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Original Article

The Shorr Versus Modified Ultrafast Papanicolaou Method for Intraoperative Diagnosis of Peritoneal Washing Cytology in Advanced Gastric Cancer: A Phase II Study

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ABSTRACT

Purpose: According to the American Joint Committee on Cancer cancer staging system, positive peritoneal washing cytology (PWC) indicates stage IV gastric cancer. However, rapid intraoperative diagnosis of PWC has no established reliable method. This study evaluated and compared the diagnostic accuracy of the Shorr and the modified ultrafast Papanicolaou (MUFP) methods for intraoperative PWC.
Materials and Methods: This study included patients with gastric cancer who were clinically diagnosed with stage cT3 or higher. The Shorr and MUFP methods were performed on all PWC specimens, and the results were compared with those of conventional Papanicolaou (PAP) staining with carcinoembryonic antigen immunohistochemistry. Sensitivity, specificity, and partial likelihood tests were used to compare the 2 methods.
Results: Forty patients underwent intraoperative PWC between November 2019 and August 2021. The average time between specimen reception and slide preparation using Shorr and MUFP methods was 44.4±4.5 minutes, and the average time between specimen reception and pathologic diagnosis was 53.9±8.9 minutes. Eight patients (20.0%) had positive cytology in PAP staining. The Shorr method had a sensitivity of 75.0% and specificity of 93.8%; the MUFP method had 62.5% sensitivity and 100.0% specificity. The area under the curve was 0.844 for Shorr and 0.813 for MUFP. In comparing the C-indices of each method with overall survival, no difference was found among the Shorr, MUFP, and conventional PAP methods.
Conclusions: The Shorr and MUFP methods are acceptable for the intraoperative diagnosis of PWC in advanced gastric cancer.

Keywords: Stomach neoplasm; Cytology; Diagnosis

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Conflict of Interest

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

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INTRODUCTION

Although the long-term survival of patients with early gastric cancer has improved over the past decade, patients with advanced gastric cancer with distant metastases have poor survival rates [1]. According to the American Joint Committee on Cancer TNM classification, the presence of metastatic gastric cancer cells on peritoneal washing cytology (PWC) tests indicates stage IV gastric cancer [2]. Positive PWC (or CY1 disease) is a significant independent factor for poor prognosis compared with negative PWC [3]. Positive PWC is related to peritoneal carcinomatosis, and methods such as intraperitoneal (IP) chemotherapy have been reported as additional means of treatment for gastric cancer with peritoneal metastasis [4]. Thus, if the cancer cells in PWC can be diagnosed intraoperatively, the surgical treatment plan may change. However, the rapid intraoperative diagnosis of PWC has no established reliable staining method.

Previously, we conducted a pilot trial comparing the Shorr staining method with conventional Papanicolaou (PAP) staining and carcinoembryonic antigen (CEA) immunohistochemistry (IHC) for the intraoperative diagnosis of PWC [5]. The Shorr method showed a high concordance rate (90.9%) with the PAP plus CEA IHC method. However, the Shorr method requires using the Shorr solution (Merck KGaA, Darmstadt, Germany), which is relatively difficult to obtain in Korea. Kamal et al. introduced the modified ultrafast Papanicolaou (MUFP) staining method [6] by modifying the previously reported ultrafast PAP staining method, which is a hybrid of the Romanowsky and PAP staining methods. The Richard–Allan hematoxylin and cytostain was replaced with the more universal Gill's hematoxylin. Thakur and Guttikonda [7] also proposed a new MUFP method that replaced Gill's hematoxylin with Harris hematoxylin, which is more readily available. This study evaluated the Shorr and MUFP methods as diagnostic tools for intraoperative PWC and compared their diagnostic accuracy with that of conventional PAP plus the CEA IHC method.

MATERIALS AND METHODS

Study design and population

This prospective study was approved by the Institutional Review Board (IRB) of Seoul National University Bundang Hospital (IRB No. B-1909-567-001). Patients with gastric adenocarcinoma clinically diagnosed as cT3 or higher disease were enrolled. Patients with other primary cancers within 5 years of treatment were excluded. For sample size calculation, the hypothesis for the sensitivity of MUFP was set at 50%, and the Shorr method was about 70%–80%, according to previous literature [5]. With a disease prevalence of <5%, the sample size needed was approximately 20–50 patients [8]. Since this was a prospective phase II feasibility study, the final sample size was decided to be 40 patients for study accrual time. Written informed consent was obtained from all the enrolled patients after a thorough description of the study, which was performed in accordance with the 1964 Declaration of Helsinki.

Processing of intraoperative PWC

After laparotomy or the creation of a pneumoperitoneum via laparoscopy, the abdomen was examined for possible peritoneal metastases. In cases of peritoneal metastasis, the peritoneal carcinomatosis index score was calculated, and a peritoneal biopsy was performed. Ascites were sampled for PWC when ascites were present. If no ascites could be sampled, 200 mL of saline was injected into the left subphrenic space and pelvis. After injection, the patient's body was shaken

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lightly for 2 minutes, and the PWC samples were aspirated. Samples were stored in conical tubes and sent directly to the pathology department. The PWC samples were first centrifuged at 2,500 rpm for 3–5 minutes, and then the sediment was set for cytospin at 2,000 rpm for 5 minutes. The samples were then separated into 3 tubes and stained according to 3 staining methods: the Shorr method, the MUFP method, and the conventional PAP plus CEA IHC method.

Staining methods

Shorr staining was performed as described in a previous pilot study [5]. Fixation was performed using 95% ethanol for 3 minutes, followed by rinsing with water. Then, the slides were dipped in hematoxylin (90 seconds) and 1% HCl (2 times, each 1 minutes) and rinsed with water (1 minute). After dipping in Shorr's solution (Merck KGaA) for 2 minutes, slides were rinsed with water (dipped 10 times), 95% ethanol, 100% ethanol, and xylene.

MUFP staining was performed with an air-dried smear according to the method reported by Thakur and Guttikonda [7], using Harris hematoxylin. Briefly, the slides were dipped in normal saline (30 seconds), alcohol formalin (10 seconds), and tap water (6 slow dips). The slides were then stained with Harris hematoxylin for 30 seconds and then dipped in tap water (6 slow dips). The slides were rinsed with 95% isopropyl alcohol and stained with EA-36 for 15 seconds. They were rinsed again in 95% isopropyl alcohol, then in 100% isopropyl alcohol, and dipped in xylene, then mounted with a coverslip for examination.

Conventional PAP staining and CEA IHC were performed for the remaining samples. For PAP staining, fixation with 95% ethanol was performed for 10–20 minutes, followed by dipping in 95%, 80%, 70%, and 50% ethanol ten times each. After rinsing with water, Harris hematoxylin was applied for 2 minutes, followed by rinsing with tap water. Then the slides were dipped in 50%, 70%, 80%, and 95% ethanol 10 times each, followed by staining with OG-6 solution for 1 minute and dipping again in 95% ethanol 10 times. EA-65 staining was performed for 10 minutes, followed by dipping in 95% and 100% ethanol ten times each. Finally, the slides were dipped in xylene and mounted on coverslips. CEA IHC was performed using an anti-CEA antibody (1:350; Dako, Glostrup, Denmark) with the Ventana Benchmark (BenchMark XT; Ventana Medical System, Inc., Tucson, AZ, USA) according to the manufacturer's instructions. Slides were reviewed immediately by a certified cytotechnologist (C.Y.) and a designated cytopathologist (N.H.Y.). Slides were examined using optical microscopy at 100× and 400× magnification.

Outcomes

The same diagnostic criteria were applied to all 3 staining methods to diagnose positive PWC. Cytological results were classified as positive for malignancy, negative for malignancy, or atypical. Those with atypical findings were considered negative when comparing sensitivity, specificity, and other predictive values. The diagnostic performances of the Shorr and MUFP methods were analyzed by comparing the results with the final diagnosis using conventional PAP staining. Measurements included the average time between sample reception and preparation of the Shorr and MUFP smears and between sample reception and a cytopathologist's reporting of the PWC results. The concordance between the 3 staining methods and patient survival outcomes was also analyzed.

Statistical analysis

Basic characteristics were compared between patients with positive and negative PWC using PAP and CEA IHC. Continuous variables were compared using Student's t-test or the Mann–

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Whitney U test, and categorical variables were compared using the χ^2 test or Fisher's exact test. Statistical significance was set at P<0.05. Diagnostic performances, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), weighted kappa, and area under the curve (AUC), were evaluated using R software (R 4.2.2, 2022; R Core Team, Vienna, Austria) with the caret package (version 6.0-93; Kuhn et al., 2022) [9]. The C-indices showing the concordance of the 3 methods and overall patient survival were calculated using R software with the Survminer package (version 0.4.9; Kassambara et al., 2021) [10], and the compareC package (version 1.3.2; Kang and Chen, 2022) [11].

RESULTS

General characteristics of the patient cohort

Forty patients were enrolled between November 2019 and August 2021. Patient demographics are shown in **Table 1**. Of the 40 patients, half (50%) were male patients. The mean age was 61.5±13.8 years. Twenty-seven (67.5%) patients underwent gastrectomy, and 13 (32.5%) received only a peritoneal biopsy or a bypass surgery. Ascites were detected during surgery in eleven (27.5%) patients. The mean tumor size was 8.6±4.3 cm. Pathologic T and N categories were evaluated among those who underwent gastrectomy, with 3 (11.1%) pT1b patients, 4 (14.8%) pT3 patients, 11 (40.7%) pT4a patients, 9 (33.3%) pT4b patients, 4 (14.8%) node-negative patients, and 23 (85.2%) node-positive patients. Sixteen (40.0%) patients had distant metastases, 14 (35.0%) had peritoneal metastasis, 1 (2.5%) had liver metastasis, and 1 (2.5%) had retroperitoneal node metastases.

Among the 40 patients, 8 (20%) were diagnosed with positive PWC using the conventional PAP plus CEA IHC method. **Table 1** compares the demographics of patients with negative and positive PWC. A greater proportion of patients with positive PWC had ascites (n=4, 50%) compared to those who had negative PWC (n=7, 21.9%), but the difference was not statistically significant (p=0.250). A greater proportion of patients showed metastatic diseases in the positive PWC group (n=7, 87.5%) compared to the negative PWC group (n=8, 25.0%; P=0.007).

Comparison of the staining quality of the 3 methods

The stained images obtained using the 3 methods are shown in **Fig. 1**. Shorr staining showed crisp nuclear features and well-preserved cell morphology with a clear background, similar to conventional PAP staining. The MUFP method showed more opaque nuclear features with moderately preserved cell morphology than the other 2 methods, with a similar transparent background. The mean time between the reception of the specimen and the preparation of the Shorr and MUFP smears was 44.4±4.5 minutes. The mean time between specimen reception and reporting of the PWC results was 53.9±8.9 minutes. The mean time between specimen reception and preparation of conventional PAP slides was 71.9±14.7 minutes.

Comparison of the diagnostic performance of the Shorr and MUFP method

The concordance of the PWC results using the Shorr and MUFP methods with conventional PAP plus CEA IHC is shown in **Supplementary Table 1**. The concordance of the results if atypical was considered negative (**Table 2**). If atypical was considered negative, the concordance was 90% for the Shorr method (36/40) and 92.5% for the MUFP method (37/40), compared with the final results of the conventional PAP plus CEA IHC method. The sensitivity, specificity, PPV, and NPV of the Shorr method were 75.0%, 93.8%, 75.0%, and

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Table 1. Patient demographics of the enrolled cohort

Patient characteristics	Total (n=40)	Negative PWC (n=32)	Positive PWC (n=8)	P-value*
Sex				0.693
Male	20 (50.0%)	17 (53.1%)	3 (37.5%)	
Female	20 (50.0%)	15 (46.9%)	5 (62.5%)	
Age (yr)	61.5±13.8	62.4±13.0	57.9±17.1	0.416
BMI (kg/m^2)	22.4±3.5	22.9±3.6	20.6±2.7	0.095
ASA score				0.404
1	6 (15.0%)	6 (18.8%)	0 (0.0%)	
2	29 (72.5%)	22 (68.8%)	7 (87.5%)	
3	5 (12.5%)	4 (12.5%)	1 (12.5%)	
Operation type				0.015
Distal gastrectomy	14 (35.0%)	14 (43.8%)	0 (0.0%)	
Total gastrectomy	13 (32.5%)	11 (34.4%)	2 (25.0%)	
Bypass or biopsy only	13 (32.5%)	6 (18.8%)	6 (75.0%)	
Operation time (min)	214.3±87.4	223.6±90.9	177.4±63.1	0.180
Estimated blood loss (mL)	62.5±101.2	70.1±111.0	31.9±33.3	0.103
Ascites present during surgery	11 (27.5%)	7 (21.9%)	4 (50.0%)	0.250
Tumor size (cm)	8.6±4.3	8.4±4.3	11.8±5.2	0.287
T stage†				0.853
T1b	3 (11.1%)	3 (12.0%)	0 (0.0%)	
T3	4 (14.8%)	4 (16.0%)	0 (0.0%)	
T4a	11 (40.7%)	10 (40.0%)	1 (50.0%)	
T4b	9 (33.3%)	8 (32.0%)	1 (50.0%)	
N stage†				0.181
N0	4 (14.8%)	4 (16.0%)	0 (0.0%)	
N1	4 (14.8%)	4 (16.0%)	0 (0.0%)	
N2	2 (7.4%)	1 (4.0%)	1 (50.0%)	
N3a	4 (14.8%)	4 (16.0%)	0 (0.0%)	
N3b	13 (48.1%)	12 (48.0%)	1 (50.0%)	
Distant metastasis				0.007
Total	16 (40.0%)	8 (25.0%)	7 (87.5%)	
Peritoneum	14 (35.0%)	7 (21.9%)	7 (87.5%)	
Liver	1 (2.5%)	1 (3.1%)	0 (0.0%)	
Retroperitoneal lymph nodes	1 (2.5%)	1 (3.1%)	0 (0.0%)	

PWC = peritoneal washing cytology; BMI = body mass index; ASA = American Society of Anesthesiologists.
*P-values generated by comparing the positive PWC and the negative PWC patients.
†T-stage and N-stage analysis for patients who had gastrectomy (total n=27).

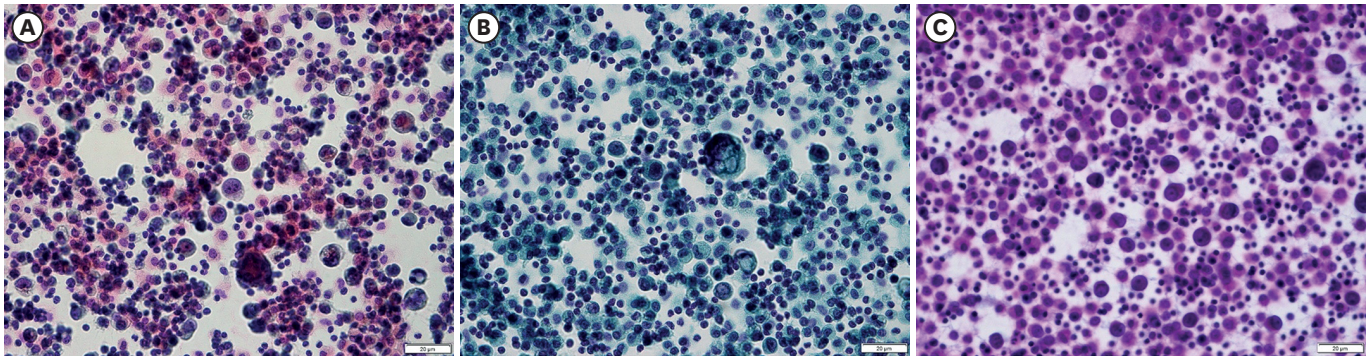

Fig. 1. Comparison of the staining quality of the 3 staining methods for peritoneal washing cytology. The Shorr staining exhibited distinct nuclear features and well-maintained cell morphology, resembling the conventional PAP staining. On the other hand, the MUFP method displayed less clear nuclear features and moderately preserved cell morphology when compared to both the Shorr and conventional PAP methods, with a comparable transparent background. (A) Conventional PAP method, (B) Shorr method, and (C) MUFP method.
PAP = Papanicolaou; MUFP = modified ultrafast Papanicolaou.

93.8%, respectively. The sensitivity, specificity, PPV, and NPV of the MUFP method were 62.5%, 100.0%, 100.0%, and 91.4%, respectively. The weighted Cohen's kappa for overall agreement of the 2 new methods was compared to conventional PAP plus CEA IHC was 0.69

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Table 2. Concordance table of the Shorr method and the MUFP method compared to the PAP plus CEA IHC method

Methods	PAP plus CEA IHC (CY1)			CY1 or P1		
	Negative	Positive	Total	Negative	Positive	Total
Shorr method						
Negative	30	2	32	24	8	32
Positive	2	6	8	1	7	8
Total	32	8	40	25	15	40
MUFP method						
Negative	32	3	35	25	10	35
Positive	0	5	5	0	5	5
Total	32	8	40	25	30	40

MUFP = modified ultrafast Papanicolaou; PAP = Papanicolaou; CEA = carcinoembryonic antigen; IHC = immunohistochemistry; CY1 = positive cytology; P1 = peritoneal metastasis.

for the Shorr and 0.73 for the MUFP method (**Supplementary Table 2**). The graph of the AUC is shown in **Supplementary Fig. 1**. The AUC for the Shorr and MUFP methods were 0.844 and 0.813, respectively.

The 2 methods were also compared using the presence of peritoneal metastasis (P1) or positive cytology (CY1) as an indication of a "positive" test (**Table 2**). The concordance was 77.5% for the Shorr method (31/40) and 75.0% for the MUFP method (30/40). The sensitivity, specificity, PPV, and NPV of the Shorr method were 46.7%, 96.0%, 87.5%, and 75.0%, respectively. The sensitivity, specificity, PPV, and NPV of the MUFP method were 33.3%, 100.0%, 100.0%, and 71.4%, respectively. The weighted Cohen's kappa for the overall agreement of the 2 new methods for CY1 or P1 was 0.47 for the Shorr method and 0.39 for the MUFP method (**Supplementary Table 3**). The AUC were 0.713 and 0.667 for the Shorr and MUFP methods, respectively (**Supplementary Fig. 2**).

Predictive value of the 3 diagnostic techniques

Fig. 2 shows the Kaplan–Meier graph of the overall survival (OS) of patients according to the results of PWC diagnosed using each staining method. Survival duration was significantly longer in patients with negative PWC according to the Shorr (P=0.043), MUFP (P=0.021), and PAP plus CEA IHC (P=0.009) methods. The 1-year survival rates of patients with negative or atypical PWC were 90.3% for the Shorr method, 87.9% for the MUFP method, and 90.0% for the conventional PAP methods. The 1-year survival rates of patients with positive PWC were 75.0% for the Shorr method, 80.0% for the MUFP method, and 75.0% for conventional PAP methods. **Supplementary Fig. 3** shows a combined Kaplan–Meier graph of the 3 diagnostic methods. The C-indices of the 3 methods for OS were 0.604 for the Shorr method, 0.591 for the MUFP method, and 0.629 for conventional PAP plus CEA IHC. No difference was found between the P-values of the 3 C-indices (**Supplementary Fig. 4**).

DISCUSSION

A positive PWC is useful for detecting peritoneal dissemination, even in the absence of visible seeding nodules [12]. A positive PWC alone is defined as distant metastasis in the Japanese classification of gastric cancer [2] and is the most significant predictor of poor survival. In a retrospective review of 371 patients who underwent R0 resection for gastric cancer at the Memorial Sloan–Kettering Cancer Center, multivariate analysis showed that a positive PWC was the most predictive factor for death from gastric cancer (relative risk, 2.7; P<0.001) [13].

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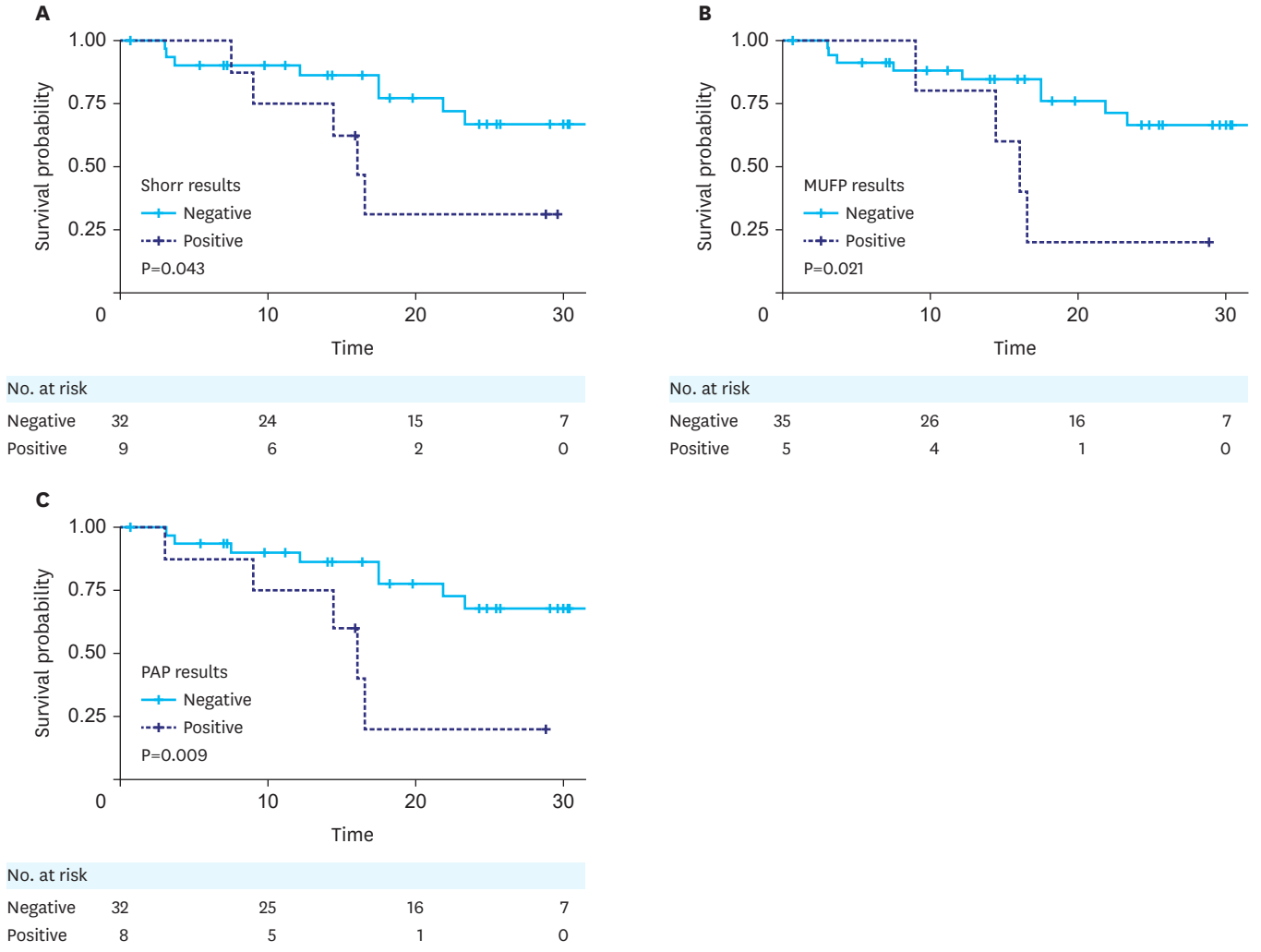

Fig. 2. Kaplan–Meier survival graphs of positive and negative peritoneal washing cytology results according to each staining method. (A) Survival according to Shorr results, (B) survival according to MUFP, and (C) survival according to PAP staining with CEA IHC results.
MUFP = modified ultrafast Papanicolaou; PAP = Papanicolaou; CEA = carcinoembryonic antigen; IHC = immunohistochemistry.

The number of cancer cells detected in the PWC specimens per slide can also be a prognostic indicator of survival after surgical resection. Miyashiro et al. investigated 417 patients with PWCs who had PWC during curative gastrectomy. The 3-year survival of patients with positive PWC who had <10 cancer cells per slide was 35%; those with ≥10 cancer cells per slide were 0% (P=0.017). Ribeiro et al. [14] evaluated 220 consecutive patients with gastric cancer who underwent gastrectomy between 1993 and 2002. Conventional PAP staining was used to determine the presence of malignant cells in the PWC. All patients with positive PWC showed pathological pT category 3 or higher, and none with positive PWC survived for >17 months after surgical treatment. Thus, when possible, PWC is highly recommended for patients with gastric cancer at clinical stage T3 or higher.

Since positive PWC affects prognosis, and the treatment plan may change accordingly. Currently, systemic chemotherapy is the standard treatment for gastric cancer with distant metastases [2]. A positive PWC is considered stage IV disease, but the choice of primary treatment for CY1P0 (positive PWC with no peritoneal metastasis) gastric cancer is debated.

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The CCOG0301 trial was designed as a phase II study, in which patients with CY1P0 gastric cancer underwent curative gastrectomy followed by S-1 monotherapy [15]. The median survival time was 705 days; the 2-year survival rate was 47%, which was greater than the historical control group used in this trial (13.3% 2-year survival).

A multicenter retrospective study by Yamaguchi et al. [16] analyzed 506 patients with gastric cancer with either positive PWC or peritoneal metastases who underwent gastrectomy. Their analysis showed that gastrectomy combined with adjuvant chemotherapy, either S-1 monotherapy or cisplatin, increased the OS of these patients. Current studies show that gastrectomy improves survival in patients with CY1P0 gastric cancer; however, surgical resection does not necessarily have to be performed first. A retrospective study by Yago et al. [17] suggested that, although whether surgery or chemotherapy was performed first has no statistical difference in survival, the prognosis of patients who underwent conversion surgery after initial chemotherapy was slightly more favorable (3-year survival rate, 23.4% vs. 27.3%).

The previously mentioned multicenter retrospective study also showed that survival was significantly greater in patients who were converted to CY0 after the initial treatment [12]. Yasufuku et al. [18] used the intraoperative diagnosis of PWC to determine the primary treatment for patients with type 4 and large type 3 gastric cancer. After staging laparoscopy, patients diagnosed as P0 and CY0 underwent curative surgery. Those diagnosed with CY1P0 cancer underwent systemic chemotherapy first, followed by conversion surgery if the PWC turned negative, with no other new lesions. The 3-year survival rate of the patients who underwent conversion surgery was 76.9%, indicating better survival rates compared to previous reports.

Various other treatment methods, such as lavage and IP chemotherapy, have been suggested for PWC-positive gastric cancer [19]. Extensive intraoperative peritoneal lavage (EIPL) has been suggested for removing free cancer cells from the peritoneal cavity [20]. In a randomized multicenter study by Kuramoto et al. [21], 88 patients with CY1P0 gastric cancer were randomly assigned to surgery only, surgery plus IP chemotherapy, or surgery plus EIPL plus IP chemotherapy groups. Their analysis showed that the 5-year survival rates of patients who received both EIPL and IP chemotherapy (43.8%) were significantly greater than the surgery-only group (0%, $P<0.0001$) and surgery plus IP chemotherapy group (4.6%, $P<0.0001$). Although a recent EXPEL trial [22] showed that EIPL did not provide a survival benefit for patients with cT3 and cT4 gastric cancers, it remains an option for CY1P0 gastric cancer. A systematic review by Cabalag et al. [19] pooled the survival data of 3 studies in which patients received IP chemotherapy and adjuvant systemic chemotherapy after radical gastrectomy. Although the data showed a trend that favored the IP group, it did not reach statistical significance (hazard ratio, 0.70; 95% confidence interval [CI], 0.47–1.04; $P=0.08$). In comparison, Ishigami et al. [23] enrolled 100 CY1 or P1 patients and used IP chemotherapy as a neoadjuvant palliative treatment. Conversion surgery was performed if cytology was converted to negative and shrinkage of the peritoneal metastasis occurred. R0 gastrectomy was achieved in 44 (69%) patients, and the median survival time was 30.5 months (95% CI, 23.6–37.7 months). IP chemotherapy can be administered before conversion surgery to prolong the survival of patients with positive PWC.

To date, conventional PAP staining has been most commonly used for diagnosing intraoperative PWC, not only in gastric cancer but also in various intra-abdominal cancers [24,25]. The conventional PAP method takes approximately 30 minutes for only the wet

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fixation before staining and may delay an intraoperative diagnosis of PWC. In comparison, the preparation of the Shorr-stained slides takes an average of approximately 10 minutes according to our protocol; other studies with variations to the Shorr [26] have reported staining times of <10 minutes [27]. The preparation of MUFP-stained slides was even faster than that of the Shorr method, with reports of approximately 130 seconds [28]. In our pilot study [5], we compared Shorr staining with conventional PAP plus CEA IHC. The Shorr method showed a high concordance rate of 90.9% (70/77) with the conventional method and a weighted kappa statistic of 0.875. Although the Shorr method produced PWC stains of reliable quality, it required the use of Shorr solution (Merck KGaA). In this prospective trial, we also tested whether the MUFP staining method using Harris hematoxylin was comparable to the results of both the Shorr and conventional staining methods. Our analysis showed that the Shorr and MUFP methods had highly balanced accuracy for the AUC (0.844 and 0.813, respectively) compared with the results of the conventional PAP plus CEA IHC method. The concordance was 90.0% for the Shorr method (36/40) and 92.5% for the MUFP method (37/40). The MUFP method showed greater specificity (100.0%); the Shorr method had greater sensitivity (75.0%). In other words, despite 0 false positives, the MUFP method had more false negatives than the Shorr method. However, the difference was not large because the number of false-negative cases for CY1 was 2 in the Shorr method and 3 in the MUFP method, while the number of false-negative cases for CY1 or P1 was 8 in the Shorr method and 10 in the MUFP method. MUFP staining showed a relatively less clear demonstration of nuclear features and cell morphology; however, the diagnostic performance was acceptable compared with the other 2 methods.

The main limitation of this study was its small sample size. Because the 3 methods had to be compared simultaneously, resources were limited. Thus, the minimum sample size was calculated, and the final sample size was small. However, although the enrolled number was 40 patients, 120 slide specimens were compared and analyzed [5]. Another major limitation of this study is that the Shorr and MUFP slides were prepared by one cytotechnologist, both slides were reviewed by one cytopathologist, and the preparation time for each slide was not observed. Due to the inability to review the slides separately, the total time for the report was longer than that in the pilot study. In a future comparative study, patients should be randomly selected to undergo either Shorr or MUFP staining, and then the results of PAP plus CEA IHC may be compared. However, this phase II study was designed to evaluate the feasibility and diagnostic performance of both methods; therefore, each patient sample was subjected to all 3 staining methods. In addition, this study considered atypical test results to be negative. This decision was made because the previous pilot study was analyzed in the same way [5] and because Ribeiro et al. [14] showed the OS of patients whose PWC stains were suspicious for malignancy (atypical) were similar to those of patients with negative PWC. Kawakatsu et al. [29] also considered atypical PWC results as negative when evaluating the prognostic significance of PWC in pancreatic ductal adenocarcinoma. Recently, several studies on the molecular detection of free cancer cells in PWC have been reported [30,31], and these novel methods may provide new insights into the best method for intraoperative PWC diagnosis for gastric cancer in the near future.

The Shorr and MUFP methods are acceptable for the intraoperative diagnosis of PWC in advanced gastric cancer.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Concordance table of the Shorr method and the MUFP method compared to the PAP plus CEA IHC method (atypical results included)

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Supplementary Table 2

Basic diagnostic performance statistics of the Shorr method and the MUFP method in comparison to PAP plus CEA IHC

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Supplementary Table 3

Basic diagnostic performance statistics of the Shorr method and the MUFP method in comparison to either CY1 or P1

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Supplementary Fig. 1

Illustration of the area under the curve compared to PAP plus CEA IHC (A) the Shorr method (0.844) and (B) the MUFP method (0.813).

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Supplementary Fig. 2

Illustration of the area under the curve compared to cytology positive or peritoneal metastasis (A) the Shorr method (0.713) and (B) the MUFP method (0.667).

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Supplementary Fig. 3

Kaplan–Meier survival graph comparing the results of all 3 staining methods for peritoneal washing cytology.

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Supplementary Fig. 4

Bar graph comparing the C-indices of the 3 diagnostic methods for overall survival.

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