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Role of Peritoneal Lavage Cytology and Prediction of Prognosis and Peritoneal Recurrence After Curative Surgery for Colorectal Cancer

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Purpose: In colorectal cancer, the role of detecting free malignant cells from peritoneal lavage is currently unclear. In this study, we investigated the positive rate of free malignant cells in peritoneal lavage fluid and their predictive value for prognosis and peritoneal recurrence after a curative resection.

Methods: From October 2009 to December 2011, in a prospective manner, we performed cytologic examinations of peritoneal lavage fluid obtained just after the abdominal incision from 145 patients who underwent curative surgery for colorectal cancer. We used proportional hazard regression models to analyze the predictive role of positive cytology for peritoneal recurrence and survival.

Results: Among total 145 patients, six patients (4.1%) showed positive cytology. During the median follow-up of 32 months (range, 8–49 months), 27 patients (18.6%) developed recurrence. Among them, 5 patients (3.4%) showed peritoneal carcinomatosis. In the multivariate analysis, positive cytology was an independent predictive factor for peritoneal recurrence (hazard ratio [HR], 136.5; 95% confidence interval [CI], 12.2–1,531.9; P < 0.0001) and an independent poor prognostic factor for overall survival (HR, 11.4; 95% CI, 1.8–72.0; P = 0.009) and for disease-free survival (HR, 11.1; 95% CI, 3.4–35.8; P < 0.0001).

Conclusion: Positive cytology of peritoneal fluid was significantly associated with peritoneal recurrence and worse survival in patients undergoing curative surgery for colorectal cancer. Peritoneal cytology might be a useful tool for selecting patients who need intraperitoneal or systemic chemotherapy.

Keywords: *Peritoneal metastasis; Cytology; Colorectal neoplasms; Survival; Prognosis*

INTRODUCTION

During the last two decades, the prognosis for patients with metastatic colorectal cancer (mCRC) has been noticeably improved because of the current combinations of chemotherapies with target agents and the development of surgical techniques. Surgical resection of hepatic and pulmonary metastatic lesions can achieve long-term survival of more than three years [1, 2]. Moreover, the survival of patients with unresectable mCRCs has been improved, up to 30 months, in the era of contemporary systemic chemotherapy with biologic agents [3]. However, these improvements in survival are not consistent among all mCRC patients. In mCRC with peritoneal carcinomatosis (PC), progress in multidisciplinary treatment has resulted in only a limited survival benefit [4, 5].

Peritoneal cytology has been considered to be useful for predicting an individual prognosis for some malignancies. Keettel and Elkin [6] introduced the technique of intraoperative peritoneal washing cytology in ovarian cancer patients for the first time in 1956. In 1975, the International Federation of Gynecologists & Obstetricians incorporated results of peritoneal cytology into the staging classification for ovarian cancer, and in 1989. It did so for endometrial cancer. Among the nongynecological adenocarcinomas, especially gastric and pancreatic adenocarcinomas, the pres-

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ence of free malignant cells in the peritoneal fluid is associated with poor survival and peritoneal recurrence and in 2009 was integrated as part of the American Joint Committee on Cancer (AJCC) TNM classification for cancers like uterine and ovarian cancers 2009 [7]. In colorectal cancer, there have been many reports on a positive correlation with poor prognosis and prediction of peritoneal recurrence [8, 9]. However, those results are still being debated [10]. New treatment modalities, including aggressive cytoreductive surgery with hyperemic intraperitoneal chemotherapy (HIPEC) and early intraperitoneal chemotherapy (EPIC) have been developed to obtain long-term control of PC and to maintain long-term PC-free survival [11, 12]. Knowing that the target for HIPEC or EPIC is intraperitoneal free cancer cells or tiny nodules, not gross disease, positive peritoneal cytology could represent an adequate selection factor following such aggressive treatments. Accordingly, revealing the correlation of intraperitoneal free cancer cells to survival and peritoneal recurrence is of primary importance and would lead to uniform therapeutic decision-making and meticulous postoperative follow-up. In this study, we investigated the tumor-positive rate of peritoneal washing cytology in patients with CRC who underwent curative surgery, and we analyzed the association of positive cytology with prognosis and peritoneal recurrence.

METHODS

Patients

From October 2009 to December 2011, we prospectively collected data on 145 patients who underwent a curative resection for colorectal cancer. Enrolled patients had no evidence of distant metastasis at preoperative staging work-up, including abdomino-pelvic computed tomography (CT), chest CT and 18-fluorodeoxyglucose positron emission tomography scan. Patients who had a previous history of treatment for malignancy, Lynch syndrome, or familial adenomatous polyposis were excluded from this study. Patients with unrecognized PC or hepatic involvement at surgical exploration were also excluded. The pathologic staging of cancer was assessed postoperatively according to the seventh edition of the AJCC TNM grading system [7]. Charlson's comorbidity index was calculated to evaluate the status of patients' comorbidity. The Institutional Review Board of Korea Cancer Center Hospital approved this study, and written informed consent for tissue collection was obtained from all patients.

Procedures

Peritoneal lavage was performed immediately after we had made a midline abdominal incision and just before we manipulated the tumor. About 100 mL of physiologic saline solution (37°C) was instilled into the abdominal cavity around the tumor with the patient in a supine position. After gentle stirring, these fluids were collected with a suction device at the Douglas pouch.

All peritoneal lavage specimens were prepared using the ThinPrep liquid-based cytology preparation system (Cytyc Co., Boxborough, MA, USA). The sample was centrifuged at 600 g for 10 minutes, and the supernatant was poured off carefully. The cell pellet was resuspended and washed with 30 mL of CytoLyt solution. The specimen was added to a PreservCyt (Cytyc Co.) solution vial and allowed to stand for 15 minutes. The vial was then placed in a Cytyc ThinPrep 2000 processor utilizing a computerized process and patented membrane technology for dispersion control, collection, and transfer of diagnostic cells from the sample to a 20-mm circular area on a glass slide. The slide was fixed

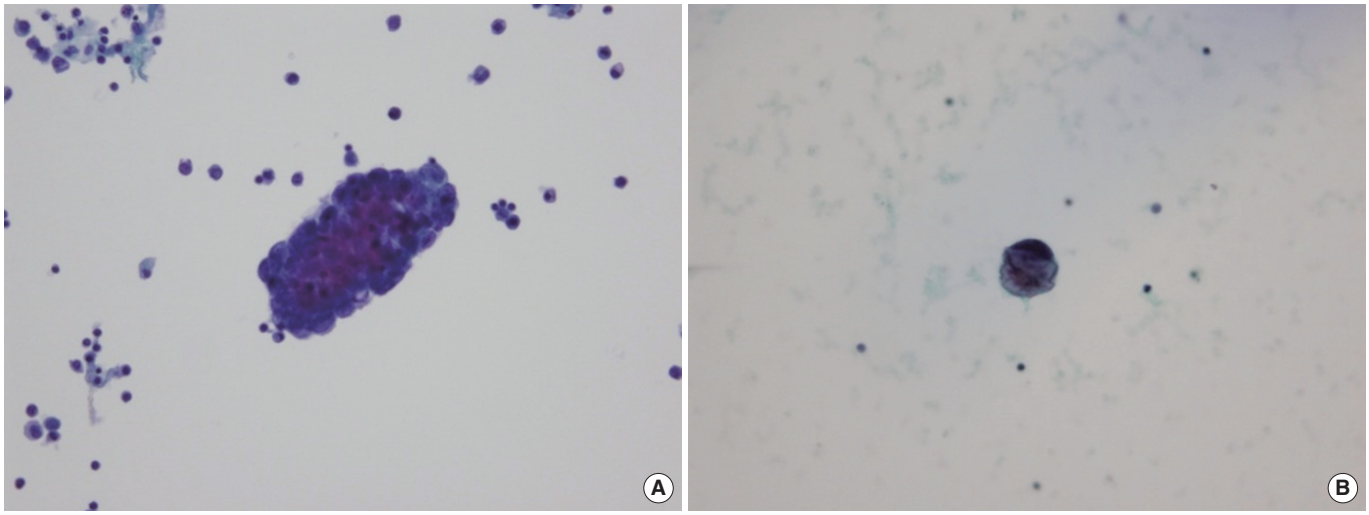

Fig. 1. (A) The malignant cells are arranged in 3-dimensional clusters with overlapping nuclei in the background of scattered reactive mesothelial cells, neutrophils and lymphocytes (Papanicolaou staining, ×400). (B) A tumor cell with high nuclear-to-cytoplasmic ratio has a hyperchromatic and coarse nucleus with an irregular eccentric contour and is 10 times larger than lymphocytes (Papanicolaou staining, ×400).

in 95% ethanol and stained by using the Papanicolaou and the diastase-periodic acid-Schiff staining methods.

All ThinPrep slides were reviewed and diagnosed by an experienced pathologist with a specialization in gastrointestinal oncology. A slide was classified as positive if malignant cells formed three-dimensional clusters or one malignant cell had a high nuclear cell ratio and over 10-fold enlargement compared to an adjacent lymphocyte (Fig. 1). A suspicion of malignancy or atypical finding was classified as negative.

Follow-up

The median duration of the follow-up period for all patients was 32 months (8–49 months). For detection of recurrence during the follow-up period, physical examinations and CEA checks were done every 3 months, and CT scans were done every 6 months in first two years after operation. Since then, carcinoembryonic antigen (CEA) checks and CT scans, including physical examinations, have been done every 6 months. Peritoneal and any systemic recurrences were diagnosed based on radiologic and/or pathologic evidence of cancer recurrence.

Statistical analysis

Statistical analyses were performed using the SPSS ver. 14.0 (SPSS Inc., Chicago, IL, USA). Associations between the clinicopathologic parameters were assessed using the chi-square test or the Fisher exact test for categorical variables. For continuous variables, the independent sample t-test or Mann Whitney U-test were done appropriately. Disease-free survivals (DFSs) and overall survivals (OSs) were calculated by using the Kaplan-Meier methods and were compared by using the log-rank test. The Cox proportional hazard model with a backward elimination method was used for multivariate analyses. In univariate analyses, variables whose P-values were less than 0.05 were selected for the multivariate analysis. P-values of less than 0.05 were considered statistically significant.

RESULTS

Overall, 6 of the 145 patients (4.1%) showed positive malignant cells in the peritoneal lavage fluid. All six positive cytology results were found in patients with more than T3 stage cancer. However, the distributions of pathologic T stages were not significantly different between the two groups according to the results of peritoneal cytology (P = 0.74). The rate of tumor perforation was relatively higher in patients with positive cytology than in patients with negative cytology, but the difference was not statistically significant. Mucinous phenotype or poor histologic grade was more frequently found in the positive-cytology group with statistical significance (P = 0.008) (Table 1).

During the follow-up period, 27 patients showed recurrence. Among them, 5 patients (18.5%, 3.4% of total) developed peritoneal recurrence. Four of these 5 patients were in the positive-cytology group. Other sites of recurrence were not significantly related with positive cytology (Table 2).

The 3-year DFS rate and OS rate for all patients were 77.0% and 93.2%, respectively. Comparing the DFS according to status of the peritoneal cytologic results, the 3-year DFS of the patients with positive cytology was 30.0%, and that of the patients with negative cytology was 78.5% (P = 0.001) (Fig. 2). Also, the 3-year OS of the patients with positive cytology was 66.7%, which was significantly

Table 1. Patients' characteristics and factors associated with positive cytology

	Cytology (+) (n = 6)	Cytology (–) (n = 139)	Total (n = 145)	P-value
Age (yr)	60.8 ± 9.7	62.4 ± 11.1	62.4 ± 11.1	0.67
Male gender	2 (33.3)	78 (56.1)	80 (55.2)	0.41
Charlson score	5.5 ± 1.5	5.6 ± 1.7	5.6 ± 1.7	0.86
Adjuvant chemotherapy	6 (100)	102 (73.4)	108 (74.5)	0.34
5-FU only	1 (16.7)	66 (47.5)	67 (46.2)	0.01
5-FU, oxaliplatin	5 (83.3)	36 (25.9)	41 (28.3)	
None	0 (0)	37 (26.6)	37 (25.5)	
Rectum	1 (16.7)	69 (49.6)	70 (48.3)	0.21
T stage				
pT0, T1, T2	0 (0)	45 (32.4)	45 (31.0)	0.17
pT3	5 (83.3)	77 (55.4)	82 (56.6)	
pT4	1 (16.7)	17 (12.2)	18 (12.4)	
Node metastasis	2 (33.3)	33 (23.7)	35 (24.1)	0.63
Stage 0 (CR)	0 (0)	7 (5.0)		0.62
I	0 (0)	34 (24.5)		
II	4 (66.7)	65 (46.8)		
III	2 (33.3)	33 (23.7)		
Lymphatic invasion	2 (33.3)	33 (23.7)	35 (24.1)	0.63
Vascular invasion	0 (0)	14 (10.1)	14 (9.7)	1.00
Perineural invasion	2 (33.3)	24 (17.3)	26 (17.9)	0.29
Differentiation (PD/MUC)	3 (50.0)	9 (6.5)	12 (8.3)	0.008
Obstruction	0 (0)	15 (10.9)	15 (10.3)	1.00
Perforation	1 (16.7)	2 (1.4)	3 (2.1)	0.12
CEA (ng/mL)	74.0 ± 159.2	8.2 ± 17.4	10.2 ± 35.9	0.36
CA 19-9, median (range)	14.9 (5.0–24.6)	9.9 (0.6–205.7)	10.1 (0.6–205.7)	0.67

Values are presented as mean ± standard deviation or number (%) unless otherwise indicated.
5-FU, 5-fluorouracil; CR, complete remission; PD, poorly differentiated adenocarcinoma; MUC, mucinous adenocarcinoma; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

worse than that of the patients with negative cytology (94.4%, P = 0.002) (Fig. 3). Regarding the peritoneal-recurrence-free rate, the patients with positive cytology showed significantly worse outcome than those with negative cytology (25.0% vs. 99.2%, P < 0.0001) (Fig. 4).

To find the factors impacting the DFS, OS and peritoneal-recurrence-free survival, we performed uni- and multivariate Cox regression analyses with clinicopathologic variables. In the univariate analysis, positive cytology was a significant poor prognostic factor that affected DFS (hazard ratio [HR], 7.15; 95% confidence interval [CI], 2.44–20.96; P < 0.0001). Pathologic T4 stage, regional lymph-node metastasis, lymphatic, perineural, and vascular invasion, and tumor perforation were also significantly associated with the DFS in the univariate analysis. In the multivariate analysis, positive cytology was one of the independent factors affecting the DFS (HR, 11.05; 95% CI, 3.41–35.8; P < 0.0001), along with regional lymph-node metastasis and the status of vascular and perineural invasion (Table 3).

In the analyses of the OS, a positive cytologic result was significantly associated with poor prognosis in the univariate analysis (HR, 8.49; 95% CI, 1.7–42.51; P = 0.009) as revealed by a former analysis. Regional lymph-node metastasis, lymphatic, vascular and perineural invasion, and tumor perforation were additional poor prognostic factors. In the multivariate analysis, a positive cytologic result (HR, 11.43; 95% CI, 1.82–71.95; P < 0.0001) was one of the independent prognostic factors for the OS, along with lymphatic and vascular invasion and tumor perforation (Table 4).

The Cox regression analyses for factors associated with peritoneal recurrence are presented in Table 5. A positive cytologic result (HR, 136.5; 95% CI, 12.17–1,531.91; P < 0.0001) and tumor perforation were the significant risk factors for peritoneal recurrence in the multivariate analysis. The pathologic T4 stage was significantly associated with peritoneal recurrence in the univariate analysis, but this significance disappeared in the multivariate analysis.

Table 2. Site of recurrence according to cytologic results

Site of recurrence	Positive cytology (n = 6)	Negative cytology (n = 139)	Total (n = 145)	P-value
Peritoneum	4 (66.7)	1 (0.7)	5 (3.4)	<0.0001
Liver	1 (16.7)	7 (5.0)	8 (5.5)	0.29
Lung	0 (0)	12 (8.6)	12 (8.3)	1.00
Distant lymph node	1 (16.7)	4 (2.9)	5 (3.4)	0.19

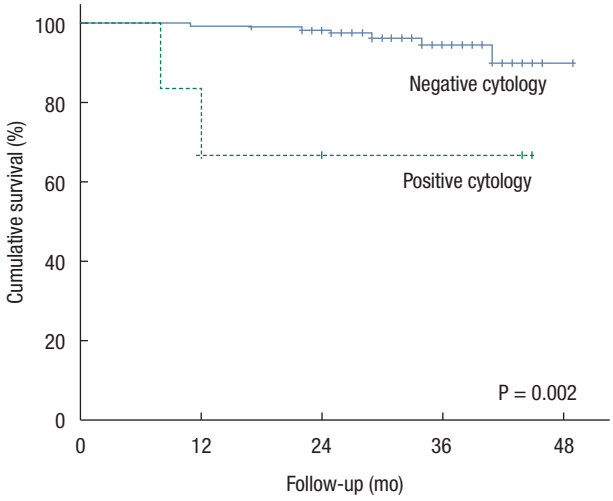

Fig. 3. Kaplan-Meier survival curves for overall survival according to the results of peritoneal cytology.

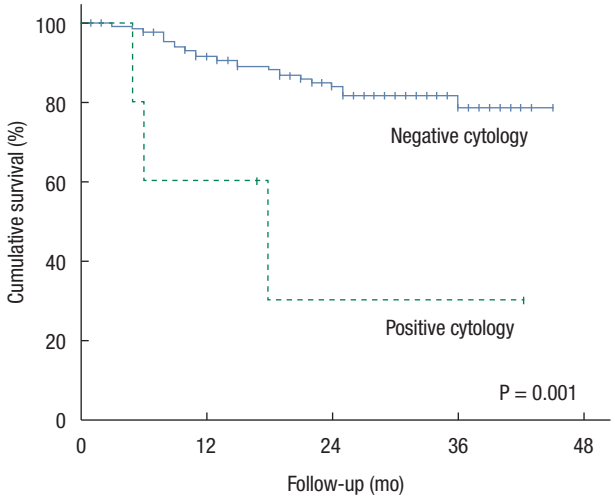

Fig. 2. Kaplan-Meier survival curves for disease-free survival according to the results of peritoneal cytology.

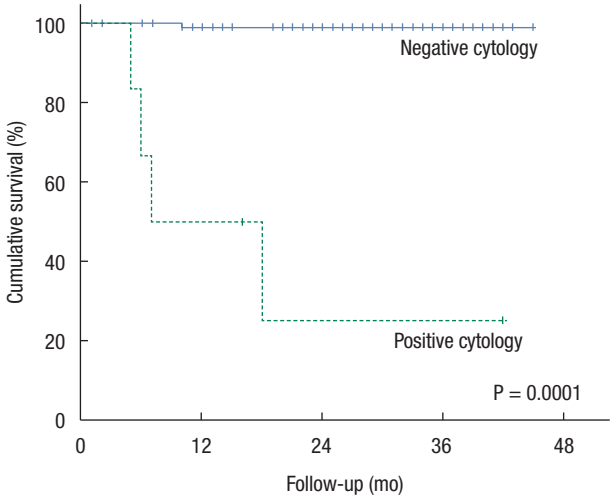

Fig. 4. Kaplan-Meier survival curves for peritoneal recurrence-free survival according to the results of peritoneal cytology.

Table 3. Cox regression analyses for factors associated with disease-free survival

Variable	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
Male sex	1.82	0.82–4.06	0.142			
Charlson score > 6	0.85	0.40–1.82	0.676			
Adjuvant chemotherapy	2.25	0.68–7.48	0.185			
Rectum	0.8	0.37–1.71	0.563			
pT4	2.93	1.18–7.28	0.021			
Node metastasis	2.82	1.32–6.04	0.007	2.90	1.33–6.33	0.007
Lymphatic invasion	3.25	1.52–6.96	0.002			
Vascular invasion	9.64	3.96–23.46	<0.0001	8.26	3.17–21.56	<0.0001
Perineural invasion	5.64	2.62–12.17	<0.0001	3.49	1.53–7.94	0.003
Differentiation (PD/MUC)	1.89	0.57–6.29	0.299			
Obstruction	1.65	0.57–4.77	0.358			
Perforation	6.00	1.41–25.55	0.015			
Positive cytology	7.15	2.44–20.96	<0.0001	11.05	3.41–35.80	<0.0001
CEA > 7 ng/mL	2.00	0.91–4.36	0.83			

HR, hazard ratio; CI, confidence interval; PD, poorly differentiated adenocarcinoma; MUC, mucinous adenocarcinoma; CEA, carcinoembryonic antigen.

Table 4. Cox regression analyses for factors associated with overall survival

Variable	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
Male sex	0.64	0.17–2.37	0.501			
Charlson score > 6	0.80	0.21–2.97	0.733			
Adjuvant chemotherapy	0.64	0.16–2.56	0.527			
Rectum	0.77	0.21–2.88	0.699			
pT4	0.97	0.12–7.74	0.974			
Node metastasis	4.43	1.18–16.56	0.027			
Lymphatic invasion	5.91	1.47–23.72	0.012	5.52	1.09–27.95	0.039
Vascular invasion	4.67	1.17–18.66	0.029	5.43	0.99–29.95	0.052
Perineural invasion	6.08	1.63–22.63	0.007			
Differentiation (PD/MUC)	3.39	0.70–16.46	0.130			
Obstruction	1.38	0.17–11.18	0.763			
Perforation	30.23	5.95–153.55	<0.0001	82.42	10.35–656.58	< 0.0001
Positive cytology	8.49	1.70–42.51	0.009	11.43	1.82–71.95	0.009
CEA > 7 ng/mL	1.54	0.38–6.16	0.540			

HR, hazard ratio; CI, confidence interval; PD, poorly differentiated adenocarcinoma; MUC, mucinous adenocarcinoma; CEA, carcinoembryonic antigen.

DISCUSSION

In this study, the rate of a positive cytologic result for peritoneal lavage fluid in patients with colorectal cancer without distant metastasis was 4.1%, and positive cytology was an independent poor prognostic factor for survival and a predictive factor for peritoneal recurrence. The rate of positive peritoneal cytology has been reported to range from 2.1% to 52% in patients with colorectal cancer [9]. This wide range across studies may be associated with heterogeneity of the techniques used to detect malignant cells in peritoneal lavage fluid. A recent systematic review for intraoperative peritoneal lavage reported mean weighted yields of 8.4%, 28.3%, and 14.5% for conventional cytology, immunocytochemistry (ICC) and polymerase chain reaction (PCR), respectively [13]. The

Table 5. Cox regression analyses for factors associated with peritoneal recurrence

Variable	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
Male sex	1.24	0.21–7.40	0.816			
Charlson score > 6	1.44	0.24–8.65	0.687			
Adjuvant chemotherapy	0.34	0.004–271.16	0.46			
pT4	5.65	0.94–33.86	0.058			
Node metastasis	4.65	0.78–27.84	0.092			
Rectum	0.26	0.03–2.33	0.229			
Lymphatic invasion	5.03	0.84–30.11	0.077			
Vascular invasion	0.04	0–9,640.64	0.646			
Perineural invasion	7.60	1.27–45.47	0.026			
Differentiation (PD/MUC)	21.50	3.57–129.53	0.001			
Positive cytology	142.54	15.57–1,305.22	<0.0001	136.52	12.17–1,531.91	<0.0001
Obstruction	0.04	0–10,967.55	0.618			
Perforation	91.08	14.62–567.31	<0.0001	33.03	1.82–599.66	0.018
CEA > 7 ng/mL	17.44	1.95–156.2	0.01			

HR, hazard ratio; CI, confidence interval; PD, poorly differentiated adenocarcinoma; MUC, mucinous adenocarcinoma; CEA, carcinoembryonic antigen.

positive rates of ICC and PCR were relatively higher than the detection rate of conventional cytology. However, their significance as a prognostic factor for survival or a predictive factor for peritoneal recurrence is not clear [14]. ICC is subjective and depends on the strength of cellular staining, and PCR-based methods have inherent problems as they detect RNA, not viable cells, and cannot delineate cancerous cells from nonmalignant cells or cellular debris [13, 15]. In addition, the targeted antigen or RNAs are heterogeneous between studies [15-19]. Therefore, further studies are required to identify which target genes/antigens detectable in peritoneal lavage fluid make the most optimal biomarkers for predicting outcome. Prospective validation of these biomarkers and validation studies to compare sensitivities and specificities between the various diagnostic criteria for positive cytology are also needed.

Conventional cytology is the most popular method because it is relatively inexpensive and requires neither the preservation of RNA nor the implementation of a complex technique. It has a high specificity, but often has a significantly lower sensitivity for detecting malignancy [20, 21]. In this study, we used liquid-based cytology with ThinPrep processing, an automated cytopreparatory method. Our cytopathology laboratory has used the ThinPrep process as a standard cytology method for the preparation of all cytologic specimens since 2000. In conventional cytology, the large numbers of blood cells and various inflammatory cells in the peritoneal lavage fluid may obscure malignant cells. The ThinPrep technique can eliminate these disturbing factors and has been reported to show good correlation with conventional preparations and to reduce the rate of false-negative diagnoses [22, 23].

The objective of being able to detect intraperitoneal free malignant cells in patients with colorectal cancer, as well as gastric and ovarian cancer [24, 25], was to evaluate its impact on survival and recurrence and to discuss intraperitoneal treatment or adjuvant systemic chemotherapy. As a prognostic factor, positive malignant cells in peritoneal lavage fluid appear to be associated with poor overall survival and recurrence-free survival and with increased risk of peritoneal recurrence [9, 13]. Although some studies have reported opposite results [10], majority of them had less than 100 patients and might be underpowered to show a difference in outcomes between positive and negative results [9].

To date, adjuvant chemotherapy has not been routinely recommended for low-risk stage-II patients. In the present study, four of the six patients with positive cytologic results were stage II. Although all of them received adjuvant chemotherapy, two patients showed recurrence: one at the peritoneum and the other at the peritoneum and the liver. If the strength of the association between positive peritoneal lavage and unfavorable outcome for recurrence and survival is considered, cytologic examination of peritoneal lavage fluid might be a useful tool to select the patients who need systemic or intraperitoneal chemotherapy and otherwise might not receive it. Noura et al. [8] reported in their analysis of 697 patients that positive peritoneal cytology was associated with poor prognosis and might be a useful marker for prediction of peritoneal recurrence. Furthermore, they showed that intraperitoneal chemotherapy with mitomycin C in positive cytology patients might reduce the peritoneal recurrence in their retrospective series [11]. In their study, postoperative intraperitoneal mitomycin C was given to 31 of the 52 patients with positive cytologic results who underwent curative surgery whereas adjuvant chemotherapy was

administered to all patients with stages II and III cancer. After a mean follow-up of 83.1 months, the 31 patients who received intraperitoneal treatment had a significantly better peritoneal recurrence-free and cancer-specific survival rate compared with those who received systemic chemotherapy only [11]. No survival benefit with systemic chemotherapy has been reported in this situation. Further large prospective clinical trials are needed to investigate the role of intraperitoneal and systemic chemotherapy in patients with isolated positive cytology.

For other risk factors associated with peritoneal recurrence, there are ongoing similar clinical trials. One involves performing adjuvant HIPEC for patients with pathologic T4a cancer to reduce the risk of peritoneal recurrence [26]. The other involves performing a second-look laparotomy and HIPEC after a 1-year follow-up for patients with synchronous PC, ovarian metastasis or perforated colorectal cancer to detect early peritoneal recurrence [27]. However, in our opinion, there were some problems in performing those trials. The former trial had no accurate preoperative diagnostic tool to identify pathologic T4a tumors, and a pathologic examination to identify the T4a tumor had to be done within 1 day after surgery to perform the adjuvant HIPEC before starting postoperative adhesion. After development of postoperative adhesion in the dissection plane, the entrapment of any free intraperitoneal malignant cells might be possible, and those tumor cells would not be exposed to intraperitoneal chemotherapy drug. For the latter trial, although the effectiveness of cytoreductive surgery (CRS) and HIPEC is clear [12], the mortality and the morbidity of these aggressive therapies are relatively high. Reported postoperative mortality rates were 3.3%–3.8%, and grades 3–4 complications occurred in 31% of the patients [12, 28]. The expertise of the center had a strong impact on the prognosis because of the presence of a significant learning curve, so this is not a procedure that can be undertaken occasionally [28, 29]. Considering these problems, we think that peritoneal lavage fluid cytology is a useful method to identify the cytologic result during surgery; therefore, intraperitoneal chemotherapy can be done immediately after resection of the tumor to reduce the peritoneal recurrence and further reduce the necessity for CRS and HIPEC, which have high mortality and morbidity. Because modern systemic chemotherapy with a biologic agent for PC has not shown improved outcomes compared with other site metastasis [4], prevention of peritoneal recurrence with early intraoperative chemotherapy might be a reasonable alternative treatment modality.

This study has some limitations of note. It is a single-institution observational study with a limited number of patients and a short-term follow-up period. The major limitation of this study is that the number of patients with positive cytology was only six. Due to this small patient number, the 95% CIs of the HR for predicting the risks of death, overall recurrence, and peritoneal recurrence were so wide that the accuracies of the estimates of the HRs were deeply impaired, despite of the significant P-values. If the accuracies of the estimates of the HRs are to be raised, an increased the sample size is needed. Also, due to this small patient number, well-known risk factors of peritoneal recurrence, like T4 cancer or tumor perforation, did not show a significant association with positive cytology and peritoneal recurrence. Among the six patients with positive cytology, only 1 patient had pathologic T4 cancer, and only 1 patient had a tumor perforation. Thus, a type-II error might exist. In addition, the possibility of under-staging on the pathologic examination should be considered because controversy persists regarding the most appropriate criteria for diagnosing serosal invasion and because practical difficulties are associated with histological assessment in some cases [30].

In spite of the previously-described limitations of small number of patients with positive cytology, positive peritoneal lavage cytology was an independent predictive factor for peritoneal recurrence and an independent prognostic factor for poor DFS and OS. For further consideration of the role of peritoneal cytology, a large, population-based, multicenter study with long-term follow-up is required.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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