Original Article

A Conventional Smear versus Cell Block Method for Malignant Cell Detection in Ascites or Peritoneal Washing in Endometrial and Ovarian Cancer

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Objective: To compare the malignant cell detection rate between the conventional smear and the cell block method in ascites or peritoneal washing in endometrial and ovarian cancer.

Materials and Methods: A cross-sectional study was conducted in 65 endometrial and presumed ovarian cancer patients who underwent surgical staging between August 2017 and February 2018 at Ramathibodi Hospital. All ascites or peritoneal washing fluids were examined by conventional smear and cell block method to detect malignant cells. The results were interpreted and reviewed by two cytologists. If the results were inconsistent or suspicious, re-interpretation and immunohistochemistry dyes were used to finalize the results.

Results: Sixty-five specimens were examined. Conventional smear revealed negative for malignancy in 57 specimens and positive for malignancy in eight specimens. The cell block method revealed negative for malignancy in 52 specimens and positive for malignancy in 13 specimens. The cell block method had a statistically significant higher rate of malignancy detection (20.0% versus 12.3%, p<0.001).

Conclusion: The cell block method increased the malignant cell detection rate. In addition, the cell block method has more advantages and the ability to perform further immunohistochemistry staining for a definitive outcome in suspicious cases.

Keywords: Ovarian cancer, Endometrial cancer, Conventional smear, Cell block, Malignancy detection rate

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Endometrial and ovarian cancer are the second and third most common gynecologic cancers worldwide[1]. The National Cancer Institute in Thailand reported 4,500 patients diagnosed with endometrial and ovarian cancer between 2010 and 2012.

Peritoneal washing or ascitic fluid collection for cytology is one important procedure in endometrial and ovarian cancer surgical staging. Many studies revealed that patients with positive cytology for malignancy had worse survival outcome[2,3]. Furthermore, cytology is beneficial in treatment planning and staging. Although the cytological results in endometrial cancer have been removed from staging, the International Federation of Gynecologists and Obstetricians (FIGO) suggests reporting the cytological results because previous studies reported that endometrial cancer patients with cytology positive for malignancy had a significantly lower survival rate over five years[3].

Nowadays, a conventional smear (CS) is the most common method for interpreting the cytology. The malignant cell detection rate in gynecologic

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cancer by the CS varies from 19% to 25%[4]. The cell block (CB) method, which requires fixing by formalin and embedding the cells in paraffin, is an interesting method to improve the detection rate. Previous studies in pleural fluid revealed that the CB method increased the detection rate of malignant cells by 10% to 15%. However, there are few studies in gynecologic cancer[5-7]. Therefore, the authors aimed to compare the malignant cell detection rate in ascites or peritoneal washing in endometrial and ovarian cancer using the conventional cytology smear and the CB method.

Materials and Methods

The present study was a cross sectional study approved by the Committee on Human Rights Related to Research Involving Human Subjects based on the Declaration of Helsinki (ID04-60-08).

Based on the present pilot study, the malignant cells detection rate between the CS and CB in ascites or peritoneal washing in endometrial cancer and ovarian cancer were 0.1 and 0.3, respectively. Type I and II errors were set at 5% and 20%, respectively. Calculating sample size of two independent groups, 62 patients were required.

The study was conducted in the Department of Obstetrics and Gynecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University on patients diagnosed with endometrial cancer or presumed ovarian cancer and underwent surgical staging between August 2017 and February 2018. The excluded patients were those who received previous treatments, i.e., neoadjuvant chemotherapy, radiation, or surgery and patients who were finally diagnosed as non-gynecological cancer. The demographic data included age, weight, height, body mass index, underlying disease, stage of cancer, and cell type of each cancer.

A total of 200 mL of ascites or peritoneal washing fluids were collected from each patient during surgical staging. The fluids were divided into two portions of 100 mL each for CS and CB preparation.

Conventional smear

First, 100 mL of ascites or peritoneal washing was centrifuged at 6,000 rpm for 10 minutes (Kokusan H-36) and the supernatant was discarded. The remaining cells were smeared on the slide and submitted for Papanicolaou staining.

Cell block

The other 100 mL of fluid was prepared for the CB method. After the fluid was centrifuged, the same as the first portion, the supernatant was removed. The cell pellets were embedded in agar using a Shandon Cytoblock kit to form a solid clot that was placed in the cassette, fixed with 10% formalin solution, and then embedded in a paraffin block. The specimens were sectioned into 3-micron thicknesses and hematoxylin and eosin staining was performed.

Interpretation

The CS and CB samples were interpreted by two cytologists blinded to the clinical data. The cytologists interpreted the results into three categories as positive for malignancy, negative for malignancy, and suspicious for malignancy. Re-interpretation or immunohistochemistry staining was used to finalize the results whenever the results were suspicious for malignancy or inconsistent.

The data were analyzed using Predictive Analytic Software Statistics 18 and compared by Pearson chi-square and Fisher's exact tests. Cohen's kappa coefficient was used to measure the inter-rater reliability of the cytologists. Continuous data were presented as mean ± standard deviation (SD) and percentage. Statistical significance was defined as p-value less than 0.05.

Results

Initially 69 patients were included but after excluding four patients diagnosed as non-gynecologic cancers, 65 patients remained in the current study with a mean age of 60.81±11.68 years. The demographic data are shown in Table 1. There were 36 (55.38%) endometrial cancers and 29 (44.62%) ovarian cancers. Two-thirds of the patients were in early stage (stage I-II) of endometrial cancer (55.38%) and ovarian cancer (51.72%). The most common cell type of endometrial cancer was endometrioid carcinoma and the most common cell type of ovarian cancer was endometrioid and serous carcinoma.

The two cytologists had high agreement in interpreting the results of both methods (Cohen's kappa coefficient 0.92). One case was suspicious for malignant cells in both methods of CS and CB. Each specimen was re-interpreted and immunohistochemistry staining for CK7 and WT1 was performed. The specimen was negative for malignant cells in the CS method but positive for malignant cells in the CB method. In the final interpretation, eight specimens were positive for malignancy in the CS method and 13 specimens were positive for malignancy in the CB method (Figure 1).

Table 1. Demographic data (n=65)

Characteristic	n (%)
Age (years); mean±SD	60.81±11.68
Weight (kg); mean±SD	61.13±16.20
Height (cm); mean±SD	153.12±6.06
BMI (kg/m^2); mean±SD	26.05±6.77
Underlying disease	
DM	16 (24.61)
HT	28 (43.08)
DLP	31 (47.69)
Other	16 (24.62)
No underlying disease	25 (38.46)
Endometrial cancer	36 (55.38)
Early stage (stage I-II)	31 (86.11)
Advanced stage (stage III-IV)	5 (13.89)
Ovarian cancer	29 (44.62)
Early stage (stage I-II)	15 (51.72)
Advanced stage (stage III-IV)	14 (48.27)

SD=standard deviation; DM= diabetes mellitus; HT=hypertension; DLP=dyslipidemia

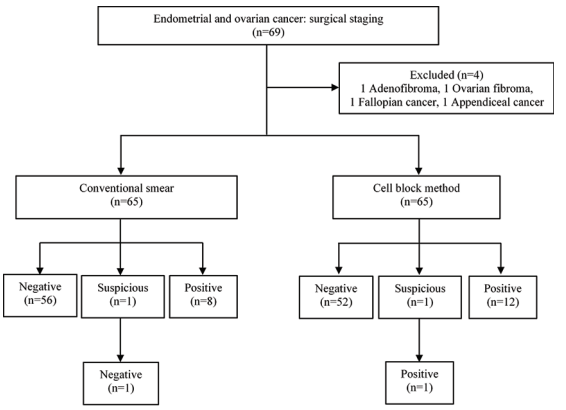

Figure 1. Flow chart of the study.

Therefore, the malignant cell detection rates in the CS and CB methods were 12.2% and 20.0%, respectively (p<0.001) (Table 2).

Moreover, the detection rates were higher in ovarian cancer, 17.2% in CS and 31.0% in CB, especially in advanced stage (stage III-IV) (Table 3). All positive cytologic cases are shown in Table 4.

Discussion

Staging of gynecologic malignancy is an important prognostic factor and is useful in treatment planning. A Gynecologic Oncology Group study[8] reported that in patients with ovarian cancer older than 60 years, higher stage, higher grade, and positive malignant cytology had worse survival and higher risk of disease recurrence. Furthermore, Seidman et al[9] confirmed that positive malignant cytology affected patient survival. The 5-year survival rates for ovarian cancer stage IA and IC were 100% and 83%, respectively[9]. The prognostic significance of positive

Table 2. Cytology interpretation of ascites/peritoneal washing in endometrial cancer and ovarian cancer (n=65)

Results	Conventional smear n (%)	Cell block n (%)	p-value
Negative	57 (87.7)	52 (80.0)	<0.001
Malignancy	8 (12.3)	13 (20.0)	

Table 3. Malignant cell detection in endometrial cancer and ovarian cancer

	Conventional smear n (%)	Cell block n (%)	p-value
Endometrial cancer	3 (8.3)	4 (11.1)	0.751
Ovarian cancer	5 (17.2)	9 (31.0)	
Endometrial cancer			0.659
Early stage	1 (2.8)	2 (5.6)	
Advanced stage	2 (5.6)	2 (5.6)	
Ovarian cancer			0.923
Early stage	1 (3.4)	2 (6.9)	
Advanced stage	4 (13.8)	7 (24.1)	

Table 4. Positive cytologic cases

Case	Cancer	Staging	Cytology interpretation	
			Conventional smear	Cell block
1	Ovary	IC3	Negative	Positive
2	Ovary	IC3	Positive	Positive
3	Ovary	IIIA	Negative	Positive
4	Ovary	IIIA	Positive	Positive
5	Ovary	IIIC	Positive	Positive
6	Ovary	IIIC	Positive	Positive
7	Ovary	IVA	Positive	Positive
8	Ovary	IVB	Negative	Positive
9	Ovary	IVB	Negative	Positive
10	Endometrium	IB	Negative	Positive
11	Endometrium	II	Positive	Positive
12	Endometrium	IIIC1	Positive	Positive
13	Endometrium	IVB	Positive	Positive

peritoneal cytology in endometrial cancer is still inconclusive[10-13]. However, much recent evidence reported abnormal peritoneal cytology in endometrial cancer, especially in early stage (stage I-II) and serous pathology was associated with decreased overall survival[10-12] and disease-free survival[12]. According to the matched cohort analysis from the National Cancer Database[10], patients with positive peritoneal cytology in early endometrial cancer had increased survival after receiving adjuvant chemotherapy. FIGO recommends peritoneal washing or ascites collection for cytology as one procedure in surgical staging in ovarian and endometrial cancer.

The malignant cell detection rate in the present study was 20%, which was comparable with previous studies that ranged between 19% and 25%[5,6]. Positive malignant cytology by the CB method was found in 11% and 31% in the endometrial and ovarian cancer groups, respectively, which corresponded with the previously reported incidence of 4.9% to 21.2% in endometrial cancer[14,15] and 25% to 50% in ovarian cancer[4,16,17]. There was a significant difference in the malignancy detection rate between the CS method and CB method ($p<0.001$). There were no discrepancies between the CS and CB methods when the CS method interpreted the cells as positive for malignancy.

The CB method had a 7.7% higher malignant cell detection rate in peritoneal washing and ascites in endometrial cancer and ovarian cancer compared with the CS method. Shivakumarswamy et al[18] studied 44 peritoneal washing samples in male and female patients diagnosed as ovarian cancer, cervical cancer, and carcinoma of the colon, liver, and urinary bladder. The CB method in that study had a 14% higher yield of malignant cell detection. Barui et al[19] reported that an additional six of 24 ascitic fluid samples detected malignant cells by the CB method. Likewise, previous research reported that the CB method was superior to the CS method for the diagnosis of malignant pleural effusion and body cavity fluids[8,20,21]. Nevertheless, the yield of malignant cell detection in the present study was lower than the previous studies because the patients in the present study were in the earlier stage since the incidence of malignant cytology in endometrial cancer stage I was reported to be 10%, whereas the incidence was 30% in advanced stage (stage III-IV)[22]. Furthermore, one patient who had ovarian cancer stage I was upstaged to stage IC from the advantage of the CB method and further treatment with chemotherapy was provided. The CB method can enhance the malignant cell detection in peritoneal washing/ascites in endometrial and ovarian cancer by providing better morphology, clearer background, and less cellular dispersal[23].

On the other hand, five cases were interpreted as negative for malignant cell in the CS group by the two cytologists (Cohen's kappa 0.932), while the interpretation was positive for malignant cells interpreted by the CB method. However, three of these patients had cancer in advanced stage (stage III-IV). There are several factors that can affect a misinterpretation in the CS method such as inadequate or improper smear, poor fixation, staining process, reactive mesothelial cell, and other artifacts[18,20].

In addition to the ability to recognize histological patterns in the CB method, the method is also favored for providing multiple sections for immunohistochemistry[18,24] to confirm a diagnosis. Recently, numerous methods of CB preparation are available like the tissue coagulum clot method, plasma thrombin clot method, agar method, HistoGel method, Shandon Cytoblock method, rapid CB method, and automated methods as well as various fixatives like formalin, heavy metal fixatives (Zenker's, B5) or acidic solutions (Bouin's solution), and microwave fixation[24]. However, the technique that is the most suitable as a standard is still controversial. The agar method and formalin fixation were used in the present study because they are less expensive and readily available. The agar method provides better orientation of the CB. Alcohol or ethanol-based formalin fixation supports preservation of the antigenicity, cytomorphological features, and DNA extraction[18,25].

Alternatively, the CS method has potential utility in immunohistochemistry studies. False positive and false negative results can occur for several reasons in immunohistochemistry staining in the CS method, i.e., 1) unavailability of serial sections for an antibody panel, 2) inadequate cells or cell loss, 3) trapped antibodies leading to non-specific staining, 4) disruption of cells and leakage of antigens during the smearing process, and 5) high background staining from blood and necrotic materials[26]. Consequently, immunohistochemistry was not used in the authors' CS method.

The CB method has some limitations. First, a gold standard does not currently exist to evaluate the efficacy of the CB method to detect malignant cells in peritoneal washing or ascites. Second, the tendency of a higher detection rate in the ovarian cancer group was found without a statistically significant difference because of the small sample size in the present group. Therefore, further studies in the ovarian cancer group with a larger sample size are suggested. Fortunately,

one patient in the early stage of ovarian cancer received adjuvant chemotherapy from the ability of the CB method to detect the malignant cells in the peritoneal washing.

Conclusion

The CB method provided a significantly higher rate of malignant cell detection than the conventional cytology smear in endometrial and ovarian cancer. In addition, the advantages of the CB method were better morphology, clearer background, and less cellular dispersal, which led to more accurate interpretations. Moreover, the CB method can provide immunohistochemistry staining that influences patient care.

What is already known on this topic?

The standard treatment for endometrial and ovarian cancer is surgical staging. The cytological report is one part of surgical staging. Nowadays, the CS is commonly used for a cytological interpretation, but the malignant cell detection rate is limited to 19% to 25%. The CB method has the advantage of providing a higher malignant cell detection rate (10% to 15%) than the CS in pleural fluid.

What this study adds?

This study demonstrated that the overall rate of malignant cell detection in ascites or peritoneal washing fluid in endometrial and ovarian cancer was 20%, and the CB method increased the detection rate by 7.7%, which correlated with prior studies in pleural fluid. Moreover, the CB method has more advantages in that immunohistochemistry staining can help finalize the cytological report in suspicious cases. Thus, the CB method is an interesting alternative for interpreting cytology results in gynecologic cancer.

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Conflicts of interest

The authors declare no conflict of interest.

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