%	10	0.06			
		D2, D3	1676	1574	24	1.5%	1550				
		Unknown	5	5	0	0.0%	5				
	Additional resection	+	159	149	1	0.7%	148	0.35			
		-	1528	1431	24	1.7%	1407				
		Unknown	11	10	0	0.0%	10				
	Bleeding volume (ml)	175≤	474	428	3	0.7%	425	0.09			
		<175	1219	1157	22	1.9%	1135				
		Unknown	5	5	0	0.0%	5				
	Operative time (min)	226≤	856	814	15	1.8%	799	0.38			
		<226	840	774	10	1.3%	764				
		Unknown	2	2	0	0.0%	2				
	Complication (all)	+	459	428	9	2.1%	419	0.30			
		-	1239	1162	16	1.4%	1146				
	Complication (Grade 3 -)	+	184	169	3	1.8%	166	0.83			
		-	1514	1421	22	1.5%	1399				
Histological factor	Histological type	Differenciated type	1567	1481	21	1.4%	1460	0.045			
		Undifferenciated type	122	100	4	4.0%	96				
		Unknown	9	9	0	0.0%	9				
	Depth of invasion	T1, T2, T3	1305	1255	14	1.1%	1241	0.004	2.13	0.89–5.06	0.09
		T4a, T4b	385	327	11	3.4%	316				
		Unknown	8	8	0	0.0%	8				
	LN metastasis	+	772	713	14	2.0%	699	0.004	1.41	0.61–3.28	0.42
		-	922	873	11	1.3%	862				
		Unknown	4	4	0	0.0%	4				
	Lymphatic invasion	0	619	601	6	1.0%	595	0.15			
		1, 2, 3	1070	980	19	1.9%	961				
		Unknown	9	9	0	0.0%	9				
	Venous invasion	0	494	475	5	1.1%	470	0.27			
		1, 2, 3	1192	1103	20	1.8%	1083				
		Unknown	12	12	0	0.0%	12				

JSCCR: Japanese Society for Cancer of the Colon and Rectum LN: lymph node

pathological findings, represent information that can only be obtained after surgery and cannot be used to determine whether PLC should be performed at laparotomy. Furthermore, PLC may have little clinical significance for Stage IV CRC because the treatment strategies, including postoperative chemotherapy and follow-up, would not be altered by the PLC results. In the present study, statistical analyses were performed for both preoperative and pathological findings for identifying preoperative factors affecting positive PLC equivalent to pathological findings. Additionally, preoperative histological type and cT were identified as factors affecting positive PLC at laparotomy and pT and pN for Stage II and III CRC with R0 resection. Preoperative histological type by the biopsy tissue was thought as an affecting factor of positive PLC at laparotomy because histological type as final diagnosis was classified by the most predominant histological type in a lesion, not by lower differentiated histological type. Furthermore, it was thought that cT and cN

Table 4. Univariate and Multivariate Analyses of Factors Affecting Positive Peritoneal Lavage Cytology after Tumor Resection for Colorectal Cancer, Which Was Curability A with Surgical Treatment According to the JSCCR Classification System among the Cases with Negative Peritoneal Cytology at Laparotomy.

	Variable	Category	Number	Performed case	Positive case		Negative case	Univariate analysis	Multivariate analysis		
					Number	%	Number	p-value	HR	95% CI	p-value
Preoperative factor	Gender	Male	999	918	10	1.1%	908	0.35			
		Female	698	625	4	0.6%	621				
		Unknown	1	1	0	0.0%	1				
	Age (year old)	<70	864	790	6	0.8%	784	0.52			
		70≤	831	751	8	1.1%	743				
		Unknown	3	3	0	0.0%	3				
	Comorbidities	+	1086	990	8	0.8%	982	0.58			
		-	640	553	6	1.1%	547				
		Unknown	2	1	0	0.0%	1				
	Neoadjuvant therapy	+	54	50	0	0.0%	50	0.49			
		-	1644	1494	14	0.9%	1480				
	Performance status	0, 1	1482	1344	10	0.7%	1334	0.02			
		2, 3, 4	148	134	4	3.0%	130				
		Unknown	68	66	0	0.0%	66				
	Tumor location	Colon	1268	1137	11	1.0%	1126	0.68			
		Rectum	430	407	3	0.7%	404				
	Histological type	Differenciated type	1529	1406	11	0.8%	1395	0.002	5.83	1.53–22.2	0.001
		Undifferenciated type	77	63	3	4.8%	60				
		Unknown	92	75	0	0.0%	75				
	Depth of invasion	T1, T2, T3	988	943	11	1.2%	932	0.18			
		T4a, T4b	710	601	3	0.5%	598				
		Unknown	0	0	0	0.0%	0				
	LN metastases	-	736	692	5	0.7%	687	0.42			
		+	959	798	9	1.1%	789				
		Unknown	3	3	0	0.0%	3				
	Number of tumor	Solitary	1613	1470	14	1.0%	1456	0.42			
		Multiple	79	68	0	0.0%	68				
		Unknown	5	5	0	0.0%	5				
	Size of tumor (mm)	<60	1183	1097	9	0.8%	1088	0.55			
		60≤	505	438	5	1.1%	433				
		Unknown	10	9	0	0.0%	9				
	Ascites	+	94	66	4	6.1%	62	<0.0001	8.62	2.58–28.8	0.0005
		-	1604	1478	10	0.7%	1468				
	Serum CEA level	Low	1055	987	8	0.8%	979	0.75			
		High	597	519	5	1.0%	514				
		Unknown	46	38	1	2.6%	37				
	Serum CA19-9 level	Low	1456	1331	11	0.8%	1320	0.61			
		High	185	165	2	1.2%	163				
		Unknown	57	48	1	2.1%	47				

were not affecting factors of positive PLC at laparotomy because the accuracies of their preoperative diagnosis were low.

The economic and clinical utilities of PLC would increase if this procedure could be performed only in those cases for which the treatment strategy depends on the PLC and that presents with factors affecting positive PLC. In the present study, preoperative histological type and cT that could be identified before surgery were identified as affecting factors for positive PLC at laparotomy. When peritoneal cytology was performed on cases that presented with at least one factor among these two identified preoperative factors, PLC

Table 4. Univariate and Multivariate Analyses of Factors Affecting Positive Peritoneal Lavage Cytology after Tumor Resection for Colorectal Cancer, Which Was Curability A with Surgical Treatment According to the JSCCR Classification System among the Cases with Negative Peritoneal Cytology at Laparotomy (continued).

	Variable	Category	Number	Performed case	Positive case		Negative case	Univariate analysis	Multivariate analysis		
					Number	%	Number	p-value	HR	95% CI	p-value
Operative factor	Laparoscopic surgey	+	667	632	9	1.4%	623	0.07			
		-	1031	912	5	0.5%	907				
	Instrument anastomosis	+	1149	1030	11	1.1%	1019	0.35			
		-	539	506	3	0.6%	503				
		Unknown	10	8	0	0.0%	8				
	LN dissection	D0, D1	17	10	0	0.0%	10	0.75			
		D2, D3	1676	1529	14	0.9%	1515				
		Unknown	5	5	0	0.0%	5				
	Additional resection	+	159	146	1	0.7%	145	0.76			
		-	1528	1388	13	0.9%	1375				
		Unknown	11	10	0	0.0%	10				
	Bleeding volume (ml)	175≤	474	420	3	0.7%	417	0.62			
		<175	1219	1119	11	1.0%	1108				
		Unknown	5	5	0	0.0%	5				
	Operative time (min)	226≤	856	791	9	1.1%	782	0.33			
		<226	840	751	5	0.7%	746				
		Unknown	2	2	0	0.0%	2				
	Complication (all)	+	459	412	5	1.2%	407	0.44			
		-	1239	1132	9	0.8%	1123				
	Complication (Grade 3 -)	+	184	159	1	0.6%	158	0.69			
		-	1514	1385	13	0.9%	1372				
Histological factor	Histological type	Differenciated type	1567	1442	12	0.8%	1430	0.20			
		Undifferenciated type	122	93	2	2.2%	91				
		Unknown	9	9	0	0.0%	9				
	Depth of invasion	T1, T2, T3	1305	1231	9	0.7%	1222	0.14			
		T4a, T4b	385	305	5	1.6%	300				
		Unknown	8	8	0	0.0%	8				
	LN metastasis	+	772	680	5	0.7%	675	0.53			
		-	922	860	9	1.0%	851				
		Unknown	4	4	0	0.0%	4				
	Lymphatic invasion	0	619	590	5	0.8%	585	0.84			
		1, 2, 3	1070	946	9	1.0%	937				
		Unknown	9	8	0	0.0%	8				
	Venous invasion	0	494	465	1	0.2%	464	0.06			
		1, 2, 3	1192	1068	13	1.2%	1055				
		Unknown	12	11	0	0.0%	11				

JSCCR: Japanese Society for Cancer of the Colon and Rectum LN: lymph node

could be omitted in almost 55% of all patients, and the positive rate increased from 2.7% to 4.5%. Of the 46 patients with positive PLC at laparotomy, two high-risk factors that were possible to know before surgery did not effectively identify 28% of patients. Among them, three patients with Stage II CRC, for whom the treatment strategy such as the use of adjuvant chemotherapy may be affected by the PLC, were included, and these three patients did not have any factors affecting positive PLC, including pathological factors. Based on these results, these preoperative factors may be useful for selecting promising candidates to undergo PLC at laparotomy, for whom PLC could provide useful information to determine proper treatment strategies.

In the present study, PLC after tumor resection was posi-

Table 5. Positive Rates of Peritoneal Cytology According to Factors Affecting Positive Peritoneal Cytology at Laparotomy.

			Performed case	Positive case (%)	
#1	Histological type (preoperative)	Undif type	76	6	(7.9)
#2	Depth of invasion (preoperative)	T4	710	33	(4.6)
#3	Depth of invasion (histological)	T4	385	25	(6.5)
#4	Lymph node metastasis (histological)	+	771	33	(4.3)
	#1 or #2		736	33	(4.5)
	#1 or #2 or #4		1131	43	(3.8)
	#1 or #3 or #4		949	42	(4.4)
	All cases		1,694	46	(2.7)

Undif type: Undiferenciated type

tive in 1.6% of patients with Stage II and III and in 0.9% of patients with Stage II and III in negative PLC at laparotomy. More than half of the cases with positive PLC after tumor resection presented negative peritoneal cytology at laparotomy, suggesting that cancer cells were exfoliated into the peritoneal cavity during surgical manipulations in some cases[26,27]. Free malignant cells may also spill out from the intestinal lumen[27] or damaged lymphatics[26]. The factors that were identified to affect positive PLC after tumor resection included preoperative parameters, such as ascites and preoperative histology, even when the postoperative information, such as pathological findings, were included in the analysis regardless of PLC at laparotomy. In the present study, PLC after tumor resection could be omitted in more than 90% of all patients by selecting patients according to the factor affecting positive PLC after tumor resection, while increasing a positive rate from 1.6% to 5.3%. The utility of PLC according to the high-risk factors was thought to be in doubt because there were few positive cases in the selected cases according to the high-risk factors in spite of the rise in positive rate. However, this result is based on the outcome of peritoneal cytology before performing peritoneal lavage after tumor resection and might be improved by performing sufficient peritoneal lavage to remove exfoliated cancer cells. Therefore, the factors affecting positive PLC after tumor resection identified in the present study may not be associated with oncological results, such as recurrence and prognosis. No oncological results associated with PLC after tumor resection have yet been reported. However, sufficient peritoneal lavage following tumor resection may be necessary for high-risk patients to remove cancer cells that have exfoliated to the peritoneal cavity. Further study of PLC after tumor resections remains necessary to validate the clinical significance, sampling method, and factors affecting positive PLC. The development of strategies designed to eliminate the exfoliation of cancer cells into the peritoneal cavity is also necessary.

This study has some limitations. First, the sample size, especially the positive cytology cases, was relatively small. Second, it is necessary for the clinical significance of PLC to confirm oncological outcomes, although the present study suggested that we can efficiently obtain clinically useful information regarding the treatment strategy by selectively performing peritoneal cytology at laparotomy on patients that present factors that were possible to know before surgery. Third, the effect of intraperitoneal lavage for eliminating exfoliated cancer cells could not be properly evaluated in the current study since performing PLC just after peritoneal lavage could not be regulated according to the protocol.

In conclusion, we found that the undifferentiated histology and/or T4 indicated by preoperative diagnosis were identified as factors affecting PLC at laparotomy. Furthermore, ascites and preoperative histological type were identified as factors affecting positive PLC after tumor resection. As factors affecting positive PLC, these preoperative findings were found to be equivalent to pathological findings.

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Conflicts of Interest

There are no conflicts of interest.

Author Contributions

Harunobu Sato, Kenjiro Kotake, Kotaro Maeda, Hirotoshi Kobayashi, Hiroshi Takahashi, and Kenichi Sugihara meet all the criteria for authorship as per the ICMJE recommendations and substantially contributed to the manuscript. Harunobu Sato is the corresponding author.

Approval by Institutional Review Board (IRB)

This study was approved by the Ethics Committee of the Fujita Health University (Number 13-125).

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