Impact of SurePath® liquid-based preparation in cytological analysis of peritoneal washing in practice of gynecologic oncology

ABSTRACT

Context: Peritoneal washing is performed for staging of gynecologic tumors to detect subclinical intraperitoneal metastases.

Aim: The aim of the present study was to assess the impact of SurePath™ liquid-based cytology (LBC) in peritoneal washing in various gynecologic malignancies.

Settings and Design: An audit of peritoneal-fluid/washing (January 2012 to July 2013) was performed with corresponding gynecologic specimens. All peritoneal washings were processed using both conventional and LBC technique. Suspicious cases on cytology were reported along with gynecologic specimens.

Results: There were a total of 393 peritoneal fluids. Eighty-three (21.1%) were positive for malignancy, and the corresponding histology was available in 352 (89.6%) cases. Sixty-nine positive samples had ovarian malignancies and 5 had uterine causes. There were 9 cases of peritoneal washings in which no histopathology was available. The most common cause of positive peritoneal cytology was ovarian serous carcinoma in 55/84 (65.5%) cases. Other causes included mucinous cystadenocarcinoma, dysgerminoma, squamous cell carcinoma in teratoma, yolk sac tumor, and granulosa cell tumor. Uterine causes included 2/45 (4.4%) cases of endometrioid adenocarcinoma, ¼ (25%) cases of clear cell carcinoma, ½ (50%) cases of carcinosarcoma, and ¼ (25%) cervix carcinoma. On review of positive cases (n = 83), 10 cases were identified, which had nil (n = 4) to low cellularity (<3 tumor clusters/smear; n = 6) on conventional smears, and were confirmed malignant on LBC.

Conclusions: The most common ovarian malignancy causing positive peritoneal cytology is papillary serous carcinoma. Endometrioid adenocarcinoma rarely leads to positive peritoneal cytology. LBC technique leads to concentration of tumor cells causing reduction in false negative cases, especially in hemorrhagic and low-cellular cases.

Key words: Endometrioid adenocarcinoma; liquid-based cytology; papillary serous carcinoma; peritoneal wash

Introduction

Peritoneal washing is performed to detect intraperitoneal metastasis in various gynecological malignancies. Peritoneal spread of tumor may lead to involvement of serosa with or without ascites. Peritoneal lavage cytology is a standard practice in initial surgical staging as well as “second look” procedures in the management of ovarian tumors. Positive peritoneal washings are considered to be an important factor
in management of patients with gynecological malignancies. Patients with positive peritoneal washings have been found to have worse prognosis than patients without malignant cells in cytology.\textsuperscript{[3,4]} Positive peritoneal washing in endometrial cancer is associated with an increased risk of recurrence and poor survival.\textsuperscript{[5-7]} Hence, it is essential to identify malignant cells in peritoneal lavage samples. False negative cytology reports can lead to undertreatment of patients with adverse clinical outcome. Factors that can lead to false negative peritoneal cytology include inadequate volume of peritoneal fluid examined, obscuration of the tumor cells by florid mesothelial reaction, blood, excessive dilution of malignant cells, or small foci of peritoneal metastasis such that the tumor cells are not easily identified.\textsuperscript{[8]} Conventional smear may display cell clumping in one corner of the smear, obscuring of cells by mucus, haemorrhage, inflammatory cells, and debris along with multilayering of cells which will hamper cytological interpretation of the smear. Liquid-based cytology (LBC) uses the principle of cell filtration (ThinPrep\textsuperscript{TM}) or cell enrichment (SurePath\textsuperscript{TM}). Consequently, the smears are homogenously stained, circular smears of 22 mm diameter in ThinPrep\textsuperscript{TM} and 13 mm diameter in SurePath\textsuperscript{TM}, which contain 50–70000 well-preserved cells per smear. The cells are uniformly distributed throughout the circular smear, which is devoid of background debris and obscuring by red blood cells (RBCs) or inflammatory cells. Because of uniform staining and absence of background debris, the nuclear morphology is clear on LBC smear. Thus, LBC preparations can overcome the shortcomings of conventional smears to a large extent and improve the diagnostic accuracy.

A similar study in the English literature has explored the efficacy of ThinPrep\textsuperscript{TM} LBC technique.\textsuperscript{[9]} The aim of the present study was to evaluate gynecological cancers causing positive peritoneal cytology and to evaluate diagnostic accuracy of LBC using BD SurePath\textsuperscript{TM} LBC technique in peritoneal cytology.

**Patients and Methods**

An audit of peritoneal fluid/washing/lavage (January 2012 to July 2013) was performed with identification of corresponding gynecologic specimens. All peritoneal washings were processed using conventional as well as SurePath\textsuperscript{TM} LBC technique. The samples were centrifuged and the smears were prepared from the sediment using a conventional technique; the samples were then processed as per the manufacturer’s instructions for SurePath\textsuperscript{TM} LBC technique. The initial cytology reporting was done without knowledge of corresponding gynecologic histopathology. However, suspicious cases at the time of initial reporting were reported in the light of corresponding histopathologic diagnosis to categorize all cytology samples as “Positive” or “Negative” only. (It is the standard practice in our Department that smears with equivocal features on cytology are reported after examining the corresponding histopathological sections). It was also attempted to further subtype positive samples. All the positive peritoneal wash samples were reviewed by two cytopathologists (RT, NG) to analyze cytological features in various gynecological malignancies and to study the difference in cytomorphology in conventional versus SurePath\textsuperscript{TM} LBC technique. Diagnostic accuracy of these techniques was evaluated using histopathological diagnosis as the Gold standard.

Peritoneal fluid/washings comprised a total of 393 samples out of 12971 samples of exfoliative cytology (excluding cervical Pap samples) received in the department during the study period. Age range for ovarian epithelial neoplasms (15–73; mean 46.6 years), germ cell tumors (14–56; mean 27 years), sex-cord stromal tumors (19–70; mean 48 years), and uterine malignancies (26–85; mean 57 years) was noted. A total of 83/393 (21.1%) were positive and 310/393 (78.9%) were negative for malignancy on peritoneal cytology. Corresponding histology was available in 352/393 (89.6%) cases.

**Results**

Out of the 393 samples of peritoneal washes, corresponding histology was available in 352 cases only. Rest of the cases either did not undergo surgery or were lost to follow-up. Out of 83 positive peritoneal washings, 69 samples had ovarian malignancies and 5 had uterine causes. No record of histopathology of the remaining 9 cases with positive peritoneal cytology was available.

Out of a total of 84 histopathologically diagnosed ovarian serous carcinoma cases, 55 (65.5%) cases had positive peritoneal cytology [Figure 1a and b]. These cases usually were easily diagnosed on cytology. Five cases also showed psammoma bodies. LBC smears were helpful to better visualize nuclear details and acted as concentrated samples. These were reported as “positive for adenocarcinoma.” Only 3 cases had a few clusters in conventional smears and the diagnosis was based mainly on LBC samples. Tumor cells were seen in peritoneal cytology in 1/8 (12.5%) cases of borderline serous tumor (atypical proliferative serous tumor) with a few tiny papillaroid clusters of tumor cells. This case was initially reported as positive for neoplasm. Four out of 9 cases (44.4%) of mucinous adenocarcinoma were positive [Figure 1c and d] with clusters as well as scattered tumor cells with mild nuclear atypia embedded in abundant mucinous material. Chicken-wire type of capillary network associated with mucin was also appreciated in 3 out of 4 cases. These
cases were reported as mucinous adenocarcinoma in fluid cytology. LBC helped to identify malignant cells in 2 cases, which were darkly stained and embedded in thick mucin in conventional smears. Pseudomyxoma peritonei was seen in two cases of ovarian mucinous cystadenoma with mucinous neoplasm involving the appendix. The tumor was present in ovary as well as the appendix; hence, ovarian involvement was not secondary. Thick mucinaceous material along with benign mesothelial cells were seen in these cases. Mucinous material was seen metachromatically staining in conventional smears; however, the same was also represented as thick greenish mucin pools in LBC smears. Three out of 4 (75%) cases of dysgerminoma, squamous cell carcinoma in teratoma \[(n = 1)\], 2/4 (50%) yolk sac tumor, and 1/13 (7.7%) granulosa cell tumor had positive peritoneal cytology. Cytology samples revealed dispersed population of intermediate-sized tumor cells with high N/C ratio, coarse chromatin, prominent nucleoli, and scanty vacuolated cytoplasm in cases of dyserginoma. These were better seen in conventional MGG smears as compared to LBC samples due to even smaller size and intense staining in LBC smear. The case of squamous cell carcinoma showed tumor cells having squamoid differentiation and marked variation in size and shapes of the tumor cells. Cases of yolk sac tumor showed obviously malignant cell clusters admixed with florid mesothelial cell proliferation. The main clue to diagnosis of a germ cell tumor in fluid cytology was round-to-oval tumor cells with very prominent nucleoli and vacuolated cytoplasm. The case of granulosa cell tumor revealed classic morphology with dispersed population of round-to-angulated tumor cells and a few tumor cells arranged in acinar-like arrangement around pink staining bodies. The cytomorphology in this case was better seen in MGG stained smear. The cases of dysgerminoma, yolk sac tumor, and granulosa cell tumor were initially reported as “positive for malignancy” on cytology.

Uterine causes included 2/45 (4.4%) cases of endometrioid adenocarcinoma, 1/4 (25%) cases of clear cell carcinoma, 1/2 (50%) cases of carcinosarcoma, and 1/4 (25%) cases of cervix carcinoma [Tables 1 and 2]. These cases were reported as “positive for carcinoma” or “positive for adenocarcinoma.” On review, 14 cases were identified in which peritoneal washings were reported as negative, both on conventional smear as well as the LBC smear, whereas histopathological examination of the omentum revealed tumor deposits. Further review of the smears (both conventional and LBC smears) of these cases failed to reveal any tumor cells. Hence, peritoneal cytology was false negative in these cases. Rest of the cases with omental deposits had positive peritoneal cytology also.

There were 2 cases of sertoli leydig cell tumor, 3 cases of fibroma thecoma, 15 cases of mature cystic teratoma of ovary, and 5 cases of leiomyosarcoma, where the peritoneal cytology was negative. The histopathological diagnoses of other cases with negative peritoneal washings included leiomyoma, adenomyosis, follicular, endometriotic and simple cyst of ovaries, serous cystadenomas, mucinous cystadenoma, cervicitis, salpingitis, etc., [Table 3].

All the positive cases \((n = 83)\) were reviewed by two cytopathologists mainly concentrating on cytologic features, which were different in two techniques (conventional and LBC Sure Path™). LBC smears were easier to study, small circular smears of 13 mm diameter, with uniformly distributed, concentrated cells. The cellular morphology was seen with considerably better clarity of nuclear details. All the LBC
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Table 1: Distribution of ovarian neoplasms

| Tumor type                      | No of cases with positive peritoneal cytology | Total cases |
|---------------------------------|-----------------------------------------------|-------------|
| Ovarian serous carcinoma        | 55                                            | 84          |
| APST                            | 1                                             | 8           |
| Mucinous cystadenocarcinoma     | 4                                             | 9           |
| Pseudomyxoma peritonei in mucinous cystadenoma | 2                                            | 2           |
| Dysgerminoma                    | 3                                             | 4           |
| Squamous cell carcinoma in teratoma | 1                                             | 1           |
| Yolk sac tumor                  | 2                                             | 4           |
| Granulosa cell tumor            | 1                                             | 13          |
| Total                           | 69                                            | 125         |

Table 2: Distribution of uterine neoplasms

| Tumor type                       | No of cases with positive peritoneal cytology | Total cases |
|----------------------------------|-----------------------------------------------|-------------|
| Endometrioid adenocarcinoma      | 2                                             | 45          |
| Clear cell carcinoma             | 1                                             | 4           |
| Malignant mixed mullerian tumor  | 1                                             | 2           |
| Cervical carcinoma               | 1                                             | 4           |
| Total                            | 5                                             | 55          |

Table 3: Distribution of cases with negative peritoneal cytology

| Histopathological diagnosis       | No of cases |
|-----------------------------------|-------------|
| Sertoli Leydig cell tumor         | 2           |
| Fibroma thecoma                   | 3           |
| Mature cystic teratoma            | 15          |
| Serous cystadenoma                | 17          |
| Mucinous cystadenoma              | 12          |
| Follicular cyst                    | 15          |
| Corpus luteal cyst                | 18          |
| Endometriotic cyst                | 13          |
| Torsion of ovary                  | 6           |
| Xanthomatoussalpingo-oophoritis    | 3           |
| Granulomatous salpingo-oophoritis  | 4           |
| Leiomyosarcoma                    | 5           |
| Leiomyoma                         | 15          |
| Adenomyosis                       | 12          |
| Proliferative endometrium         | 7           |
| Secretory endometrium             | 5           |
| Endometrial polyp                 | 11          |
| Simple hyperplasia of endometrium | 3           |
| Cervicitis                        | 4           |
| Endocervical polyp                | 2           |

LBC smears showed presence of tumor cell clusters. These cases on follow-up histopathology were diagnosed as ovarian high-grade serous carcinoma with omental metastases. There were 6 cases whose conventional smears had low cellularity (less than three tumor cell clusters in the entire smear), however, LBC smears displayed uniformly present tumor cells [Figure 2c and d], indicating a better diagnostic accuracy of the LBC technique.

Discussion

Peritoneal wash cytology helps in the management of gynecological malignancies by identifying microscopic intraperitoneal metastatic deposits and helps in the surgical staging of these tumors. During “second-look” laparotomy with negative peritoneal cytology, the patient may be considered disease free and chemotherapy may be discontinued accordingly. Serous ovarian carcinoma was found to be the most common gynecological malignancy resulting in positive peritoneal cytology in the present study. Positive peritoneal cytology can be found in 30–40% of serous borderline tumors. We had one such case with positive peritoneal cytology in the present study. We also observed positive peritoneal cytology in 3 cases of dysgerminoma, 2 cases of yolk sac tumor, and 1 case of granulosa cell tumor. One case showed squamous cell carcinoma arising in teratoma, which led to shedding of malignant squamoid cells in the peritoneal wash fluid. These tumors with positive peritoneal cytology are rarely described previously.

Jacques et al. in 1991 studied multiple peritoneal cytologies collected during laparotomy for gynecologic malignancies and showed 23/61 cases of positive peritoneal cytology due to ovarian causes. All sex-cord stromal tumors had negative peritoneal cytology samples in their study.

Positive peritoneal cytology is associated with high recurrence rate and decreased survival in endometrial cancer. Szpak et al. (1981) found that stage I endometrial carcinoma cases with negative peritoneal cytology did not recur, whereas tumor recurrence at shorter interval was observed in cases with positive peritoneal cytology. However, according to Yazigi et al. (1983), positive peritoneal cytology did not influence prognosis in stage I endometrial carcinoma. Tubal transportation, exfoliation from extraterine tumor deposits, contamination from manipulation during endometrial sampling or intracavitary therapy or other procedures, and lymphatic spread have been proposed as factors contributing to the spread of malignant cells in the peritoneal cavity.

One out of two cases of carcinosarcoma of the uterus also showed positivity for malignant cells in peritoneal lavage in the present study. Tumor cells are commonly observed from...
the carcinomatous component of carcinosarcoma because this component is less cohesive and is easily shed into washing samples.\(^{[10,17]}\)

Positive peritoneal washing was noted in 1 out of 4 cases of cervical carcinoma. Selvaggi and Jacques (1991) reported negative peritoneal cytology in all 33 cases of cervical carcinoma in their study.\(^{[13]}\) Positive peritoneal cytology has been associated with higher recurrence of cervical carcinoma.\(^{[18]}\)

False positive peritoneal washings, where peritoneal washings are reported as positive in absence of any known tumor in the patient has been described previously.\(^{[4]}\) We did not observe any such case with false positive peritoneal cytology, similar to Ziselman et al. (1984).\(^{[10]}\) This observation may be explained by the fact that all the suspicious cases were reported along with corresponding gynecologic specimens. There were 14 false negative cases (negative peritoneal washings but had omental tumor deposits on histology) in the present study. False negative peritoneal cytology is more commonly associated with peritoneal washings than ascitic fluid samples.\(^{[8,19,20]}\) This could be because of dilution of sample with blood and less number of malignant cells in fluid. Four cases in the present study had only blood in conventional smears, and malignant cells could be identified on LBC smears only. In another 6 cases, conventional smears had less than three clusters of malignant cells, whereas LBC smears helped to concentrate and better delineate the tumor cells. The negative peritoneal cytology may be explained by poor distribution or infrequent exfoliation of tumor cells. Excessive blood in hemorrhagic samples may obscure the tumor cells in conventional smears.\(^{[20]}\) This shortcoming is overcome in LBC technique where preservative solution lyses the RBCs and cell enrichment process results in uniform homogenous concentration of cells within the circular smear. The excessive amount of fluid may also dilute the tumor cells, making their identification difficult in conventional preparations.\(^{[21]}\) These factors may result in false negative peritoneal cytology which can be detrimental to the patient’s clinical outcome. LBC preparation offers advantage in hemorrhagic and paucicellular samples improving the diagnostic accuracy of peritoneal washings. Residual material from LBC samples can also be utilized for ancillary techniques such as immunocytochemistry (ICC). ICC can directly be done on LBC smears.\(^{[22]}\) However, in our study, we did not perform immunocytochemistry on LBC sample for diagnosis. LBC smears also reduce the time of observer to hunt for tumor cells because cells are uniformly distributed and concentrated in the circular smear. These factors result in improved productivity by reducing the inadequate rate and increasing the diagnostic accuracy of peritoneal cytology.

**Conclusion**

In conclusion, papillary serous carcinoma is the most common ovarian malignancy leading to positive peritoneal cytology and presenting at advanced stages. Endometrioid adenocarcinoma rarely leads to positive peritoneal cytology, especially when myometrial invasion is more than 50% and/or with parametrical involvement. We compared conventional technique with LBC for evaluation of peritoneal cytology samples. Further studies are needed to compare cytocentrifuged smears with LBC technique in effusion fluids. There are previous studies evaluating different LBC techniques in effusion cytology.\(^{[21,24]}\) LBC technique leads to concentration of tumor cells causing reduction in false negative cases, especially in hemorrhagic and paucicellular cases, thereby improving the diagnostic accuracy of peritoneal washings. The correct identification of malignant cells in peritoneal wash fluids is essential for the proper management of gynecological cancer, especially early stage carcinomas.

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**Conflicts of interest**

There are no conflicts of interest.

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