

Approaches to the Interpretation of Transabdominal Fine-Needle Aspiration Biopsy

The interpretation of fine-needle aspiration biopsy specimens is very different from that of tissue sections. In aspirate preparations, the histologic pattern and cellular arrangements of various lesions seen in tissue sections cannot be visualized. The relationship between the cellular components of a lesion and the normal structures of an organ is also distorted. Another disadvantage of fine-needle aspiration biopsy is that it provides a small amount of material for examination. Moreover, there are numerous look-alikes and pitfalls involved in the cytomorphologic interpretation. Any pathologist who simply applies histopathologic approach to cytopathology and who is unfamiliar with the cytologic features and unaware of the pitfalls in the cytologic interpretation will be expected to make frequent mistakes or end up with many “suspicious” reports. This provides no help to clinical management. As transabdominal fine-needle aspiration biopsy has not been widely practiced in North America until the 1980s, pathologists should prepare themselves in this field and have adequate training before attempting to make cytologic diagnoses.

TEAM APPROACH TO DIAGNOSIS

The use of transabdominal fine-needle aspiration biopsy has made the diagnosis of malignancies much easier for clinicians and much easier on patients

but often poses a serious challenge to pathologists. In the hospital setting, most patients with an intraabdominal mass or masses who undergo transabdominal aspiration biopsy have no known diagnosis. In many such patients, few clinical investigations have been done at the time of aspiration biopsy. In fact, many aspiration biopsies are not scheduled in advance but are performed on an outpatient basis. This usually happens when a lesion is found at ultrasonography, and the referring clinician is contacted. It is most efficacious for clinical management and beneficial for the patient to have the biopsy done at the same visit and to receive the report on cytology specimens after a short time interval.^{11,16} Under such conditions, a close working relationship among the clinician, the radiologist, and the pathologist is essential for the accurate interpretation of transabdominal fine-needle aspiration biopsy specimens, particularly in problem cases.¹¹ Some avoidable mistakes have been made because of insufficient radiographic information or a lack of clinical information. To minimize these mistakes, the radiologist and clinician must communicate with the pathologist.¹² The clinician should take final responsibility for correlating the clinical, laboratory, radiologic and cytopathologic data to make a final diagnosis and recommend treatment. If a positive cytology report is totally unexpected or distinctly at odds with the clinical or radiologic data, the matter should be discussed with the cytopathologist to ensure an error has not occurred.

In some cases, radiographic information about an intraabdominal lesion provides important diagnostic clues and is, therefore, helpful in differential diagnosis. Useful information obtainable from a radiologist who performs an aspiration biopsy should include:

1. Anatomic location and size of the lesion under investigation, as well as the duration of the disease.
2. Radiographic appearance of the lesion, for instance, cavitory, cystic, partially cystic, solid or multilocular.
3. Consistency of the lesion, for example, a firm, soft, or empty sensation felt during aspiration biopsy.
4. Gross appearance of the aspirate, for example, mucous, purulent, cheesy or clear fluid.

In other cases, clinical information, including signs and symptoms, laboratory data, and a history of malignant diseases, as well as clinical

impressions, are useful for the cytologic differential diagnosis. In our institutions, most patients with an intraabdominal lesion or lesions who undergo transabdominal aspiration biopsy have no diagnosis. In many such patients, few clinical investigations have been done at the time of aspiration biopsy. It is not uncommon for the clinical information and radiographic impressions provided on the requisition forms to be incorrect and thus misleading, putting the examiner on the wrong track. Therefore, one should always examine aspirate smears before reading the clinical and radiographic information so as to avoid being bias in interpretation. The only information a cytopathologist needs to make an initial examination of an aspirate preparation is the patient's age and sex, the exact site of the aspiration biopsy, and a gross description of the aspirated material.

Although clinical data and impressions are sometimes helpful in establishing the cytologic diagnosis, especially for the differential diagnosis, one should use this information only to ascertain whether it agrees with the cytologic diagnosis.¹² If the information does not correlate with the diagnosis, a careful reexamination of the aspirate preparations may reveal the cause of discrepancy. If the cytomorphologic findings are definitive and the cytologic diagnosis is conclusive, one should not go along with a clinical impression. If the preliminary cytologic diagnosis is based on inadequate evidence and is not conclusive, a conservative approach is recommended. One should not try to make a cytologic diagnosis on the basis of unsatisfactory specimens. There should be no guessing in the interpretation of aspiration biopsy specimens.

To establish a cytologic diagnosis with high accuracy, the examiner should have full knowledge of the anatomy, histology, and cytologic characteristics of the cellular components of the organ to be investigated, as well as pathologic features of various conditions of that organ. Although a thorough understanding of the histopathology of the organ under study is important for the correct interpretation of a cytology specimen, it is also crucial that the examiner be familiar with the cytologic features of different lesions seen in that organ and the cytologic criteria for the interpretation of aspiration biopsy specimens. Because the cytologic appearances of various lesions in aspirate preparations are different from the morphologic appearances as seen in tissue sections, the criteria used for the cytologic diagnosis are also different from those for the histologic diagnosis.¹⁵

CYTOLOGIC CRITERIA FOR THE INTERPRETATION OF ASPIRATION BIOPSY

In our practical work, the so-called “cytologic criteria for malignancy” as generally described in pathology and cytology books are not applicable to the interpretation of transabdominal aspiration biopsy specimens in many instances because malignant cells from some tumors (e.g. some renal cell carcinomas and well-differentiated ductal adenocarcinoma of the pancreas have a benign appearance (Fig. 3.1), and irritated epithelial cells (e.g. injury, or chemotherapy and irradiation effects) and reactive macrophages may meet the cytologic criteria for malignancy¹² (Fig. 3.2). Thus, there are cytologic criteria only for various types or subtypes of tumors. Neoplasms seen in transabdominal fine-needle aspiration biopsy are highly heterogeneous and include tumors derived from various epithelial, neuroepithelial, mesenchymal and mesothelial cells. Oversimplification of cytologic criteria for the interpretation of aspiration biopsy specimens only misleads the examiner.

The cytomorphologic interpretation of transabdominal aspiration biopsy specimens is very different from the interpretation of tissue biopsy samples. Interpretation of a tissue biopsy sample involves pattern diagnosis. What appears on the slides are cross-sections of the tissue sample. All of the histologic structures and cellular arrangements in histologic sections are in the same plane, not in three dimensions. In aspirate preparations, histologic patterns are not visualized. All of the histologic features and cellular arrangements seen in tissue sections do not exist in aspirate preparations. Therefore, the criteria used to arrive at a histologic diagnosis are not applicable to the establishment of a cytologic diagnosis.^{8,14} On the basis of our experience in dealing with thousands of intraabdominal and intrathoracic lesions¹² and published material,^{1-4,6-11} the cytologic criteria for the interpretation of aspiration biopsy specimens can be summarized in the following sections.

Cohesion Factor (Intercellular Cohesion)

This cytologic feature is related to cohesion between tumor cells. If tumor cells are scattered all over the slides, occur singly, or are present in loose groups, there is poor cohesion between the cells. This poor cohesion is typical of malignant lymphomas and most sarcomas. In general, most carcinomas

Table 3.1 Cohesion between Tumor Cells

Cohesion Factors	Solitary Cells	Loose Groupings or Noncohesive Clusters of Cells	Cohesive Clusters or Cell Fragments
0	++++	—	—
1	+++	++	—
2	+++	++	+
3	++	+++	++
4	+	+++	+++
5	—	+	++++

have good intercellular cohesion, forming tightly packed cell clusters. Exceptions include squamous cell carcinoma of the keratinizing large cell type; small cell anaplastic carcinoma; giant cell carcinoma and well-differentiated neuroendocrine carcinoma (carcinoid, islet cell tumors, medullary thyroid carcinoma); adrenocortical carcinoma; lobular carcinoma of the breast, signet ring cell gastric carcinoma of the linitis plastica type; and fibrolamellar hepatocellular carcinoma. This cytologic feature cannot be appreciated or assessed by studying histologic sections.

In general, the intercellular cohesion for any type or subtype of tumor is relatively constant and consistent in different aspirate preparations from the same tumor. Also, for any type or subtype of tumor, the degree of intercellular cohesion is fairly consistent among tumors from different patients and even from tumor metastases of the same type or subtype.¹² Thus, this cytologic feature is useful in the interpretation of aspirate preparations, especially for the differential diagnosis. In this book, the authors will use the term “cohesion factor,” which is graded from 0 to 5 to designate the degree of intercellular cohesion for any type or subtype of tumor,¹² as illustrated in Table 3.1.

Average Nuclear Size in Tumor Cells

The average nuclear size of any type or subtype of tumor cell tends to be relatively constant. The nuclear sizes of tumor cells are readily obtainable by comparison with those of red blood cells (7 μm in diameter) that are present on virtually every slide in Diff-Quik stained smears. In general, primary and metastatic cancers seen in transabdominal aspiration biopsy specimens can be divided into three groups on the basis of nuclear size (Fig. 3.3).

1. Tumor cells that have small nuclei ($< 20 \mu\text{m}$ in diameter), including those of abdominal desmoplastic small round cell tumor, Wilms' tumor, neuroblastoma, Ewing's sarcoma, carcinoid, metastatic small cell anaplastic carcinoma of the lung and malignant lymphomas of the small lymphocytic type.
2. Tumor cells that have medium-sized nuclei (20 to $30 \mu\text{m}$ in diameter), including those of well-differentiated adenocarcinoma of the pancreas, prostate, and endometrium; borderline serous tumor of the ovary; cholangiocarcinoma; large cell lymphomas; metastatic bronchioloalveolar carcinoma; and metastatic ductal carcinoma of the breast.
3. Tumor cells that have large nuclei ($> 30 \mu\text{m}$ in diameter), including those of most poorly differentiated adenocarcinomas of various origins, most squamous cell carcinomas, and most sarcomas. The nuclei of tumor cells in this group tend to be more variable in size.

General Nuclear Shape of Tumor Cells

For most types or subtypes of tumor cells, there are certain tendencies regarding nuclear shape. Tumors of various origins can be divided into five groups on the basis of nuclear shape (Fig. 3.4):

1. Tumor cells that have round nuclei. The tumor cells of most adenocarcinomas frequently have round nuclei, especially those of well-differentiated renal cell carcinoma, and adenocarcinoma of the prostate. The tumor cells of well-differentiated hepatocellular carcinoma also have round nuclei.
2. Tumor cells that have mixed ovoid and round nuclei, including those of most well-differentiated adenocarcinomas of various origins, epithelial malignant mesothelioma, and metastatic bronchioloalveolar carcinoma.
3. Tumor cells that have elongated and spindle-shaped nuclei, including those of sarcomatoid carcinoma; and spindle cell variants of carcinoid; gastrointestinal stromal tumor; spindle cell sarcoma (e.g. fibrosarcoma, leiomyosarcoma, liposarcoma, malignant fibrous histiocytoma, and hemangiopericytoma); fibrous malignant mesothelioma; peripheral nerve sheath tumors; and desmoplastic malignant melanoma.
4. Tumor cells that have irregular nuclei, including those of most poorly differentiated carcinomas and high-grade sarcomas and lymphomas.

5. Tumor cells that have multinucleated nuclei, including those of osteogenic sarcoma; rhabdomyosarcoma of the pleomorphic type; malignant fibrous histiocytoma; and giant cell carcinoma of the lung, pancreas and thyroid.

Arrangement of Tumor Cells

This cytologic feature is also helpful in typing some tumors in aspirate preparations, i.e. tumors which cannot be appreciated or assessed by examining histologic sections (Fig. 3.5):

1. Tumor cells in a monolayer arrangement (sheet arrangement). Primary or metastatic bronchioloalveolar carcinoma of the nonsecretory type is often composed of many groups of tumor cells in a monolayer arrangement in aspirate preparations. Well-differentiated ductal carcinoma of the pancreas may also show many groups of tumor cells in a monolayer arrangement.
2. Tumor cells in a multilayer arrangement (three-dimensional), including those of most adenocarcinomas of various origins and squamous cell carcinoma of the nonkeratinizing large cell-type.
3. Tumor cells forming papillary structures, including those of solid-pseudopapillary neoplasm of the pancreas; papillary serous carcinoma of the ovary; papillary renal cell carcinoma; and malignant mesothelioma of the papillary type. However, not all papillary carcinomas identified in tissue sections are found to have papillary structures in aspirate preparations. Papillary transitional cell carcinoma is one such neoplasm. In such tumor cells, cohesion is low between the tumor cells, as cohesion of the tumor cells around the fibrovascular cores is disrupted by the force of smearing.

A Unique Cytologic Feature or Special Structure

Any tumor that has a unique cytologic feature, special structure, or secretory product can be readily identified, and its origin can often be determined. Examples include keratinization in squamous cell carcinoma; an endothelial lining wrapping around a group of tumor cells in well-differentiated hepatocellular carcinoma and psammoma bodies in papillary serous carcinoma of the ovary, and mesothelioma of the papillary type (Fig. 3.6).

Other Cytologic Criteria

Some minor cytologic criteria are also helpful in the interpretation of aspirate preparations in some cases. These include:

1. The location of nuclei in tumor cells. In tumor cells from some tumors, especially those with moderate or large amounts of cytoplasm, the usual locations of nuclei are characteristic. For instance, the nuclei in tumor cells from adrenocortical carcinomas are usually eccentrically placed (Fig. 3.7D), whereas those in tumor cells from hepatocellular carcinoma of the well-differentiated type are centrally located (Fig. 3.6C).
2. The amount of cytoplasm in tumor cells. Tumor cells from some tumors have an abundance of cytoplasm, e.g. hepatocellular carcinomas of the pleomorphic large cell type (Fig. 4.33), whereas tumor cells from other cancers have no recognizable cytoplasm, e.g. small cell carcinoma (Fig. 4.51) and some sarcomas (Fig. 8.32; Fig. 10.3).
3. The texture of cytoplasm of tumor cells. This cytologic feature also helps in identifying various tumor cells. For instance, the cytoplasm of mucin-secreting tumor cells appears multivacuolated, and tumor cells from epithelial mesotheliomas of the noncohesive epithelial cell type have abundant dense cytoplasm. Ultrastructurally, multivacuolated (Fig. 3.7A,B) correlates to mucin droplets; fibrillary (Fig. 3.7C) correlates to long cytoplasmic extensions in peripheral nerve sheath tumors; dense (Fig. 3.7E) correlates to tonofilaments in mesothelioma; ground glass (Fig. 3.7D) correlates to lipid droplets; and mitochondria in adrenal cortical carcinoma, brown cytoplasmic inclusion (Fig. 2.5) correlates to sacromeres,¹⁷ and granular correlates to mitochondria-filled cytoplasm in oncocytic tumors (Fig. 3.7F).
4. The size and number of nucleoli in tumor cells. Tumor cells from different cancers may have a single prominent nucleolus or several conspicuous nucleoli, or have no recognizable nucleoli. For instance, lymphoma cells of the immunoblastic type have a single prominent nucleolus (Fig. 11.16), and those of the large noncleaved cell type have several conspicuous nucleoli (Fig. 11.14). Tumor cells from islet cell tumors (Fig. 5.36) or carcinoid tumors (Fig. 10.21C) have no recognizable nucleoli.

When examining aspirate preparations, one should always observe the gross characteristic prior to mounting the best smear of the case on the

microscope and always scan the smears at the lowest available magnification to have a general impression. The examiner can often narrow down the differential diagnosis to a few tumors and then study the tumor cells in greater detail at a higher magnification to make a final interpretation. The whole range of objectives should be used, including 100× oil immersion lens for difficult cases. The final diagnosis should always be made on the basis of the overall findings. One should not give too much consideration to a few abnormal-looking cells, which can confuse the diagnosis and be alert for floaters from an earlier case with large tumor load. A few abnormal cells or groupings of abnormal cells could be reactive or irritated epithelial cells, or they could simply be an artifact of the sampling technique employed.

PITFALLS AND LIMITATIONS

The technique of transabdominal fine-needle aspiration biopsy is considered easy. It must not be forgotten, however, that the method ceases to be so easy at the microscope. An aspirate smear is a much more difficult specimen to assess than a histologic section. Often the cytologic diagnosis is not straightforward and is missed or misinterpreted by inexperienced examiners. The difficulties in the cytomorphologic interpretation of transabdominal fine-needle aspiration biopsy specimens^{14,15} arise because:

1. Neoplastic cells aspirated from different malignant tumors have variable cytomorphologic appearances, ranging from benign to bizarre-looking, in aspirate preparations. In our daily work, bizarre-looking cells (e.g. atypical hepatocytes in the cirrhotic liver) are not necessarily malignant and, on the contrary, malignant cells from some tumors (e.g. well-differentiated renal cell carcinoma) often look benign.
2. Different types of tumors may have similar cytomorphologic appearances. There are many look-alikes in transabdominal aspiration biopsy cytology. For instance, clear tumor cells from renal cell carcinoma and adrenal cortical adenoma may look alike in aspirate preparations. Other examples, such as an islet cell tumor, as opposed to well-differentiated adenocarcinoma of the pancreas; carcinoid to well-differentiated adenocarcinoma; and serous cystadenoma to serous cystadenocarcinoma, may also present the same diagnostic problem.

3. Tumors of the same origin may have different cytomorphologic appearances. Most tumors have several cytomorphologic patterns. For instance, on the basis of cytomorphologic features, the neoplastic cells of renal cell carcinoma can have a clear or granular cytoplasm, arranged in loosely cohesive groupings or cohesive papillary fragments, and can have a sarcomatoid appearance. Therefore, a large number of cytomorphologic patterns may be seen in transabdominal aspiration biopsy cytology.
4. Samples from a tumor obtained by different cytologic methods may have variable cytomorphologic appearances. For instance, cell balls are a prominent finding in metastatic ductal carcinoma of the breast in effusions (Fig. 3.8); however, no cell balls are seen if a specimen is directly aspirated from the breast nodule.
5. A specimen prepared by different cytologic techniques shows variable cytomorphologic appearances. Aspirated material can be made into different preparations for cytomorphologic study, for example, direct smears, liquid based preparations, and cell blocks. In general, the cohesion factor is best demonstrated in direct smears rather than liquid-based preparations, where the aspirated samples are immediately fixed in the transport medium. Histology features are best appreciated in cell blocks.
6. Irritated epithelial cells and reactive cells may meet the so-called “cytologic criteria for malignancy (Fig. 3.2).” Atypical pancreatic ductal cells (e.g. in pancreatitis); atypical type 2 pneumocytes (e.g. in alveolar hemorrhage, pneumonia, or infection); reactive macrophages (e.g. in tuberculosis lesion); and atypical mesothelial cells (e.g. irradiation effects) may have highly abnormal appearances in aspirate preparations.

From our combined experience in dealing with more than 30,000 transabdominal and transthoracic fine-needle aspiration biopsy, these pitfalls in the cytomorphologic interpretation of transabdominal aspiration biopsy specimens, which often account for unsuccessful attempts, can be readily avoided with experience.¹³ It is also well-recognized that there are some limitations in aspiration biopsy cytology, as in any other method dealing with morphology.^{5,15} The limitations in aspiration biopsy cytology are different in different sites and also in different types of tumors. For instance, no conclusive diagnosis can be made on purely cytomorphologic grounds for follicular neoplasms of the thyroid because the cytomorphologic features of

endocrine tumors are not a reliable indicator of malignancy. It is not possible to assess capsular and vascular invasion on cytology. In the cytomorphologic interpretation of transabdominal aspiration biopsy specimens, the differentiation between reactive lymphoid hyperplasia and low grade small cell lymphomas of various types can be difficult without immunophenotyping by flow cytometry. Any cytopathologist who is fully aware of the limitations in aspiration biopsy cytology can avoid erroneous interpretations by triaging such cases for further studies, e.g. flow cytometry, immunocytochemistry, or tissue assessment.

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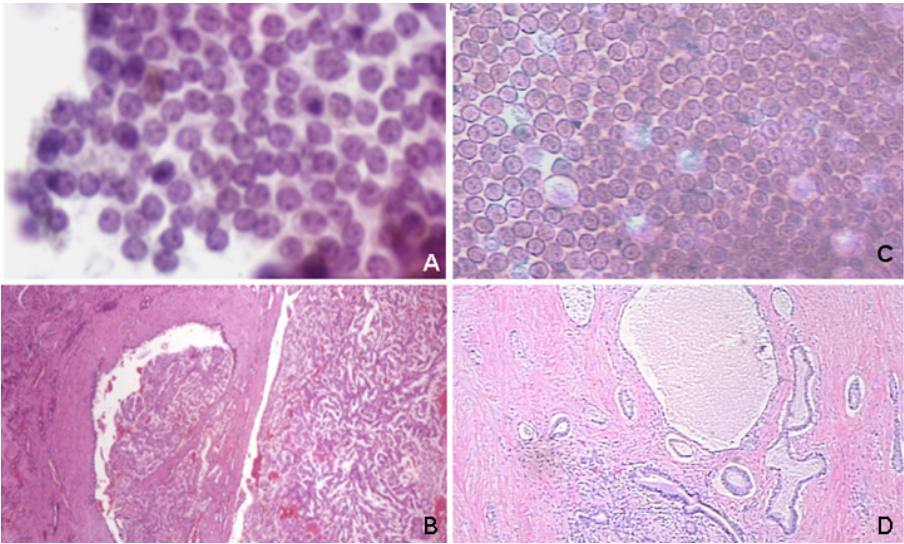


Fig. 3.1 Malignant cells that have a benign nuclear features. (A) Many cohesive epithelium with uniform, small bland nuclei. UFP, 1000 \times ; (B) Nephrectomy shows renal cell carcinoma with vascular invasion. H&E, 40 \times ; (C) Sheets of ductal epithelium with equal-sized bland nuclei. UFP, 1000 \times ; (D) Surgery shows pancreatic ductal carcinoma. H&E, 400 \times .

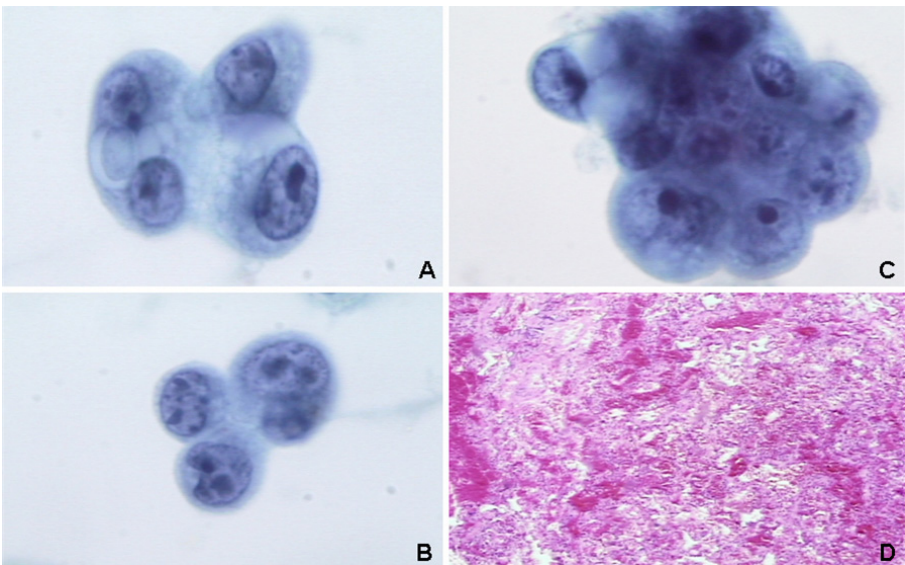


Fig. 3.2 Irritated benign cell that have a malignant nuclear features. Scanty marked atypical glandular cells with coarse chromatin and prominent irregular nuclei, reported as “suspicious for adenocarcinoma.” (A–C) UFP, 1000 \times ; (D) Wedge resection showed alveolar hemorrhage lined by irritated hyperplastic type 2 pneumocytes. H&E, 40 \times .

TRANSABDOMINAL FINE-NEEDLE ASPIRATION BIOPSY (Second Edition) A Color Atlas and Monograph (With CD-ROM)

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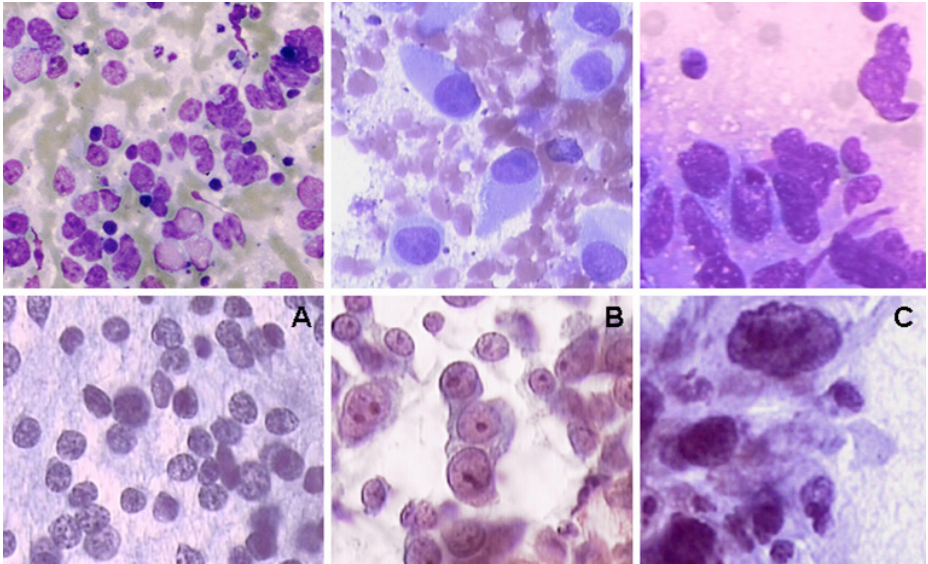


Fig. 3.3 Average nuclear size in tumor cells. (A) Small ($< 20 \mu\text{m}$); (B) Medium ($20\text{--}30 \mu\text{m}$); (C) Large ($> 30 \mu\text{m}$). *Top row:* Nuclear size is best measured in DQ due to background RBCs ($= 7 \mu\text{m}$). *Bottom row:* Nuclear and nucleolar features are best seen in UFP, $400\times$.

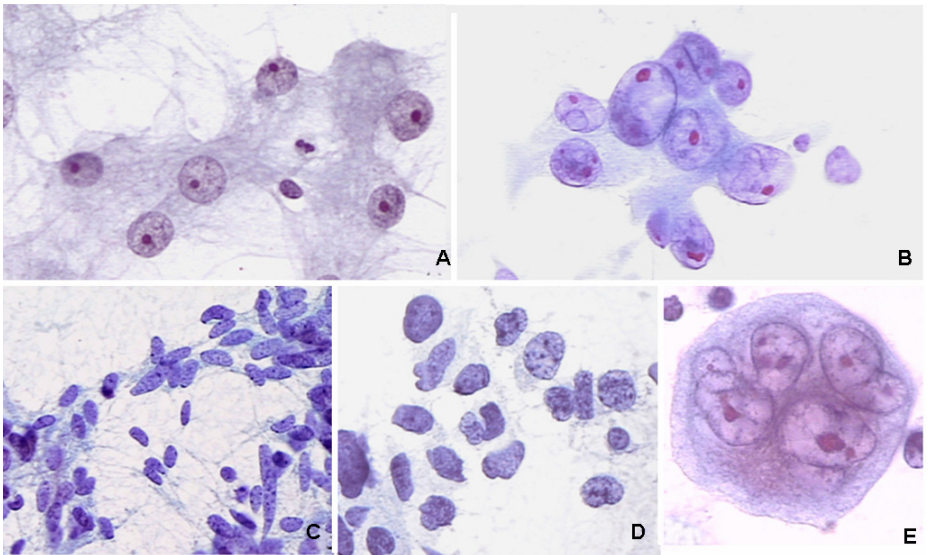


Fig. 3.4 General nuclear shape in tumor cells. (A) Round; (B) Mixed ovoid and round; (C) Elongated and spindle-shaped; (D) Irregular; (E) Multinucleated. UFP, $400\times$.

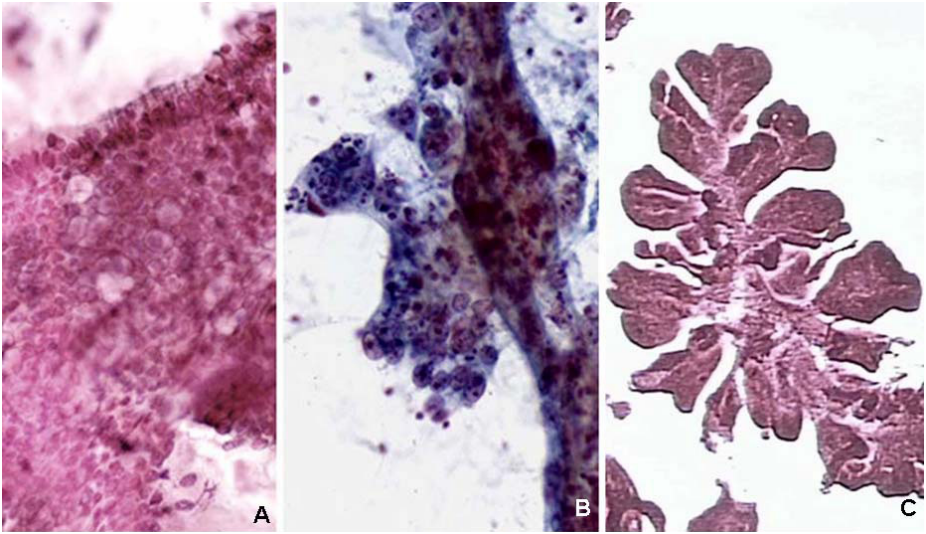


Fig. 3.5 Arrangement of tumor cells. (A) Monolayer; (B) Multilayer; (C) Papillary. UFP, 400 \times .

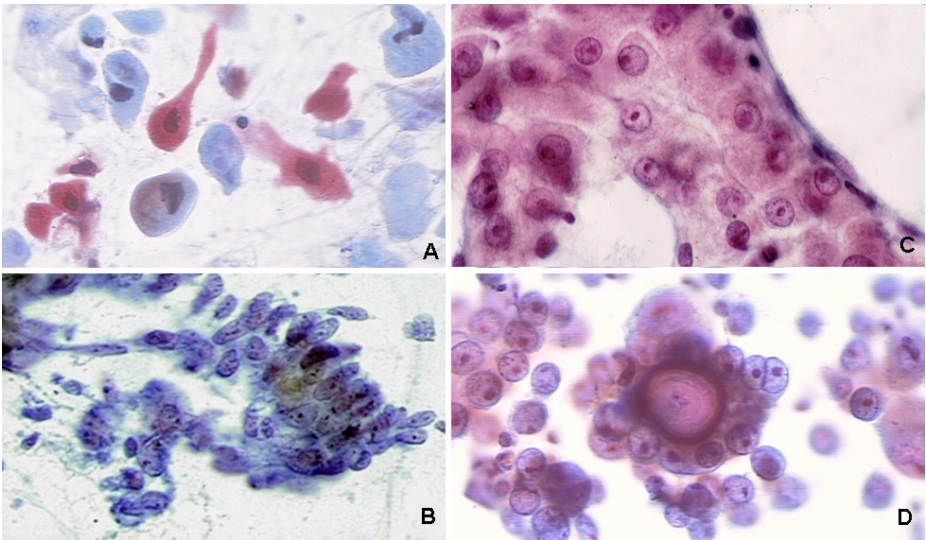


Fig. 3.6 Unique features of tumor cells. (A) Keratinization in squamous cell carcinoma. UFP, 400 \times ; (B) Parallel cigar-shaped nuclei in colonic adenocarcinoma. UFP, 400 \times ; (C) Endothelial wrapping in hepatocellular carcinoma. UFP, 400 \times ; (D) Psammoma bodies of papillary serous carcinoma of the ovary. UFP, 400 \times .

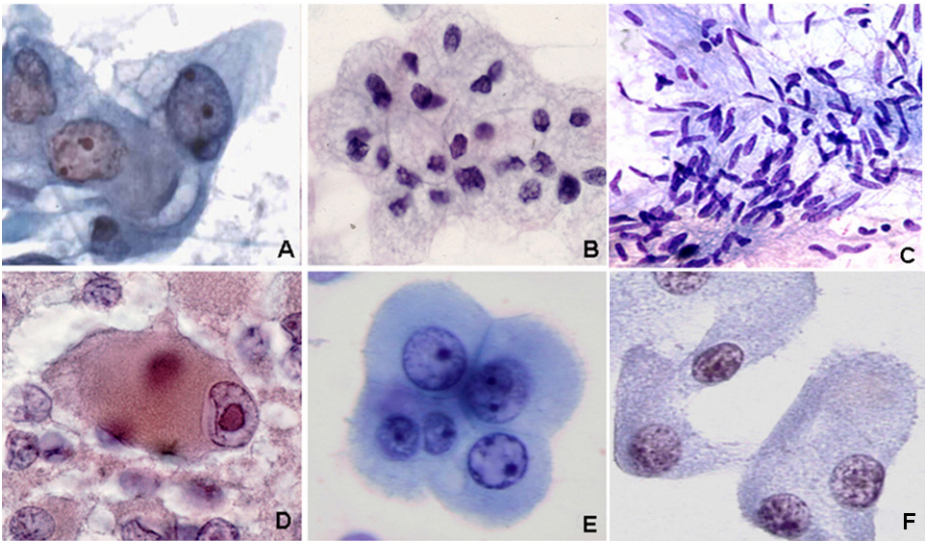


Fig. 3.7 Cytoplasmic texture of tumor cells. UPE, 400 \times . (A & B) Multivacuolated in adenocarcinoma. UFP, 400 \times ; (C) Fibrillary in peripheral nerve sheath tumor. (D) Ground glass in adrenal cortical carcinoma. (E) Dense in mesothelial cells; (F) Granular, mononocytic cells.

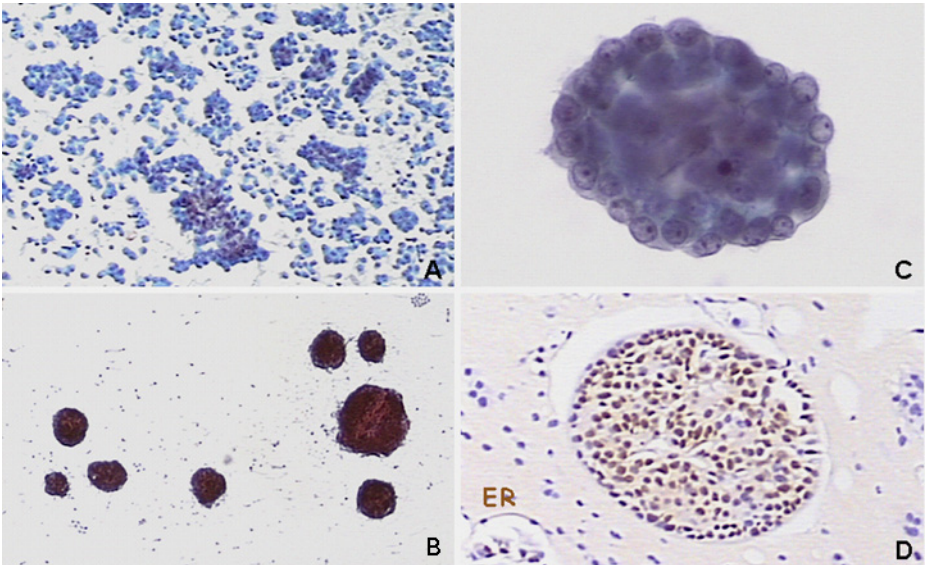


Fig. 3.8 Ductal carcinoma of the breast in different environment. (A) Loosely cohesive cells aspirated from the breast nodule. UFP, 100 \times ; (B) Cell balls in a pericardial effusion due to surface tension. UFP, 40 \times ; (C) High power shows closely packed tumor cells. UFP, 100 \times ; (D) Estrogen receptor positivity confirms breast origin. Cell block, 100 \times .