Intraoperative Scrape Cytology from Ovarian Mass Lesions: A Study of 81 Cases

Amita Jain Gupta, Meeta Singh, Jenna B. Bhattacharya, Anusha S, Shyama Jain, Nita Khurana
Department of Pathology, Maulana Azad Medical College, Lok Nayak Hospital, New Delhi, India

Abstract

Background: Intraoperative evaluation of an ovarian mass is of crucial importance in its further management, accomplished by frozen section (FS) and scrape smear (SS) examination. Aim: To evaluate utility of SS over FS and to study scrape cytological features of a variety of ovarian neoplasms. Materials and Methods: The study comprised ovarian tumors studied over a period of 1 year (2014–2015) that were submitted for intraoperative assessment. SS and FS were examined and evaluated independently. The results were compared with final pathological diagnosis in each case, and cases with discordant diagnoses were reviewed. All the SSs of ovarian tumors were re-evaluated with Giemsa-stained smears, and cytological features were described. Results: The results of SS and FS were 100% concordant. On histopathology, of 81 cases, 43 were epithelial [(categorized further as serous, mucinous, or malignant mixed Mullerian tumor (MMMT)] along with subcategorization of benign, borderline, and malignant), 16 were germ cell (categorized as teratoma: mature/immature and yolk sac tumor), 11 were sex cord stromal tumors (fibroma, granulosa cell tumor, Sertoli–Leydig cell tumor), 8 cases were hemorrhagic cysts (torsion, endometroid, corpus luteal cyst, etc.), and 3 were metastasis. There were 61 benign, 2 borderline, and 18 malignant cases on FS and scrape. Combining all the values, sensitivity and specificity were 76% and 98.21%, respectively. The diagnostic accuracy in diagnosing malignant lesions was 91%. Conclusion: Adequate knowledge on cytohistological correlation of ovarian scrape cytology may phase out the use of cryostat in intraoperative diagnosis of ovarian neoplasms, and thus be a boon for resource-deprived settings.

Keywords: Frozen section, intraoperative cytology, ovarian mass lesions, scrape smears

INTRODUCTION

Intraoperative evaluation of neoplastic lesions is becoming an essential tool in deciding further management of patient and in many cases preventing unnecessary radical surgery. Ovarian mass is frequently subjected to the same with the use of scrape smear (SS) along with frozen section (FS) examination. Various studies have compared the diagnostic accuracy of SS and FS with final histopathological diagnosis.[1-4] However, an attempt to phase out FS has rarely been made in intraoperative management of ovarian masses. Thus, the aim this study is to detail cytomorphological features of a variety of ovarian masses on scrape cytology and to study the concordance of FSs and scrape cytology with histopathology. And to propose limited usage of cryostat in intraoperative management of ovarian masses.

MATERIALS AND METHODS

Our study comprised 81 ovarian tumors studied over a period of past 1 year that were submitted for intraoperative assessment in which both SSs and conventional FSs were examined. Each technique was evaluated independently. The results were grouped into benign, borderline, and malignant categories on SS and FS. The results were compared with final histopathological diagnosis. A review of discordant cases diagnosed was done. All the toluidine-blue-stained SSs of ovarian tumors were re-evaluated with Giemsa-stained smears, and cytological features were described, and cytohistological correlation was detailed. Fresh gross specimens were immediately examined for presence of cysts, any capsular breach, papillary growth, or any solid tumor tissue. The material was obtained by scraping widely all the representative areas in the mass, that is, solid areas, papillary areas, hemorrhagic, necrotic, and other minor areas different from main mass from fresh cut
surface of tumors and was directly deposited on a glass slide and then spread gently over another slide. Half the slides were stained with toluidine blue and with hematoxylin–eosin (H&E) and some were left unstained. Specimens were then fixed in 10% formalin and were sampled for histopathological evaluation. All malignant and suspicious cases had at least one section per centimeter tumor diameter. Giemsa staining was performed simultaneously on unstained slides whenever extra smears were available or else destain Giemsa was performed on toluidine-blue-stained slide. This was done to detail the cytomorphological features of each and every neoplasm in this study. The SSs were fixed in methanol and stained with toluidine blue and were available for microscopy much before FS. Each technique was evaluated independently and the results were recorded separately. Both the techniques (SS and FS) were combined to make final intraoperative impression. Histopathological diagnosis was regarded as the final diagnosis, and accuracy of the intraoperative diagnosis was compared with it. As classified by Stewart et al., 1 the cases were regarded as concordant or discordant as follows. Concordant: The cases were classified as concordant if the category of tumor classification was correct regarding its nature that is benign/malignant as intraoperative surgical management of tumor did not change with further subtyping. In addition, the cysts/masses which were given nonspecific and benign cytological diagnosis on SS and FS and were later recorded as benign on histopathology (endometriotic, hemorrhagic) were included under miscellaneous category and not regarded as discordant. Discordant: The cases where incorrect intraoperative assessment affected the intraoperative surgical management. In some cases, an equivocal diagnosis was provided intraoperatively; if the final correct diagnosis was included in the intraoperative differential diagnosis, then the case was not regarded as discordant but such cases were noted separately. The tumors were graded histologically according to World Health Organization classification. All malignant and borderline lesions were included in positive category and all benign neoplasms were included in negative category. The cases which were concordant on intraoperative cytology and histopathology were regarded as true positive. False-negative cases included histology-positive and scrape-negative cases. False-positive cases included cases where SS was wrongly interpreted as malignant; however, it actually turned out to be benign on examination of paraffin-embedded sections. Sensitivity, specificity, and diagnostic accuracy were calculated using descriptive statistics.

**Observations and Results**

The total number of cases studied was 81, and cases that correlated with histopathology were 74. There were 61 benign, 2 borderline, and 18 malignant cases on FS and SSs. The results of SS and FS were 100% concordant. On histopathology, of 81 cases; 43 were epithelial (categorized further as serous, mucinous, MMMT) along with subcategorization of benign, borderline, and malignant), 16 were germ cell (categorized as teratoma: mature/immature and yolk sac tumor), 11 were sex cord stromal tumors (fibroma, granulosa cell tumor, Sertoli–Leydig cell tumor), 8 cases were hemorrhagic cysts (torsion, endometroid, corpus luteal cyst, etc.), and 3 were metastasis. Cytohistological correlation of ovarian neoplasm is described in Table 1.

Comparing the diagnosis of cytological smears with histological sections, 74 of 81 cases were concordant. There were 19 true-positive, 1 false-positive, 6 false-negative, and 55 true-negatives. Of the seven discordant cases, one case which was diagnosed as benign papillary tumor on FS was reported as papillary serous cystadenocarcinoma on H&E, four cases which were diagnosed as benign mucinous tumor on SS/FS were reported as mucinous cystadenocarcinoma, one case of benign mucinous tumors was reported as borderline mucinous neoplasm on histopathological examination (HPE), and one case of SS/FS benign mucinous tumor on HPE was falsely diagnosed as malignant tumor on FS. For the purpose of statistical calculation, all borderline malignancies were included in the malignant group.

Specificity of FS/SS in detecting malignancy was 98.21%. Sensitivity of FS/SS in detecting malignancy was 76%. Positive predictive value (PPV) for detecting malignancy was 95%. Negative predictive value was 90.16%. The diagnostic accuracy in diagnosing malignant lesions was 91%. Combining all the values, sensitivity and specificity were 76% and 98.21%, respectively. P value by Fisher’s exact for FS/SS to diagnose benign versus malignant is significant (<0.005). In addition, the results of Giemsa-stained smears matched those of H&E-stained smears providing better cellular details.

On SS, the cytological spectrum of the epithelial tumors which were diagnosed as malignant showed definitive atypia, pleomorphism, necrosis, and atypical mitosis, whereas on FS invasion was the most useful to diagnose the lesion as malignant. The scrape cytomorphological features of some of the prominent lesions are detailed as follows:

Endometriotic cysts showed abundant hemosiderin-laden macrophages and endometrial stroma. The background was formed by altered blood and cellular debris. The endometrial cells were scanty in number showing scant cytoplasm and the nuclei were round to oval with fine granular chromatin. A suspicion of these lesions, hemorrhagic cysts (torsion, endometroid, corpus luteal cyst), was attempted at time of FSs only after strong clinical suspicion, per operative details and radiological findings.

The benign serous tumors showed monolayered sheets and small cohesive groups of uniform cuboidal cells with round to oval nuclei; amphiophilic cytoplasm. Cilia were identified only in few cells in a clean background in the absence of necrosis. There was absence of atypia, mitosis, and necrosis. Papillary serous carcinoma showed papillary fronds and clusters with...
fibrovascular core along with psammoma body calcifications. The cells showed anisocytosis and anisonucleosis with hyperchromasia, irregularity of nuclear outlines, nucleoli, and mitotic figures in a hemorrhagic and often necrotic background [Figure 1a–e].

Benign mucinous tumors: Grossly cysts were multiloculated with papery thin walls or uniloculated with mucoid fluid. The benign cysts showed presence of columnar, mucus-containing cells with small, basally located nuclei, similar to endocervical cells arranged in a honeycomb pattern in a mucinous background giving a pale pink hue to the smear. The cells resembled endocervival cells with small uniform staining nucleus, tiny nucleolus, and moderate amount of mucin-filled pink cytoplasm. The cells had end-on appearance showing basally placed nucleus with apical mucinous cytoplasm in picket fence arrangement.

Borderline mucinous: The SSs were composed of small to large monolayered cohesive sheets of polygonal cells with honeycomb pattern showing anisocytosis and hyperchromatic nuclei lining the papillary fronds. These showed singly scatter cells along with groups and sheets of cells. Large areas of necrosis, marked nuclear atypia frequent mitosis, and vacuolated cytoplasm were the features that indicated that lesion was mucinous adenocarcinoma.

Some borderline and malignant mucinous tumors in the study were missed due to largely heterogeneous areas as detailed sampling was not possible in rapid analysis. One case reported as malignant on SS was a 45-year-old lady which turned out to be benign mucinous tumor; two borderline cases reported correctly on FS were 45 and 50 years; one case reported as benign mucinous which turned out to be borderline on HPE was 35 years old. All the four cases reported as benign which later turned out to be malignant mucinous adenocarcinoma were between 45 and 40 years of age which may suggest that pathologists tend to give underdiagnosis in younger patients.

Granulosa cell tumors: Tumors showed small- to medium-sized cells with scanty cytoplasm arranged in nests or trabecular or follicular patterns, with few cells showing nuclear grooves and one case showing cells in acinar arrangement with central acellular eosinophilic bodies suggestive of Call–Exner bodies.

Yolk sac tumor: Cellular smears with cells in papillary groups and tight cell clusters and formed acinar structures. Cells showed pleomorphic, hyperchromatic nucleus, and moderate amount of cytoplasm with few cells showing cytoplasmic

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**Table 1: Cytohistological correlation of ovarian neoplasm**

| Number of cases | Final diagnosis (HPE and Giemsa) | Cytological diagnosis (FS=SS) | Cytology/intraoperative and histopathological correlation |
|-----------------|----------------------------------|------------------------------|----------------------------------------------------------|
| **Epithelial tumors** |                                  |                              |                                                          |
| 13              | Serous cystadenoma                | Serous cystadenoma           | 13/13 correlated                                          |
| 3               | Papillary serous carcinoma        | Papillary serous carcinoma (2) | 2/3 correlated                                           |
| 1               | Cystadenocarcinoma                | Cystadenocarcinoma           | 1/1 correlated                                           |
| 15              | Benign mucinous tumor             | Benign mucinous tumor (14)   | 14/15 correlated                                          |
|                 |                                  | Malignant mucinous adenocarcinoma (1) |                                          |
| 7               | Mucinous adenocarcinoma           | Mucinous adenocarcinoma (3)   | 3/7 correlated                                           |
| 3               | Borderline mucinous tumor         | Borderline mucinous tumor (2) | 2/3 correlated                                           |
| 1               | Primary MMMT                      | High grade carcinoma         | 1/1 correlated                                           |
| **Germ cell tumors** |                                  |                              |                                                          |
| 1               | Yolk sac tumor                    | Malignant germ cell possibly yolk sac tumor (1) | 1/1 correlated |
| 13              | Mature teratoma                   | Mature teratoma               | 13/13 correlated                                          |
| 2               | Immature teratoma                 | Immature teratoma             | 2/2 correlated                                           |
| **Sex cord stromal tumors** |                                  |                              |                                                          |
| 3               | Sertoli-Leydig cell tumor         | Sertoli-Leydig cell tumor     | 3/3 correlated                                           |
| 4               | Granulosa cell tumor              | Granulosa cell tumor          | 4/4 correlated                                           |
| 4               | Fibroma                           | Fibroma (4)                   | 4/4 correlated                                           |
| **Secondary tumors** |                                  |                              |                                                          |
| 3               | Metastatic adenocarcinoma         | Metastatic adenocarcinoma     | 3/3 correlated                                           |
| **Cysts**       |                                  |                              |                                                          |
| 8               | Miscellaneous cysts (torsion, endometriotic, hemorrhagic) | Miscellaneous cysts (torsion, endometriotic, hemorrhagic) | 8/8 correlated |

FS: Frozen section; HPE: Histopathological examination; SS: Scrape smear; MMMT: Malignant mixed Mullerian tumor
vacuolation. The characteristic Schiller–Duval body was present in histopathological sections.

Teratoma: On macroscopic observation had keratinous debris along with hair tufts in few cases. Smears consisted mostly of debris comprising keratin, grease along with squames, and hair shafts on SS, whereas FS comprised intact stratified squamous epithelium along with keratin flakes. In three cases, mature respiratory and intestinal epithelium were also identified on SS/FS. Solid areas of immature teratoma [Figure 2a–c] showed clusters of small round cells in addition to the above findings while cystic areas showed only benign areas.

Sertoli–Leydig cell tumor: It showed an admixture of Sertoli (cuboidal cells with indistinct outline, vacuolated cytoplasm, and prominent single nucleolus) and Leydig cells (cells with central nucleus and moderate amount of granular cytoplasm) [Figure 3a–c]. The cells were distributed in sheets or singly scattered with bland chromatin and absence of nuclear irregularities. Reinke’s crystals though pathognomonic of Leydig cells were not observed. There was absence of atypia, necrosis, and mitosis.

Metastasis in all cases showed features of adenocarcinoma (pleomorphic cells arranged in acinar pattern along with loosely cohesive individual dispersed cells, intracytoplasmic mucin with background of mucin). Papillae and significant pleomorphism were distinctly absent. One of the cases also showed signet ring cells. In this study, a history of primary in patient was known; otherwise, it is difficult to distinguish these lesions from primary mucinous ovarian carcinoma.

Fibroma cases showed scanty cellularity comprising spindled out cells in compact clusters and bundles, also dispersed singly with elongated bland nucleus, coarsely granular chromatin with no or minimal pleomorphism, and inconspicuous nucleoli. No mitosis or necrosis was noted. There was absence of vacuolated cytoplasm which was later confirmed by absence of staining with Oil Red O.

MMMT which could be diagnosed intraoperatively only as malignant epithelial tumor as sarcomatous element was not readily appreciated in SS and FS at time of intraoperative consultation. However, the SS when retrospectively studied after the diagnosis of MMMT was rendered on histopathology; the sarcomatous areas could be delineated on SS.

In all the cases, clinical and radiological findings along with gross appearance of the lesion were helpful in reaching a diagnosis on SS and FS.

**Discussion**

Ovarian masses are frequently being subjected to SS and FS examination so as to determine the extent of surgery required, and complete resection along with removal of draining lymph nodes can be done in a single operation. Also in cases which are benign, the extensive surgical resection can thus be avoided. Cytomorphological examination of SS alone along with appropriate clinical details can play a crucial role in resource-limited setting where FS is not possible as part of intraoperative consultation.

Various studies have compared the diagnostic accuracy of SS and FS with final histopathological diagnosis.[1-13] The benefits of intraoperative diagnosis by cytomorphology over FS have been described. SSs are less time-consuming, inexpensive, and lack freezing artefacts; various heterogeneous areas can be examined in little time and tissue loss can be avoided.[1-7]

In addition, adipose and necrotic tissues are easier to examine on SS as these are difficult to embed in FS.[4-7] Dudgeon and Patrick were the first to describe the use of imprint smears
of fresh tissues in rapid microscopic diagnosis of tumors.\cite{9}

Cytology of ovarian neoplasms is difficult to interpret alone; gross and clinical data including tumor markers are essential for diagnosis. Various morphological patterns seen in various ovarian neoplasms on SS need to be understood and familiarized by the pathologist to avoid an over/underdiagnosis.

The characteristic cytopathological morphology and pattern help in reaching a conclusive diagnosis.

The cytological preparations are also superior over FS as sampling of multiple sites is a possibility in limited time as seen in seven of our cases. The possible reason for discordance between SS and HPE in mucinous tumors is associated with characteristics of these tumors such as larger sizes and presence of benign, borderline, and malignant components in the same tumor in contrast to serous tumors.\cite{9,11,12} The sampling thus should be done from solid-looking areas of cystic neoplasms. In addition, metastatic tumors especially mucinous carcinomas maybe challenging in FS if there is inadequate clinical information and communication with the surgeon. Tumor size less than 10 cm, bilaterality of the tumor, involvement of ovarian surface, and nodular infiltration pattern are diagnostic clues for metastasis.\cite{10}

Stewart \textit{et al.} demonstrated that FS is useful in all categories of ovarian mass (benign, borderline, and malignant). Intraoperatively, borderline tumors are not diagnosed accurately on FS.\cite{11,12} This finding is similar to the present case series.

Intraoperatively, a definitive diagnosis in benign lesions is not always possible by SS unlike FS as observed in this study and by Stewart \textit{et al.},\cite{11} this does not actually hamper intraoperative management.

The diagnosis which is frequently missed on FS and SS is that of primary mucinous adenocarcinoma as described in various studies in literature\cite{9,11,12} similar to the present case series. This has been mainly based on the sampling error that is due to multiplicity of heterogeneous areas especially in mucinous tumors and also to the fact that these are large in size and only routine paraffin embedding enables sampling from a number of areas. Hence, multiple smears from heterogeneous areas on gross should be sampled which is only possible through study of SS. Similar problem arose in present series where a single case of MMT as sarcomatous areas could be delineated on SS only on retrospective analysis.

Stewart \textit{et al.} in their study found that FSs are more sensitive (97%) compared with cytology diagnosis (93%), whereas both modalities yielded similar specificity (100%) compared with histopathological diagnosis.\cite{11} Rao \textit{et al.} (50 cases each) found an accuracy of 92%. Whereas Vijayakumar found a diagnostic accuracy of 90\%.\cite{9,11} Khunamornpong \textit{et al.} examined 131 cases with a diagnostic accuracy maximum for malignant (98%) > benign (95%) > borderline cases (47%).\cite{11}

In this study, comparing SS and FS, there was 100\% concordance rate among them. However, we observed that specificity and PPV in diagnosing benign/malignant lesion on SS were 98.21\% and 95\%, respectively; however sensitivity was 76\%. The reason for a lower sensitivity value in this study was probably due to inability of accurately diagnosing borderline/malignant mucinous tumor for the above-said reasons; however, specificity was similar to other studies\cite{9,10,13} based on SS/FS diagnosis alone and thus confirm the overall reliability of a malignant intraoperative diagnosis. In this study, 91.3\% of cases were diagnosed correctly on FS and SS. Nevertheless, difficulties arise, including the distinction of category of mucinous tumors, borderline, mixed tumors, and primary ovarian carcinoma from metastasis.

### Conclusion

In this study, high specificity and PPV were observed on both SS/FS during intraoperative cytology for ovarian neoplasms although HPE remains the gold standard. Also, the results of SS and FS were 100\% concordant with each other; thus adequate knowledge on cytohistological correlation of ovarian scrape cytology may phase out the use of cryostat in ovarian masses. This will be a boon for resource-deprived settings since intraoperative diagnosis helps in determining the extent of surgical intervention required. Thus study also re-emphasizes on the characteristic cytopathological findings for the practicing cytopathologist.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.
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Conflicts of interest
There are no conflicts of interest.

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