Review Article

Fine Needle Aspiration Cytology's Role in the Diagnosis of Ovarian Tumor

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Abstract

Fine needle aspiration cytology (FNAC) is a cost-effective, minimally invasive technique for diagnosing a wide range of benign and malignant lesions. However, there are a number of reasons why its use is limited in the diagnosis of ovarian cancer, such as the fear of tumor cells spilling into the peritoneal cavity and the difficulty of subtyping with cytology alone. In experienced hands, FNAC is a safe, cost-effective procedure with acceptable diagnostic accuracy. In ovarian cystic lesions, secondary degenerative changes and the sample's low cellularity were the primary causes of false negative FNAC results. Preparing cell block can partially avoid this, so we recommend doing so. All of the clinical and sonographic findings, in addition to the FNAC findings, the preparation of the cell block, and the application of immunohistochemistry, need to be taken into consideration in order to arrive at an accurate diagnosis.

Keywords: *Fine needle aspiration cytology, immunohistochemistry, ovarian tumor*

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Introduction

The ovary is a key reproductive organ that is involved in the evolution of progeny. Ovarian lesions encompass a wide range of complicated neoplasms with diverse histological patterns emanating from epithelial tissue, connective tissue, specialized hormone-secreting germinal and embryonal cells.[1] Ovarian cancer accounts for about 3% of all cancer in women. Ovarian lesions are solid and/or cystic in nature. Ovarian lesions are most common in women of reproductive age. Fine needle aspiration cytology (FNAC) had a significant impact on the diagnosis of ovarian neoplasm. Interventional radiology and ancillary techniques such as immunohistochemistry (IHC) have a greater impact on accurate diagnosis and subsequent management these days.

Diagnostic evaluation

A complete medical evaluation requires a menstrual history, which includes the last menstrual period and the presence or severity of dysmenorrhea. The most common symptoms are abdominal pain, irregular menstruation (dysmenorrhea), and fever. Sexual history and any additional risk factors, such as a family history of ovarian, fallopian tube or breast cancer. Pelvic examination includes abdominal distention, ascites, signs of endometriosis, infection, or malignancy in physical examination. The pelvic examination findings confirmed with pelvic imaging like transvaginal ultrasound (USG) and computed tomography (CT) in advance case.

Obtaining specimen

Always perform cytological procedures under radiologic guidance, preferably USG. The transvaginal route is the most common. Other approaches include transabdominal, transrectal, laparoscopic and aspiration during laparotomy. Large masses with omental deposits benefit from a transabdominal approach. The method used is determined by the size of the lesion, where it is located and the person performing the aspiration's resources.[2] Complications are rare. Although common belief holds that a malignant tumor puncture can result in peritoneal cavity seeding, documented cases are

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extremely rare.[3] If any USG features are concerning or suspicious of malignancy, it is advisable to prepare a cell block and apply IHC for the categorization of malignancy.

Preparing the Specimen and Reporting

Specimens are usually cyst fluids. Smears are made from sediment. Both wet-fixed and air-dried smears are prepared and stained with Hematoxylin and Eosin, May Grunwald Giemsa stain, periodic acid-Schiff, Papanicolaou, and Ziehl–Neelsen stain. Microscopically, the slides were examined for cellularity, the predominant cell type, size, architecture, nuclear and cytoplasmic characteristics, chromatin, the degree of inflammation, reactive changes, and other background characteristics. The obtained data were then summarized and examined. Additionally, cell block preparation is recommended. IHC is applicable when and where it is required. Blood tumor marker levels and a portion of fresh fluid can be used to measure the level of E2 or tumor-associated antigens; cancer antigen 125 (CA-125), carcinoembryonic antigen (CEA) and alpha fetoprotein. Some ovarian lesions have elevated levels, which can be used in addition to cytologic testing.[4,5] The tumor marker and its diagnostic value in ovarian neoplasms are shown in Table 1. FNA of the ovary is generally reserved for small, incidental masses that appear benign on sonographic or laparoscopic examination.[6,7] Incidental ovarian cysts are often discovered in women with infertility or during pregnancy.[2] Aspiration cytology, in combination with a benign USG appearance, is used to reassure the patient that an oophorectomy is not necessary.[3] USG, cytology and E2 levels or other tumor marker levels can form an effective "triple test" for distinguishing benign from malignant ovarian cysts. Nondiagnostic specimens consist of those that are virtually acellular.[8,9] There is a wide variation in the nondiagnostic percentage of ovarian FNAs. The cytology report should mention whether malignant cells are present (positive for malignant cells) or absent (no malignant cells found). If the result is ambiguous, the report should mention whether atypical or suspicious cells are present.

Table 1: Common serum tumor markers for ovarian cancer diagnosis and monitoring

Type of ovarian cancer	Tumor markers
Epithelial cell tumor	
Serous tumor (high grade, low grade)	CA-125, HE4
Endometrioid tumor	CA-125, HE4
Mucinous tumor	CA-125, CEA, CA 19-9, CA-125/CEA ratio
Clear cell tumor	CA-125, CA 19-9
Transitional cell tumor	CA-125
Nonepithelial tumor	
Sertoli-Leydig cell tumor	Inhibin A and B
Granulosa cell tumor	Inhibin A and B, AMH
Dysgerminomas	LDH
Yolk sac tumor	AFP
Carcinosarcoma	CA-125

AFP: Alpha-fetoprotein, AMH: Anti-mullerian hormone, CA 19-9: Cancer antigen 19-9, CA-125: Cancer antigen 125, CEA: Carcinoembryonic antigen, HE4: Human epididymis protein 4, LDH: Lactate dehydrogenase

Nonneoplastic Lesion

The majority of benign ovarian cysts are discovered incidentally during a USG, laparoscopy or laparotomy. The most common are functional cysts, while nonfunctional cysts derived from ovarian surface epithelium or endometriosis are the second most common. Cytological examination does not always allow for precise classification, particularly when only cyst contents (fluid and macrophages) are obtained.[10] Cystic lesions of the ovary are one of the most common lesions; they arise from an ovarian follicle and are not neoplastic, but rather physiologic. Follicle cysts can be solitary or multiple and their diameters can reach 8 cm or more. Follicle cysts are treated conservatively in order to preserve the ovary because they appear benign on sonography and laparoscopy.

Tubo-ovarian abscess is an advanced complication of acute salpingitis, known clinically as pelvic inflammatory disease. The majority of cases are caused by an ascending infection of the lower genital tract with sexually transmitted pathogens, the most common of which are *Neisseria gonorrhoeae*, *Chlamydia trachomatis,* and *Mycoplasma genitalium*.[11]

Benign Surface Epithelial–Stromal Tumor

The most common ovarian neoplasms are tumors derived from the ovarian surface epithelium. Histologically, they are classified as serous (the most common), mucinous, endometrioid, clear cell, Brenner, seromucinous, and undifferentiated.

Benign Serous Tumor

Benign cystic serous tumor aspirates clear fluid that is sparsely cellular on FNA. The cysts are lined by cuboidal cells with a uniform round to oval nucleus and are arranged in crowded clusters. Psammoma bodies and detached ciliary tufts are sometimes present.

Benign Mucinous Tumor

Mucinous cystadenoma and the uncommon mucinous adenofibroma are cystic neoplasms lined by a single

layer of mucinous epithelium of gastric foveolar type or intestinal type. They can be very large and multiloculated. The fluid is either gelatinous or watery. Lining cells are isolated or arranged in clusters or honeycomb-like sheets with well-defined cell membranes, typically retaining their columnar structure. They resemble either benign gastric foveolar cells or goblet cells. Mucin-filled macrophages and extracellular mucin are present. Chemical analysis of the fluid shows a high CEA level and low estradiol and CA-125 levels, which are characteristic of mucinous cysts and help to distinguish them from follicle and serous cysts.[12,13]

Benign Brenner Tumor

Brenner tumor constitutes about 5% of benign ovarian epithelial neoplasms. It composed of nests of transitional epithelium (resembling urothelial cells) embedded in a dense fibrous stroma. Aspirates reveal sheets of transitional-type cells with oval nuclei and a prominent longitudinal groove, resembling coffee beans. Brenner tumors express CK7, p63 and GATA3. A granulosa cell tumor, the cells of which also exhibit longitudinal grooves, is one of the possible diagnoses. Granulosa cell tumors do not express keratin proteins.[13]

Malignant Surface Epithelial-Stromal Tumor

FNA is recommended for diagnosing advanced-stage ovarian tumors and documenting the recurrence or metastasis of ovarian cancer.[14-16] Ovarian tumors can be diagnosed and classified using IHC. All epithelial ovarian tumors express CK7, the absence of CK7 staining indicates a nonepithelial ovarian tumor or metastasis. The majority of ovarian tumors test negative for CK20 and CDX2 (Caudal-type homeobox 2). WT1 (Wilms' tumor 1) is expressed by serous ovarian carcinomas. PAX8 is an extremely sensitive marker for ovarian serous tumor. Phosphatase (PLAP), SALL4 (Sal-like protein 4), Oct-3/4 and NANOG are sensitive and relatively specific markers of germ cell tumor. Inhibin is a sensitive and relatively specific marker of sex cord-stromal tumors, but it is also expressed in adrenocortical neoplasms. Calretinin is a more-sensitive but less-specific marker of sex cord – stromal tumor than inhibin.

Serous Borderline Tumor and Serous Carcinoma

Histologically, serous carcinomas can be divided into low-grade and high-grade types. The high-grade serous carcinoma is by far more common; aspirates are usually very cellular, composed of atypical cells in papillary clusters. The nuclei are large and pleomorphic with prominent nucleoli and the cytoplasm contains large vacuoles. Many atypical bare nuclei are present.[13] In some cases, psammoma bodies are seen with a rim of malignant cells. Low-grade serous carcinomas account for only 5% of all serous carcinomas and are composed of a more uniform population of cells with minimal pleomorphism.

It is very important to distinguished between low grade and high grade serous cystadenocarcinoma due to their different management. IHC is essential for distinguishing it. Table 2 summarizes the IHC markers that are useful in determining diagnosis of ovarian carcinoma. Cell block shows a tumor arranged as cluster composed of polyhedral atypical cells with oval nuclei, exhibiting marked anisokaryosis, coarse chromatin, visible nucleoli and moderate to abundant vacuolated eosinophilic cytoplasm. Many tumor cells display dark eccentric nuclei with abundant vacuolated cytoplasm [Figure 1a]. IHC shows tumor cells strong and diffusely positive for PAX8 [Figure 1b] and WT1 [Figure 1c] and negative for TP53 [Figure 1d] confirm the diagnosis of high grade serous carcinoma.

Mucinous Borderline Tumor and Mucinous Carcinoma

Mucinous carcinoma aspirates are cellular, with isolated cells, cells in sheets, and cells grouped in irregular clusters. Cells from well-differentiated tumor are columnar, contain mucin and have mild nuclear atypia.[13] Poorly differentiated tumor nuclei are pleomorphic and indistinguishable from high grade serous carcinoma nuclei; some tumors show a spectrum of differentiation.

Table 2: Immunohistochemistry markers utilised in the diagnosis of ovarian carcinoma

IHC marker	HGSC	LGSC	Endometrioid	Clear cell	Mucinous
WT1	Diffuse positive	Diffuse positive	Negative	Negative	Negative
p53	Mutation type	Wild type	Wild type or mutation type	Wild type	Wild type or mutation type
p16	Diffuse positive	Focal positive	Focal positive	Variable	Variable
ER	Diffuse or focal positive or negative	Diffuse positive	Diffuse positive	Negative	Negative or focal positive
Napsin A	Negative	Negative	Negative	Diffuse or focal positive	Negative

IHC: Immunohistochemistry, ER: Estrogen receptor, HGSC: High-grade serous carcinoma, LGSC: Low-grade serous carcinoma

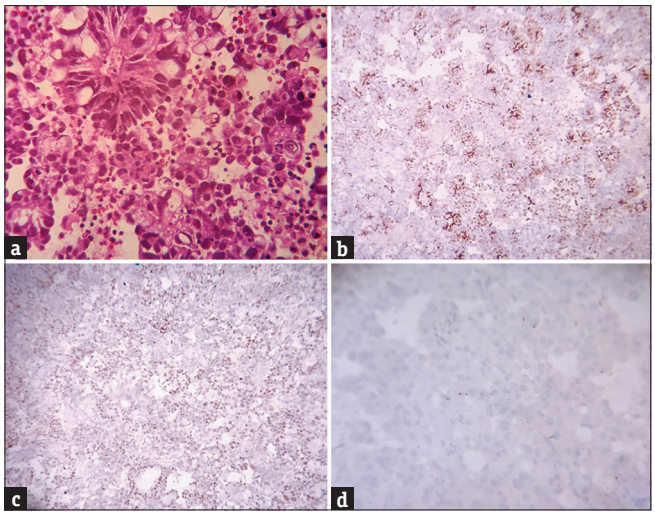

Figure 1: Cell block of high grade serous carcinoma shows a tumor arranged as cluster composed of polyhedral atypical cells with oval nuclei, exhibiting marked anisokaryosis, coarse chromatin, visible nucleoli and moderate to abundant vacuolated eosinophilic cytoplasm with abundant vacuolated cytoplasm (a) (H and E, ×400). Immunohistochemistry shows tumor cells are strong and diffusely positive for PAX8 (b) (×100) and WT1 (c) (×100), and negative for TP53 (d) (×200)

Endometrioid Carcinoma

Endometrioid carcinoma is the second-most common form of ovarian epithelial malignancy, comprising 10% to 15% of ovarian carcinomas. Aspirates contain intact or "broken" glands, small cell strips and many isolated, elongated cells with hemorrhagic background contain hemosiderin-laden macrophages. Cell block sections are helpful for application of ancillary testing and confirmation of diagnosis.[13]

Clear Cell Carcinoma

Tumor cells have a large, pleomorphic nucleus that is often eccentrically placed and has a prominent nucleolus. The cytoplasm can be abundant and vacuolated or it can be sparse and eosinophilic. There may be hyaline extracellular material that stains pink-purple within or adjacent to tumor cell clusters. Necrosis can be seen in the background. PAX8 is almost always positive in clear cell carcinomas and WT1 is negative in clear cell carcinoma.[17,18]

Germ Cell Tumor

Germ cell tumor can develop at any age; however, they are more common during reproductive age. They account for 30% of all ovarian tumor and most common germ cell derived neoplasm.

Teratoma

Mature teratoma

Mature cystic teratomas also known as dermoid cysts are mostly cystic in nature and derived from ectoderm, endoderm and mesoderm. The most mature teratoma is composed of skin, hair, and sebaceous glands which are ectodermal derivates. Monodermal teratoma known as Struma ovarii is entirely composed of mature thyroid tissue. Mature teratomas are rarely aspirated because their sonographic features (particularly the tooth) are diagnostic. FNA shows predominantly anucleated squamous cells. Ciliated cells, detached ciliary tufts, mucinous cells, and hair are seen in some cases.[13]

Immature teratoma

FNA shows immature or embryonal tissue, usually admixed with benign mature elements is characteristic of these tumors.

Dysgerminoma

Dysgerminoma is the most common type of malignant germ cell tumor; it accounts for only 1%–2% of all malignant ovarian tumor.[18] Dysgerminoma aspirates are highly cellular, consisting predominantly of isolated tumor cells with occasional loose, syncytium-like clusters composed of a large, round, centrally located nucleus with one or more conspicuous, irregularly shaped nucleoli with clear or granular cytoplasm. There may be necrosis and haemorrhage. Mitoses are present. A distinctive "tiger-stripe" background, similar to that seen in seminomas, can be seen on air-dried preparations. Dysgerminomas are immunoreactive for cytoplasmic and membrane staining of placental alkaline PLAP, CD117 (c-kit; membrane staining), as well as the stem cell-related proteins, which show positive nuclear staining for Oct-3/4, NANOG and SALL4. PLAP and SALL4 are expressed in most germ cell tumor, whereas Oct-3/4 and NANOG are only expressed in dysgerminoma/seminoma and embryonal carcinoma.[13,18]

Embryonal carcinoma and other malignant germ cell tumor

Embryonal carcinoma tumor cells have a centrally located, large, round or highly irregular nucleus with several nucleoli. The cytoplasm is indistinct and pale. Bizarrely shaped cells and mitoses are common. The cells of Yolk sac tumors are similar to those of adenocarcinomas that are poorly differentiated. They are cohesive, pleomorphic cells with prominent nucleoli.[11] Some tumor cells contain intracytoplasmic dense hyaline globules composed of α-fetoprotein.[19] Mucoid and basement membrane-like material may be present in the background.[20] Choriocarcinomas are composed of malignant cytotrophoblast and syncytiotrophoblast. Human chorionic gonadotropin is secreted by these tumors.

Embryonal carcinoma, Yolk sac tumor and choriocarcinoma are keratin-positive but distinguishable from epithelial malignancies due to PLAP and SALL4 staining. Embryonal carcinoma, like dysgerminoma

is also immunoreactive for Oct-3/4 and NANOG. Dysgerminoma distinguishes from embryonal carcinoma in that it exhibits distinct membranous staining pattern for CD117.

Granulosa cell tumor

FNA smears are highly cellular arranged in clusters, sheets or trabeculae having small-to medium-sized cells, round, monomorphic nuclei, nuclear grooves and the ill-defined cytoplasm. Call-Exner bodies are tumor cells that are arranged around small cavities filled with eosinophilic fluid. Inhibin and calretinin show strong positivity for granulosa cell tumor and negative for CK7 and epithelial membrane antigen.

Metastatic tumor

The most common tumor that metastasize to the ovaries originate in the colon, stomach, appendix, breast and elsewhere in the female genital tract. Krukenberg tumors are characterized by mucin-filled signet ring-shaped cells metastatic to the ovary.[21] Most arise in the stomach, but tumor of the colon, appendix and breast also cause this pattern of spread. Cytomorphology alone is often insufficient to distinguish between a metastasis and a primary ovarian carcinoma. Because primary ovarian epithelial tumors are generally CK7-positive and CK20-negative, the reverse immunophenotype (CK7-negative, CK20-positive) suggests metastasis, probably from the appendix or intestine. Table 3 shows Immunohistochemical profile to distinguished primary carcinoma from Krukenberg tumor. FNA smear shows signet ring cell arranged in cluster with a large clear cytoplasmic vacuole pushed the nucleus to the periphery [Figure 2] in a case of Krukenberg tumor.

Intraoperative cytology has many advantages over traditional frozen section procedures, including simplicity, speed, precision and great cellular resolution. While the patient is sedated, it provides accurate results within minutes. Before performing a major resection, the surgeon often requires microscopic confirmation of the clinical diagnosis. Microscopic diagnosis is required for future management of irresectable lesions. Intraoperative cytology allows for microscopic examination of lesions that are not suitable for surgical biopsy and can be used as an alternative to surgical biopsy in certain cases. Furthermore, FNAC allows for more accurate sampling of deep-seated lesions. Unfortunately, it is not being utilized to its full potential. The use of the aspiration technique for diagnosing malignant ovarian tumors is highly controversial due to the risk of rupturing the cancer capsule and spillage of malignant cells into the peritoneal cavity. However, the risk of seeding can be reduced by using a thin-bore needle and avoiding multiple aspirations. If a mass is smaller than 5 cm and does not have a proper tumor marker or radiological correlation, then a FNAC is recommended since tuberculosis is commonly found in our country. Very few medical colleges and institutes have frozen section facilities in India. Hence, if a frozen section facility is available, it is undoubtedly beneficial for diagnosis confirmation as well as margin or peritoneal implants.

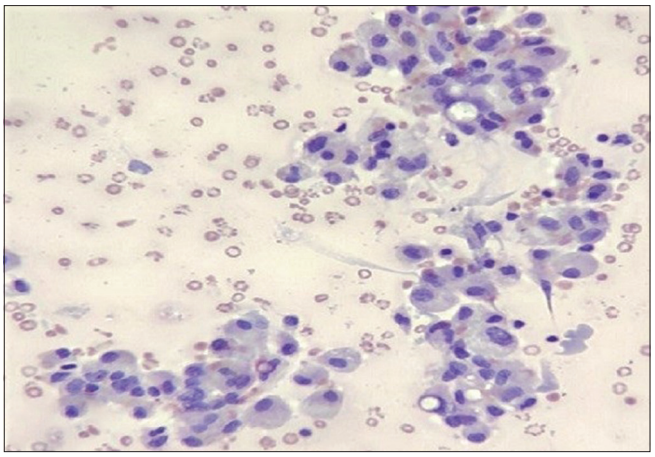

Figure 2: Fine needle aspiration smear of Krukenberg tumor shows signet ring cells arranged in clusters with peripherally pushed nuclei and abundant clear cytoplasmic vacuoles (H and E, ×400)

Table 3: Immunohistochemical profile

	Krukenberg tumor	Primary mucinous tumor of ovary	Mucinous carcinoid tumor
Special stain: PAS	Positive	Positive	Positive
IHC			
CK 7	Positive	Positive	Negative
CK 20	Positive	Negative	Negative
Chromogranin	Negative	Negative	Positive

PAS: Periodic acid Schiff, IHC: Immunohistochemistry

Conclusion

Cytology has been underutilized as a diagnostic modality for ovarian neoplasms; however, it should not be used as a routine. It has great value for evaluating metastatic tumors and for recurrence. But not advisable for the primary diagnosis of the malignancy when there is confirmation by tumor markers and radiology. With the availability of modern techniques, USG and CT-guided FNAC and cell block preparation in a precise subclassification and application of IHC on cell blocks for a definite diagnosis and to rule out metastatic ovarian neoplasms, as well as the assessment of recurrent malignant tumors, have a significant impact on patient management.

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Conflicts of interest

There are no conflicts of interest.

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