

# **ESSENTIALS OF ABDOMINAL FINE NEEDLE ASPIRATION CYTOLOGY**

**Gia-Khanh Nguyen**

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Gia-Khanh Nguyen, M.D.  
Professor Emeritus  
Department of Laboratory Medicine and Pathology  
Faculty of Medicine and Dentistry  
University of Alberta  
Edmonton, Alberta, Canada

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# PREFACE

Fine needle aspiration for cytologic evaluation of intra-abdominal and retroperitoneal mass lesions in adult patients now is practiced worldwide. However, this diagnostic procedure is not commonly practiced in infants and children in general community hospitals. Therefore, cytology of pediatric tumors is excluded from this monograph on *Essentials of Abdominal Fine Needle Aspiration Cytology*. The book is written for practicing pathologists in community hospitals, residents in pathology and cytotechnologists who are interested in acquiring a basic knowledge on fine needle aspiration cytology of abdominal tumors in adults. The text is concise and illustrations are abundant, and only commonly encountered tumors or uncommon lesions with characteristic cytologic manifestations are discussed. A large number of histopathologic images are included for cytohistologic correlation. In the first edition of the monograph cytodiagnostic criteria were emphasized. In this revised edition value and limitations of immunocytochemistry in tumor typing and differential diagnosis are stressed. A small number of important references are listed in alphabetic order at the end of each chapter for further consultation.

For improvement of the future editions of this monograph, constructive comments and suggestions from the readers will be highly appreciated.

Gia-Khanh Nguyen, M.D.  
Surrey, BC, Canada  
khanhnguyen1730@hotmail.com  
Summer 2008

To my family with love.

## **RELATED MATERIAL BY THE SAME AUTHOR**

Essentials of Needle Aspiration Biopsy Cytology. Igaku-Shoin, New York, 1991  
Essentials of Exfoliative Cytology. Igaku-Shoin, New York, 1992  
Essentials of Cytology: An Atlas. Igaku-Shoin, New York, 1993  
Critical Issues in Cytopathology. Igaku-Shoin, New York, 1996  
Essentials of Lung Tumor Cytology. UBC Pathology, Vancouver, 2008

## **KEY TO ABBREVIATIONS**

FNA: fine needle aspiration or fine needle aspirate  
TFNA: trans-abdominal FNA  
EUS-FNA: endoscopic ultrasound guided FNA  
Pap: Papanicolaou stain  
HE: hematoxylin and eosin stain  
ABC: avidin-biotin complex technique

# PANCREAS AND AMPULLARY REGION

## INDICATIONS, CONTRAINDICATIONS, TECHNICAL CONSIDERATIONS AND COMPLICATIONS OF PANCREATIC FNA

### Indication and Contraindications

Pancreatic mass lesions, solid or cystic, constitute the main indication for FNA. Contraindications for TFNA of the pancreas include bleeding disorders, bowel distention and intraabdominal hydatid cysts, as leaking of the cyst contents into the peritoneal cavity may cause a fatal anaphylactic reaction. Pancreatic mass lesions can also be directly aspirated by a surgeon at laparotomy. In recent years, FNA of small mass lesions (< 3 cm) of the pancreas and ampulla of Vater region may be accurately performed by a gastroenterologist via EUS-FNA. There are no contraindications for pancreatic EUS-FNA other than bleeding diathesis.

### Technical Considerations

TFNA of pancreatic masse is a safe, economical and accurate diagnostic procedure. It is performed under guidance of an abdominal US or CT scanning. Usually a 22-gauge and 20-cm-long, spinal-type needle is used. There are no objective criteria for an adequate or representative needle aspirate of the pancreas, and obtaining a representative cell sample depends largely on the skills of the radiologist or a gastroenterologist who performs the FNA.

Direct smears are usually prepared from the needle aspirate and cytopsin smears are made from liquid cystic contents. The smears are either fixed in 95% ethanol or air-dried and stained either with the Papanicolaou method or Diff-Quik technique (modified Wright stain), respectively. Minute tissue fragments obtained by FNA are removed and fixed in formalin for histologic examination or in 2% glutaraldehyde for ultrastructural study. Excess of aspirated material is fixed in formalin for preparation of a cell block that may also provide valuable histologic information and that can be used for immunohistochemical studies. Immunocytochemical staining can also be done on a Papanicolaou-stained smear without prior de-staining.

## **Complications**

Complication rates of pancreatic FNA are low and include intra-abdominal bleeding (2.6%) and acute pancreatitis (1%). Tumor spreading on peritoneal surface occurs more commonly with TFNA than with EUS-FNA (16.3 % versus 2.2%).

## **DIAGNOSTIC ACCURACY**

The success of pancreatic FNA is the result of the collaboration between a radiologist or a clinician who is familiar with the biopsy technique and a cytopathologist with experience in interpreting FNA cytology. US- or CT-guided TFNA of pancreatic tumors has an average sensitivity and specificity of 80% and 90%, respectively. For EUS-FNA a sensitivity of 80-94% and a specificity of 82-100%, have been reported. Intra-operative pancreatic FNA has diagnostic sensitivity and specificity approaching 100%. Sampling error is the most common cause of false-negative cytodiagnoses of pancreatic cancers and false-positive cytodiagnoses are rare.

## **NORMAL PANCREAS**

Histologically, the pancreatic tissue is composed of excretory ducts, acini and islet cells (Figures 1.1-1.3). A representative cell sample from normal pancreatic tissue commonly shows numerous acinar and ductal epithelial cells. Islet cells are rarely identified. Ductal epithelial cells are columnar in shape and show basally located oval nuclei, small nucleoli and clear or vacuolated cytoplasm. These cells are commonly seen in large- or medium-sized monolayered sheets with a honeycomb pattern or singly. The acinar cells are pyramidal in shape and show basally located oval nuclei, conspicuous nucleoli and granular cytoplasm. They are commonly seen in small clusters or acini consisting of 6-10 cells. Hepatocytes are commonly present in pancreatic FNAs as the biopsy needle passes through the liver. Small sheets of mesothelial cells with oval nuclei are also commonly seen. (Figures 1.4-1.7).

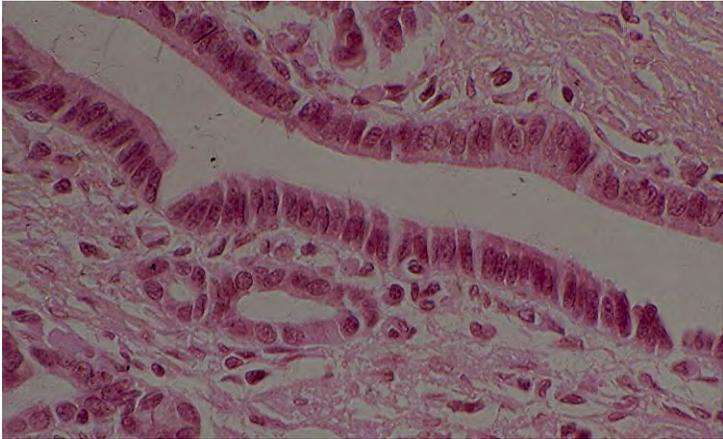


Figure 1.1. Normal pancreatic parenchyma showing a larger excretory duct lined by a layer of mucus-secreting columnar epithelial cells and a few ductules lined by cuboidal epithelial cells. (HE, x 250).

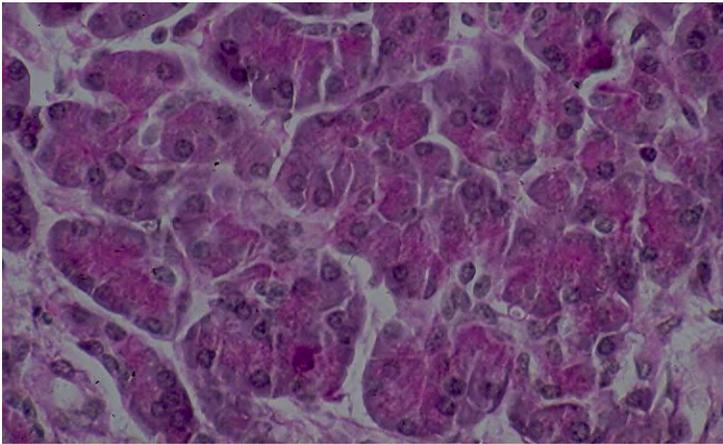


Figure 1.2. Normal pancreatic parenchyma showing acini consisting of pyramidal acinar cells with basally located round nuclei. (HE, x 250).

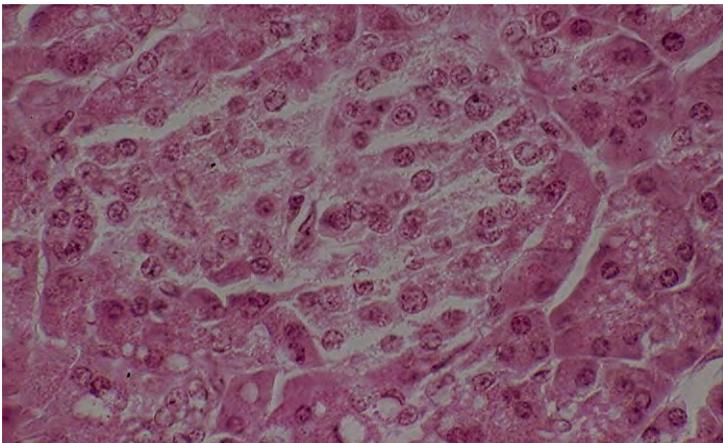


Figure 1.3. Normal pancreatic tissue showing a nest of islet cells surrounded by acinar cells. (HE, x 250).

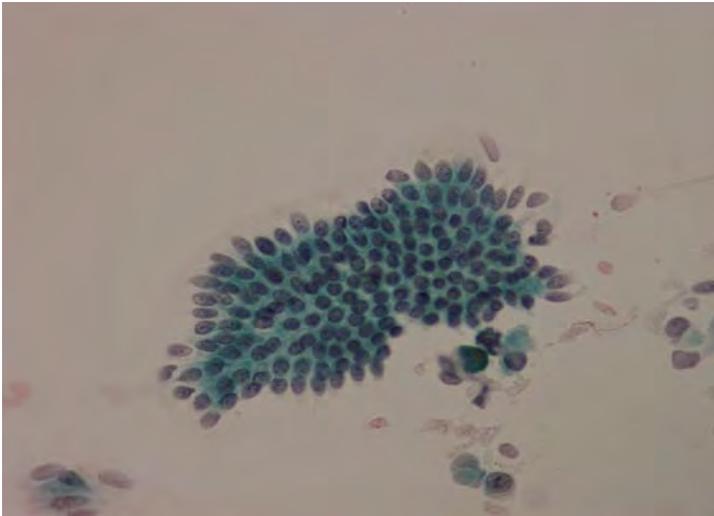


Figure 1.4. A monolayered sheet normal pancreatic ductal columnar epithelial cells with honeycomb pattern in a pancreatic TFNA. (Pap, x 100).

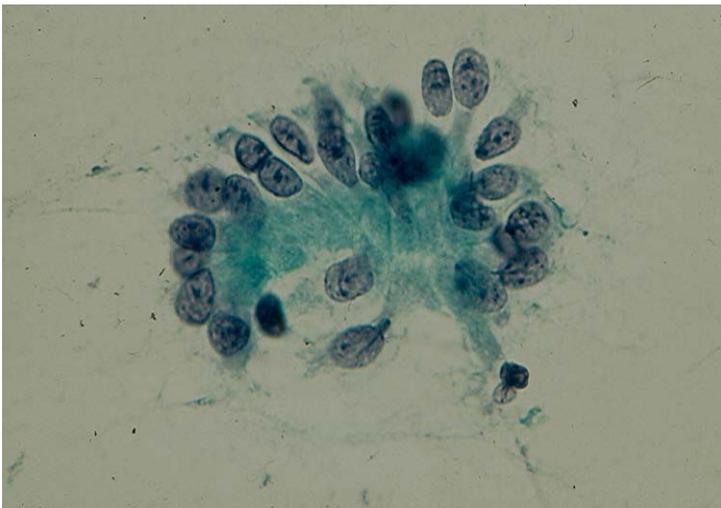


Figure 1.5. A small fragment of pancreatic ductal epithelium showing columnar cells with basally located oval nuclei, small nucleoli and ill-defined cytoplasm. (Pap, x 500).

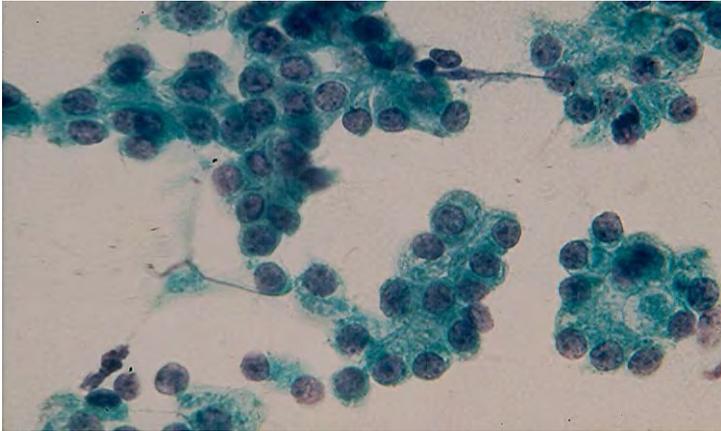


Figure 1.6. Pancreatic TFNA showing normal cuboidal acinar cells forming acini and in small clusters. (Pap, x 500).

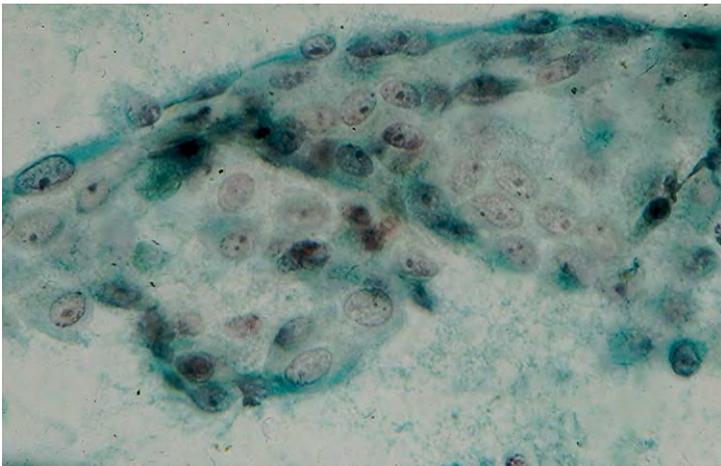


Figure 1.7. A sheet of mesothelium with honeycomb pattern and folding showing oval nuclei with conspicuous nucleoli. (Pap, x 500).

## **PRIMARY PANCREATIC NEOPLASMS**

In the United States and Canada pancreatic carcinoma is the fourth most common cause of cancer death in men and the fifth in women. About half of the patients die within 6 months after the diagnosis and the overall 5-year survival rate is less than 1%. The most frequent location of the tumor is the head (60%) followed by the body (10%) and the tail (5%) of the organ. In the remaining 25% the pancreas is diffusely involved.

### **1. Pancreatic ductal carcinoma**

Pancreatic ductal carcinoma accounts for over 90% of all primary pancreatic cancers and 75% of them are adenocarcinomas. (Figures 1.8 and 1.9).

**1.1. A well-differentiated ductal adenocarcinoma** yields in FNA large, folded sheets of fairly monomorphic epithelial cells showing a vague honeycomb pattern with focal nuclear crowding and overlapping. The tumor cell nuclei are oval in shape with regular or irregular nuclear contours and conspicuous nucleoli. (Figures 1.10-1.13). **A mucus-rich ductal adenocarcinoma** shows tumor cells in honeycomb sheets with well-defined and angulated cell borders. (Figure 1-14). **A poorly differentiated ductal adenocarcinoma** shows in FNA more pleomorphic malignant cells in irregular groups or clusters with marked nuclear overlapping and crowding and single malignant glandular cells. (Figures 1.15 and 1.16).

Cells derived from a well-differentiated pancreatic ductal adenocarcinoma may mimic reactive ductal epithelial cells aspirated from a chronic pancreatitis.

Robins et al. have identified 3 major criteria (nuclear crowding and overlapping, irregular chromatin distribution and irregular nuclear contours) and 3 minor criteria (nuclear enlargement, single malignant cells, necrosis/mitosis) for cytodiagnosis of pancreatic ductal adenocarcinoma. A combination of 2 or more major or 1 major and 3 minor criteria are diagnostic of pancreatic ductal adenocarcinoma with 100% sensitivity and specificity.

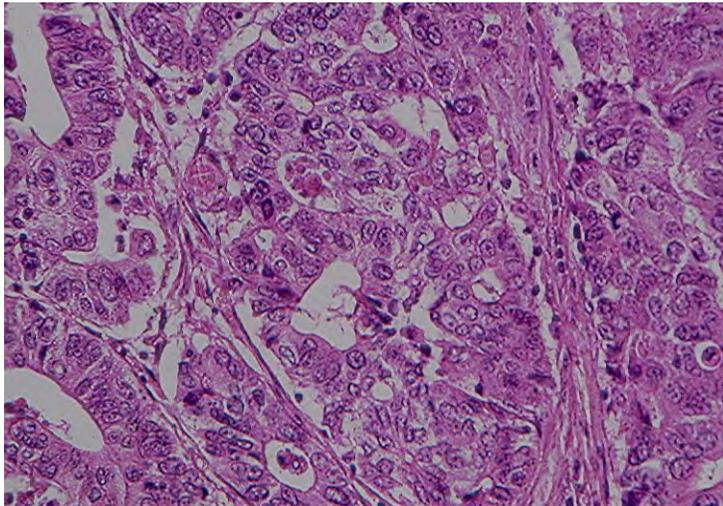


Figure 1.8. Histology of a well-differentiated pancreatic ductal adenocarcinoma. (HE, x 250).

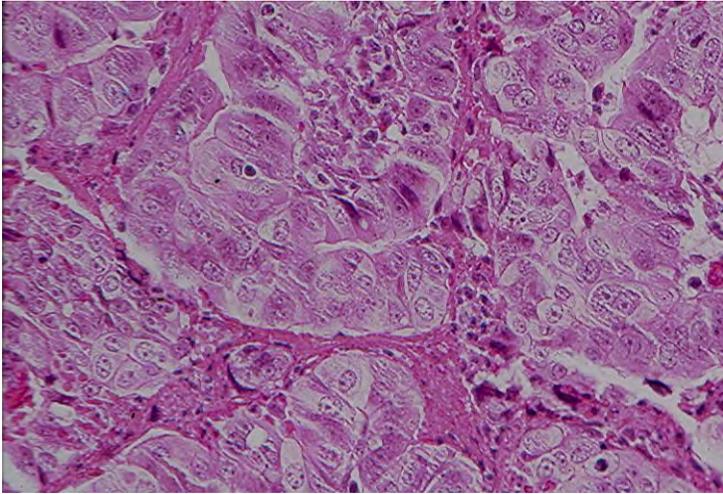


Figure 1.9. Histology of a poorly differentiated pancreatic ductal adenocarcinoma. (HE, x 250).

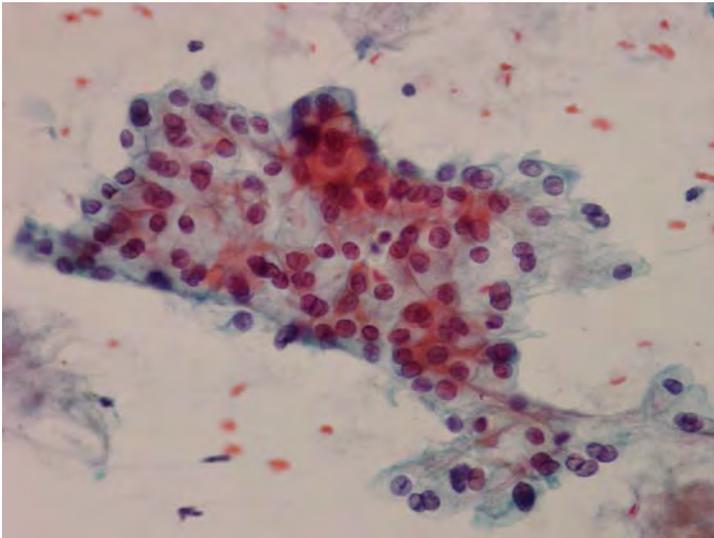


Figure 1.10. Cells aspirated from a well-differentiated pancreatic ductal adenocarcinoma are seen in sheet with honeycomb pattern and focal nuclear crowding. (Pap, x 250).

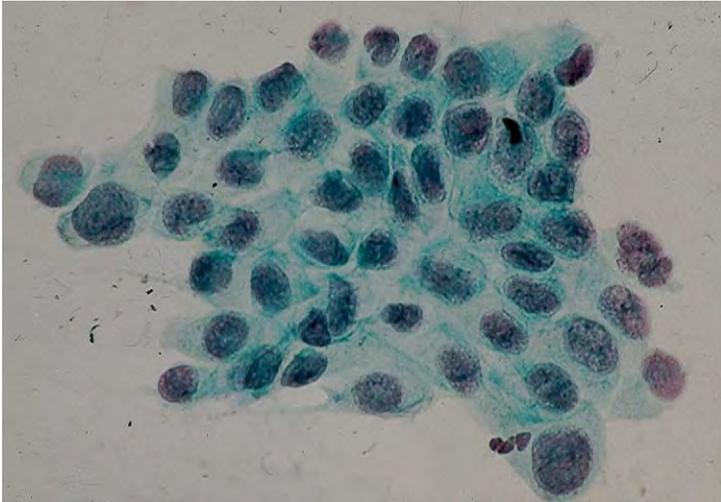


Figure 1.11. A well-differentiated pancreatic ductal adenocarcinoma showing in TFNA a monolayered sheet of tumor cells with honeycomb pattern, enlarged and hyperchromatic nuclei with irregular nuclear contours and minimal, focal nuclear crowding. (Pap, x 500).

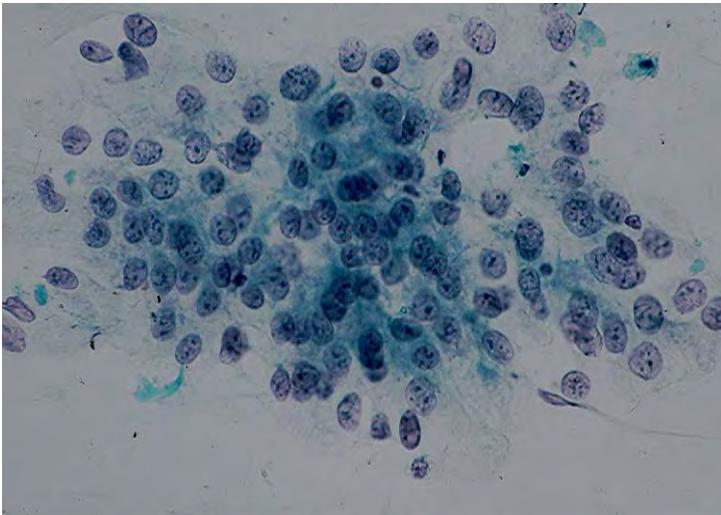


Figure 1.12. A sheet of tumor cells aspirated from a well-differentiated pancreatic ductal adenocarcinoma showing a vague honeycomb pattern, nuclear crowding/overlapping and prominent nucleoli. (Pap, x 400).

Cells of a pancreatic ductal and those of a biliary tree adenocarcinomas usually express CK7 and CEA and they are commonly CK20 and CDX2 negative.

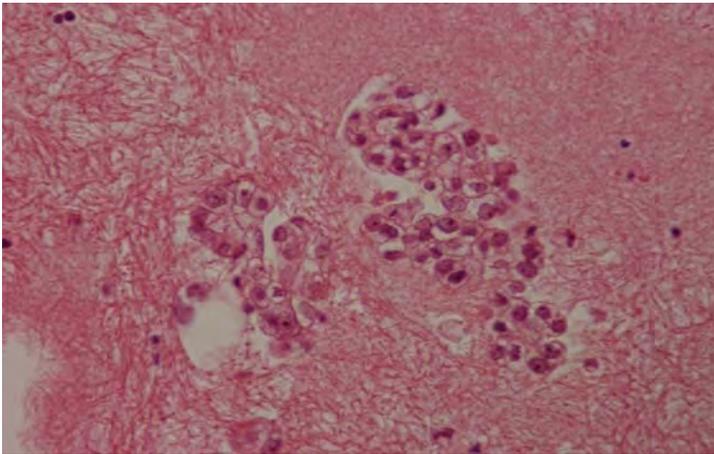
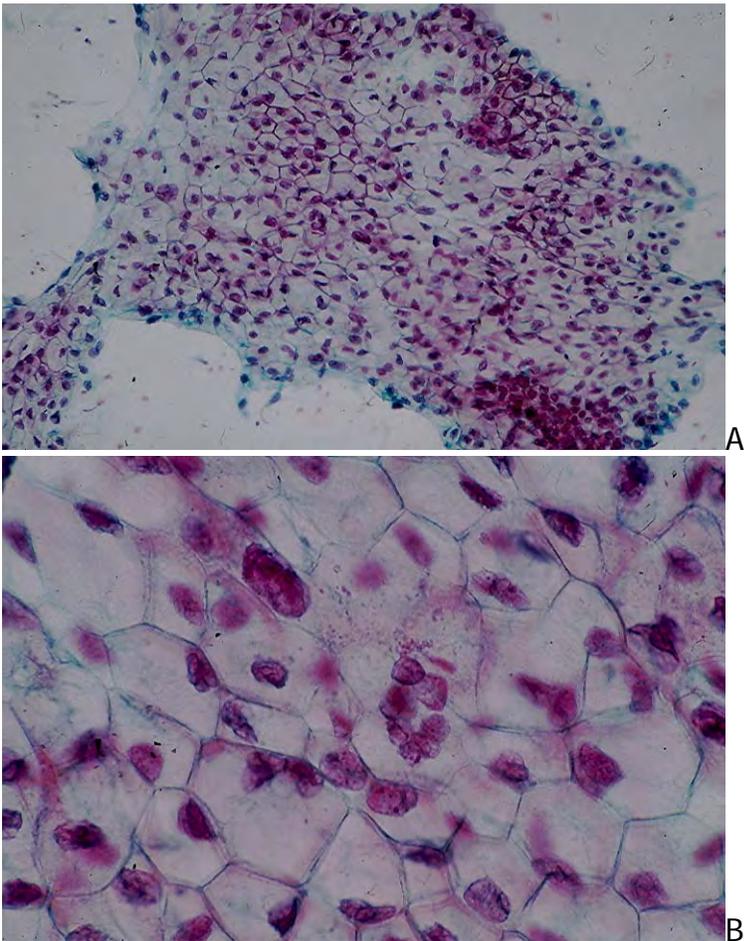


Figure 1.13. Cell block prepared from the FNA of a well-differentiated pancreatic ductal adenocarcinoma showing minute fragments of tumor tissue. (HE, x 250).



Figures 1.14. A large sheet of tumor cells from a mucus-rich, well-differentiated pancreatic ductal adenocarcinoma showing an exaggerated honeycomb pattern, nuclear crowding and angulated nuclei. (Pap, A x 100, B x 500).

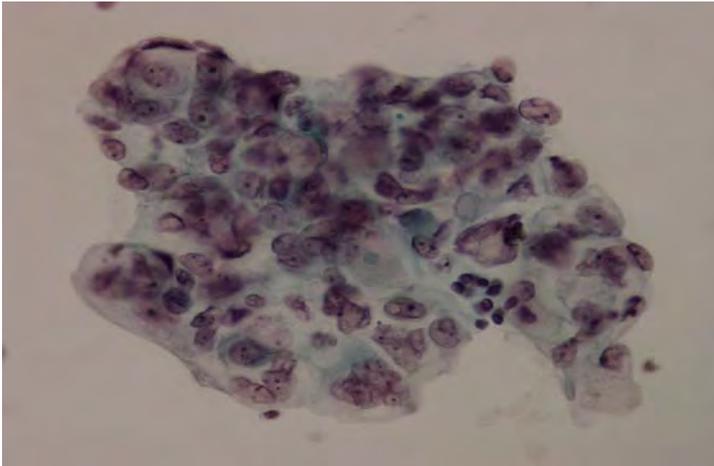
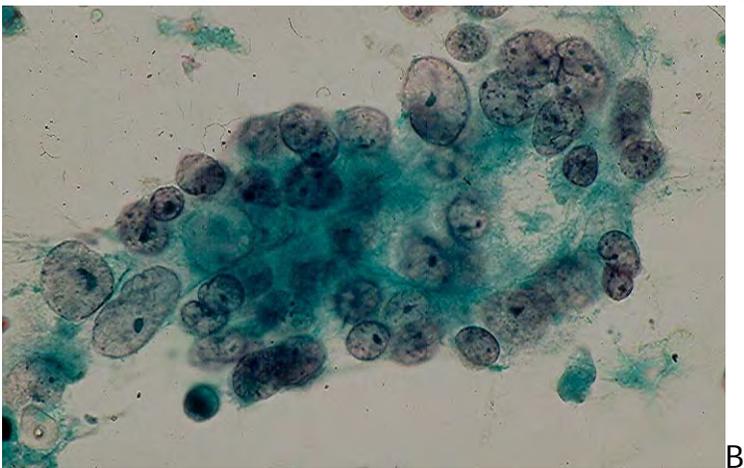
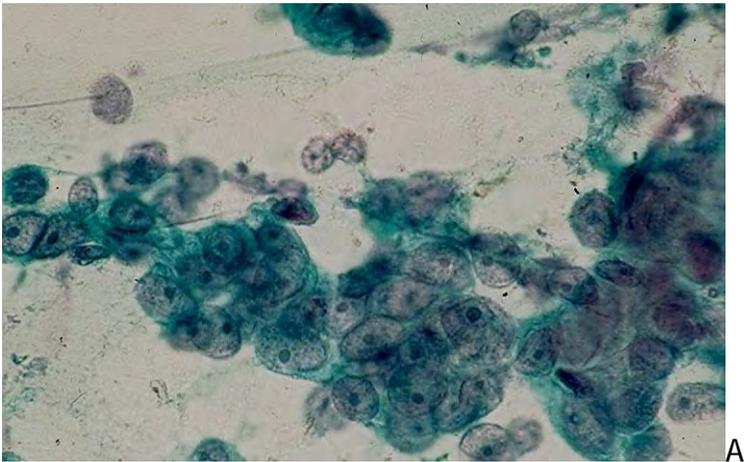


Figure 1.15. A cluster of pleomorphic tumor cells with marked nuclear crowding and overlapping from a poorly differentiated ductal adenocarcinoma. (Pap, x 400).



Figures 1.16. Clustered pleomorphic tumor cells showing marked nuclear crowding and overlapping and prominent nucleoli aspirated from a poorly differentiated pancreatic ductal adenocarcinoma (Pap, x 500).

Histologic variants of pancreatic ductal carcinoma such as mucinous, mucoepidermoid, large cell, small cell and osteoclastic giant cell carcinomas are uncommon and they account for about 25% of all ductal carcinomas, with each variant accounting for 1 to 7% of all tumors. These tumors can be easily identified in representative cell samples as they show distinctive cytologic features.

**1.2. Mucinous carcinoma** is characterized by clusters or sheets of malignant epithelial cells with well- or ill-defined, vacuolated cytoplasm in a mucous background that stains positively with periodic-acid Schiff (PAS) reagent and with PAS with prior diastase digestion (PAS-D) or with mucicarmine stain. (Figures 1.17 and 1.18).

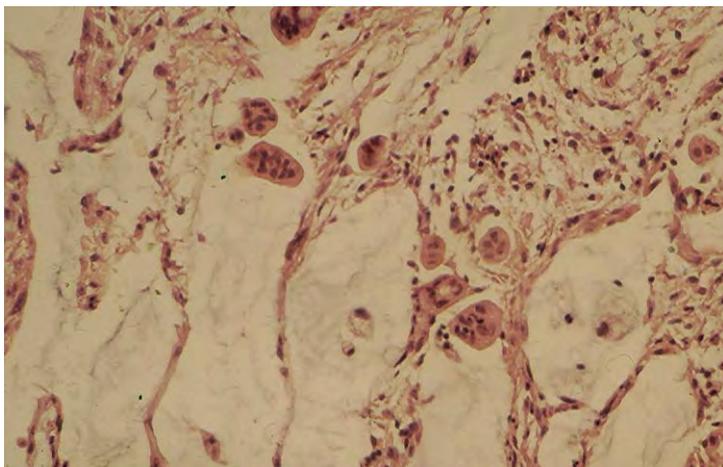
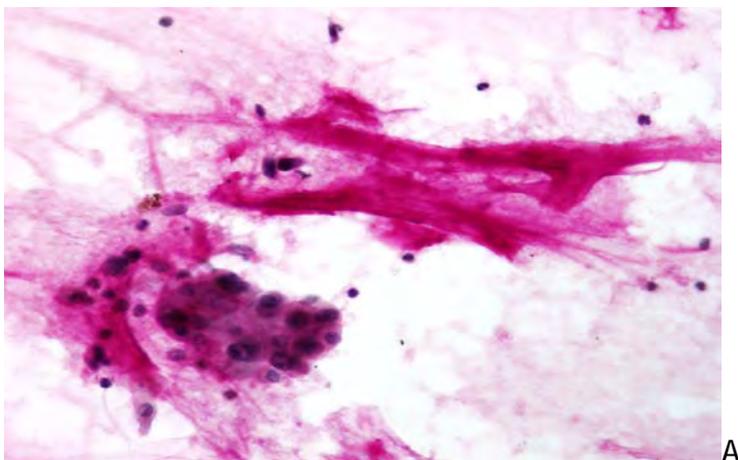
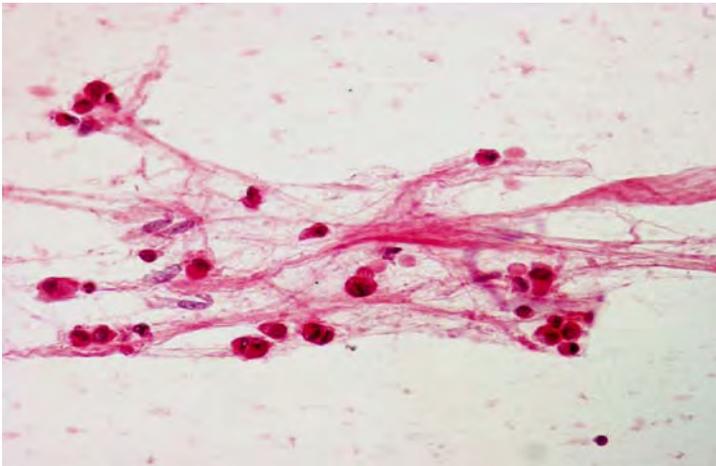


Figure.1.17. Histology of a pancreatic mucinous adenocarcinoma (HE, x 250).



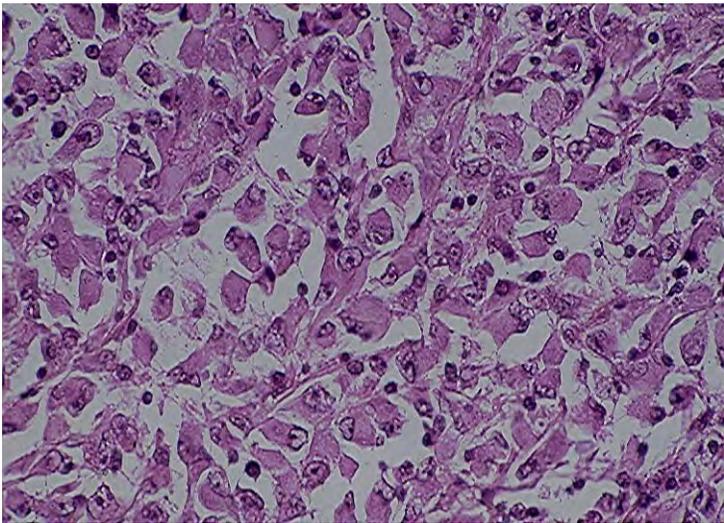
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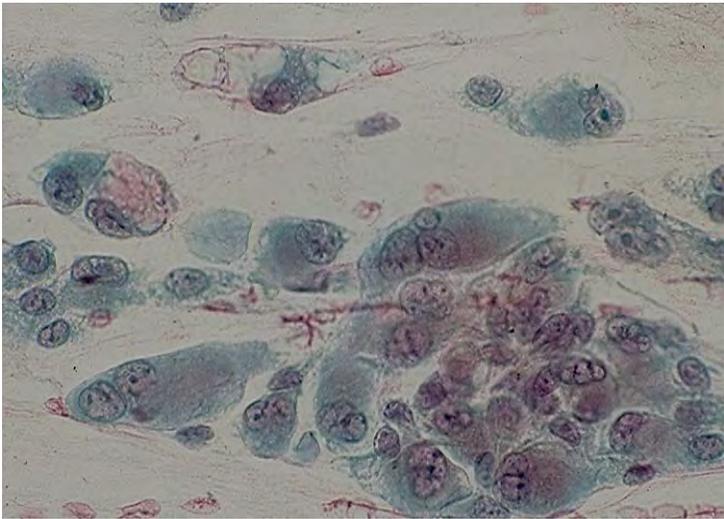
B

Figures 1.18. Pancreatic mucinous adenocarcinoma showing in (A) a cluster of tumor cells and thick mucus and in (B) single and clustered tumor cells in a mucous background (A, PASD, x 500; B, Mucicarmine x 250).

**1.3. Mucoepidermoid carcinoma** is a very rare pancreatic cancer. It yields in TFNA isolated cells showing a well-defined, granular and thick or “hard” cytoplasm with mucous globules that may be visualized by mucicarmine or PAS-D stain. (Figures 1.19).

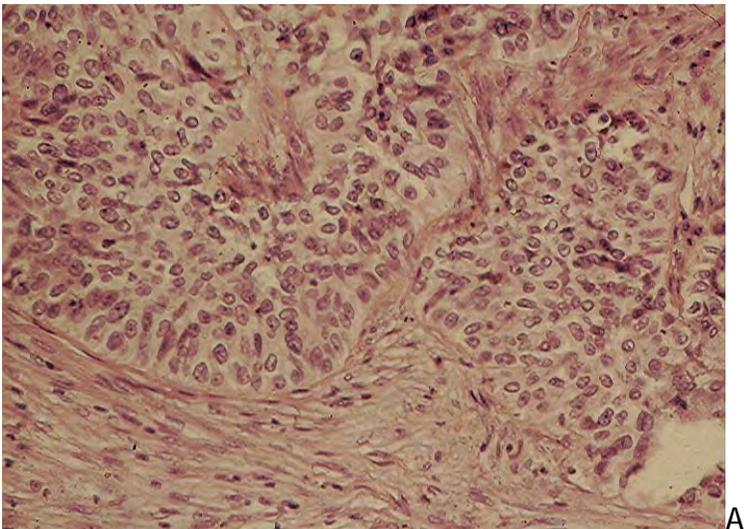


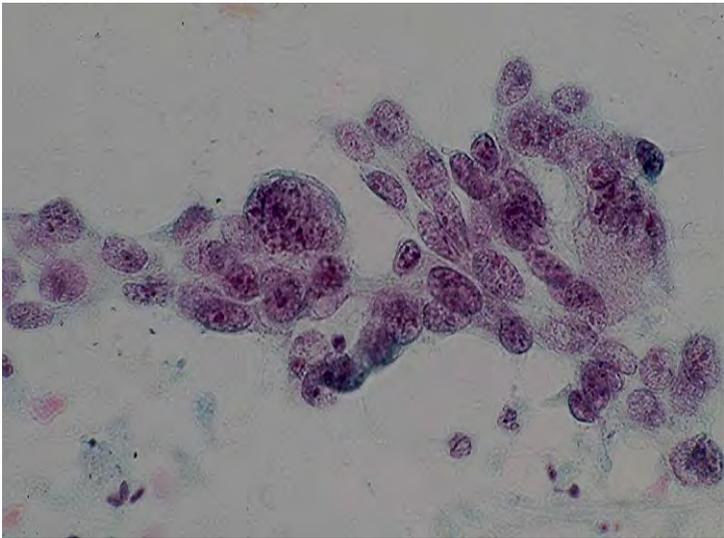
A



Figures 1.19. A. Histology of a pancreatic mucoepidermoid carcinoma consisting of pleomorphic squamoid cells in solid pattern. (HE, x 250).  
 B. TFNA of the above tumor showing pleomorphic malignant epithelial cells with abundant, thick, granular cytoplasm and oval nuclei with conspicuous nucleoli. Intracytoplasmic pink mucus is noted in some tumor cells. (Pap, x 500).

**1.4. Squamous cell carcinoma** is a very rare variant of pancreatic ductal carcinoma. It can be nonkeratinizing or keratinizing. A nonkeratinizing tumor shows in FNA single and loosely clustered malignant epithelial cell with oval and pleomorphic nuclei and variably abundant granular cytoplasm with ill-defined cytoplasm. (Figures 1.20).





B

Figures 1.20. Histology and FNA cytology of a pancreatic nonkeratinizing squamous cell carcinoma (A. HE, x 250; B. Pap, x 500).

**1.5. Osteoclastic giant cell carcinoma** is characterized by loosely clustered pleomorphic malignant epithelial cells with well-defined cytoplasm admixed with benign large multinucleated giant cells. (Figures 1.21 and 1.22)

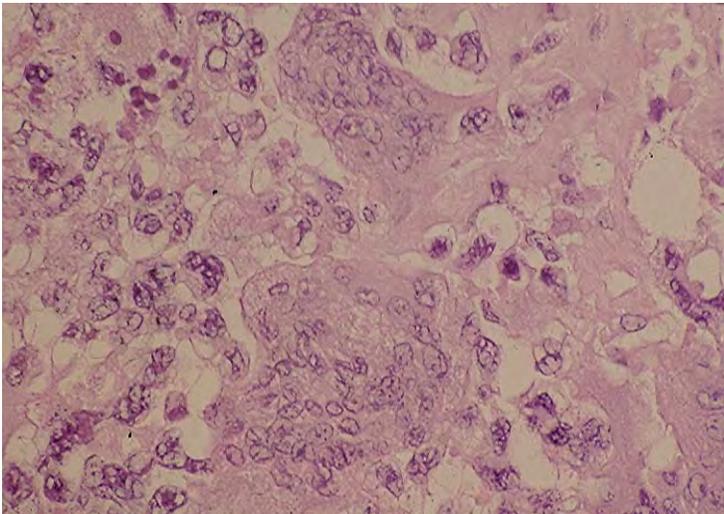
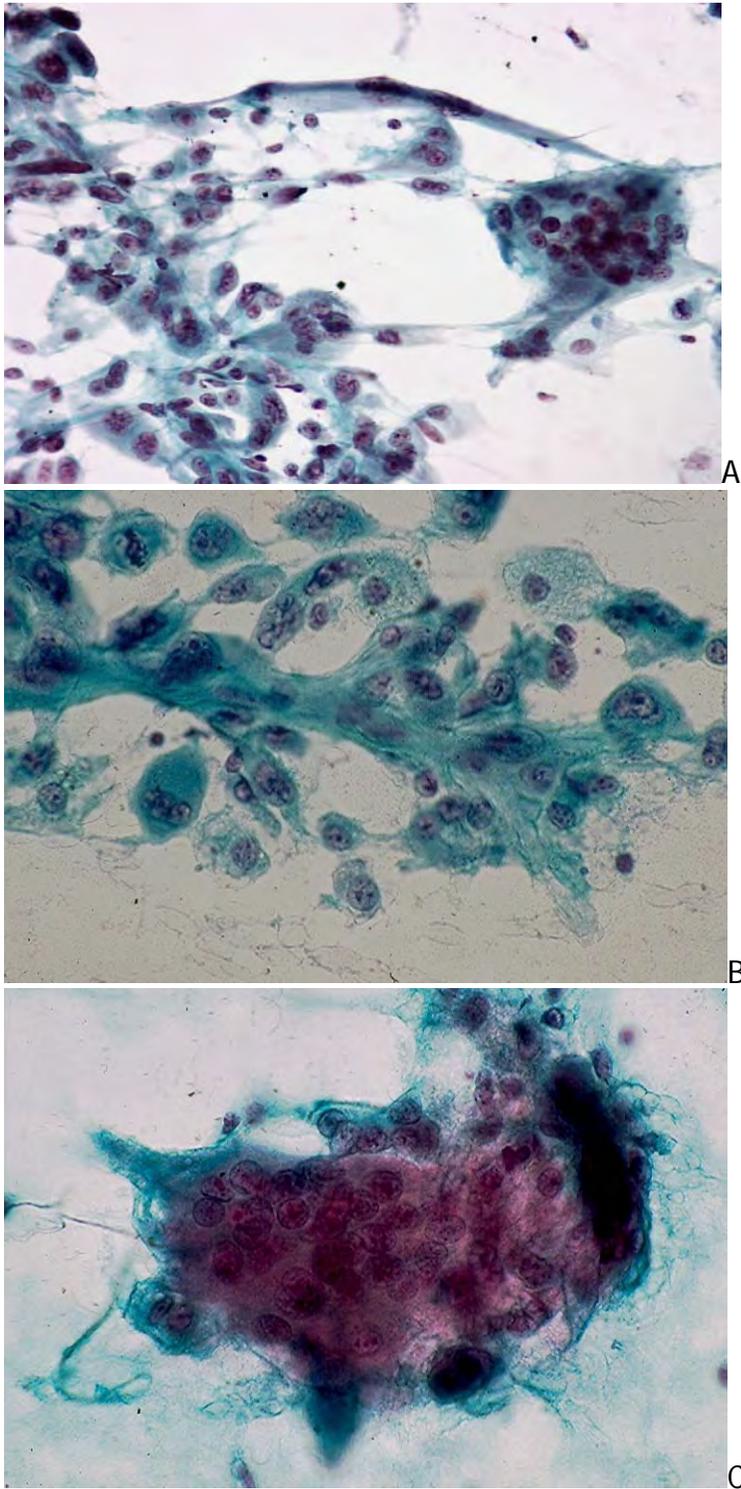


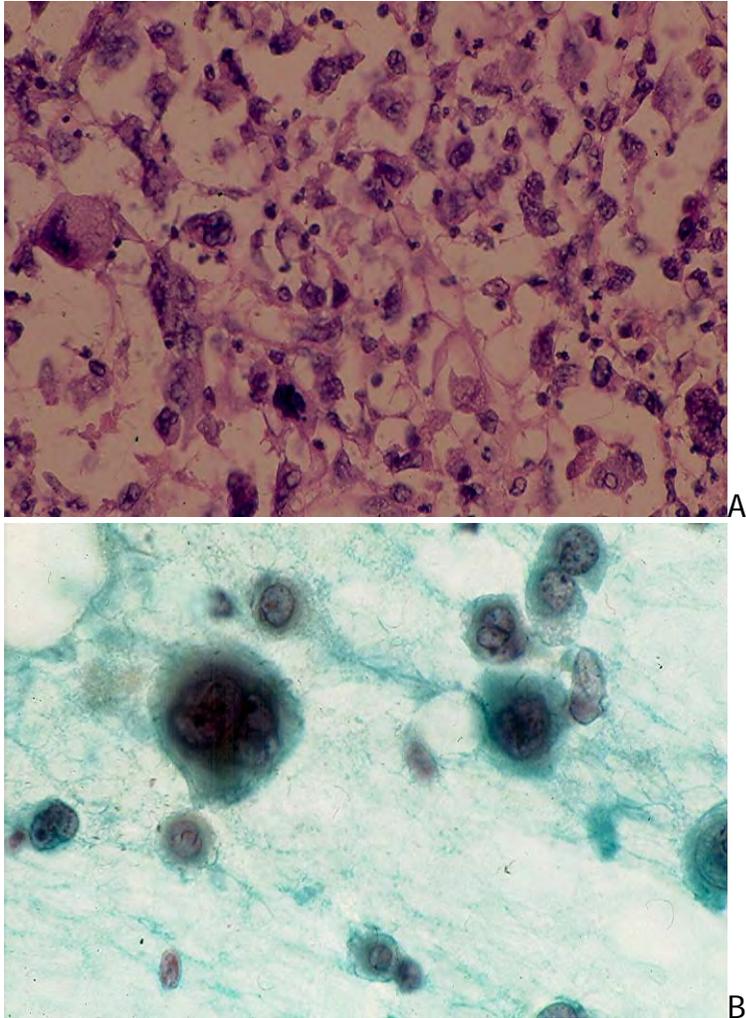
Figure 1.21. Histology of a pancreatic osteoclastic giant cell carcinoma. (HE, x 250).



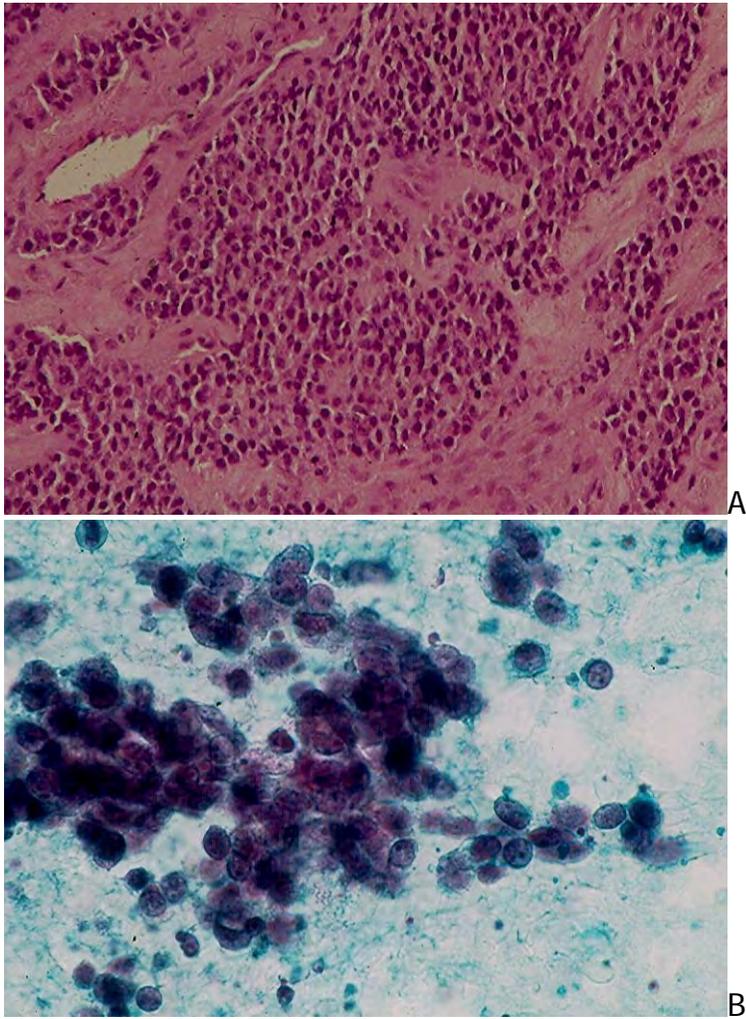
Figures 1.22. FNA of a pancreatic osteoclastic giant cell carcinoma showing in:  
A. An admixture of single and loosely clustered pleomorphic malignant cells and a benign multinucleated giant cell.

- B. Higher magnification of pleomorphic tumor cells.
  - C. Higher magnification of a benign-appearing multinucleated osteoclastic giant cell.
- (Pap, A x 250, B and C x 500).

**1.6. Large and Small cell carcinomas** of the pancreas in FNAs are cytologically similar to those of the lung and other organs. (Figures 1.23 and 1.24)



Figures. 1.23. A. Histology and FNA cytology of a pancreatic large cell carcinoma. B. Tumor FNA showing dyshesive large, pleomorphic malignant epithelial cells. (A, HE, x 250, B. Pap x 500).



Figures. 1.24. Histology and FNA cytology of a pancreatic small cell carcinoma. The FNA reveals predominantly clustered small tumor cells with scant cytoplasm and nuclear molding. (A. HE, x 250, B. Pap, x 500).

## 2. Acinar cell carcinoma

This is an uncommon tumor and accounts for about 1% of all pancreatic carcinomas. A well-differentiated acinar cell carcinoma shows in FNA clustered polygonal or pyramidal malignant cells with granular cytoplasm, basally located oval nuclei and prominent nucleoli. A poorly differentiated acinar cell carcinoma is characterized by clustered pleomorphic malignant cells with clear, granular or vacuolated cytoplasm. (Figures 1.25 and 1.26). The tumor cell cytoplasm stains positively with PAS and expresses trypsin, chymotrypsin, lipase and rarely amylase. Electron microscopic study of aspirated minute tumor tissue fragments may reveal glandular epithelial cells with characteristic

intracytoplasmic zymogen granules. Molecular analysis of pancreatic acinar cell carcinoma may show multiple loss of heterozygosity without k-ras gene mutation.

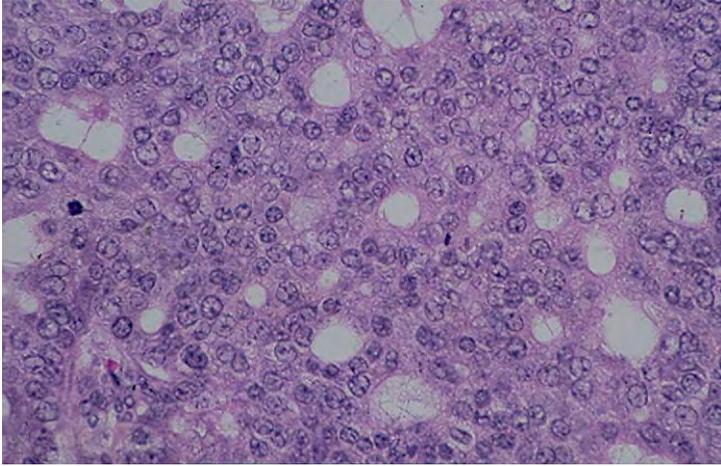
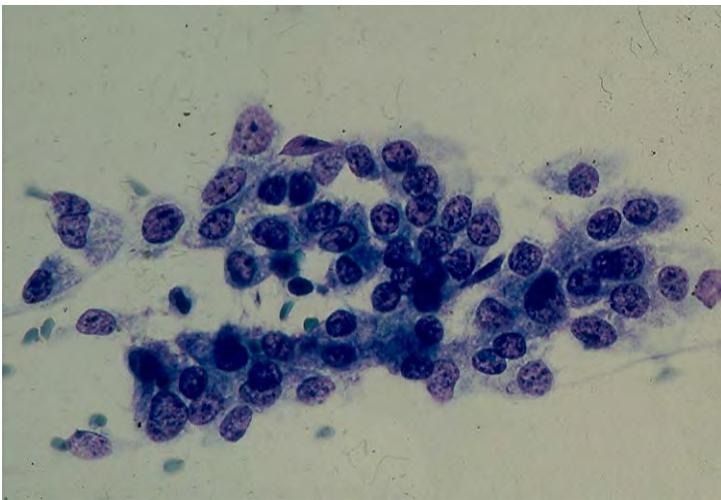
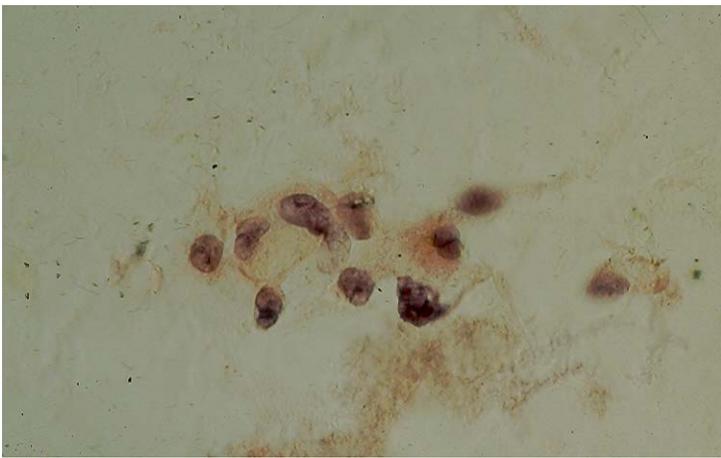


Figure. 1.25. Histology of a pancreatic well-differentiated acinar cell carcinoma.(HE,x250).



A



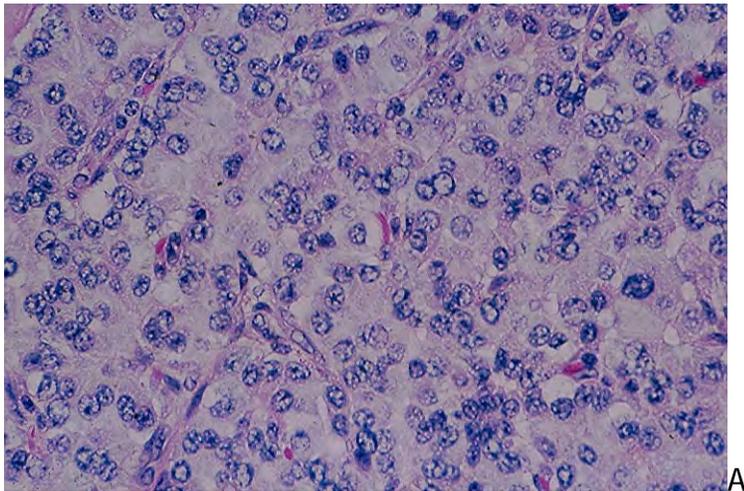
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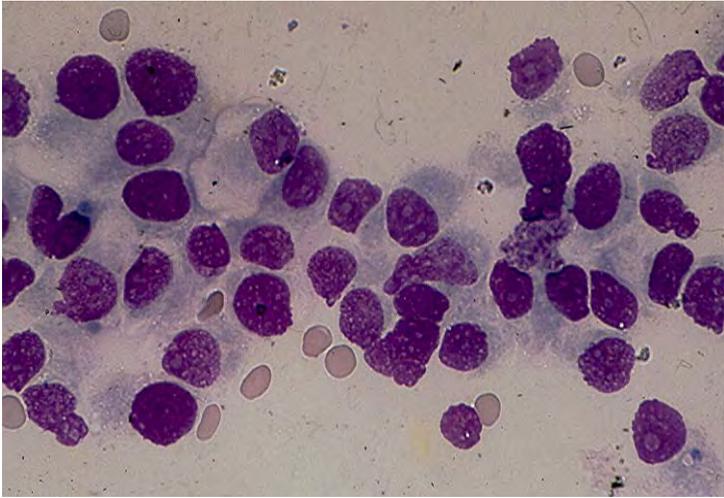
Figures 1.26. A. A pancreatic well-differentiated acinar cell carcinoma showing in FNA clustered polygonal or pyramidal malignant epithelial cells with granular cytoplasm, round nuclei and conspicuous nucleoli. (Diff-Quik, x 400).

B. A pancreatic poorly differentiated acinar cell carcinoma yields in FNA loosely clustered malignant epithelial cells with clear, vacuolated or granular cytoplasm and pleomorphic nuclei with conspicuous nucleoli. (Pap, x 400).

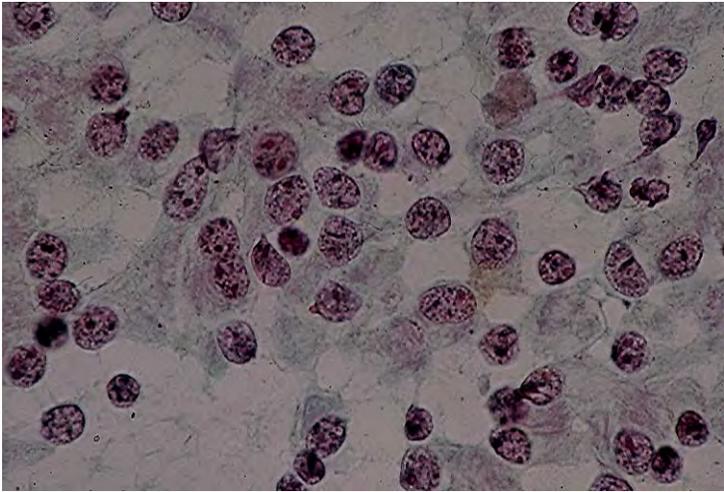
### 3. Neuroendocrine carcinoma

This neoplasm accounts for about 1% of all primary pancreatic cancers. The aspirated tumor cells usually have a plasmacytoid configuration with eccentrically located oval nuclei, conspicuous nucleoli and granular cytoplasm. These cells occur singly and in small clusters or monolayered sheets. The tumor cell cytoplasm stains positively with neuron-specific enolase, CD56 and chromogranin antibodies. (Figures 1.27 and 1.28). Rarely the tumor cells show a signet ring cell configuration. Electron microscopic examination of aspirated minute tumor tissue fragments may show characteristic intracytoplasmic membrane-bound and electron-dense neurosecretory granules.





B



C

Figures 1.27. Pancreatic neuroendocrine carcinoma:  
A. Histology of a pancreatic neuroendocrine carcinoma showing plasmacytoid tumor cells in solid pattern. (HE, x 250).  
B and C. FNA from a pancreatic neuroendocrine carcinoma showing single and loosely clustered tumor cells with plasmacytoid configuration, finely nuclear chromatin clumping and conspicuous nucleoli. (B, Diff-Quik,x500; C, Pap,x500).

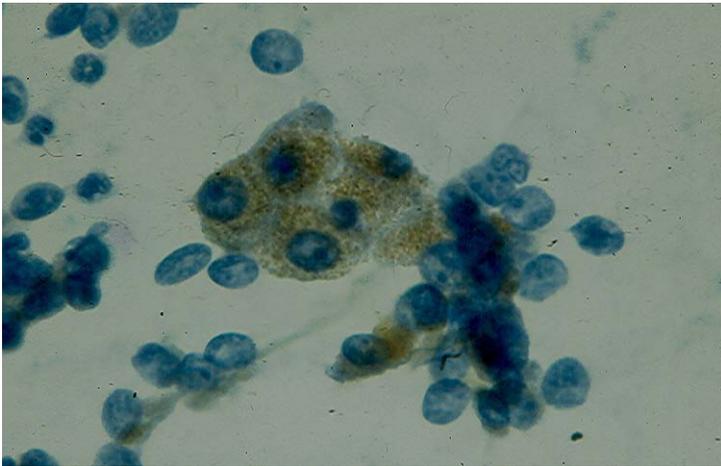


Figure 1.28. Neuroendocrine tumor cells showing immunopositive cytoplasmic reaction to chromogranin antibody. (ABC,x500).

**4. Pancreatic Cystic Tumors** comprise 5% of all pancreatic neoplasms and consist of three lesions:

**4.1. Papillary solid-cystic tumor.** This uncommon low-grade neoplasm has an uncertain histogenesis and occurs mainly in young women. The tumor yields in FNA abundant small and uniform tumor cells present singly and in loose aggregates. Tumor cells wrapping around capillary vessels may be seen. This “Chinese characters” cytologic pattern is highly characteristic for the tumor. Nuclear indentation is commonly observed. (Figures 1.29 and 1.30). Tumor cells from a papillary solid cystic neoplasm should be differentiated from those of a neuroendocrine carcinoma of the pancreas. They express alpha-1-antichymotrypsin and vimentin.

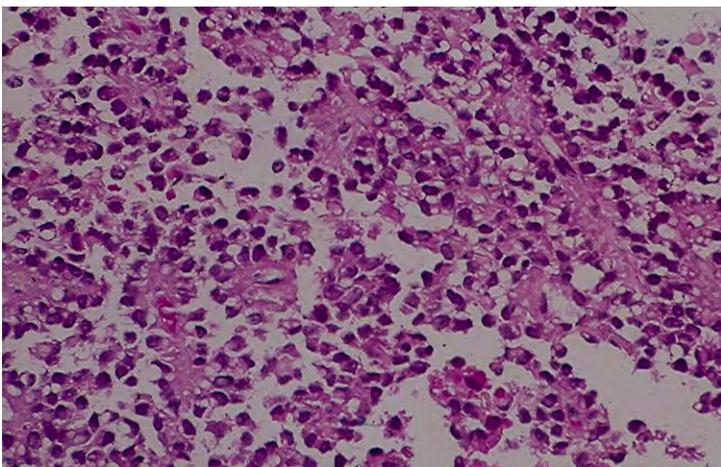
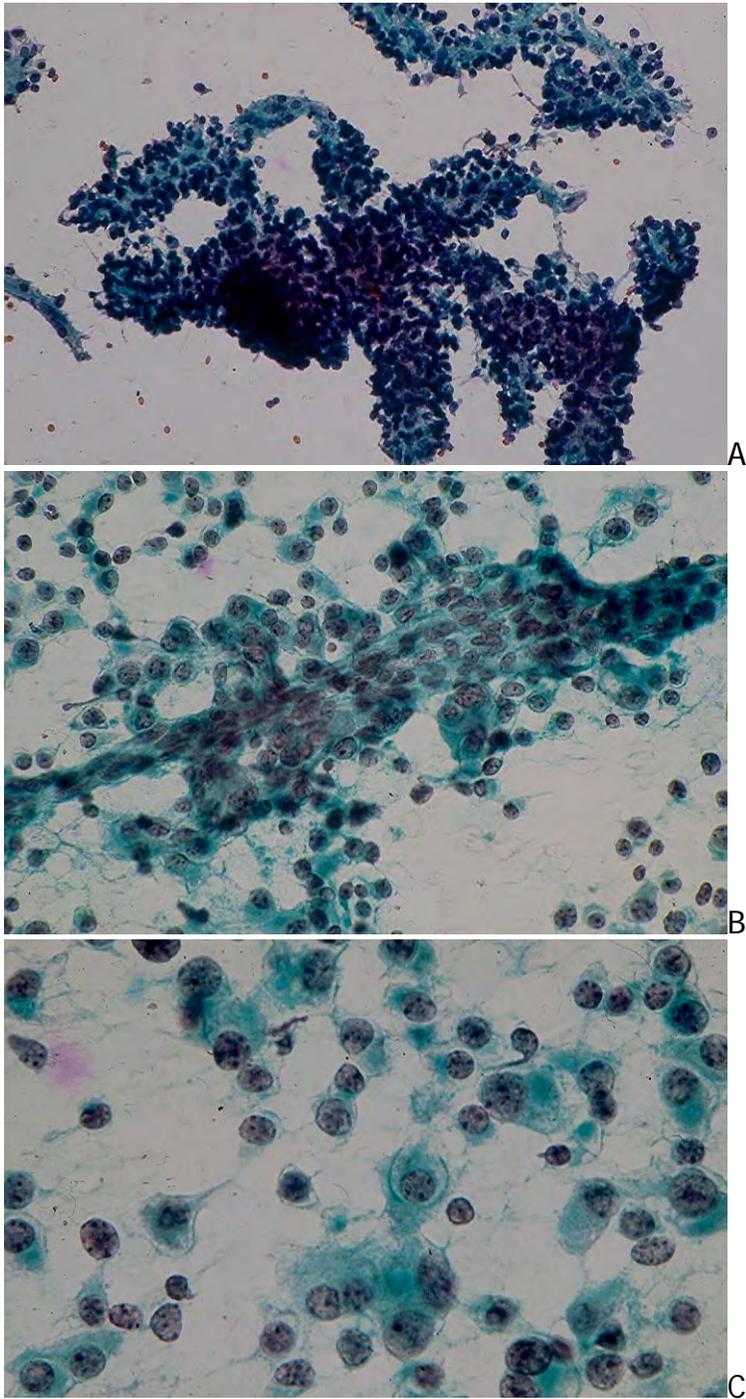
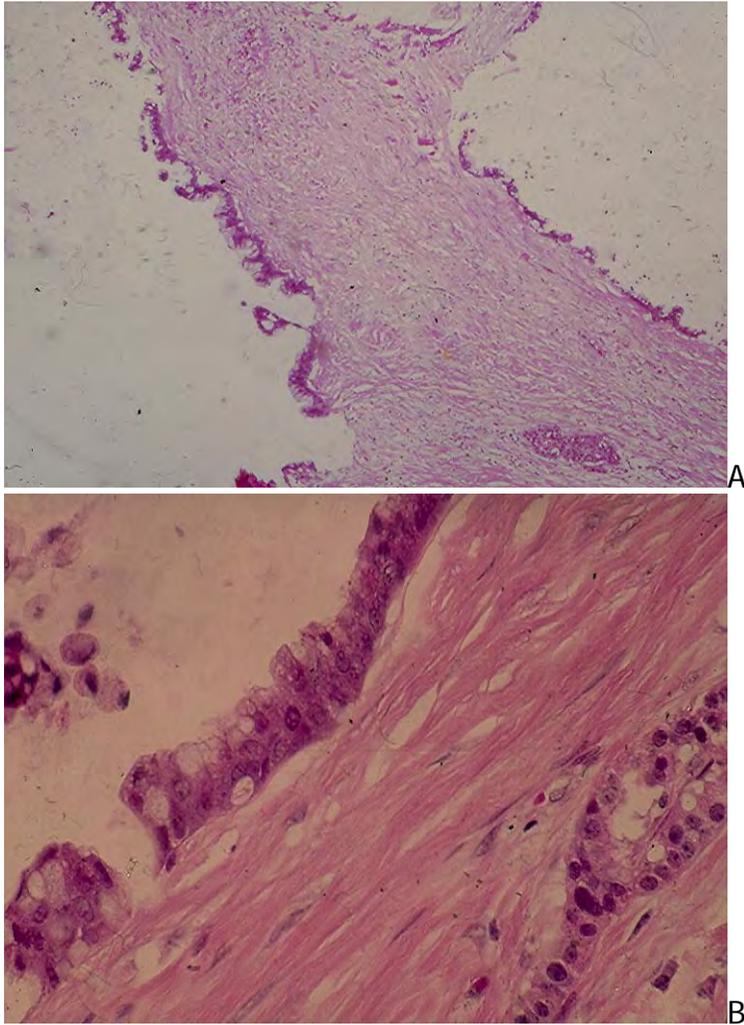


Figure 1.29. Histology of a papillary solid-cystic tumor of the pancreas. (HE,x250).

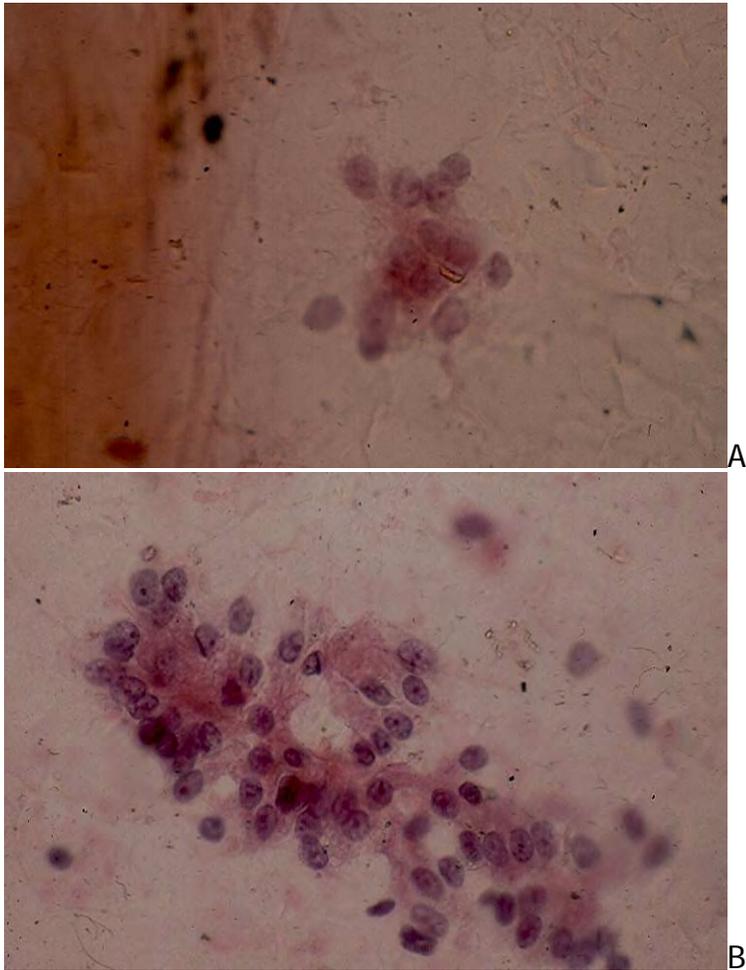


Figures 1.30. FNA of a pancreatic papillary solid-cystic tumor of the pancreas showing:  
A. Anastomotic tumor tissue fragments consisting of thick fibrovascular cores wrapped with tumor cells in characteristic "Chinese characters" pattern. (Pap, x 100).  
B. Single and clustered tumor cells with vascular transgression. (Pap, x 250).  
C. Monomorphic polygonal tumor cells with some having a plasmacytoid configuration. (Pap, x 500).

**4.2. Mucinous cystic neoplasm** of the pancreas usually harbors foci of a mucus secreting adenocarcinoma. (Figures 1.31 and 1.32). Therefore, when the tumor is detected by FNA, it should be removed by a total pancreatectomy. Abundant mucus and clusters of benign, atypical or malignant glandular cells may be seen in the tumor FNA. If only mucus material is identified a total pancreatectomy is also indicated.

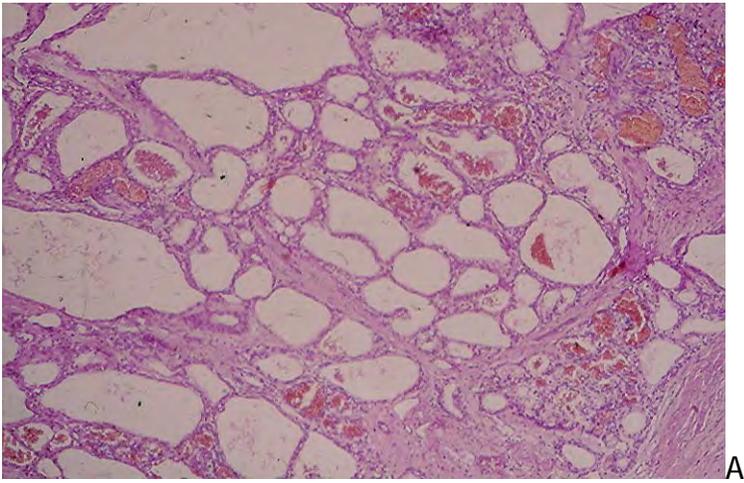


Figures.1.31. Histology of a mucinous cystic tumor of the pancreas showing a thick fibrous septum invaded by mucus secreting malignant cells. (HE, A x 100, B x 250).

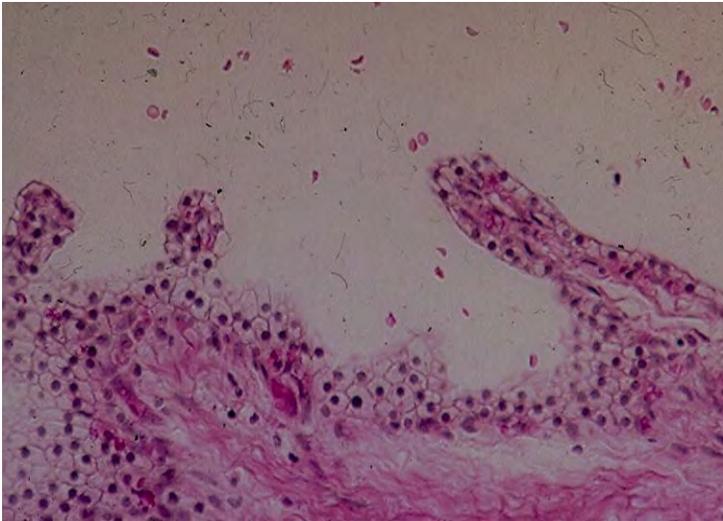


Figures 1.32. FNA of a pancreatic mucinous cystic tumor showing in:  
 A. A cluster of benign epithelial cells and thick mucinous material.  
 B. A fragment of slightly atypical columnar epithelium with conspicuous nucleoli.  
 (Pap, x 400)

**4.3. Serous cystadenoma** is composed of two morphologic variants: microcystic and macrocystic. (Figures 1.33 and 1.34). Pancreatic serous adenomas are glycogen-rich and yield in FNAs serous fluid that is scanty cellularity. The tumor cells are benign cuboidal or slightly pleomorphic epithelial cells that occur in small monolayered sheets. These cells stain positively with PAS and negatively with PAS with prior diastase digestion (PAS-D). Cells of a serous cystadenoma should be differentiated from benign mesothelial cells. A staining with calretinin antibody is helpful in this case, as mesothelial cells express calretinin while cells derived from a serous adenoma do not.



A



B

Figures 1.33. Histology of a pancreatic microcystic serous adenoma. (HE, A x 100, B x 250).

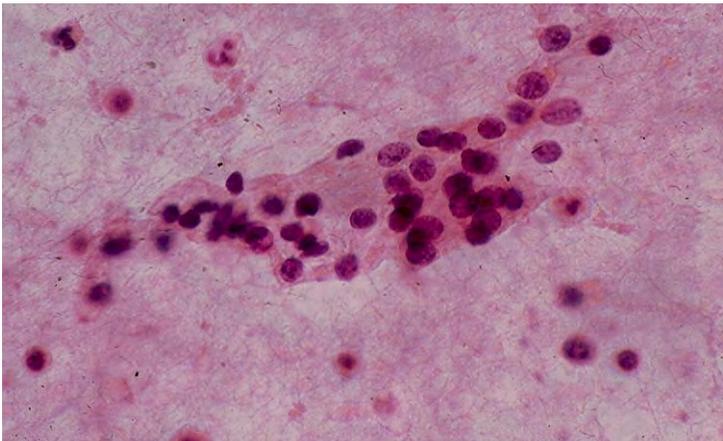
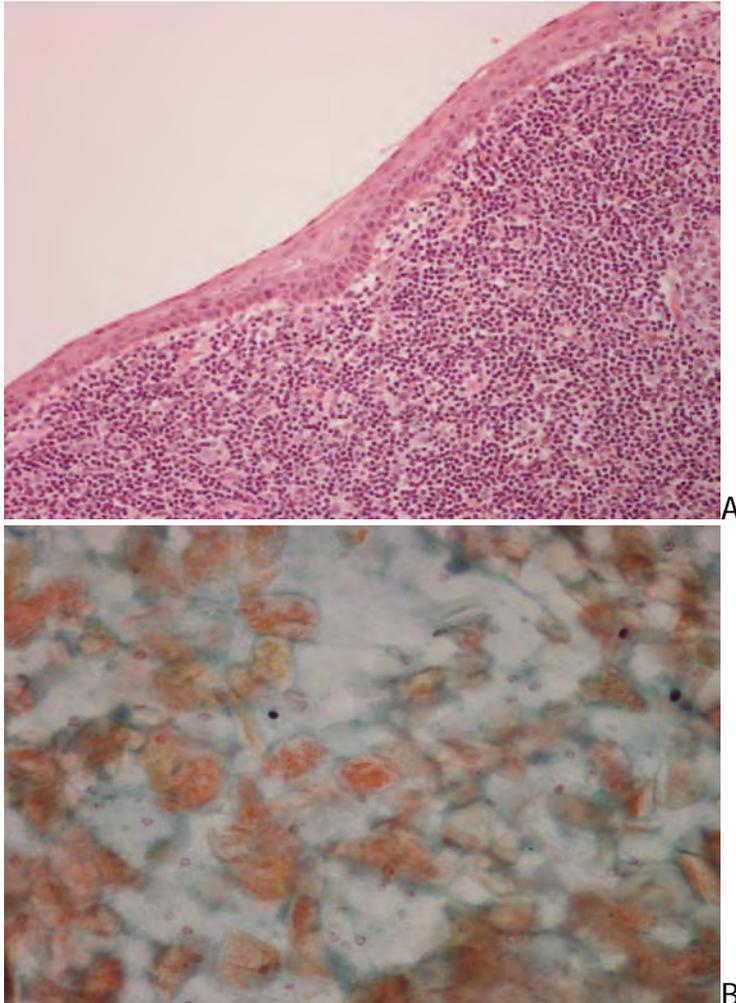


Figure 1.34. A sheet of benign epithelial cells with round nuclei and "opaque" or clear cytoplasm seen in TFNA of a pancreatic microcystic adenoma. (Pap, x 500).

## OTHER PANCREATIC MASS LESIONS

**1. Lymphoepithelial cyst** of the pancreas is a rare lesion that can mimic a pancreatic pseudocyst. It shows in FNA abundant benign squamous cells and anucleated squames. A variable number of benign lymphoid cells may be present. (Figures 1.35).



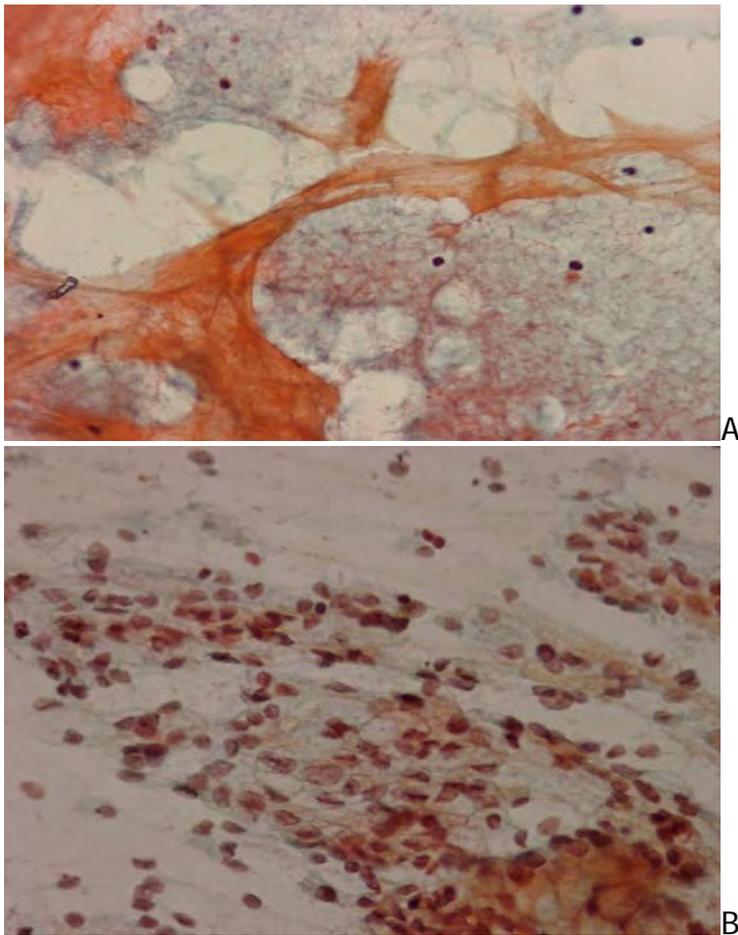
Figures 1.35. Lymphoepithelial cyst of the pancreas.

A. Histology of the cyst wall showing a squamous epithelial lining and lymphocyte-rich subepithelium. (HE, x 200).

B. The aspirated cystic contents showing numerous anucleated squames, a few benign squamous cells and rare lymphocytes. (Pap, x 200).

**2. Intraductal papillary mucinous tumor** is a neoplasm with a good or better prognosis. It occurs in adults of both sexes and involves predominantly the main pancreatic duct. The lining epithelium is mucinous and papillary. By endoscopy, the ampulla of Vater is seen patulous and draining mucus. Biopsy of the lesion is almost

always done by EUS-FNA. It is characterized by abundant thick mucus containing tight, large sheets and papillary clusters of cuboidal and columnar, mucus-secreting benign or slightly atypical cells. (Figures 3.36). In a recent study by Michaels et al. it was found that the presence of thick, "colloid-like" mucin was noted in about 50% of the cases, but was not found to be specific to grade. The absence of such mucin did not exclude this tumor, and the presence of tight epithelial cell clusters was consistent with a neoplasm of at least moderate dysplasia. Abundant background inflammation and parachromatin clearing correlated with the presence of at least carcinoma in situ and necrosis was the only feature found to be strongly suggestive of tumor invasion.

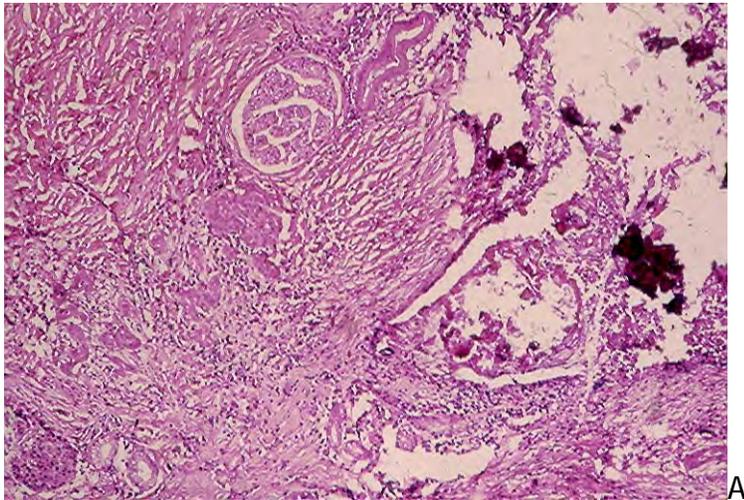


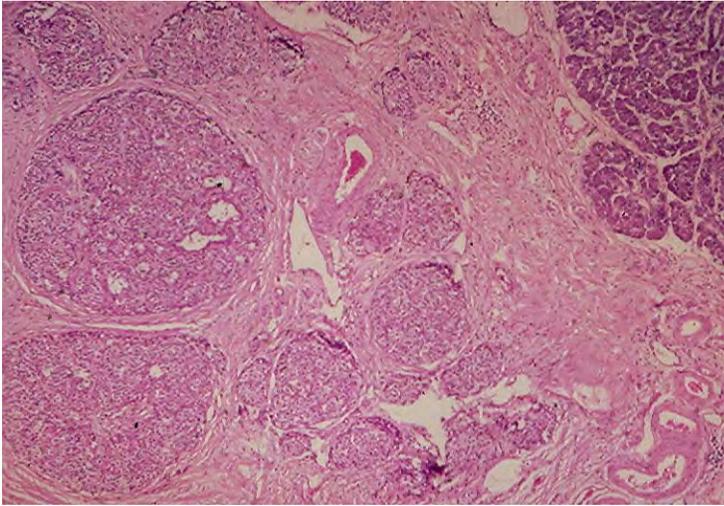
Figures 3.36. EUS-FNA of an intraductal mucinous papillary tumor showing abundant thick "colloid-like" mucin in A and irregular sheets of benign mucus-secreting epithelial cells with some cells displaying minimal nuclear atypia in B. (Pap, A x 400, B x 400).

**3. Secondary Pancreatic Cancers** account for about 10% of all pancreatic cancers. The tumors may develop as the result of a direct invasion by an adjacent cancer such as gastric or colonic cancer and retroperitoneal malignancies such as a lymphoma or a sarcoma. True

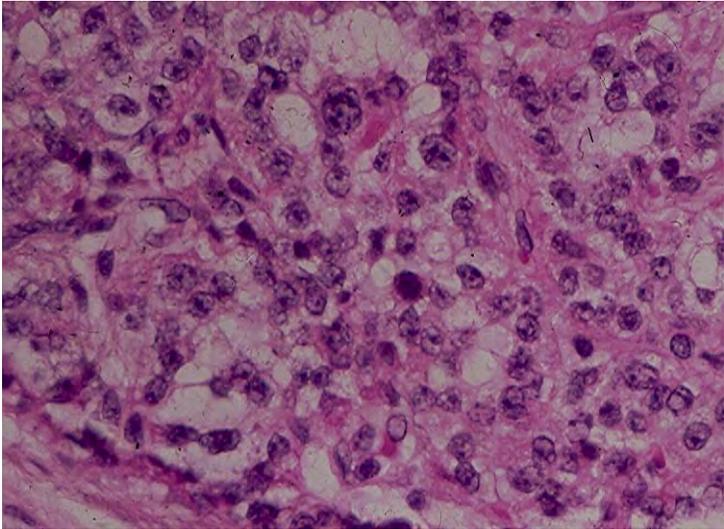
metastatic cancers to the pancreas are very rare, and cancers arising from any organs may metastasize to the pancreas

**4. Chronic pancreatitis** may mimic a pancreatic cancer clinically, radiologically and macroscopically. Chronic pancreatitis commonly follows an acute pancreatitis. It yields in FNA irregular sheets and clusters of benign ductal and acinar epithelial cells admixed with chronic inflammatory cells. (Figures.1.37 and 1.38). The ductal epithelial cells may display mild cytologic atypias. However, the degrees of nuclear atypias as seen in a well-differentiated ductal adenocarcinoma are not present. Staining with of the cells in question with MUC1 antibody may be helpful in identifying a pancreatic duct carcinoma, as cells derived from the tumor show either apical or diffuse membranous staining with variable cytoplasmic staining, while those of a chronic pancreatitis and benign lesions do not generally express this marker or may display only a weak apical membranous staining. Hyperplastic endocrine cell nodules are common in fibrotic pancreases and the cells derived from these nodules may be mistaken for those of a well-differentiated pancreatic ductal adenocarcinoma. Staining of the cell sample with neuron-specific enolase or chromogranin antibody is helpful for identifying pancreatic endocrine cells.



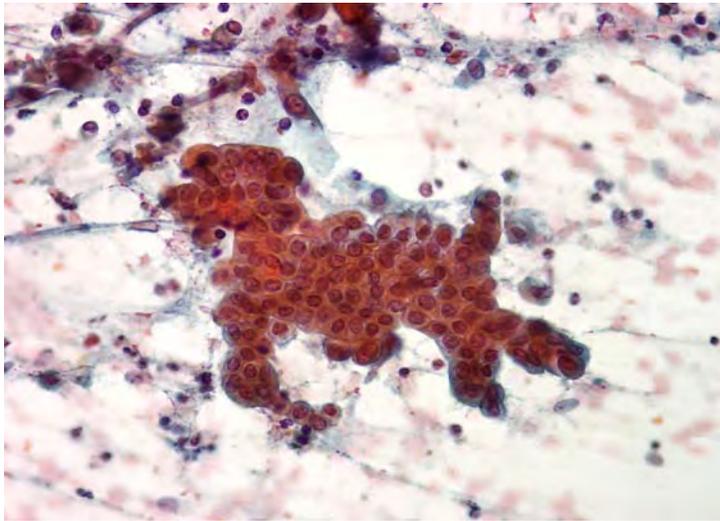


B

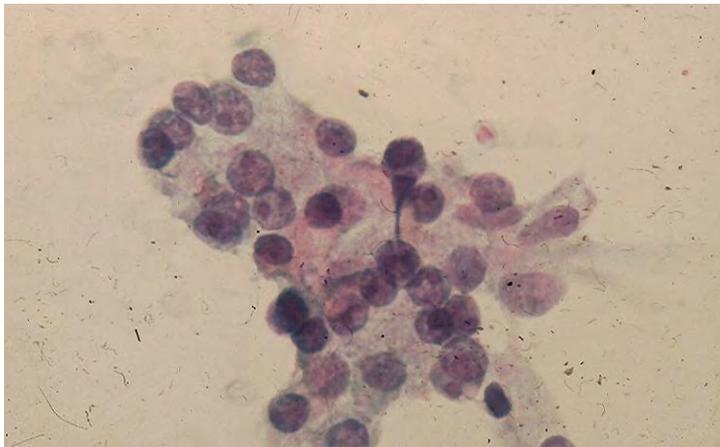


C

Figures 1.37. Histology of chronic pancreatitis:  
A. Fibrotic pancreatic tissue with focal calcification.  
B. Hyperplastic islet cell nodules in a fibrotic stroma.  
C. Hyperplastic islet cell nodule showing cells in acinar arrangement with slightly pleomorphic nuclei. (HE, A x 100, B x 100, C x 400).



A



B

Figures 1.38. FNA of a chronic pancreatitis showing in:  
 A. A sheet of ductal epithelial cells with honeycomb pattern admixed with lymphocytes, macrophages and necrotic debris.  
 B. A cluster of hyperplastic islet cells with clear cytoplasm and oval nuclei with conspicuous nucleoli. (Pap, A x 200, B x 800).

In recent years, molecular genetics studies of cytologic and histologic materials to differentiate a pancreatic ductal carcinoma from a chronic pancreatitis have been conducted. A detection of k-ras mutation at codon 12 in a pancreatic FNA has an adjunctive value to the diagnosis of ductal adenocarcinoma, as a detection of mutation at a high amount is present in over 95% of all pancreatic ductal cancers. Its presence in a chronic pancreatitis suggests a high risk for developing cancer. However, its absence increases the possibility of a chronic pancreatitis.

**5. Pancreatic pseudocyst** usually follows an acute pancreatitis. It yields in FNA foamy histiocytes and necrotic debris, and small sheets of benign or reactive mesothelial cells

may be seen. (Figure 1.39). A diagnosis of a pancreatic pseudocyst can only be made with certainty by histologic examination of the cyst wall, as any pancreatic neoplasm may undergo central necrosis with cystic formation.

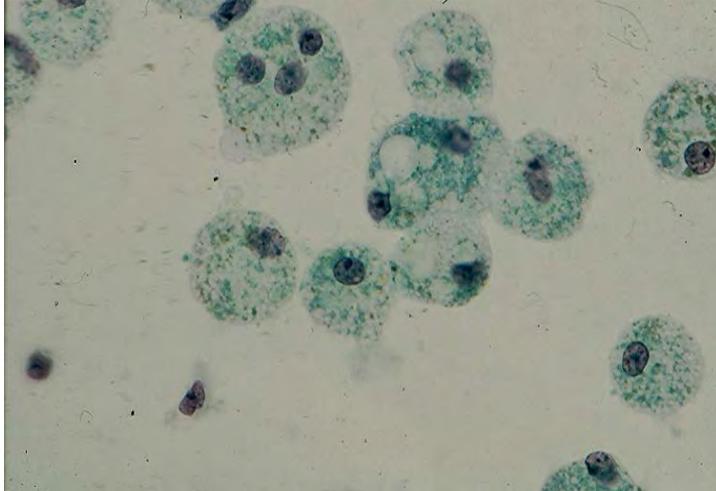


Figure 1.39. Numerous benign foamy macrophages seen in the liquid cystic contents of a pancreatic pseudocyst .(Pap, x 400).

## **SUMMARY**

FNA of pancreatic mass lesions for cytologic evaluation is a safe and reliable diagnostic procedure. A definitive diagnosis may be made in the cases of ductal, acinar and neuroendocrine carcinomas, papillary solid-cystic tumor, serous cystadenoma, lymphoepithelial cyst, mucinous neoplasm of the pancreas or a metastatic cancer to the organ. However, a suggestive or probable diagnosis can only be made in patients with chronic pancreatitis and pancreatic pseudocyst, and clinico-pathologic correlations are important for making a final diagnosis in these situations.

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# LIVER AND BILIARY TREE

## INDICATIONS, CONTRAINDICATIONS, TECHNICAL CONSIDERATIONS AND COMPLICATIONS OF LIVER FNA

### Indications and Contraindications

FNA for cytologic evaluation of the liver has a limited value in diagnosing medical diseases of the liver. Single and multiple mass lesions or diffuse enlargement of the liver that are suspected to be neoplastic in nature are targets of FNA. TFNA of the liver is contraindicated in patients with bleeding diathesis and bowel distention. Intraabdominal hydatid cysts are generally regarded as a contraindication for liver FNA, as leaking of the cyst contents into the peritoneal cavity may cause fatal anaphylactic reaction. However, several cases of liver hydatid cyst had been safely sampled by FNA.

### Technical considerations

TFNA of the liver is performed under guidance of an abdominal diagnostic imaging method, such as ultrasonographic and computed tomographic scans. A 22- or 23-gauge, spinal-type needle, a syringe and a syringe holder or pistol are used to obtain cell samples from the liver lesion. Depending on the tumor location and size, the length of needle used can vary from 8 to 20 cm. Some smaller liver mass lesions located in the left lobe or on the inferior aspect of the liver can be safely and successfully sampled by EUS-FNA.

Direct smears prepared from the needle aspirates and are either fixed in 95% ethanol or air-dried and stained either with the Papanicolaou method, hematoxylin and eosin or Diff-Quik technique (modified Wright stain), respectively. Aspirated minute tissue fragments, if found in the needle wash, are removed and fixed in formalin for histologic, histochemical and immunohistochemical studies or fixed in a vial 2% glutaraldehyde for electron microscopy. Excess of aspirated material is fixed in formalin for preparation of a cell block that can be used for histologic and immunohistochemical studies. There are no objective criteria for an adequate needle aspirate of the liver, and obtaining an adequate or representative cell sample for cytologic evaluation depends largely on the skills of the radiologist or gastroenterologist who performs the FNA.

## Complications

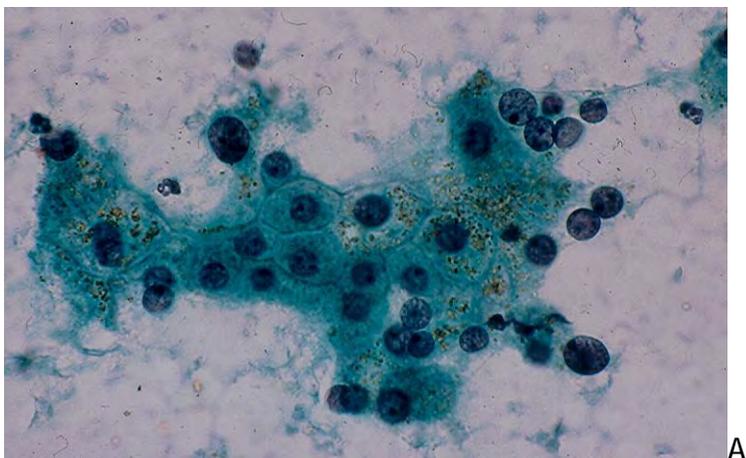
Complications of TFNA of the liver are rare in experienced hands and include intra-peritoneal bleeding secondary to liver laceration, bile peritonitis due to perforation of the gallbladder, peritonitis caused by gastric or bowel perforation and tumor seeding along the needle tract.

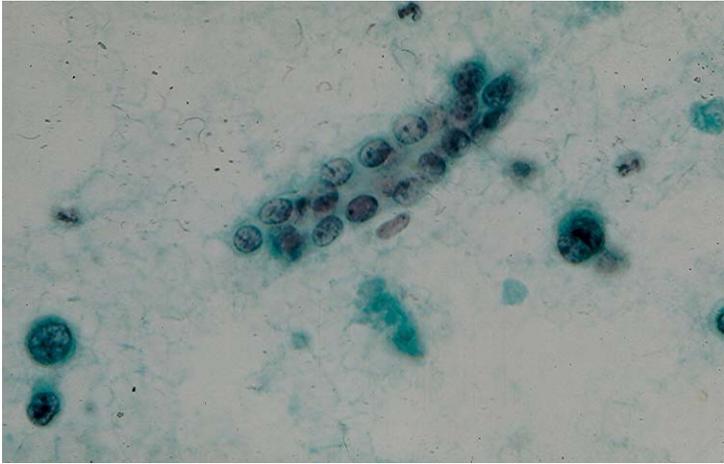
## CYTODIAGNOSTIC ACCURACY

The success of liver FNA is the result of the collaboration between a radiologist or a clinician who is familiar with liver TFNA techniques and a cytopathologist with experience in interpreting liver cytology. TFNA of liver cancers has a success rate varying from 92 to 96%. Sampling error is the most common cause of a false-negative cytodiagnosis of liver cancer and false-positive cytodiagnoses are rare.

## NORMAL LIVER

FNA from normal liver tissue is usually cellular and shows numerous hepatocytes admixed with a few small sheets or clusters of bile duct epithelial cells. Normal hepatocytes are polygonal in shape and show a well-defined, granular cytoplasm, centrally located oval nuclei and small nucleoli. Intracytoplasmic bile pigment and lipofusin granules may be seen as well as rare small fat droplets. Normal hepatocytes are present singly, in sheets and in clusters consisting of a variable number of cells. Hepatocytes arranged in small anastomotic cords are rarely observed. Bile duct epithelial cells are columnar in shape with a clear cytoplasm, basally located oval nuclei and inconspicuous nucleoli. These cells are commonly seen in monolayered sheets with honeycomb pattern and rarely singly. Fragment of small bile duct may be seen as a short tubule consisting of cuboidal cells. (Figures 2.1).





Figures 2.1. FNA of normal hepatic tissue showing in:  
A. A sheet of normal hepatocytes with intracytoplasmic brown lipofuscin granules.  
B. A segment of small bile duct. (A and B, Pap, x 400)

## PRIMARY HEPATIC CANCERS

### 1. Hepatocellular Carcinoma

The incidence of hepatocellular carcinoma (HCC) varies from country to country. HCCs are common in Asia and Africa, and rare in North America and Europe. Its associated etiologic factors vary. Hepatitis B and C and aflatoxin are common associated etiologic factors of HCCs in Asian countries, while Hepatitis C and alcoholic cirrhosis are the main diseases associated with the tumor in North America and Europe. Worldwide, HBV probably accounts for 70 to 80%, HCV for 10 to 15%, and aflatoxin and alcohol for 5 to 10% of HCC cases. A combination of more than one factor may be operative in a minority of cases: HBV and/or HCV with or without cirrhosis are frequent combinations.

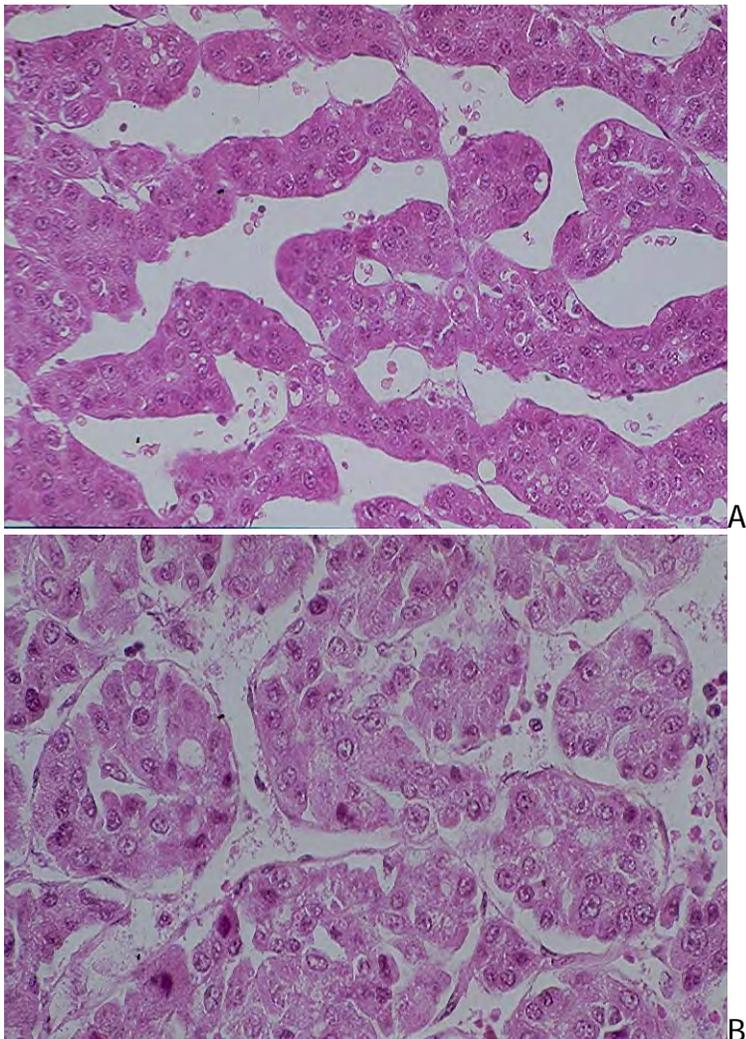
HCCs account for about 80% of all primary liver cancers in North America. The tumor occurs predominantly in adult patients with ages varying from 50 to 70 years and is more common in male than in female (3 to 5:1). 70 to 90% of HCCs are associated with an elevated serum level of alpha-fetoprotein. The surgical resectability rate of HCCs is about 10 to 30%, and the 5-year survival rate of patients with this type of liver cancer is less than 5%.

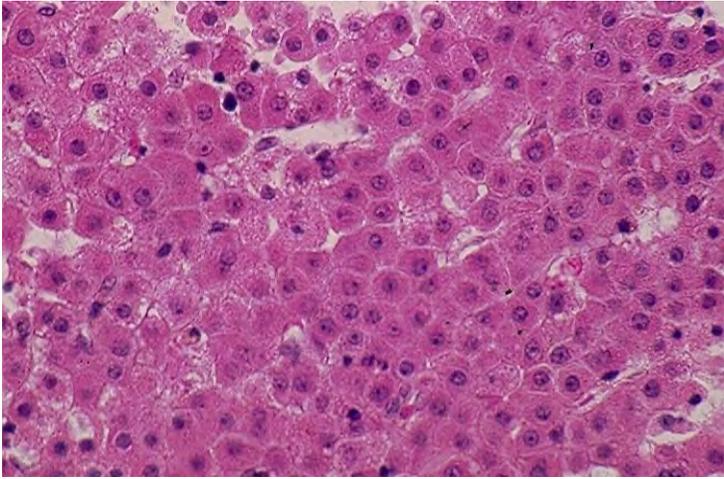
Macroscopically, HCC may have a nodular, massive or diffuse growth pattern. The nodular growth pattern, the most common one, is typically seen in HCCs associated with cirrhosis and it is characterized by multiple nodules scattered across the liver, with one dominant mass. In HCC with massive growth pattern, a large single mass occupies a large part of a hepatic lobe, and this pattern is mainly seen in HCC occurring in non-cirrhotic liver. The

diffuse growth pattern is characterized by a widespread infiltration by numerous small tumor nodules.

### 1.1. Usual Hepatocellular Carcinoma

Histologically, HCC is characterized by a varying degrees of hepatocellular differentiation. Studies have demonstrated that most HCCs have a combination of histologic patterns, including trabecular, acinar and solid, with the trabecular pattern being the most common one. (Figures 2.2 and 2.3). Depending on the tumor differentiation, HCCs may be graded as well- and poorly differentiated. Bile production or stasis is a common finding, as well as intracytoplasmic globular and Mallory-type inclusions. Intranuclear cytoplasmic inclusions are commonly observed, but they are non-specific, and cytoplasmic fatty changes are not uncommonly found.





Figures 2.2. Histology of well-differentiated HCC showing a trabecular pattern (A), a pseudoglandular pattern (B) and a solid pattern (C). Flat endothelial cells are seen covering trabeculae and acini of neoplastic cells as in (A) and (B). (HE, A-C x 250).

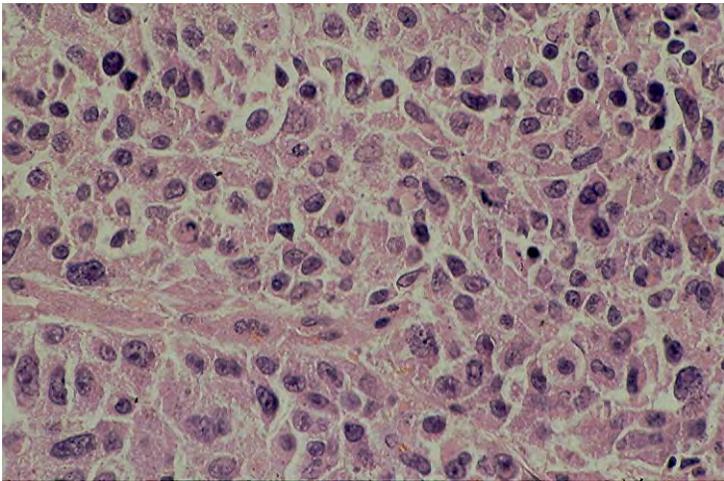
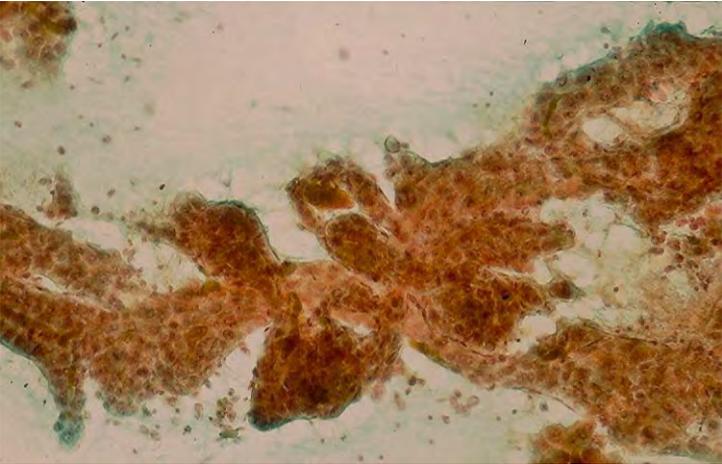


Figure 2.3. Histology of a poorly differentiated HCC showing pleomorphic tumor cells in solid pattern. (HE, x 250)

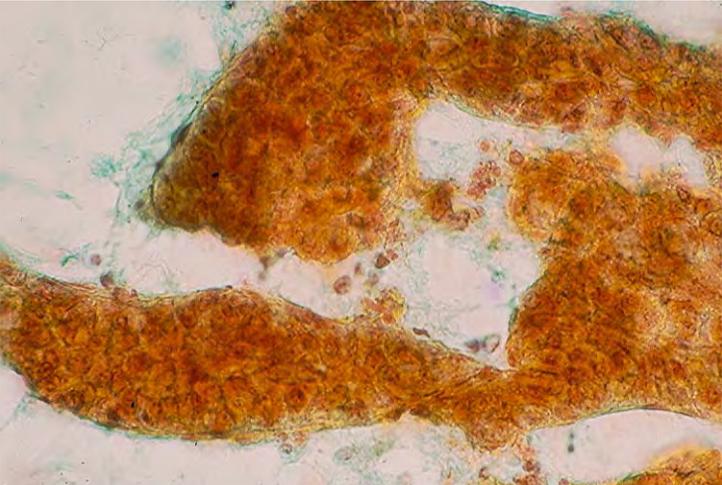
### **FNA Cytology of Usual HCC**

Four smear patterns may be identified cytologically: trabecular, pseudo-glandular or adenomatoid, sheet-like and dissociated cell. The trabecular pattern is commonly seen in FNA from a well- or moderately differentiated HCC. It is diagnostic of the tumor and present in 70-80% of the cases. The other three patterns are less common and are usually seen as a minor component of the needle aspirate.

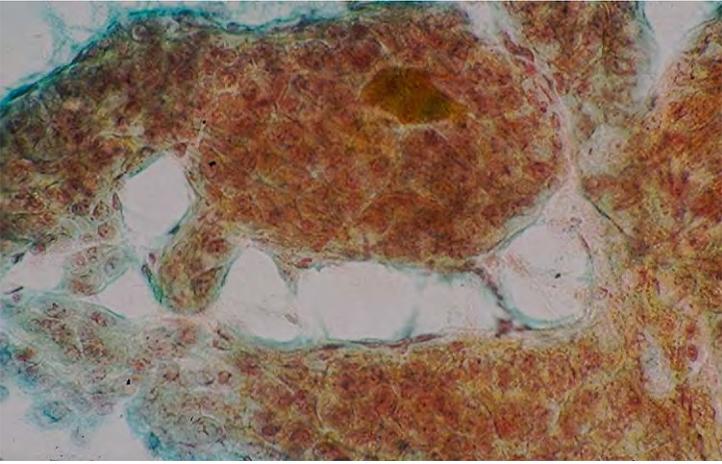
A well-differentiated tumor is characterized by benign-appearing hepatocytes arranged in anastomotic thin or thick trabeculae surrounded by endothelial cells. (Figures 2.4-2.6).



A



B



C

Figures 2.4. FNA of a well-differentiated HCC with trabecular pattern showing in:  
A. Anastomotic cords of tumor cells.

B. Higher magnification of 2 tumor cell cords showing thin endothelial cells covering the outer aspects of the tumor cell cords.

C. A bile plug is noted within a tumor cell cord. (Pap, A x 100, B and C x 250)

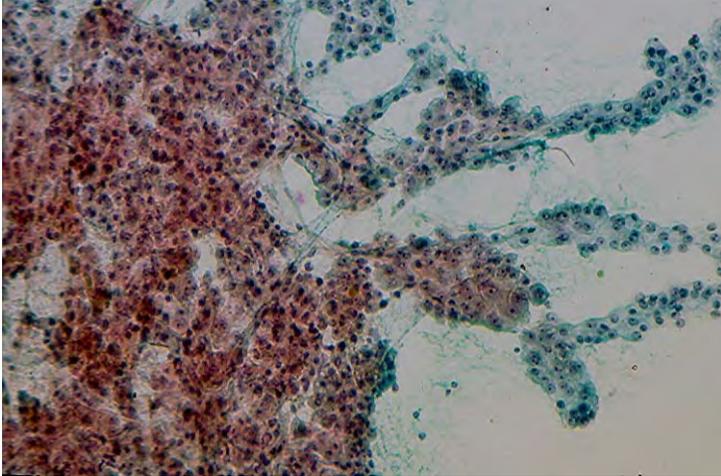


Figure 2.5. Thin anastomotic cords of tumor cells in FNA of a well-differentiated HCC (Pap, x 100).

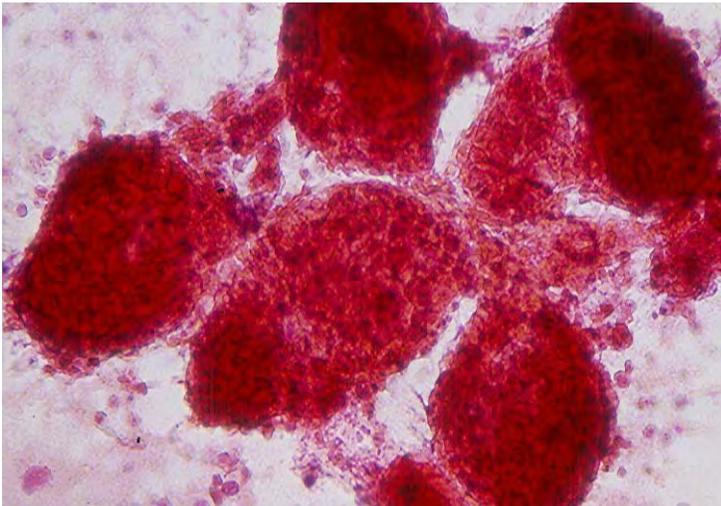


Figure 2.6. FNA of a well-differentiated HCC showing round nests of tumor cells wrapped peripherally by flat endothelial cells (Pap, x 100).

Vascular transgression (blood vessels surrounded by tumor cells) may be present but is non specific, as it can be seen in FNA from a hepatocellular adenoma or a focal nodular hyperplasia of the liver. (Figure 2.7).

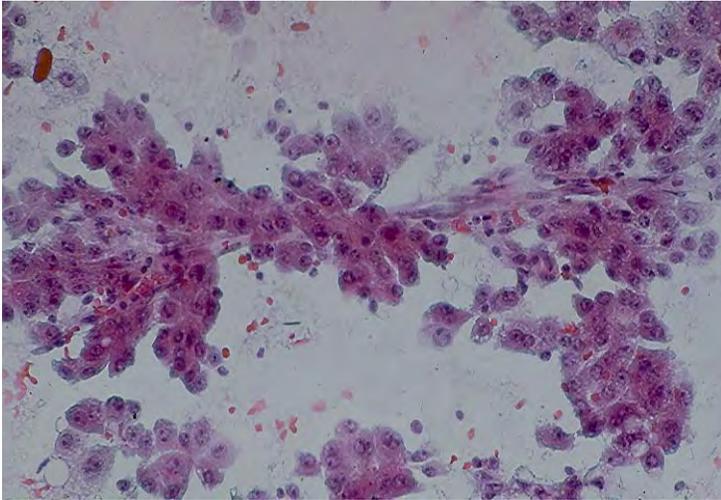
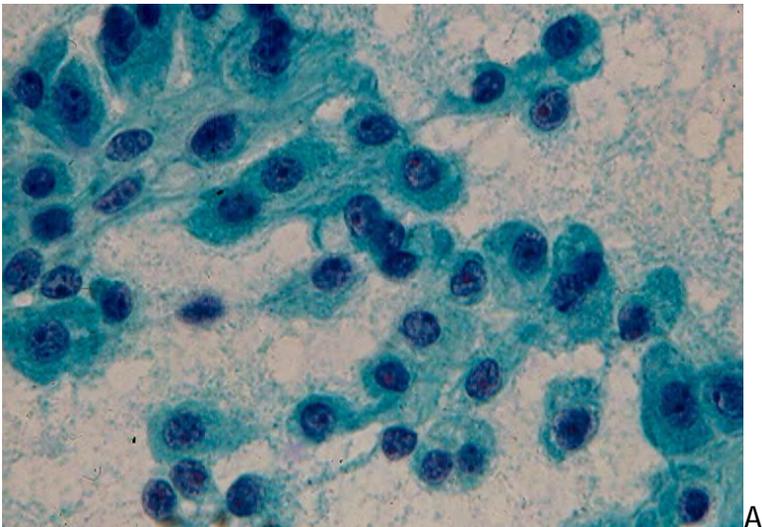
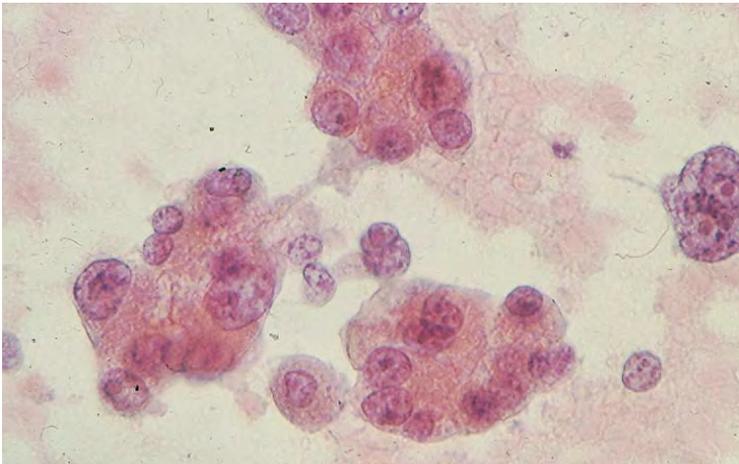


Figure 2.7. Clusters of tumor cells with vascular transgression. (Pap, x 200).

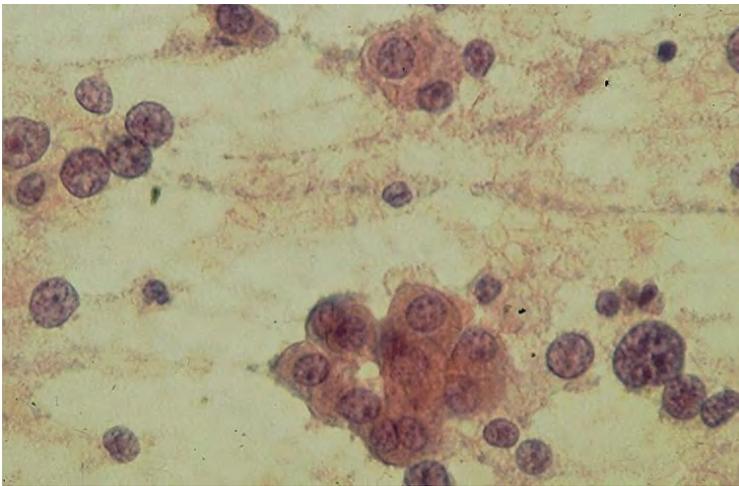
Single, clustered tumor cells admixed with stripped tumor cell nuclei are commonly found. Globular intracytoplasmic inclusions may be observed. (Figures 2.8 and 2.9). Rarely, HCC cells have a spindle shape but these cells are usually seen admixed with usual HCC cells. (Figure 2.10). These inclusions may appear as basophilic globular bodies in the smear background, stain positively with periodic acid-Schiff reagent with prior diastase digestion and contain alpha-fetoprotein, alpha 1-antitrypsin and alpha 1-antichemotrypsin. Rare Mallory-type bodies may be seen. Intracytoplasmic pale bodies may occasionally be present in ordinary HCCs, but they are more common in cells derived from a fibrolamellar HCC. Fatty change of the tumor cells is a common finding, as well as bile plugs (bile stasis).



A

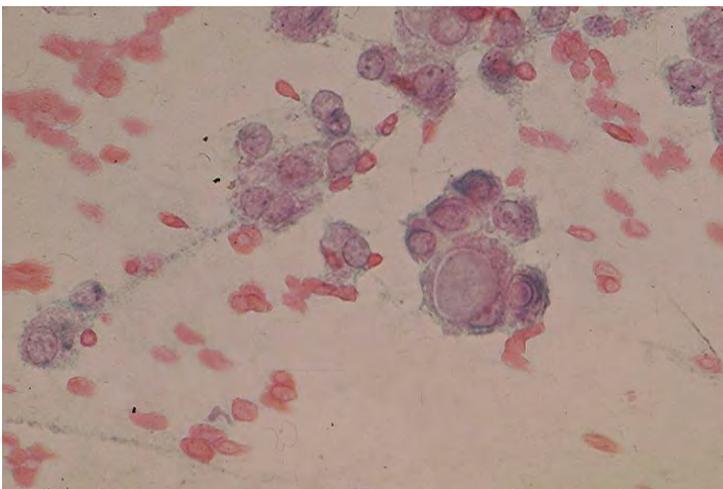


B

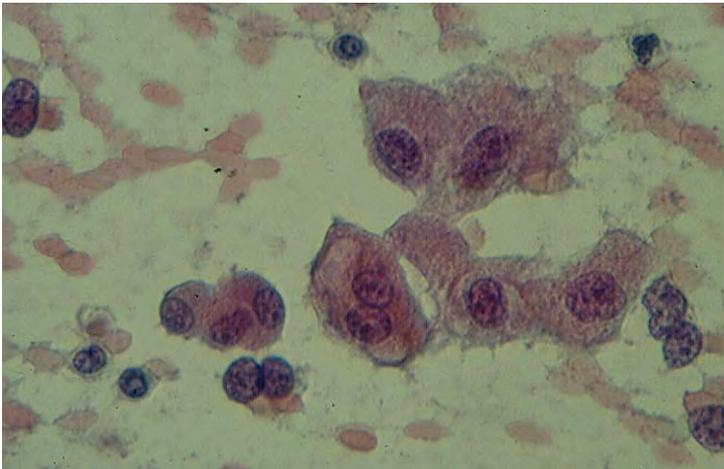


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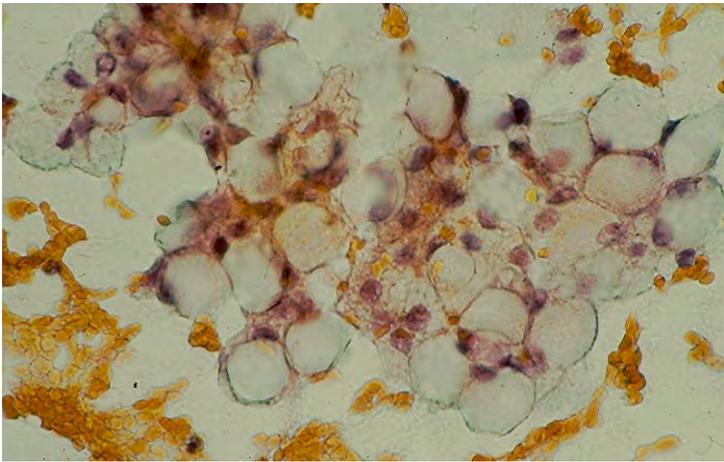
Figures 2.8. Hepatocellular carcinoma showing in FNA. (Pap, A-C x 500):  
A. Tumor cells with minimal nuclear atypia, singly and in sheets.  
B. Clustered pleomorphic tumor cells with prominent nucleoli.  
C. Single and clustered tumor cells admixed with naked tumor cell nuclei.



A



B



C

Figures 2.9: Hepatocellular carcinoma showing in FNA. (Pap, A-C x 500):  
 A. Tumor cells with one cell showing a large intracytoplasmic globular inclusion.  
 B. Tumor cells with 2 cells showing an intracytoplasmic pale bodies.  
 C. A group of tumor cells showing extensive fatty change.

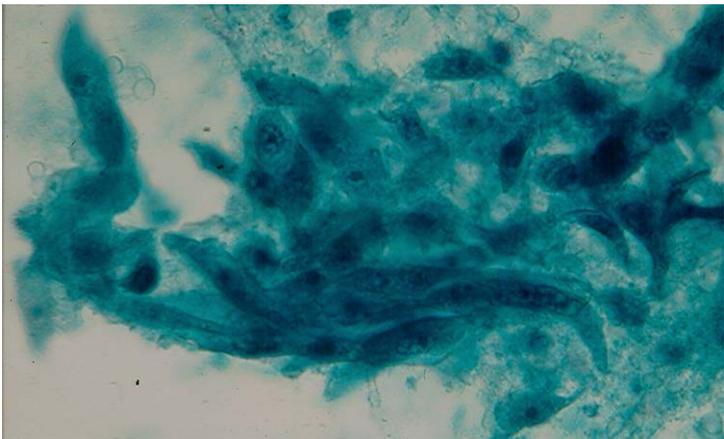


Figure 2.10. A cluster of spindle neoplastic hepatocytes from a HCC. (Pap, x 500).

A poorly differentiated HCC usually shows a dissociated cell pattern with pleomorphic malignant cells. Stripped or naked tumor cell nuclei are commonly found. Tumor cells containing intracytoplasmic globular bodies and stripped round bodies may be seen. (Figure 2.11).

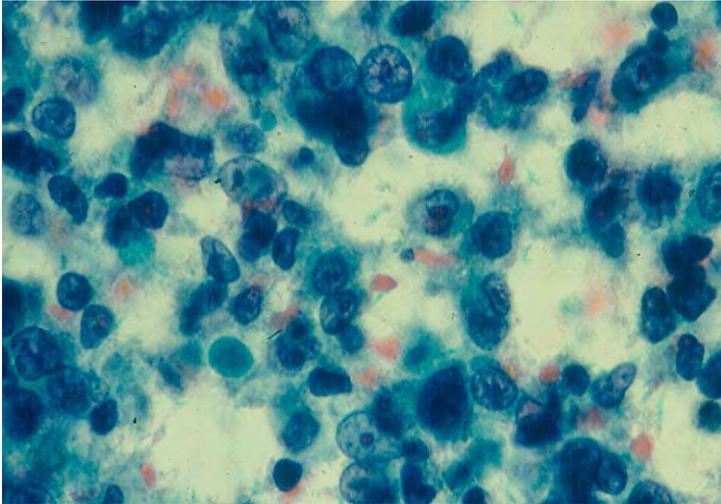
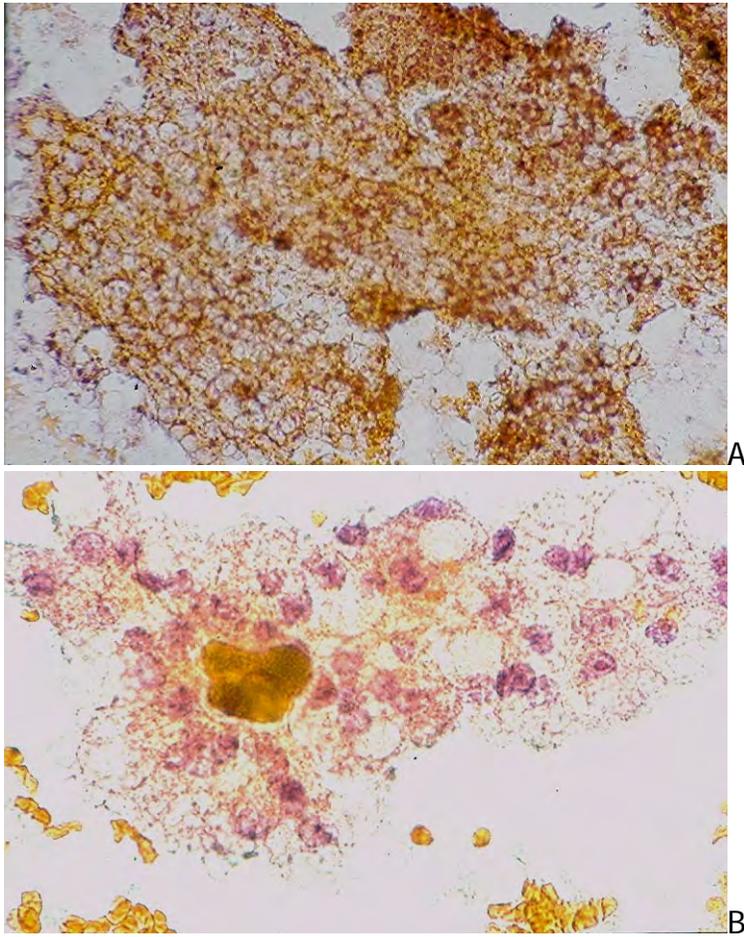


Figure 2.11. Poorly differentiated HCC shows in FNA single and clustered pleomorphic tumor cells displaying intracytoplasmic globular inclusions that may be seen laying free in the smear background (Pap, x 500).

## 1.2. Histologic variants of Hepatocellular Carcinoma

**Clear cell HCC** accounts for about 9% of all HCCs. It shows an extensive clear cell change that is due to the presence of a large amount of intracytoplasmic glycogen or fat. In FNA the tumor is characterized by large sheets of tumor cells with clear or finely vacuolated cytoplasm. Bile plug or stasis may be observed. (Figures 2.12).



Figures 2.12. FNA from a clear cell HCC showing sheets of tumor cells with clear cytoplasm. A tumor tissue fragment with a bile plug is seen in B. (Pap, A x 100, B x 250).

**Small cell HCC** is a rare variant and characterized by small cuboidal cells with scant cytoplasm arranged in trabecular and/or solid pattern. It yields in FNA small malignant cells singly, in sheets or trabeculae. A positive staining of the tumor cell cytoplasm with alpha-fetoprotein and HepPar-1 antibodies is necessary for a correct cytodiagnosis.

**Fibrolamellar HCC** accounts for about 5% of all HCCs and is seen primarily in young patients younger than 35 years of age, with a mean age of about 25 years. Over 90% of fibrolamellar HCCs arise in non-cirrhotic livers, and has no particular predisposing factors such as HBV, oral contraceptive use or alcohol abuse. An elevated serum level of alpha-fetoprotein is present in only about 10% of the cases. The surgical resectability rate of fibrolamellar HCC is 50 to 75%, and the 5-year survival rate after surgical resection is 40 to 55%. Most fibrolamellar HCCs are histologically low grade; mitotic figures are rare and nuclear pleomorphism is infrequent. The tumor cell cytoplasm is oncocytic, granular and abundant. Intracytoplasmic fibrinogen rich pale bodies are commonly found, but eosinophilic cytoplasmic globules and Mallory bodies are rare. (Figure 2.13).

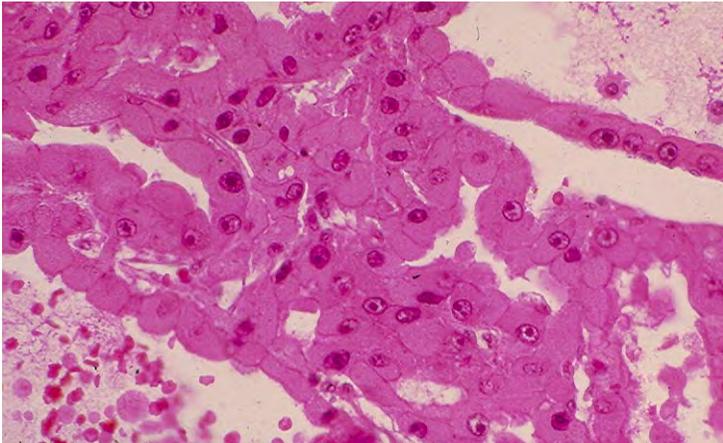
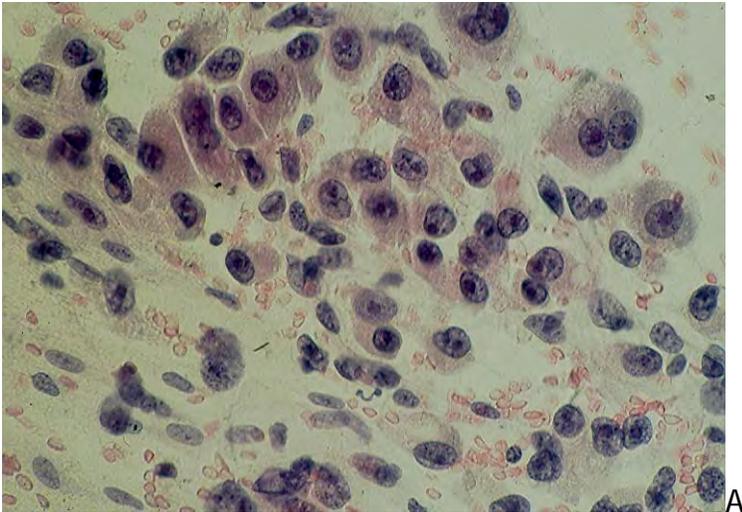
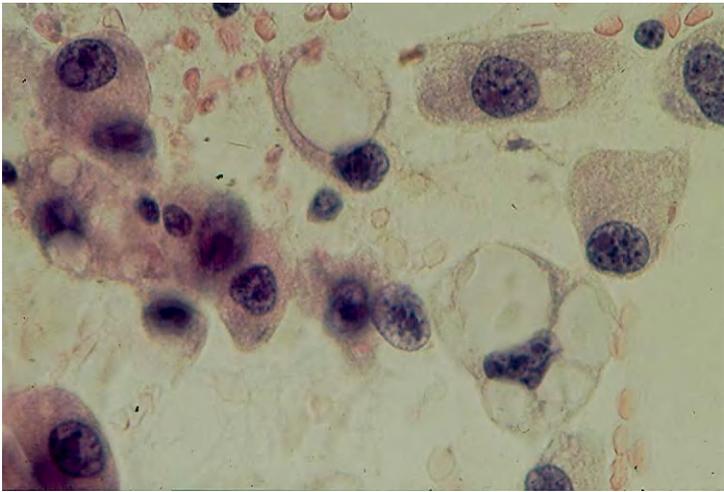


Figure 2.13. Histology of a fibrolamellar HCC showing tumor cells with extensive oncocytic change. (HE, x 200).

The tumor yields in FNA dyshesive large polygonal epithelial cells with abundant, granular and eosinophilic cytoplasm, hyperchromatic nuclei and prominent nucleoli. Intracytoplasmic ground-glass pale bodies are a common finding. Fragments or bundles of spindle-shaped fibroblastic cells may be seen. (Figures 2.14).

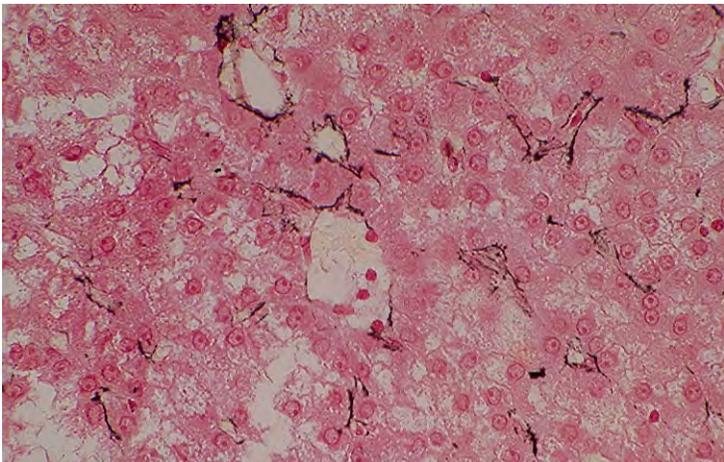




Figures 2.14. FNA from a fibrolamellar HCC showing in:  
 A. Dyshesive polygonal tumor cells with abundant, eosinophilic, granular cytoplasm admixed with spindle fibroblastic or endothelial cells.  
 B. Tumor cells with intracytoplasmic pale bodies.  
 (HE, A x 250, B x 500).

### HCC Cytochemistry

HCC cells contain no iron (Prussian blue negative). Reticulin staining of aspirated tumor tissue fragments shows a marked loss of reticulin framework that is characteristic for HCC. An increase in reticulin fibers or a normal reticulin pattern is seen only in non-HCC liver tissue. (Figures 2.15 and 2.16).



Figures 2.15. Reticulin staining of a minute tissue fragment aspirated from a well-differentiated HCC with solid pattern showing a marked loss of reticulin fibers.  
 (Reticulin stain x 250).

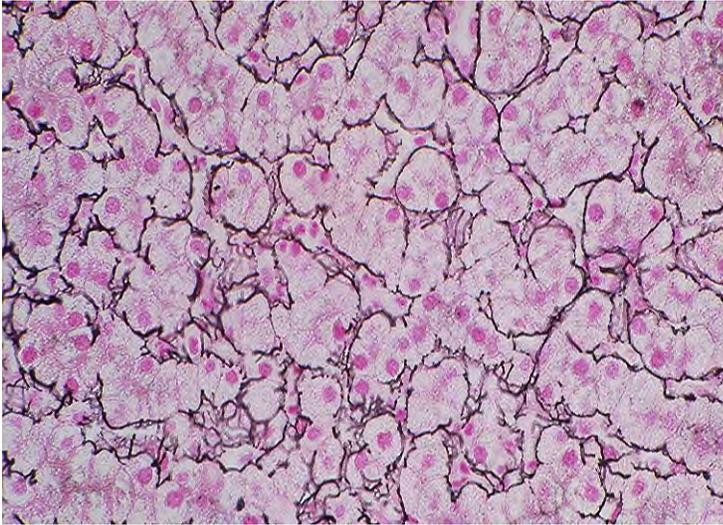


Figure 2.16. An increased number of reticulin fibers is present in a section from a regenerative liver cell nodule. (Reticulin stain, x 250)

### HCC Immunocytochemistry

HCC cells usually stain positively with alpha-fetoprotein, HepPar- 1 and negatively with monoclonal carcinoembryonic antigen (CEA) antibodies. Polyclonal CEA antibody stains bile canaliculi (cross reaction with biliary glycoprotein 1), and therefore aids in the identification of hepatocellular neoplasms. (Figure 2.17).

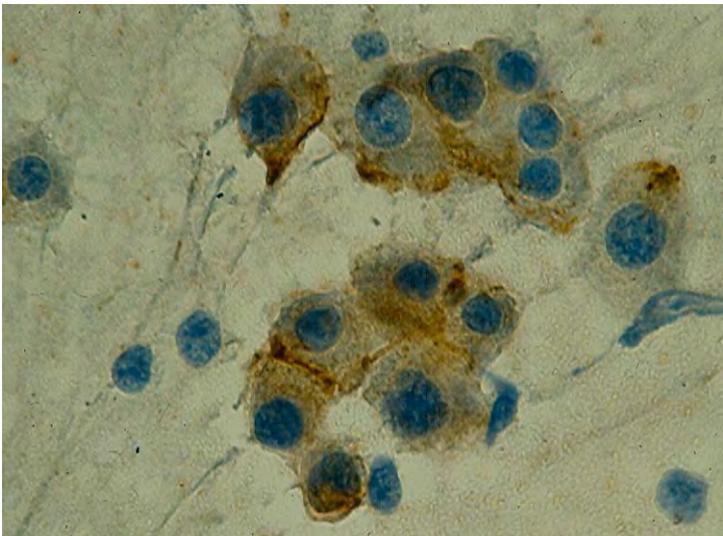


Figure 2.17. Bile canaliculi between 2 adjacent tumor cells stain positively with polyclonal CEA antibody. (ABC, x 500).

## Differential Diagnosis

Well-differentiated HCC should be differentiated from those of benign liver lesions, as hepatocytes arranged trabecular pattern may be seen in benign liver lesions as well as intranuclear cytoplasmic inclusion. Cells derived from a poorly differentiated HCC should be differentiated from those of a metastatic poorly differentiated adenocarcinoma. Immunocytochemical staining of aspirated tumor cells with polyclonal CEA, MOC-31, alpha-fetoprotein and HepPar-1 antibodies may be helpful in this case, as HCC cells usually express alpha-fetoprotein and HepPar-1. Polyclonal CEA antibody stains bile canaliculi on the surface of HCC cells. HCC cells are usually CK7/CK20 negative. A differential diagnosis of this type of liver cancer with other spindle cell sarcomas, such as leiomyosarcoma, fibrosarcoma, malignant Schwannoma, malignant fibrous histiocytoma and Kaposi sarcoma should be considered. Immunocytochemical studies of the tumor cells with appropriate antibodies and/or electron microscopy may yield useful information for a more accurate tumor typing. Fibrolamellar HCCs have immuno-cytochemical features similar to those of usual HCCs.

## Diagnostic Accuracy of HCC

Cohen et al used a step-wise logistic regression analysis of FNAs from 52 HCCs and 30 non-neoplastic lesions of the liver and identified three features that had predictive value in the diagnosis of HCC: increased nuclear/cytoplasmic ratio, trabecular pattern and atypical hepatocytic nuclei, and when these three criteria were used, the sensitivity of diagnosing HCC by FNA was 100% and the specificity was 87%.

## 2. Intrahepatic Cholangiocarcinoma

Intrahepatic cholangiocarcinoma is composed of about 7 to 25% of all primary liver cancers, depending on the geography. It is a disease of older adults with the mean age of about 65 years. Most cases of intrahepatic cholangiocarcinoma are not associated with any underlying factors. A few underlying predisposing conditions have been documented: liver fibropolycystic disease, primary sclerosing cholangitis, recurrent pyogenic cholangitis and parasitic infections with *Clonorchis* and *Opisthorchis* organisms. The tumor may be unifocal or multifocal and is a mucus secreting adenocarcinoma. It yields in FNA mucus secreting malignant glandular cells singly, in acinar arrangement or in sheets. The tumor cell cytoplasm stains positively with mucicarmine, periodic-acid Schiff (PAS) reagent and with PAS with prior diastase digestion. (Figure 2.18).

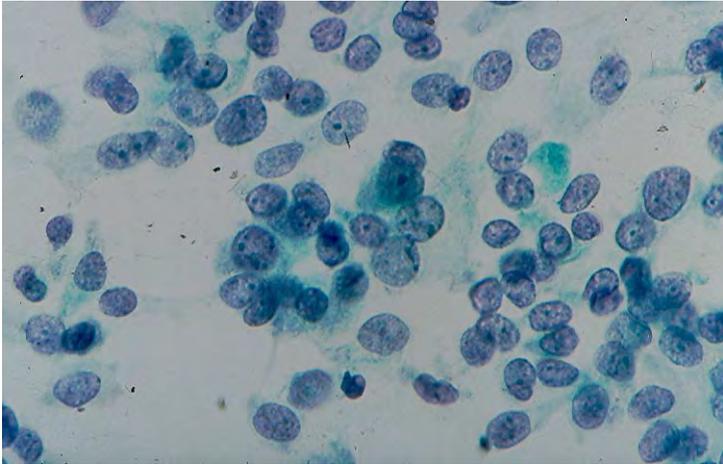


Figure 2.18. Monomorphic malignant glandular cells singly and in acinar arrangement seen in FNA of a well-differentiated intrahepatic cholangiocarcinoma (Pap, x 500).

Differential cytodiagnosis between a cholangiocarcinoma and other metastatic adenocarcinomas to the liver is extremely difficult, if not impossible, even with the availability of numerous epithelial antibodies. Clinical information and diagnostic imaging data may be of diagnostic help in some cases. Rarely, a cholangiocarcinoma associated with a HCC, and FNA from this type of tumor reveals an admixture of malignant glandular cells and HCC cells. Cells from a cholangiocarcinoma usually express AE1/AE3, CK19, monoclonal CEA, CK7, CA19-9, CDX2 and MOC31; and they are CK20 and polyclonal CEA negative.

### 3. Hepatoblastoma

Hepatoblastoma is the most common hepatic tumor in children and accounts for 50% of all pediatric malignant liver tumors. It rarely occurs in adults. The tumor is an embryonal neoplasm arising from multipotential blastematos cells with the ability to differentiate into epithelial or mesenchymal cell lines. There are 3 basic histologic types of hepatoblastoma: epithelial, mixed (epithelial and mesenchymal) and anaplastic types. The epithelial tumor cells can be anaplastic, embryonal or fetal in types. The anaplastic tumor yields small round tumor cells similar to those of a neuroblastoma or Ewing sarcoma. The embryonal hepatoblastoma may yields cells similar to those of a HCC in a trabecular pattern. (Figures 2.19 and 2.20). A fetal type tumor may show in FNA larger tumor cells in clusters. A mixed hepatoblastoma is characterized by an admixture of epithelial tumor cells and mesenchymal tumor cells that are often primitive. (Figures 2.21 and 2.22). But well-formed osteoids, chondrocytes and muscle cells may be seen. Cells of hepatoblastomas display immunocytochemical features similar to those of unusual HCCs.

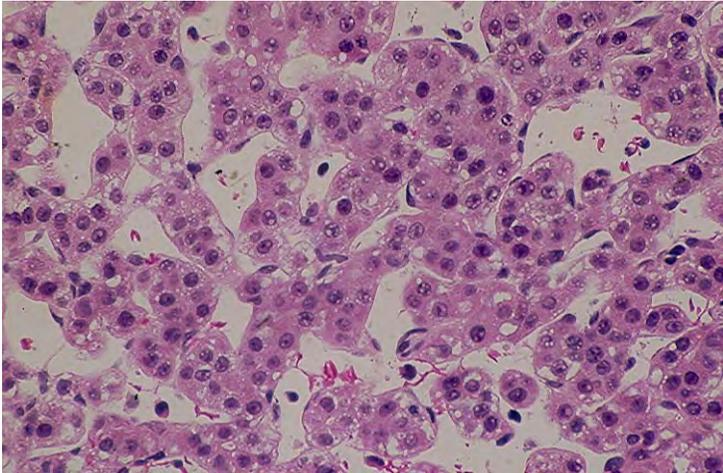
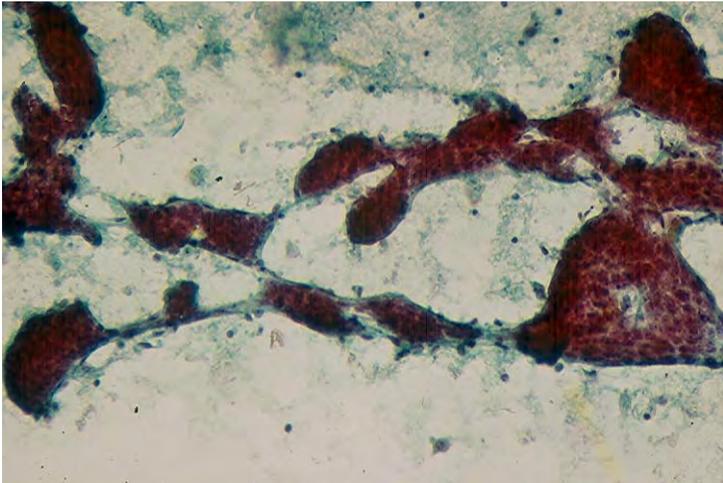
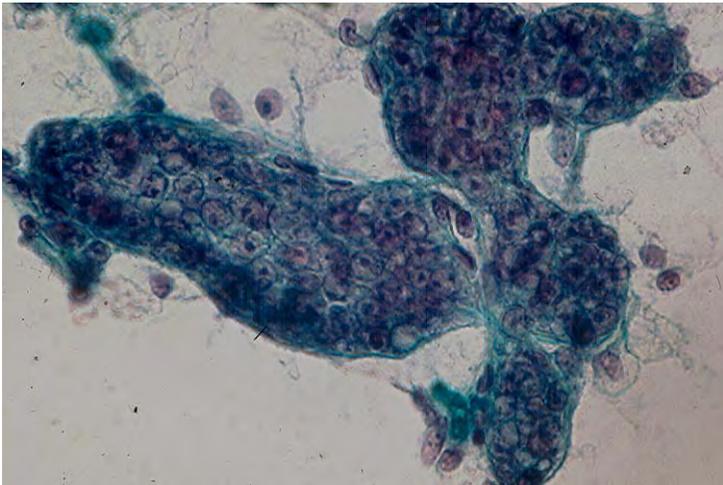


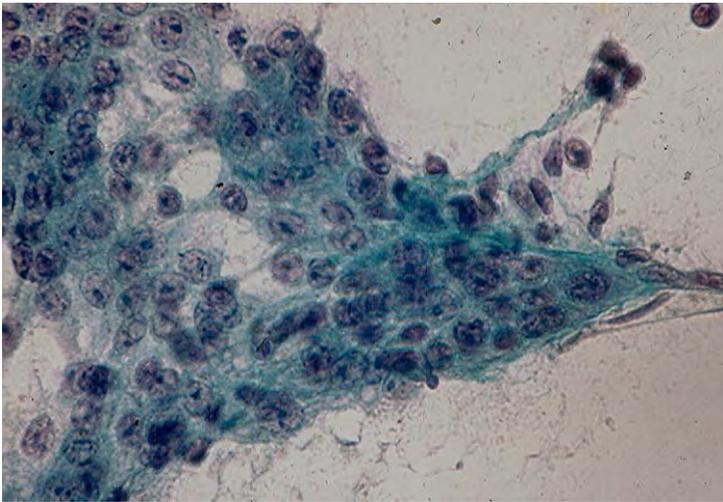
Figure 2.19. Histology of an epithelial hepatoblastoma, embryonal type showing a trabecular pattern. (HE, x 250).



A



B



Figures 2.20. FNA of an epithelial hepatoblastoma, embryonal type showing:  
A. Anastomotic cords of tumor cells.  
B. Higher magnification of a tumor cell cord showing endothelial cells wrapping on outer aspects of tumor cell cords.  
C. A sheet of tumor cells showing some cytologic features of normal hepatocytes.  
(Pap, A x 100, B x 250, C x 500).

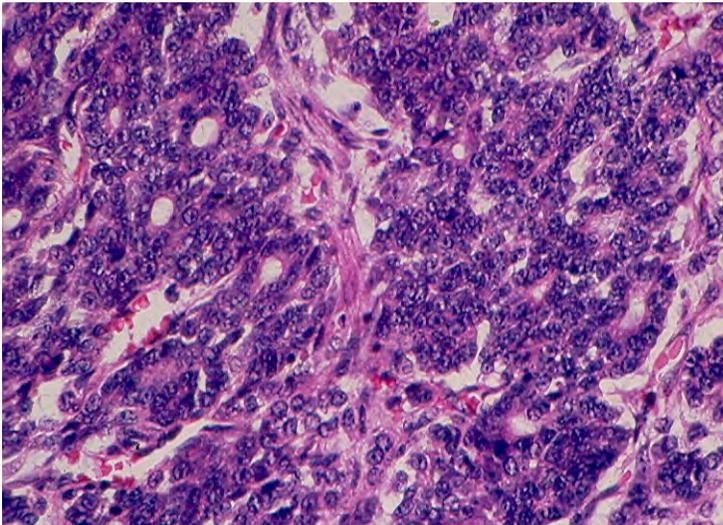
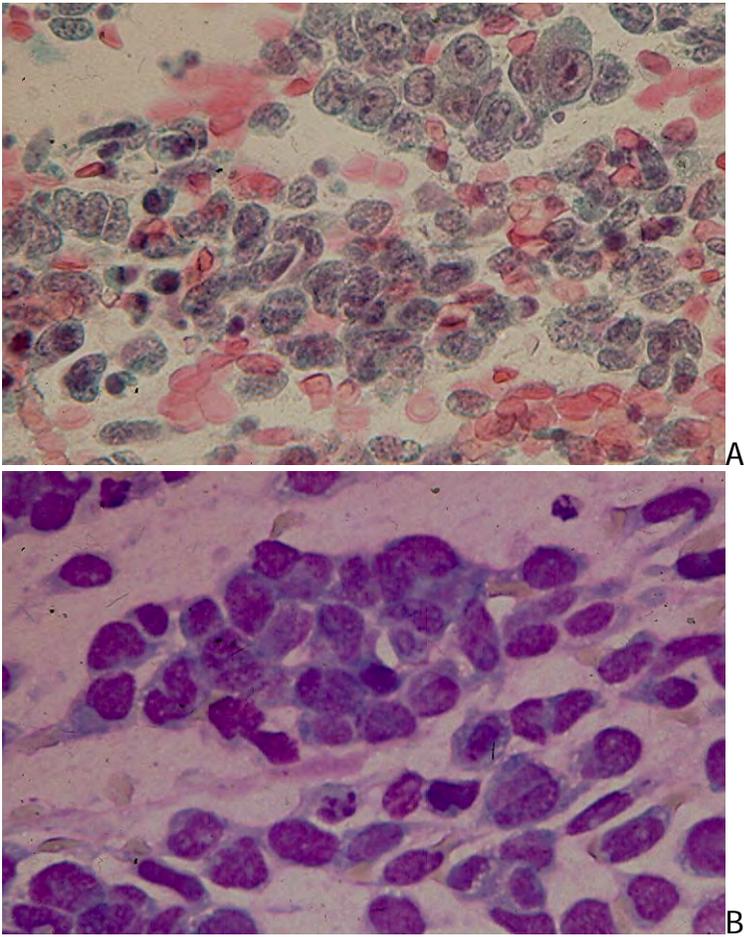


Figure 2-21. Histology of a mixed hepatoblastoma. (HE, x 250).



Figures 2.22. FNA of a mixed hepatoblastoma showing an admixture of clustered epithelial tumor cells and spindle mesenchymal-like cells (A, Pap, x 500; B, Diff Quik, x500).

#### 4. Malignant vascular tumors

**Angiosarcoma** is a very rare and highly malignant hepatic tumor that occurs more commonly in patients with history of thorotrast or vinyl chloride exposure. It accounts for only 2% of all malignant primary liver cancers seen at autopsy, with an estimated 25 to 30 cases occurring annually in the United States. The tumor has a peak incidence in the sixth and seventh decades of life and about 75% of patients are men. It has a diffuse growth pattern, commonly involving the whole liver. FNA from the lesion may reveal clustered spindle-shaped malignant endothelial cells with focal luminal formation. A positive staining reaction of the tumor cell cytoplasm with factor VIII-related-antigen, CD31 and CD34 antibodies is of diagnostic help. (Figures 2. 23 and 2.24).

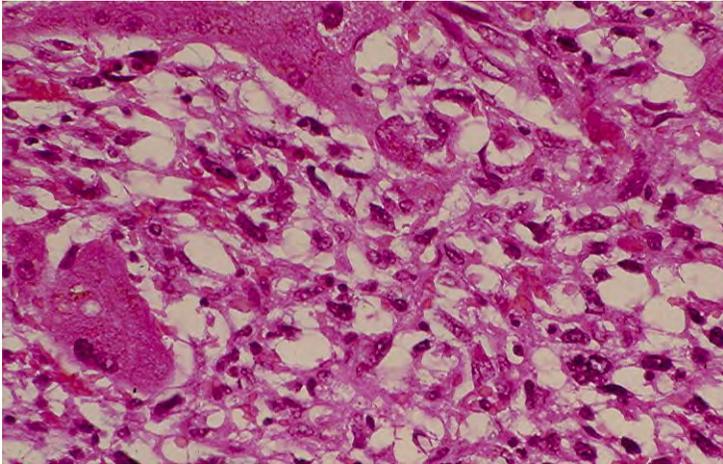
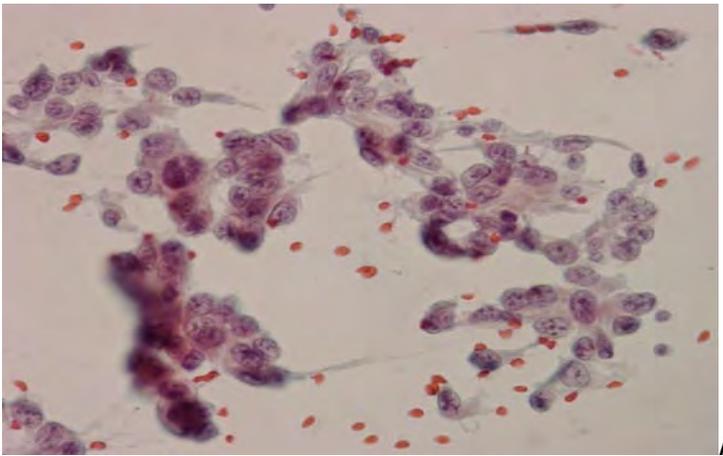
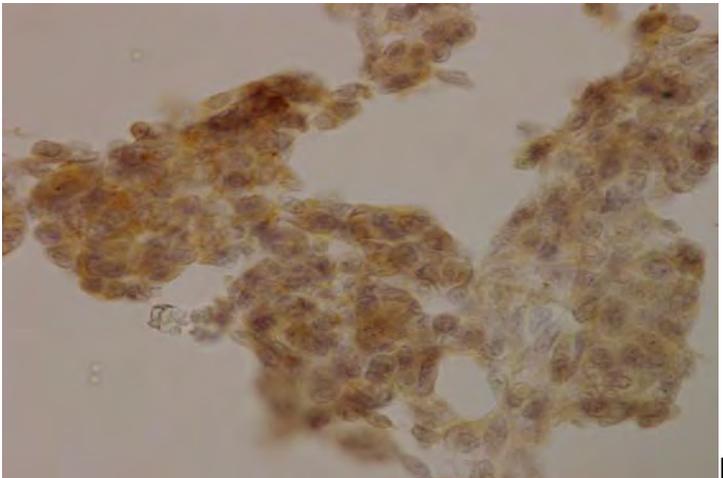


Figure 2.23. Histology of a hepatic angiosarcoma. (HE, x 250).



A



B

Figures 2.24. FNA of a hepatic angiosarcoma showing in:  
A. Malignant spindle and cuboidal cells with vasoformative arrangement.  
B. Tumor cells staining positively with Factor VIII related antigen antibody.  
(A, Pap, x 500; B, ABC, x 500).

**Epithelioid hemangioendothelioma** is a low-grade malignant vascular tumor of the liver with a favorable prognosis, and it can be successfully treated by liver transplantation. The tumor occurs in all age groups, but 60% of them occur in women in their fourth or fifth decades of life. No strong etiologic association or predisposing factors have been found. The cancer is characterized a diffuse liver involvement by pleomorphic epithelial-like cells with a round cytoplasmic profile, oval nuclei and small nucleoli. Intracytoplasmic lumens containing blood cells are not uncommonly seen, and an immuno-positive reaction of the tumor cell cytoplasm with Factor VIII-related antigen, CD31 and CD34 antibodies will confirm the diagnosis. They may express cytokeratins. (Figures 2.25 and 2.26)

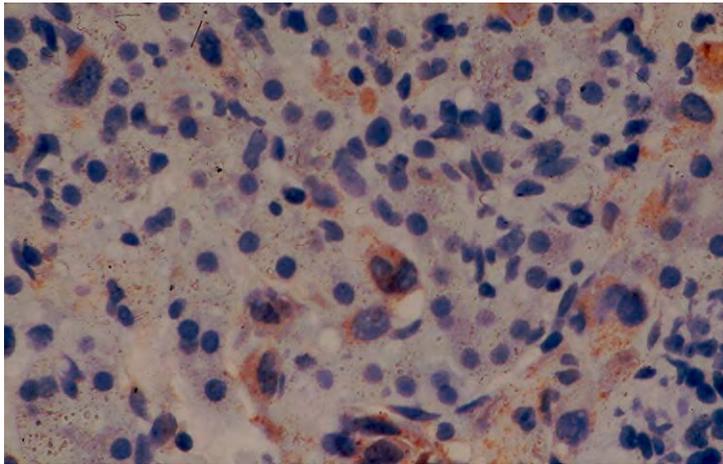
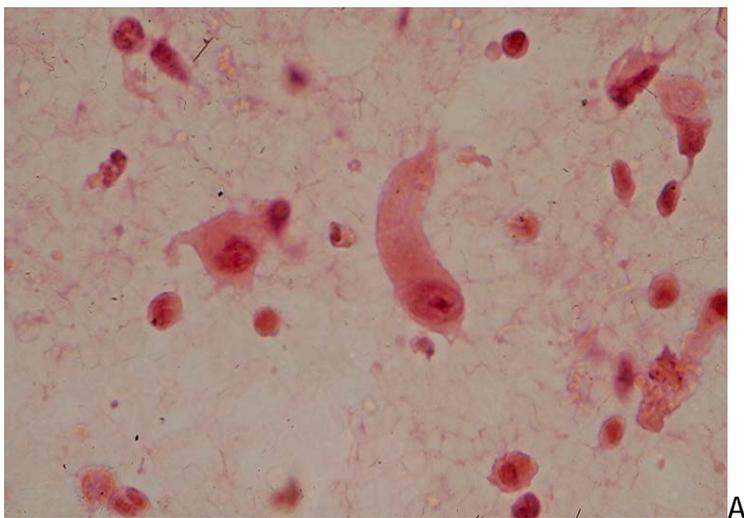
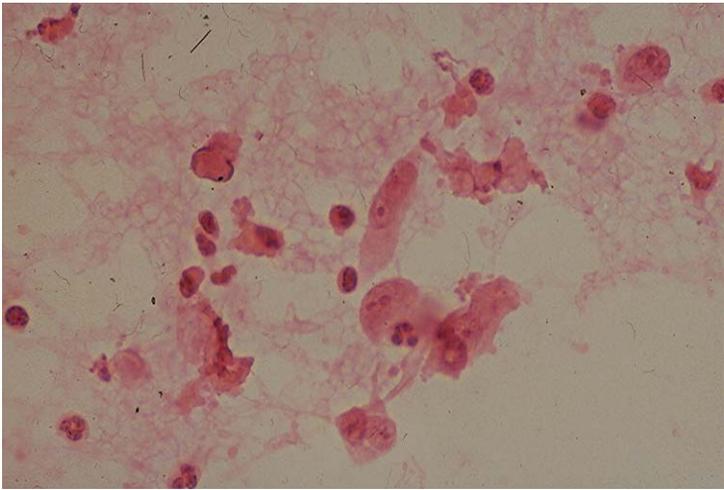


Figure 2.25. Hepatic epithelioid hemangioendothelioma showing tumor cells displaying immunopositive staining reaction with Factor VIII related antigen antibody. (ABC, x 250).





Figures 2.26. FNA of a hepatic hemangioendothelioma showing large, pleomorphic, epithelioid tumor cells with abundant cytoplasm and prominent nucleoli. (A, B:HE, x 500). (Courtesy of Dr. Kenneth Suen, Vancouver, B.C., Canada).

## 5. Undifferentiated (embryonal) sarcoma

This is the most common pediatric liver sarcomas. The tumor typically occurs in children, but it may also be seen in adults. In children this neoplasm is the third most common tumor after hepatoblastoma and HCC and accounts for 6% of all liver cancers in this age group. It shows in FNA malignant spindle cells and round bodies, as seen in histologic sections of the tumor. These globules are PAS positive and resistant to prior diastase digestion. (Figures 2.27 and 2.28).

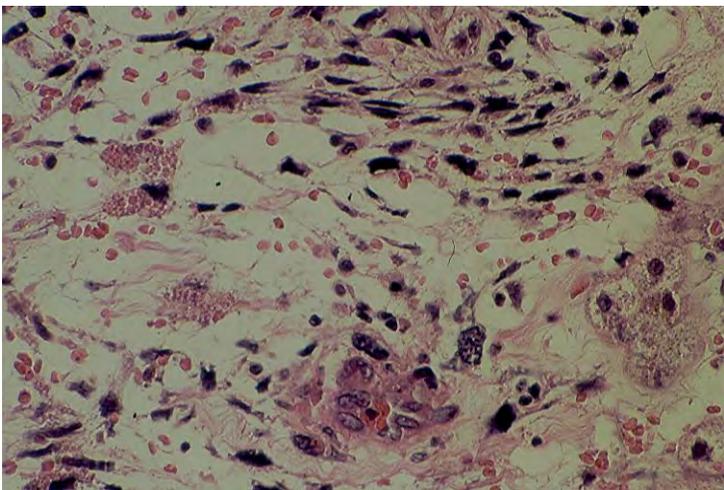


Figure 2.27. Histology of an embryonal sarcoma of the liver showing spindle tumor cells in a myxoid stroma. Intracytoplasmic or extracytoplasmic globules are seen. (HE, x 250).

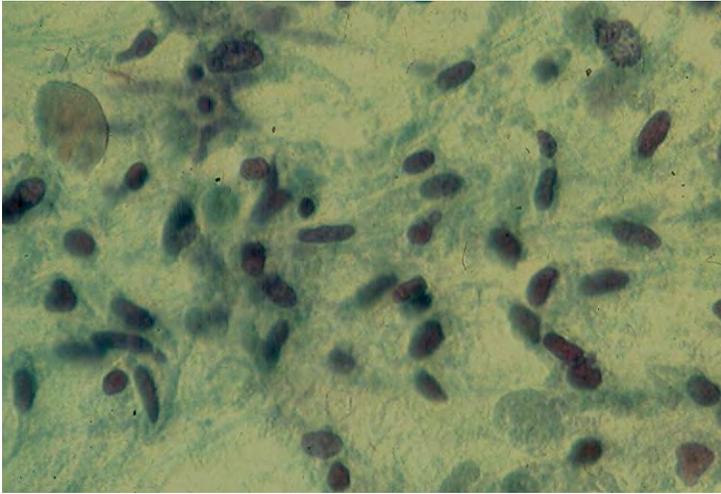


Figure 2.28. FNA of a hepatic embryonal sarcoma showing spindle malignant cells in no specific arrangement, myxoid background and a globular body. (Pap, x 500).

## **METASTATIC CANCER TO THE LIVER**

Metastatic cancers occur more frequently in the liver than in any other organs, and metastatic sarcomas are less common than metastatic carcinomas. Cancers arising from any anatomic sites can metastasize to the liver, and most liver metastases arise from abdominal organs drained by the portal vein. Primary cancers of the gallbladder, extrahepatic bile ducts and pancreas may invade the liver by direct extension. When the patient is known to have a cancer, the cytologic diagnosis of a metastatic deposit in a liver FNA is not difficult, and a firm diagnosis can be made by comparing the histology or cytology of the primary tumor with the cellular composition in the liver needle aspirate.

The most challenging problem is to suggest the location of a clinically occult cancer that has metastasized to the liver. The first step is to identify and classify the metastatic tumor cells in broad categories such as adenocarcinoma, squamous cell carcinoma, anaplastic carcinoma, lymphoma, melanoma or sarcoma. Once the tumor cells have been identified, the search for the primary cancer can be directed to the organ where that type of cancer is more commonly associated with liver metastasis. According Douglas the most common site of occult cancer with liver metastasis is the lung followed by the pancreas, colon and stomach. It should be born in mind that the failure rate for finding an occult primary cancer at autopsy averages about 28%.

Diagnosis of the primary site of metastatic adenocarcinoma in the liver can be very difficult and in many cases impossible. Some adenocarcinomas have a specific cytologic manifestation that could be helpful in localizing the primary cancers.

**Colonic well- or moderately differentiated adenocarcinomas** usually yield sheets of malignant columnar cells with elongated nuclei arranged in palisade at the periphery of epithelial tissue fragments and a large amount of necrotic debris. A poorly differentiated colonic adenocarcinoma yields cancer cells that show no specific pattern. The presence of signet ring malignant cells commonly indicates a gastric primary. Staining of the malignant glandular cells with TTF-1, CDX2, MUC-2, MUC-5, CK20 and CK7 would be helpful in identifying their origins. CDX2 (-) or weakly (+), CK7 (+), MUC-1 and MUC-5 (+) cells are most likely derived from a **pancreas or biliary tract adenocarcinoma** while strongly CDX2 (+), CK20 (+), CK7 (-) and MUC-2 (+) cells are most likely derived from a **colorectal adenocarcinoma**. Metastatic deposits of **bronchogenic adenocarcinomas** show malignant glandular in no specific patterns. A positive immunostaining of the tumor cell nuclei with TTF-1 antibody will favor a lung primary. Metastatic **renal cell carcinoma** commonly shows cohesive sheets of polygonal cells with clear or granular cytoplasm that express RCC. A metastatic **mammary duct carcinoma** commonly shows small glandular cells that are ER (+) and PR (+). And tumor cells in linear arrangement may be observed. A metastatic **neuroendocrine carcinoma** usually yields loosely clustered polygonal cells with plasmacytoid configuration that react positively with chromogranin, synaptophysin, CD56 and neuron-specific enolase antibodies. The primary neuroendocrine cancer is most commonly located in the pancreas, gastrointestinal tract or ovary. Metastatic small cell carcinoma to the liver is commonly arising from a lung primary. A metastatic **prostatic adenocarcinoma** consists of prostatic specific antigen positive small glandular cells arranged in sheets or acinar pattern. A metastatic high-grade **transitional cell carcinoma** yields pleomorphic cells with several cells showing elongated and tapering cytoplasm (cercariform cells). Urothelial cancer cells usually express CK7, CK20, Uroplakin III (UROIII), thrombomodulin and p63. Illustrations on metastatic cancers can be found in Chapter 5 in the author's monograph on Essentials of Lung Tumor Cytology, 2008.

## **BENIGN HEPATIC TUMORS AND OTHER MASS LESIONS**

**Hepatocellular adenoma** is commonly associated with the use of oral contraceptives and it is characterized by benign liver cells arranged in solid pattern. (Figure 2.29). The individual cells may show focal fatty changes. Intracytoplasmic globular bodies, as seen in HCC cells may rarely be seen. The lesion yields in FNA benign hepatocytes in sheets and singly, and no bile duct epithelial cells are seen. (Figure 2.30). Hepatocellular adenoma may show extensive fatty changes (clear cell change).

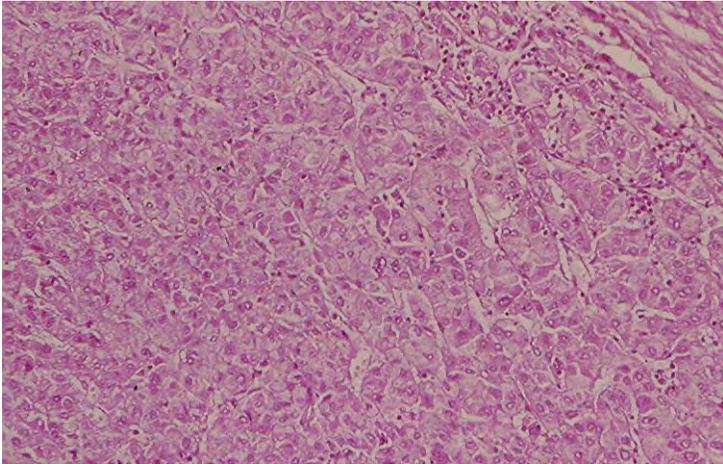


Figure 2.29. Histology of a hepatocellular adenoma. (HE, x 250).

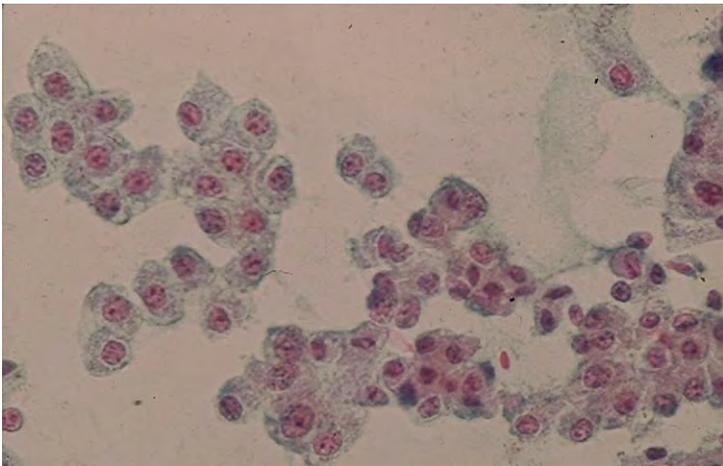


Figure 2.30. FNA of a hepatocellular adenoma showing single and clustered benign hepatocytes. (Pap, x 400).

**Focal nodular hyperplasia** presents as a solitary liver mass with a central stellate scar. It yields in FNA benign liver cells, but bile duct epithelial cells and fibroblastic cells may also be seen. The presence of a central stellate scar detected by computed tomographic scan will favor a focal nodular hyperplasia of the liver.

**Cavernous hemangioma** is a common hepatic lesion and is usually multifocal. It is rarely investigated by FNA unless it mimics a metastatic cancer radiologically. FNA from a cavernous hemangioma shows a large amount of blood, and sheets of benign endothelial cells. (Figures 2.31 and 2.32). Fragments of fibrous stroma lined by endothelial cells may be seen in a cell block section.

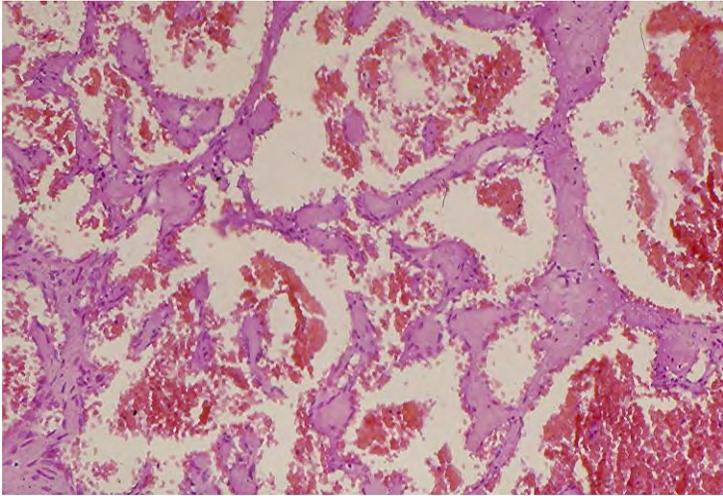


Figure 2.31. Histology of a hepatic cavernous hemangioma. (HE, x 100).

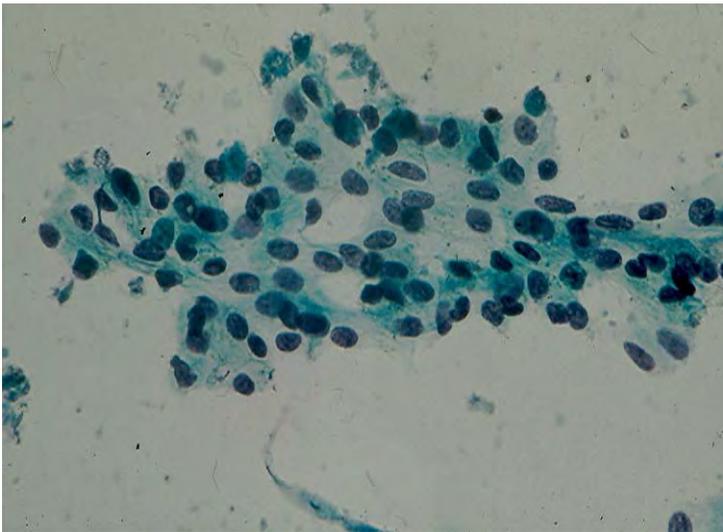


Figure 2.32. FNA of a hepatic cavernous hemangioma reveals a fragment of endothelium with spindle endothelial cells. (Pap, x 400).

**Angiomyolipoma** is a very rare benign hepatic lesion that may mimic a liver cancer by imaging study. It is characterized histologically by an admixture of benign fatty tissue, smooth muscle fiber bundles and blood vessels (Figure 2.33). In FNA the lesion is characterized by clusters of benign fat cells admixed with bundles of smooth muscle cells that may show nuclear atypia. (Figures 2.34).

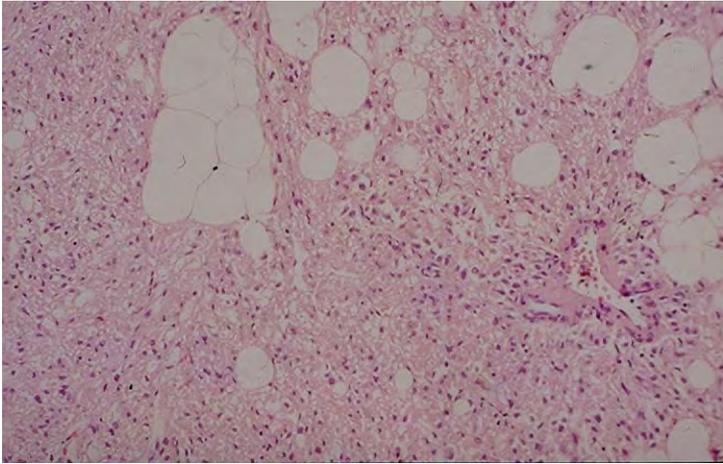
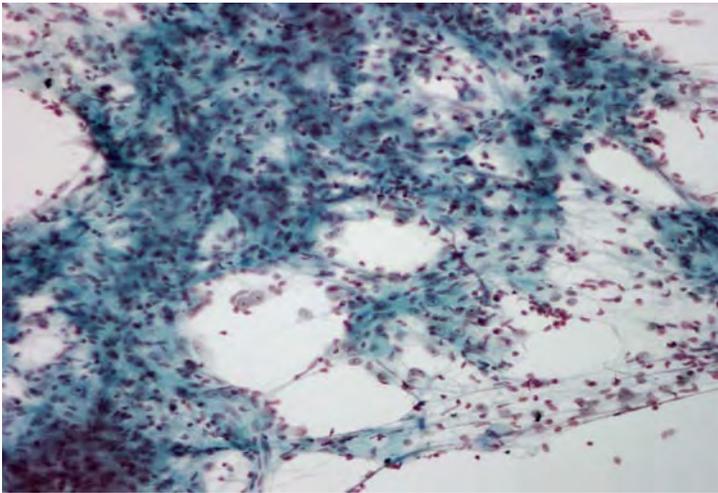
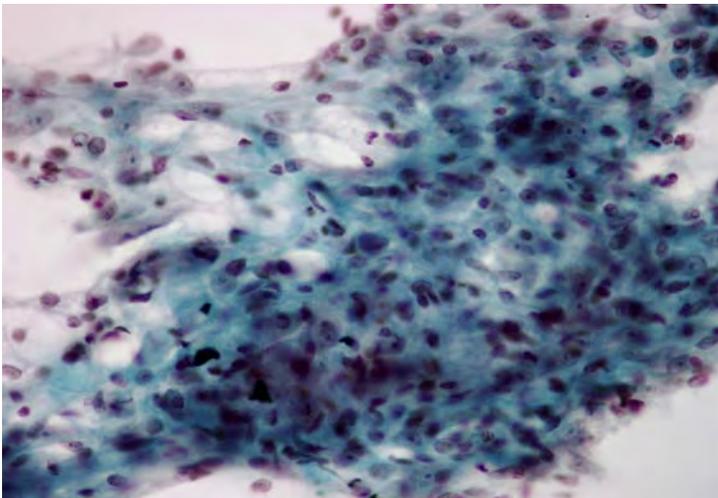


Figure 2.33. Histology of a hepatic angiomyolipoma. (HE, x 100).



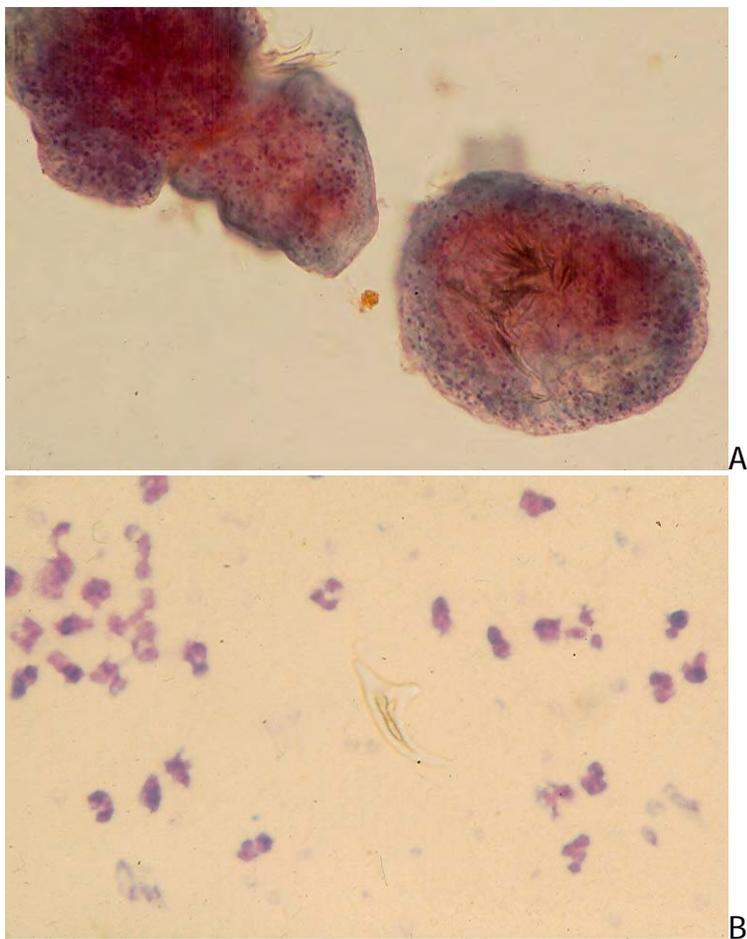
A



B

Figures 2.34. FNA of a hepatic angiomyolipoma reveals thin and thick bundles of spindle cells surrounding round spaces containing benign fat cells. Spindle tumor cells with nuclear atypia are noted in (B). (Pap, A x 100, B x 400).

**Hydatid cyst.** Echinococcal cyst (hydatid cyst) is caused by infection with *Echinococcus granulosus*, a dog tapeworm. The disease is endemic in countries bordering the Mediterranean and Baltic seas, but it has been sporadically reported in other countries worldwide. It is characterized by cystic lesions in a number of organs in human body such as liver and lung... Several cases of Hydatid cyst accidentally and correctly diagnosed by transabdominal FNA have been reported. Parasite scolices and hooklets are easily identified in routinely stained cytologic preparations. (Figures 2.35).

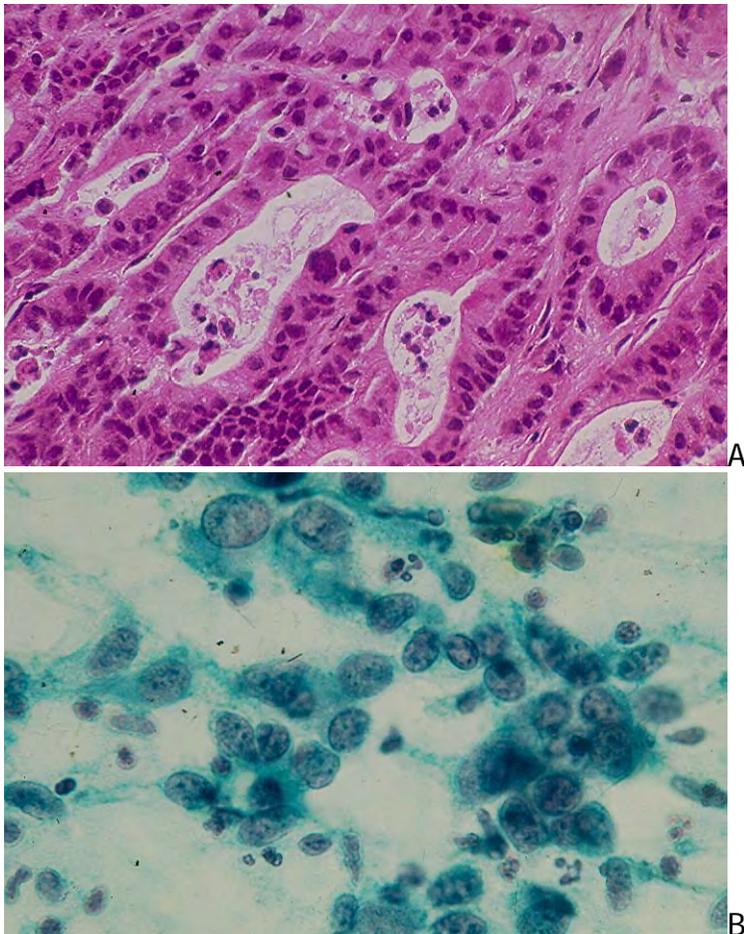


Figures 2.35. Liver hydatid cyst yields in FNA scolices with hooklets (A) and hooklets of *Echinococcus granulosus* (A, Pap,x 250; B, Diff-Quik, x 400).

## BILIARY TREE MASS LESIONS

Mass lesions of the bile duct and gallbladder can be safely aspirated for cytologic evaluation by transabdominal or EUS-guided FNA.

**Adenocarcinoma** is the most common cancer arising from extrahepatic bile ducts and gallbladder. Tumors of large extrahepatic bile ducts may be associated with a marked desmoplastic reaction and yield in FNA scanty cellular materials. FNA from a gallbladder adenocarcinoma reveals malignant glandular cells singly and in clusters. (Figures 2.36). Cytodiagnosis of a well-differentiated adenocarcinoma of extrahepatic bile ducts may be challenging as the tumor cells may display subtle malignant nuclear changes. A demonstration of aneuploidy by DNA image analysis and staining of the tumor cells with k-ras oncogen antibody may be helpful in this case. Diagnosis of a poorly differentiated adenocarcinoma is usually straightforward as its cancer cells are readily identifiable. Cells derived from a bile duct adenocarcinoma are generally TTF-1 and CK20 negative and CA19-9, CK19, CK7, MOC-31, CEA, CDX2 and MUC-5 positive.



Figures 2.36. A well-differentiated adenocarcinoma of the gallbladder (A) showing in TFNA single and clustered malignant glandular cells with oval nuclei and conspicuous nucleoli. (A, HE, x 250; B, Pap, x 400).

**Xanthogranuloma** of the gallbladder may mimic a neoplasm clinically and by diagnostic imaging. It shows in FNA abundant foamy macrophages, lymphoid cells and plasma cells. It should be born in mind that the diagnosis of this lesion is made by exclusion, as it may occur in association with an obstruction of the cystic duct caused by a cancer arising in this location.

## SUMMARY

FNA for cytologic evaluation of a liver mass lesion is a safe, economical and effective diagnostic procedure. Hepatocellular carcinoma, angiosarcoma, epithelioid hemangioendothelioma, hepatoblastoma, embryonal sarcoma and angiomyolipoma display fairly characteristic cellular and/or immunocytochemical features, permitting a correct cytodiagnosis in the majority of cases. However, only a suggestive or probable diagnosis may be made in cases of hepatocellular adenoma and focal nodular hyperplasia, and correlations of the cytologic findings with the patient's clinical and diagnostic imaging data are important for more accurate diagnoses of the two lesions. Cytodiagnosis of metastatic cancers is relatively straightforward as malignant cells are readily identifiable.

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# KIDNEY AND RENAL PELVIS

## INDICATION, CONTRAINDICATION, TECHNICAL CONSIDERATIONS AND COMPLICATIONS OF KIDNEY AND RENAL PELVIS FNA

### Indication and Contraindication

Mass lesions of the kidney and renal pelvis associated with negative urine cytology are targets of FNA for cytologic investigation. Kidney and renal pelvis FNA is contraindicated in patient with bleeding disorders.

### Technical considerations

The biopsy needle most often used is a 22-gauge, spinal-type needle, 8- to 20-cm long. For a palpable mass lesion the biopsy is performed directly through the abdominal wall with manual fixation of the skin over the lesion, and an 8-cm-long needle is used. For a non-palpable lesion a 15- to 20-cm-long needle is used and it is guided by either CT or US scanning of the abdomen. In the majority of cases the FNA is performed through the anterior abdominal wall with the patient in supine position or via a lumbar approach with the patient in prone or lateral position.

Direct and cytospin smears are prepared from the FNA and stained either by the Papanicolaou or Diff-Quik method. Excess of aspirated material should be fixed in formalin for preparation of a cell block for histologic immunohistochemical studies, if indicated.

### Complications

Complications of FNA of the kidney and renal pelvis are rare and no death has been reported. Transient gross or microscopic hematuria is the most common complication of renal FNA. In a review of 5674 renal cyst aspirations, Lang noted that perirenal hemorrhage was the most common complication (0.63%), followed by pneumothorax (0.17%), infection (0.15%), arteriovenous fistula (0.14%), and urinoma (0.1%).

## **CYTODIAGNOSTIC ACCURACY**

The high cytodiagnostic accuracy rates of FNA of the kidney and renal pelvis tumors is the results of the efforts of a team consisting of a radiologist with experience in FNA techniques and a cytopathologist with expertise in interpreting FNA cytology. In a review of 10 large series of FNA of renal tumors totalling 922 cases Katz found that the sensitivity and specificity of the technique were 79% and 99% respectively. The success rate of FNA of transitional cell carcinomas of the renal pelvis has been about 85%.

## **NORMAL KIDNEY AND RENAL PELVIS**

Normal cellular elements of the kidney may be seen in needle aspirates of a renal mass lesion. Renal glomeruli are lobular structures consisting of loops of capillary blood vessels. Cells from proximal convoluted tubules are seen in small sheets. They are characterized by abundant, eosinophilic, granular, ill-defined cytoplasm and oval nuclei with conspicuous nucleoli. Cells from distal convoluted tubules and collecting ducts are smaller than those of proximal tubules. Loops of Henle appear as thin tubular structures consisting of small cuboidal cells with scant cytoplasm and oval nuclei and are rarely seen in renal FNA. Needle aspirate from the renal pelvis reveals only a few drops of urine containing a small number of normal urothelial cells with well-defined, elongated cytoplasm with or without "tail" and regular, oval nuclei with inconspicuous nucleoli.

## **BENIGN RENAL SPACE-OCCUPYING LESIONS**

### **Renal Cyst**

Renal cysts may be congenital or acquired. Congenital renal cysts may be multiple as in polycystic kidneys. Solitary renal cysts are found in about 50% of patients over their fifth decade of life and they are particularly common in patients on chronic renal dialysis. The lesions are usually asymptomatic and may be large, up to 10 cm in diameter. They may be diagnosed by US or CT scans. Most solitary renal cysts contain a clear straw-colored fluid. If the cystic content is bloody a renal cell carcinoma should be suspected, as about 30% of all renal cell cancers undergo extensive hemorrhagic cystic degenerative changes. On the other hand, a cystic renal cell carcinoma may have a clear cyst content. Therefore, cytologic examination of all renal cyst contents is mandatory. FNA of the base of the cyst should be done as residual renal cell carcinoma is almost always found in this location. FNA from a benign renal cyst usually yields a several foamy histiocytes. Hemosiderin laden macrophages may be present.

## **Renal Angiomyolipoma**

Renal angiomyolipoma (RA) accounts for about 2% of all renal tumor. RAs may occur as multiple and bilateral lesions in 50 to 80% of patients with tuberous sclerosis or they may be found as a solitary lesion in patient without tuberous sclerosis. Diagnosis of isolated RA is challenging, and the differential diagnosis between a RA and a renal cell carcinoma is not always possible despite of the US and CT characteristic features of the former. A correct preoperative diagnosis of a RA is desirable as it may be managed conservatively. Histologically, renal angiomyolipoma is characterised by an intimate mixture of smooth muscles cells, mature fatty tissue and blood vessels. The proportions of these cellular components may vary considerably. The smooth muscle cell component of the lesion may have bizarre morphology mimicking that of a leiomyosarcoma.

FNA from a renal angiomyolipoma may reveal bundles of smooth muscle cells admixed with benign fat cells. Cytodiagnosis of a RA can be difficult if its smooth muscle component contains bizarre-appearing nuclei that may be mistaken for those of a leiomyosarcoma or a sarcomatoid renal cell carcinoma. When the cytodiagnosis of a RA is uncertain by FNA, frozen section examination of a tissue sample of the lesion taken intraoperatively is helpful for solving this diagnostic dilemma. For illustrations, please see Chapter 2.

## **Renal Oncocytoma**

Renal oncocytoma (RO) probably arises from the intercalated cells of the collecting duct in the renal medulla. It accounts for about 5% of all renal epithelial neoplasms and may rarely be multifocal. It is more common in men than in women and occurs mainly in their 7th decade of life. ROs are usually asymptomatic and incidentally found by abdominal US and CT scans done for investigation of an unrelated disorder. About 50% of ROs have peculiar angiographic patterns, but they may show angiographic features similar to those of a renal cell carcinoma (RCC). ROs are well-circumcribed, mahogany brown solid tumors with a central stellate scar, and they are composed of polygonal cells with abundant, granular cytoplasm arranged in solid, trabecular or acinar patterns. The nuclei are small, oval and show inconspicuous nucleoli. (Figure 3.1). Cytodiagnosis of a RO may only be suggested by FNA, since a RCC may show areas with oncocytic features and cells derived from a chromophobe RCC are similar to those of a RO. A firm diagnosis of RO requires a careful macroscopic and microscopic examination of the tumor. Thus, when a RO is suspected the lesion should be removed by partial nephrectomy if technically feasible and examined by frozen section. If a RCC is present a radical nephrectomy will follow.

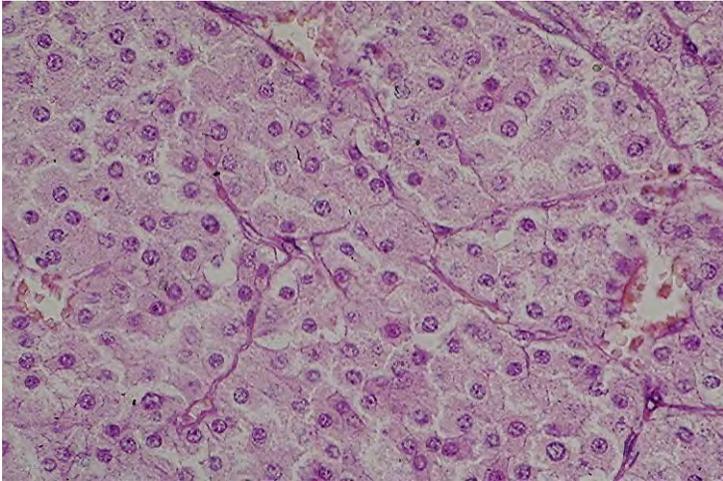


Figure 3.1. Histology of a renal oncocytoma. (HE, x 250).

FNA from a RO shows single and clustered polygonal cells with abundant, granular, well-defined cytoplasm and oval nuclei without or with inconspicuous nucleoli. Occasional tumor cells may show bizarre-appearing nuclei. (Figure 3.2). RO cells may be mistaken for those of a chromophobe RCC. Positive staining reaction with Hale colloidal iron is a characteristic feature of the chromophobe tumor cells.

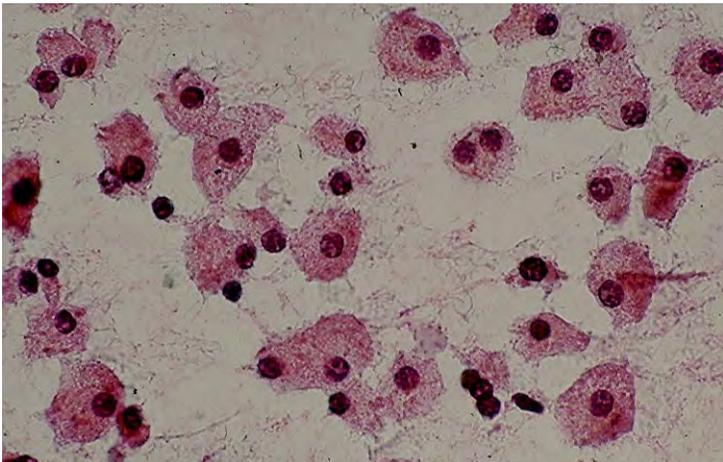


Figure 3.2. FNA of a renal oncocytoma showing single and loosely clustered polygonal tumor cells with abundant, granular cytoplasm (Pap, x 500).

### Other Benign Renal Mass Lesions

*Renal abscess and Xanthogranulomatous pyelonephritis* are two inflammatory lesions presenting with a renal mass. Renal abscess in adults is usually caused by a Gram-negative bacterial infection in a kidney with stones. FNA of a renal abscess yields purulent material. *Xanthogranulomatous pyelonephritis* is a rare condition associated with an urinary tract

obstruction or calculi. It is commonly caused by *E. Coli*, *Pseudomonas* or *Proteus*. The lesion shows in FNA numerous histiocytes with bean-shaped nuclei and foamy histiocytes. Proliferating histiocytes may show bizarre-appearing nuclei and may mimic cells of an anaplastic carcinoma, and lipid-laden macrophages may be mistaken for cells of a renal cell carcinoma.

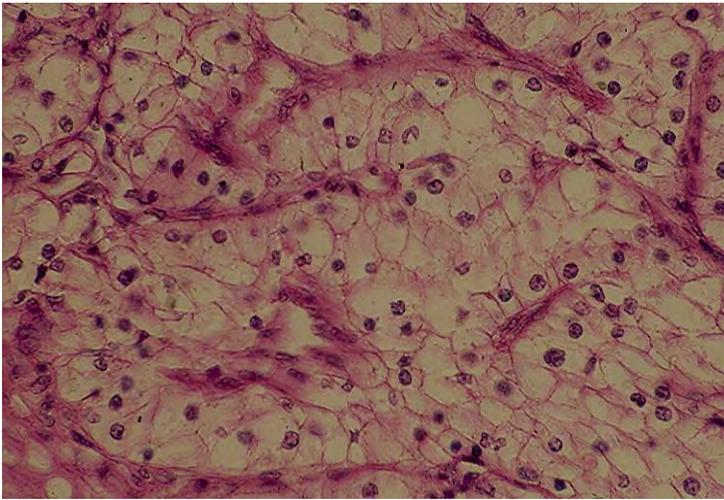
## **MALIGNANT TUMORS OF THE KIDNEY**

### **1. Renal Cell Carcinoma**

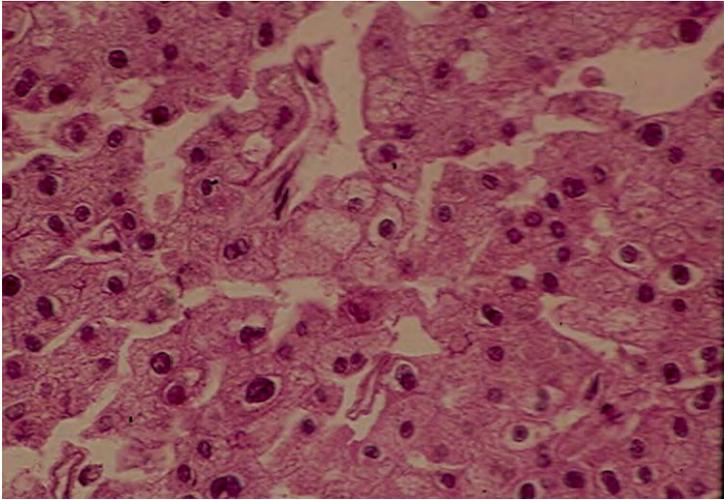
Renal cell carcinomas (RCC) account for about 3% of all malignant solid tumors in adults. RCCs are more common in men than in women, and their peak incidence is in the fifth and sixth decades of life. The clinical diagnosis of RCC is challenging. The classic clinical triad of gross hematuria, palpable mass and flank pain is a late manifestation and is present in about 9% of the cases, and about 1/3 of patients with RCC have distant metastases when they seek medical attention. Other paraneoplastic syndromes of RCC include hypercalcemia, hypertension and Cushing syndrome. Some clinical conditions such as von Hippel-Lindau disease, acquired cystic disease of the kidney in patients with long term renal dialysis and adult polycystic kidney are associated with a high risk in developing RCC. About 40 to 50% of patients with von Hippel-Lindau syndrome develop RCCs in their adulthood.

#### **1.1. Conventional RCC**

RCCs are histologically classified into clear cell or conventional and papillary types. Clear cell tumors account for about 85% of all RCCs. Fuhrman nuclear grading system is commonly used in surgical pathology. However, it is more convenient to use 3 nuclear grades to grade RCCs histologically and cytologically, with grade 3 equivalent to grade 3 and 4 in Fuhrman grading system. In grade 1 tumors the nuclei are small and regular, and the nucleoli are inconspicuous or absent. (Figures 3.3). Grade 2 tumors are characterised by larger and slightly irregular nuclei with conspicuous nucleoli. Large pleomorphic nuclei with prominent nucleoli are features of grade 3 RCCs. Multiple nuclear grades coexist in about 15% of all RCCs. A papillary RCC is characterized by the presence of fibrovascular cores covered with epithelial tumor cells with similar cytologic features to those of a conventional RCC. (Figure 3.4). Cells of RCCs express RCC, keratin, vimentin, epithelial membrane antigen and CEA.



A



B

Figures 3-3. Histology of conventional renal cell carcinomas:  
A. Tumor with nuclear grade 1. (HE, x 250).  
B. Tumor with nuclear grades 1 and 2. (HE, x 250).

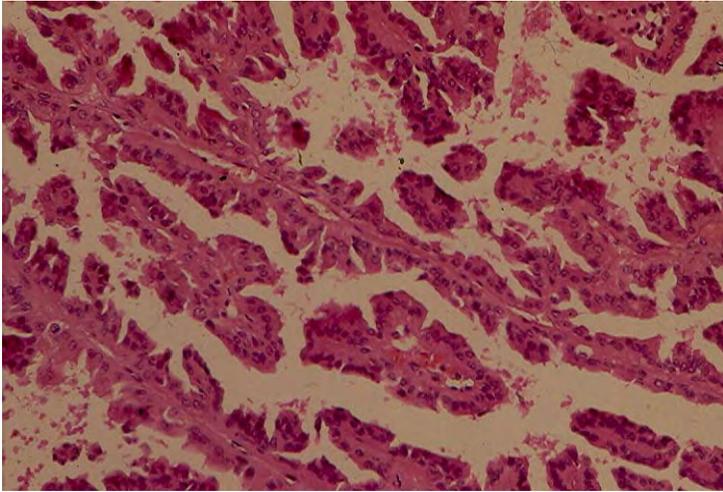


Figure 3.4. Histology of a papillary renal cell carcinoma. (HE, x 250).

FNA from a RCC is usually hypercellular. Grade 1 tumors show large, cohesive, monolayered sheets of tumor cells with clear and or granular cytoplasm and small nuclei containing inconspicuous or no nucleoli. Single tumor cells and naked tumor cell nuclei are rarely observed. (Figure 3.5). Grade 2 tumors yield similar tumor cells, but their nuclei are larger and contain conspicuous nucleoli. (Figure 3.6). Grade 3 RCCs show malignant tumor cells singly and in small clusters or sheet. (Figures 3.7 and 3.8). Abundant naked tumor cell nuclei. The tumor cell nuclei are pleomorphic, irregular, large and contain prominent nucleoli. Intracytoplasmic eosinophilic globular or Mallory-like bodies may be seen. The cytologic manifestations of a papillary RCC are usually similar to those of a clear-cell RCC. However, on rare occasions, papillary tissue fragments with fibrovascular core are observed, as well as abundant foamy histiocytes. (Figures 3.9).

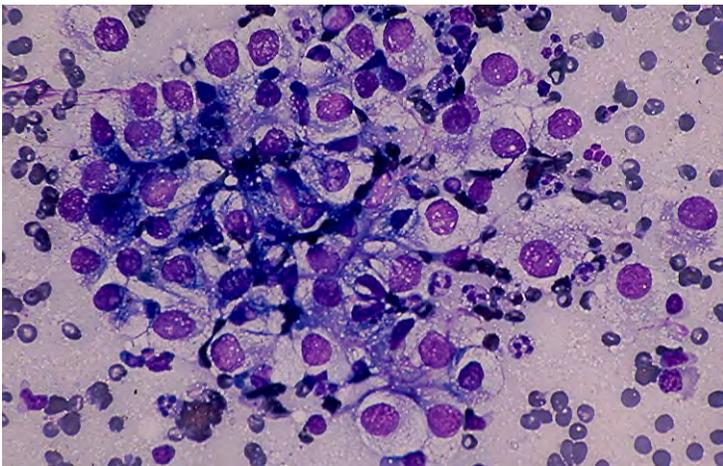


Figure 3.5. FNA of a RCC, grade 1 showing a sheet of tumor cells with clear cytoplasm and round nuclei with inconspicuous nucleoli. (Diff-Quik, x 400).

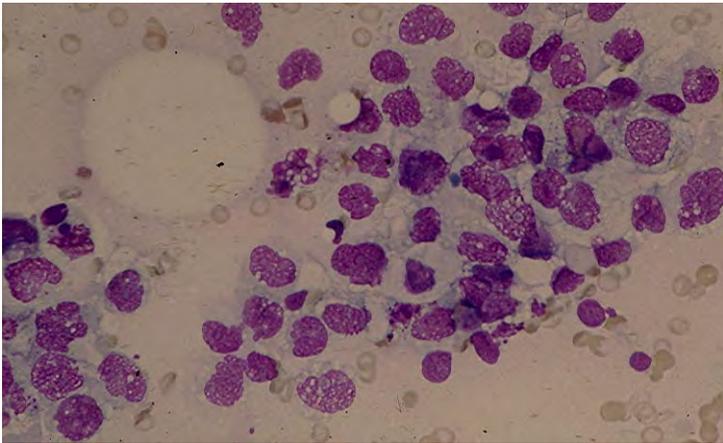


Figure 3.6. FNA of a conventional RCC, grade 2 showing a monolayered sheet of tumor cells with clear cytoplasm and enlarged, more pleomorphic nuclei with conspicuous nucleoli. (Diff-Quik, x 400).

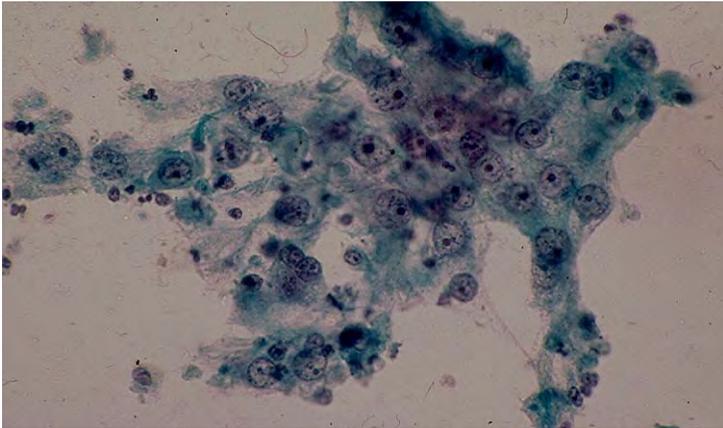


Figure 3.7. FNA of a conventional RCC, grade 3 showing a cluster of tumor cells with clear or granular cytoplasm and oval nuclei with prominent nucleoli. (Pap, x 400).

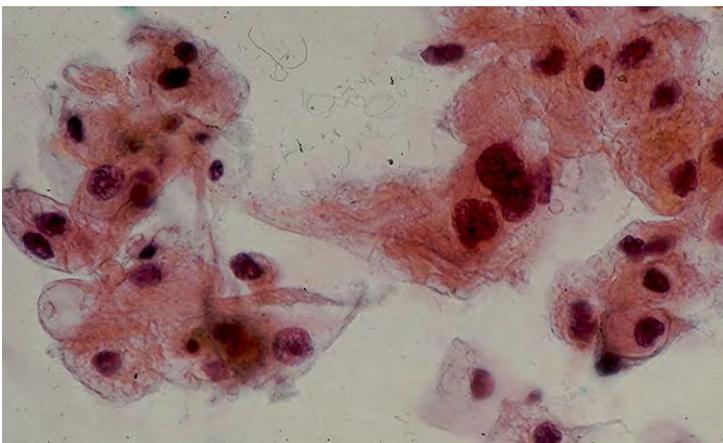
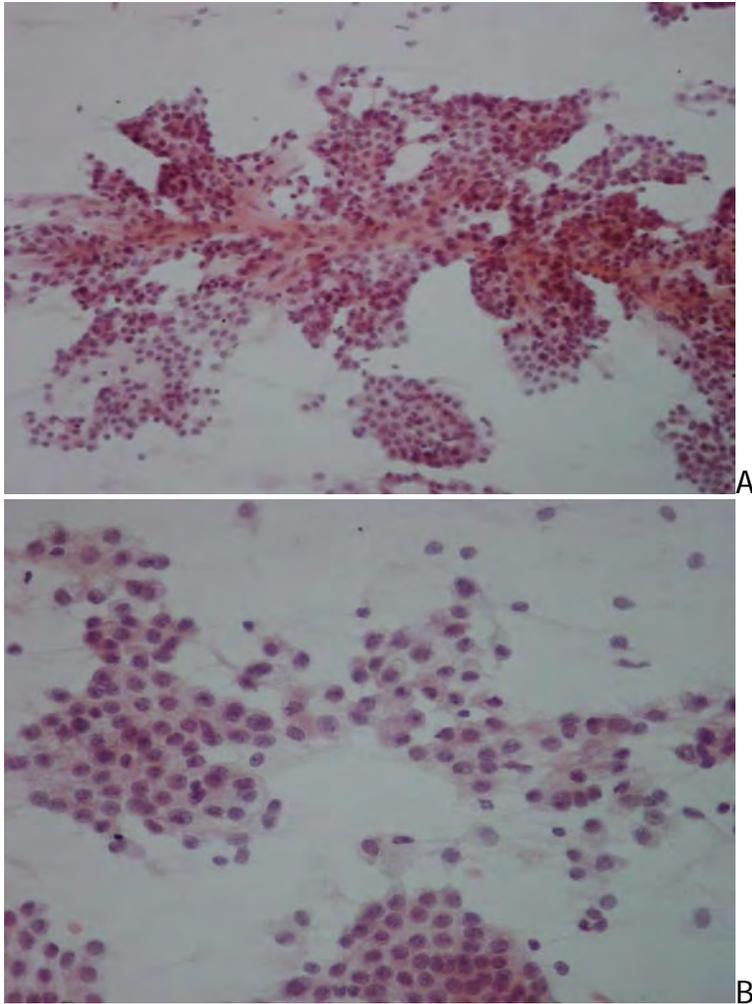


Figure 3.8. A conventional RCC, grade 3 showing in FNA single and clustered tumor cells with hyperchromatic, pleomorphic nuclei and prominent nucleoli. (Pap, x 400).



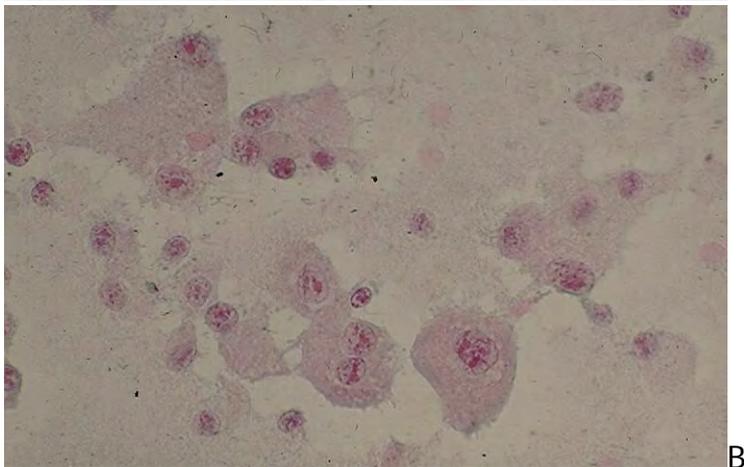
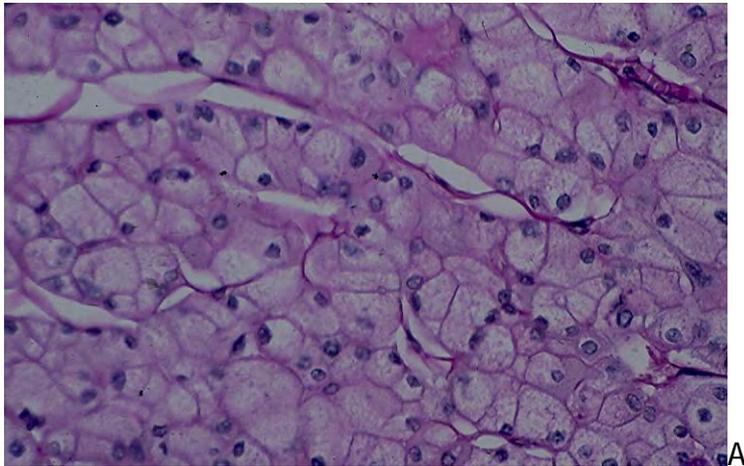
Figures 3.9. A papillary RCC showing in FNA:  
A. Papillary tumor tissue fragment with fibrovascular cores.  
B. Monolayered sheets of tumor cells with honeycomb pattern showing monomorphic nuclei. (Pap, A x 100, B x 250).

Cells of a RCC should not be mistaken for those of a hepatocellular carcinoma or a renal oncocytoma. Cells of a hepatocellular carcinoma do not express CEA, but CEA antibody cross-reacts with biliary glycoprotein 1 and stains bile canaliculi between adjacent cells of a hepatocellular carcinoma.

## 1.2. RCC histologic subtypes

Three main histologic subtypes of RCCs are identified: chromophobe RCC, collecting duct carcinoma and sarcomatoid RCC.

- **Chromophobe renal cell carcinoma** accounts for about 5% of all RCCs. It probably arises from the intercalated cells of the collecting ducts and are usually solitary. Histologically, a chromophobe RCC consists of an admixture of tumor cells with well-defined, abundant, transparent or granular, eosinophilic cytoplasm with a clear perinuclear region that is due to the presence of numerous intracytoplasmic vesicles. The tumor cells are arranged in an alveolar pattern and stain positively with Hale colloidal iron indicating the presence of an acidic mucin. Chromophobe RCCs yield in FNA abundant tumor cells singly and in clusters. The tumor cells show abundant, granular cytoplasm, perinuclear clear space and vesicular nuclei containing small nucleoli. (Figures 3.10). Cells of a chromophobe RCC mimic those of a renal oncocytoma. Prominent nucleoli and positive cytoplasmic staining reaction with Hale colloidal iron are features of chromophobe RCCs.



Figures 3.10. A. Histology of a chromophobe RCC (HE, x 250).

B. FNA of a chromophobe RCC showing single and loosely clustered epithelial tumor cells with abundant, pale cytoplasm and inconspicuous nucleoli (Pap, x 500).

- **Collecting duct carcinomas** account for about 1 to 2% of all RCCs. The tumor is characterized by pleomorphic epithelial cells arranged in tubular pattern and a remarkable desmoplastic reaction. It commonly has a papillary component within the renal pelvis. The tumor cells express high-molecular-weight keratin and lectin. It shows in FNA single and tightly cohesive clusters of pleomorphic glandular cells with prominent nucleoli.

- **Sarcomatous RCC** is a rare tumor and constitutes about 1% of all RCCs. The tumor is highly aggressive and characterized by an admixture of areas of conventional RCC and areas consisting of spindled and pleomorphic sarcomatoid cells. The sarcomatoid RCC cells variably express keratin and show strong immunopositive reaction with vimentin antibody. It yields in FNA pleomorphic malignant cells singly and in loose aggregates. Cells with features of a clear cell RCC may be seen. Sarcomatous tumor cells should be differentiated from those of a leiomyosarcoma or fibrosarcoma by immunocytochemistry and/or ultrastructural study.

## 2. Wilms Tumor

Wilms tumor (WT) or nephroblastoma is the most common kidney tumor in children but it may rarely occur in adult patients. It has no sex predilection, and in 5 to 10% of patients the tumor is bilateral. About 50% of WTs occur before the age of 3 years, and 90% of these neoplasms are detected before the age of 6 years. Some risk factors have been identified in patients with WT such as Wilms-aniridia-genital anomaly-retardation syndrome, omphalocele macroglossia (Beckwith-Wiedemann syndrome), and Denys-Drash syndrome. Clinically, the patients present with an abdominal mass, and hematuria is a rare initial manifestation.

Histologically, a typical WT has 3 cellular components: undifferentiated blastematos, epithelial and stromal cells. The stromal elements vary considerably in composition and structure. In the majority of WTs the stroma is myxomatous, but smooth and striated muscle cells, fat cells, neural elements and cartilage may be present, however. Epithelial elements may form glandular structures with various degrees of differentiation with some having basaloid, squamous or glandular appearances.

A classic WT yields in FNA undifferentiated cells or blastematos elements and epithelial cells in acinar or glandular arrangement. Benign appearing smooth muscle cells, skeletal muscle cells and chondrocytes may be seen. Blastematos cells of a WT should be

differentiated from those of other small blue cell tumors of the childhood such as Ewing sarcoma, rhabdomyosarcoma, peripheral neuroectodermal tumor and lymphoma.

### **3. Other Malignant Renal Tumors**

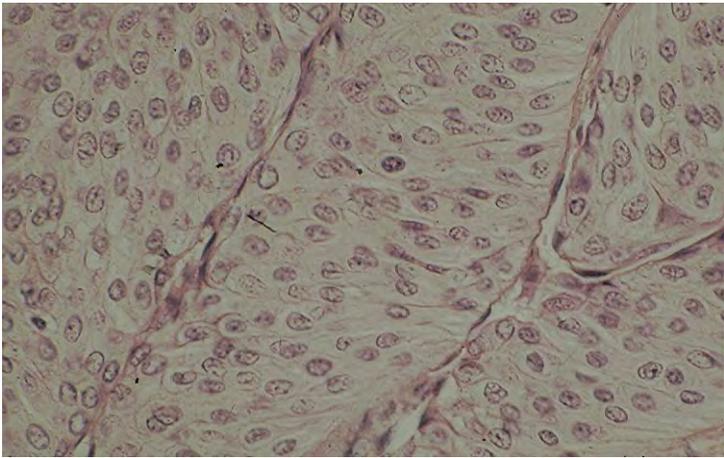
*Small cell carcinoma* is a rare renal neoplasm. It yields in FNA single and clustered small malignant cells with scant cytoplasm and nuclei with finely granular chromatin pattern and nuclear molding. Linear basophilic debris is a common finding. The kidney is occasionally the site of *metastatic cancers* arising from other organs such as breast, lung, gastrointestinal tract, contralateral kidney, gastrointestinal tract, ovary, testis and cutaneous melanoma. These metastatic tumors are often small and multiple but they may be large and mimic a primary renal tumor clinically.

## **TUMORS OF RENAL PELVIS**

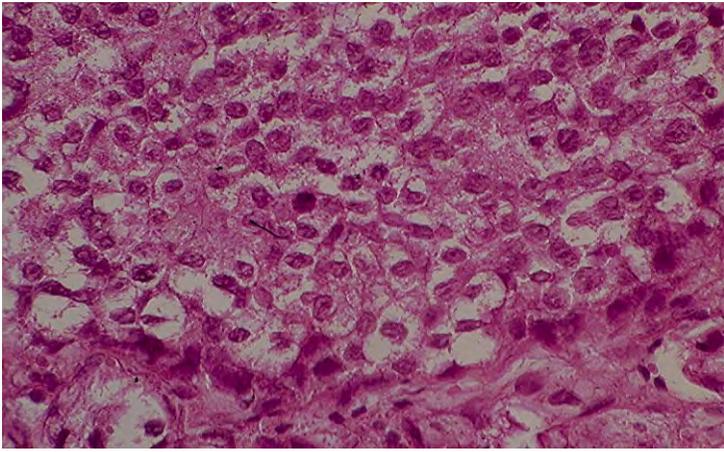
Carcinomas arising from the renal pelvis are similar to those of the urinary bladder and account for less than 5% of all urothelial tumors. Benign and malignant mesenchymal tumors arising from this anatomic site are very rare. The most common clinical manifestation of renal pelvis cancers is gross hematuria (90%), and flank pain is present in 20% of patients.

### **1. Transitional Cell Carcinoma**

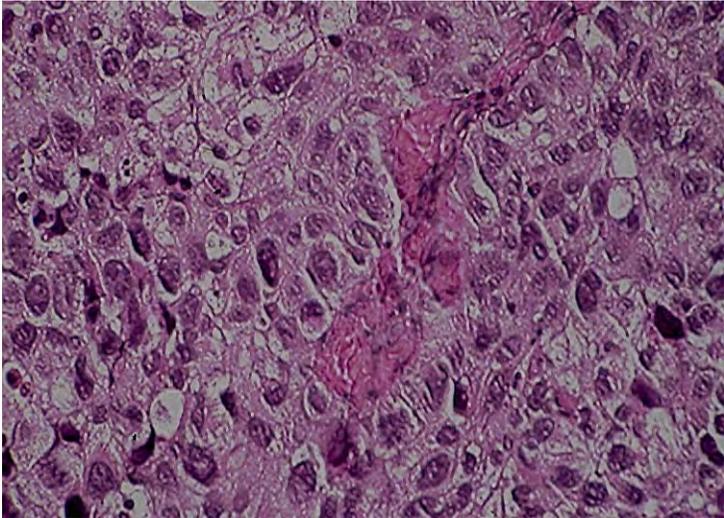
Transitional cell carcinoma (TCC) of the renal pelvis account for 90% of all renal pelvis tumors, and 85% of them are papillary. The tumor occurs mainly in adults aged 50 to 70 years, but pediatric cases have been documented. A history of analgesic abuse, urinary stone or urinary tract infection is present in about 25% of the cases. Depending on the degrees of cellular atypia the tumors can be divided into 3 grades. Grade 1 TCCs show only mild nuclear enlargement, inconspicuous nucleoli. Grade 2 tumors display slightly hyperchromatic, enlarged nuclei. Grade 3 TCCs are characterized by pleomorphic malignant cells with prominent nucleoli. Focal squamous and/or glandular differentiation are commonly present. (Figures 3.11). Grade 2 and 3 are much more common than grade 1 TCCs in this location.



A



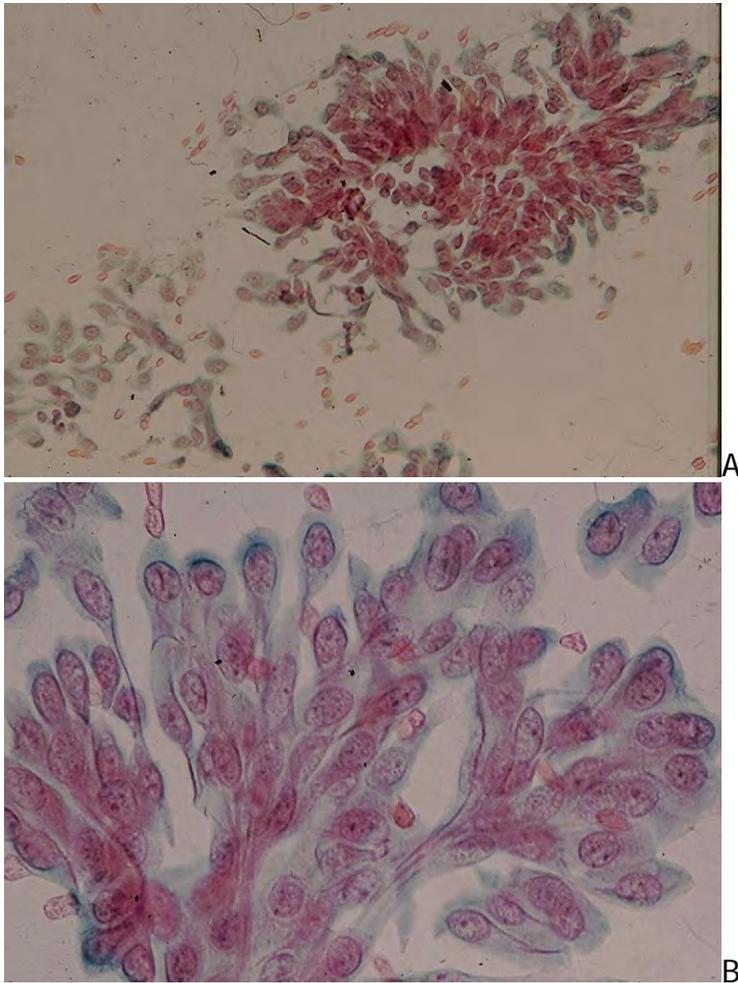
B



C

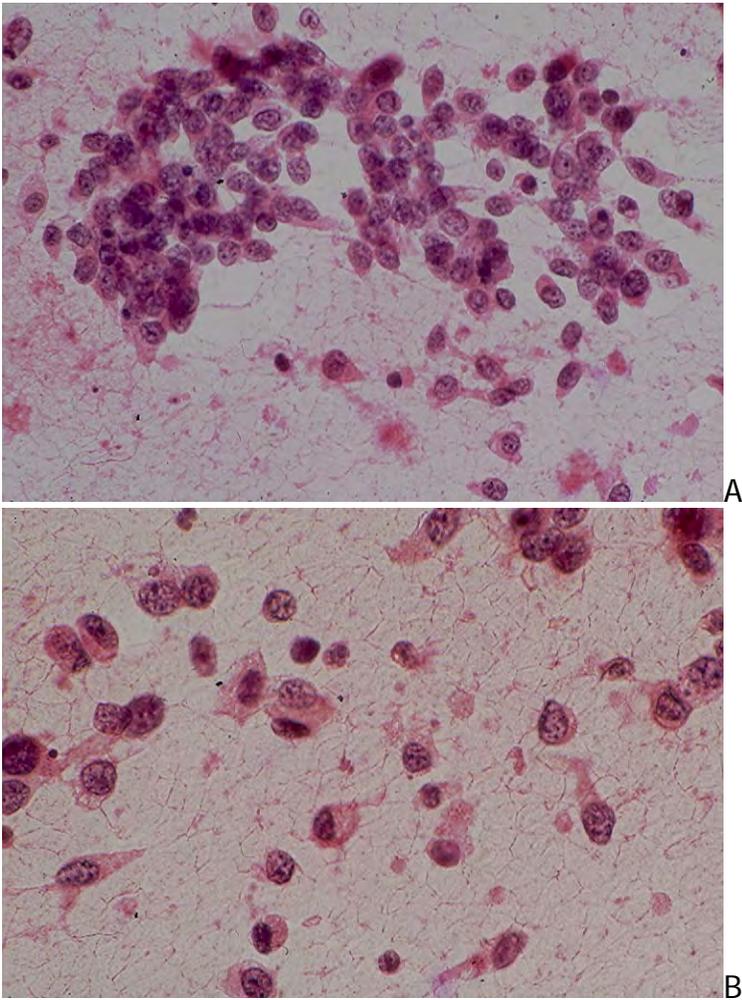
Figures 3-11. Histology of papillary transitional cell carcinoma of the renal pelvis.  
A. A grade 1 tumor showing a thick epithelium with inconspicuous nucleoli.  
B. Grade 2 tumor showing slightly pleomorphic nuclei with conspicuous nucleoli.  
C. Grade 3 tumor showing pleomorphic cells with prominent nucleoli.  
(HE, A-C x 250).

Grade 1 TCCs exfoliate benign appearing urothelial cells in urine sediments while grade 2 and 3 tumors exfoliate malignant tumor cells in urine. If the ureteropelvic junction is obstructed by a tumor mass, no neoplastic cells may be detected in urine sediments, and FNA of the tumor is necessary for establishing its diagnosis. FNA from a grade 1 TCC reveals thick papillary clusters or sheet of benign appearing urothelial cells, and single and loose aggregates of tumor cells with cytoplasmic tails (cercariform cells) are commonly noted. (Figure 3.12).



Figures 3.12. FNA of a grade 1 TCC of the renal pelvis showing in:  
A. Dyshesive and cohesive clusters of tumor cells.  
B. Tumor cells showing "cercariform" configuration with oval nuclei, inconspicuous nucleoli and cytoplasmic "tails" or extensions (Pap, A x 40, B x 400).

A grade 2 TCCs shows in FNA small sheets, loose clusters and syncytial groups of malignant cells with ill- or well- defined cytoplasm, enlarged nuclei with conspicuous nucleoli. Cercariform tumor cells are often present. (Figure 3.13).



Figures 3.13. A and B. FNA of a grade 2 TCC of the renal pelvis showing single and clustered "cercariform" tumor cells with cytoplasmic "tails" or extensions and conspicuous nucleoli (Pap, x 400).

A grade 3 TCC is characterized by single and clustered malignant cells with well-defined cytoplasm, hyperchromatic, pleomorphic nuclei containing prominent nucleoli. Tumor cells with "cercariform" configuration and with squamous or glandular differentiation may be present. (Figure 3.14).

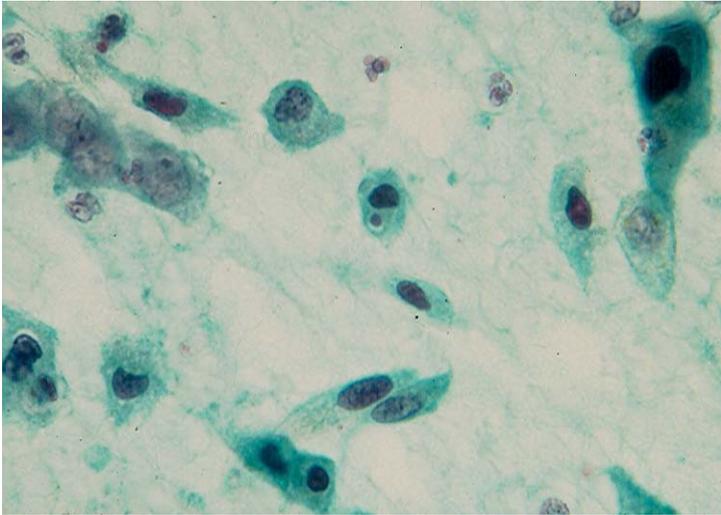


Figure 3.14. FNA cytology of a grade 3 TCC of the renal pelvis showing pleomorphic malignant cells with prominent nucleoli. Tumor cells showing cytoplasmic "tails" or extensions (cercariform cells) are noted (Pap, x 400).

From the immunocytochemical point of view, TCC cells may express Uroplakin III, CK7/CK20, thrombomodulin, P63 and K903, and they are negative for WT-1.

## 2. Other Renal Pelvis Carcinomas

**Squamous cell carcinoma** accounts for about 7% of all carcinomas arising from the renal pelvis, and it is often associated with calculi and infection of the same kidney and renal pelvis. The tumor is commonly associated with pyonephrosis and is often at an advanced stage at the time of diagnosis. It shows in FNA malignant squamous cells in a purulent background. (Figure 3.15).

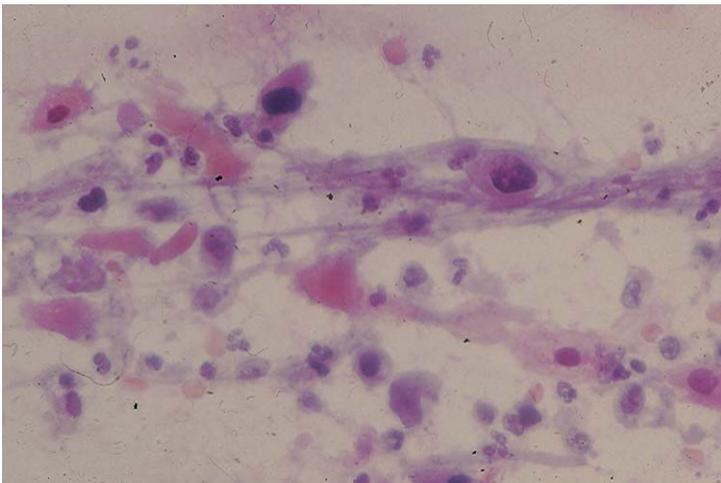


Figure 3.15. FNA of a renal pelvis squamous cell carcinoma showing single malignant keratinizing squamous cells in an inflammatory exudates. (Pap, x 400).

**Adenocarcinoma** and **small cell carcinoma** rarely arise from this location. These two tumors show in FNA single and clustered mucus secreting malignant glandular cells and aggregates of small malignant cells and basophilic linear nuclear debris, respectively. (Figure 3.16).

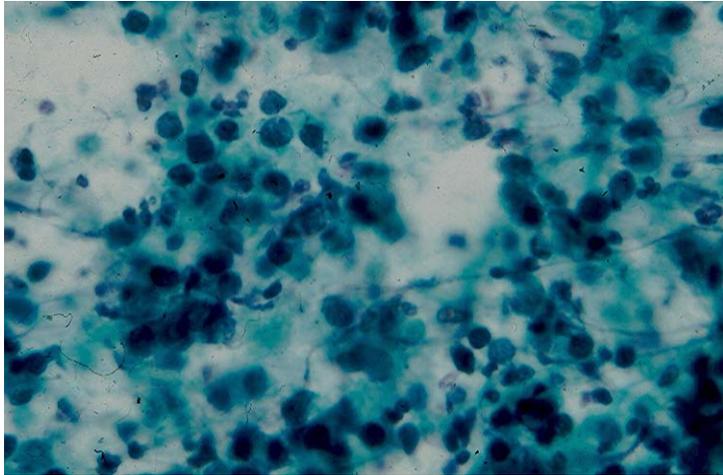


Figure 3.16. FNA of a small cell carcinoma of the renal pelvis reveals single and clustered malignant small cells with hyperchromatic nuclei and focal nuclear molding is noted in a few tumor cell group. (Pap, x 400).

## SUMMARY

FNA of the kidney and renal pelvis for cytologic evaluation is a safe and accurate diagnostic procedure. An angiomyolipoma, renal cell carcinoma, Wilms tumor, metastatic cancer of the kidney and low- and high-grade transitional cell carcinomas of the renal pelvis have characteristic cytologic manifestations in the majority of cases and they may be correctly diagnosed. However, a renal cyst and a renal oncocytoma may only be suggested by FNA.

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## Chapter 4

# ADRENAL GLAND

In this chapter FNA of adrenal mass lesions in adult patients is discussed. This method of investigation is generally not used in infants and children in community general hospitals.

## **INDICATION, CONTRAINDICATION, TECHNICAL CONSIDERATIONS AND COMPLICATIONS OF ADRENAL FNA**

### **Indication and Contraindication**

In adult patients adrenal mass lesions are target of FNA for cytologic evaluation. The only contraindication of adrenal FNA is the presence of a bleeding disorder. FNA proves to be a reliable diagnostic procedure to determine the nature of an adrenal mass lesion that can be neoplastic, inflammatory or degenerative. Adrenal cortical nodules greater than 1 cm in diameter are found in 1-3% of patients undergoing abdominal diagnostic imaging investigation for unrelated disorders, and in 2-9% of all autopsies. Routine work-ups of cancer patients commonly reveal adrenal mass lesions and only about 50% of them are metastatic cancers. The exact nature of an adrenal mass lesion is difficult to diagnose by clinical, diagnostic imaging and biochemical findings. The size and weight of lesions have been practically used to differentiate a benign from a malignant lesion.

### **Technical considerations**

For a palpable lesion the FNA is performed directly through the abdominal wall with manual fixation of the skin over the lesion, using an 8-cm-long needle placed in a syringe holder or pistol. For a non-palpable lesion a 15- to 20-cm-long needle is used and the FNA is guided by either a US or CT abdominal scan. The biopsy may be performed through the anterior abdominal wall or via a lumbar approach. In recent years, FNA of adrenal mass lesions has been successfully performed via endoscopy under US guidance.

Direct smears are prepared from the needle aspirates and stained by either the Papanicolaou method or Diff-Quik stain (modified Wright stain). Minutes tissue fragments found in the FNA should be removed for histologic, immunohistochemical or ultrastructural evaluations. Excess of aspirated material should be fixed in formalin for cell block preparation for histologic and immunohistochemical studies, if indicated. In recent years, electron microscopy is rarely used for tumor typing, as numerous and highly specific antibodies are commercially available. However, it may provide valuable morphologic for this purpose when IM studies yield inconclusive results.

## **Complications**

Complications of adrenal FNA are uncommon. Retroperitoneal hemorrhage has been reported to occur in about 3% and small pneumothorax in about 1% of patients undergoing adrenal mass FNA. Acute pancreatitis may occur following the biopsy of a left adrenal mass via the anterior abdominal wall. FNA of an adrenal pheochromocytoma may cause a fatal catecholamine storm. However, several cases of this tumor that were safely sampled by TFNA have been reported.

## **ADRENAL MASS LESIONS**

Adrenal mass lesions are not uncommon and can be of different natures: neoplastic, inflammatory or degenerative. FNA proved to be a safe, economical, minimally invasive and reliable diagnostic procedure to determine the nature of AMLs, obviating a surgical biopsy to obtain tissue sample for histologic confirmation on numerous occasions. Adrenal cortical nodules greater than 1 cm in diameter are found in 1-3% of patients undergoing abdominal CT for unrelated clinical symptom and in 2-9% of all autopsies; and about 50% of AMLs in cancer patients are benign. The exact nature of an AML is difficult to diagnose by clinical, diagnostic imaging and biochemical findings. The tumor size has been practically used as a criterion to differentiate a benign from a malignant epithelial neoplasm arising from the adrenal: tumors smaller than 3 cm in greatest dimension are usually benign and neoplasms greater than 6 cm in diameter are usually malignant. However, rare exceptions to this criterion do exist.

## **CYTODIAGNOSTIC ACCURACY**

FNA of adrenal tumors has an adequacy rate ranges between 67-100%. The rate of adequate or satisfactory cell samples increases with the presence of a cytopathologist to assess the specimen adequacy. It has an overall sensitivity rate varying between 85 and 94% and a specificity rate approaching 100% in experienced hands. The high cytodiagnostic accuracy rate of FNA of adrenal tumors is the result of the efforts of a

team consisting of a radiologist with experience in FNA techniques and a cytopathologist with expertise in interpreting FNA cytology.

## **NORMAL ADRENAL**

Normal adrenal cells are rarely seen in FNA of AMLs. These cells are seen singly, in loose aggregates or in small sheets. Two types of adrenal cortical cells are usually recognized. Cells from the zona fasciculata are large and contain intracytoplasmic fat droplets. They show an abundant, vacuolated cytoplasm and small oval nuclei with inconspicuous nucleoli. Cells from the zona glomerulosa are not commonly seen and these cells are difficult to differentiate from those of the zona fasciculata. Cells from the zona reticularis are smaller, polygonal in shape and show granular, eosinophilic cytoplasm without intracytoplasmic vacuoles. Their nuclei are similar to those of cells derived from the zona fasciculata. Cells from the adrenal medulla are characterized by a slightly abundant, basophilic cytoplasm and large, eccentrically located nuclei displaying a finely granular chromatin pattern and conspicuous nucleoli.

## **ADRENAL TUMORS**

### **Adrenal Myelolipoma**

Adrenal myelolipoma is a rare tumor. It is usually asymptomatic and incidentally discovered by diagnostic imaging procedures performed during the investigation of an unrelated condition, and a large adrenal myelolipoma may cause abdominal discomfort. It shows in FNA large fragments of benign fatty tissue admixed with bone marrow cells such as myelocytes, metamyelocytes, megakaryocytes and erythroblasts. (Figures 4.1 and 4.2).

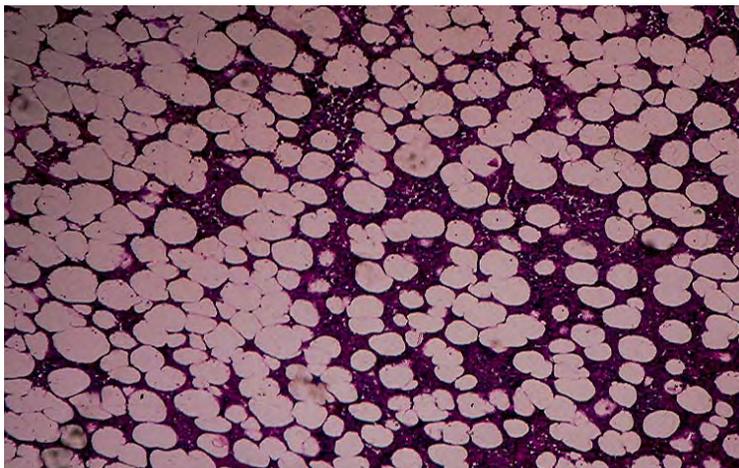
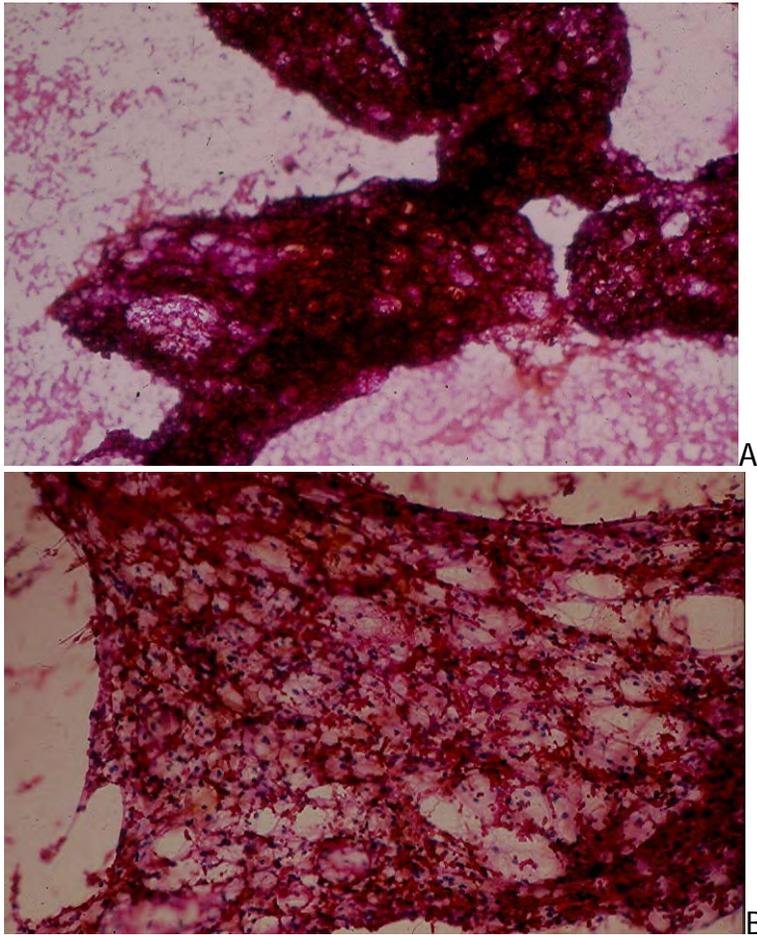


Figure 4.1. Histology of an adrenal myelolipoma. (HE, x 100).



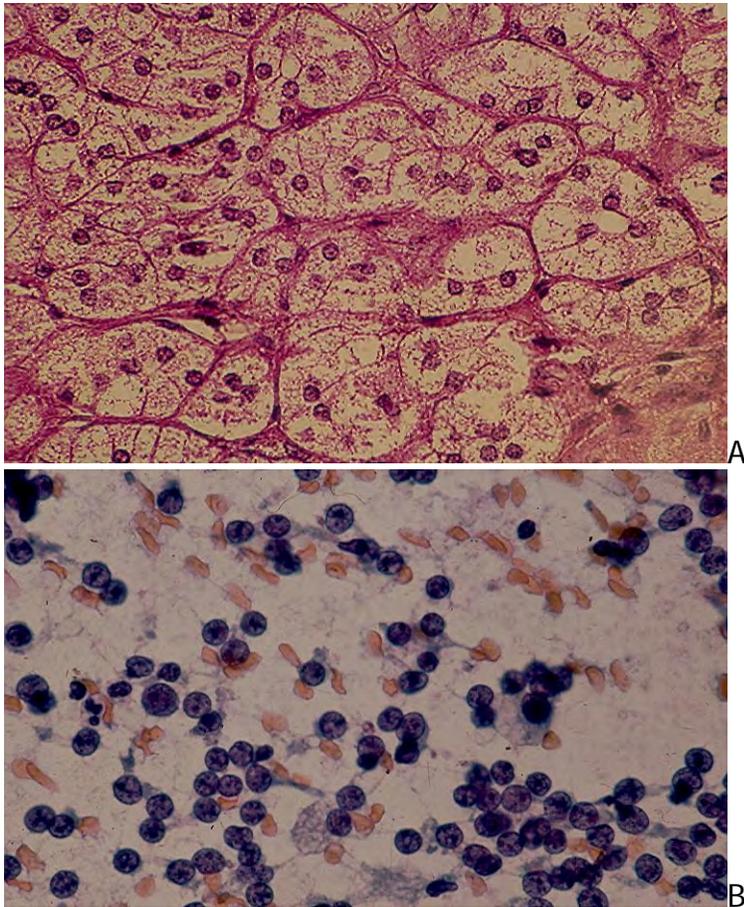
Figures 4.2. FNA of an adrenal myelolipoma showing fragments of benign fatty tissue admixed with bone marrow cells. (HE, A x 40, B x 100).

### **Adrenal Cortical Adenoma**

Adrenal cortical adenoma (ACA) is common lesion and can be found in 2-9% of autopsies. It is usually discovered incidentally during clinical investigation of an unrelated condition. ACA is usually non-functioning, and on rare occasions it may be associated with aldosteronism or Cushing syndrome. ACA usually measures less than 3 cm in greatest dimension. Identification of an ACA is important in patients with malignant diseases as the adrenal is a common site of metastases from bronchogenic and breast carcinomas as well as cutaneous melanomas, and about 50% of cancer patients have AMLs that are ACAs.

Histologically, an ACA is a well-circumscribed or encapsulated lesion that is composed of zona fasciculata- or zona glomerulosa-type cells with occasional cells having

pleomorphic nuclei. It shows in FNA single and clustered polygonal epithelial cells with vacuolated cytoplasm and oval nuclei containing conspicuous nucleoli. (Figures 4-3). Mutinucleate tumor cells are present as well as numerous stripped tumor cell nuclei that may be mistaken for those of a metastatic small cell carcinoma to the adrenal. The neoplastic cell cytoplasm stains strongly positively with low molecular weight keratin and vimentin antibodies, does not express epithelial membrane antigen (EMA) but reacts positively with adrenal 4 binding protein, A103 (melan A) and inhibin antibodies.



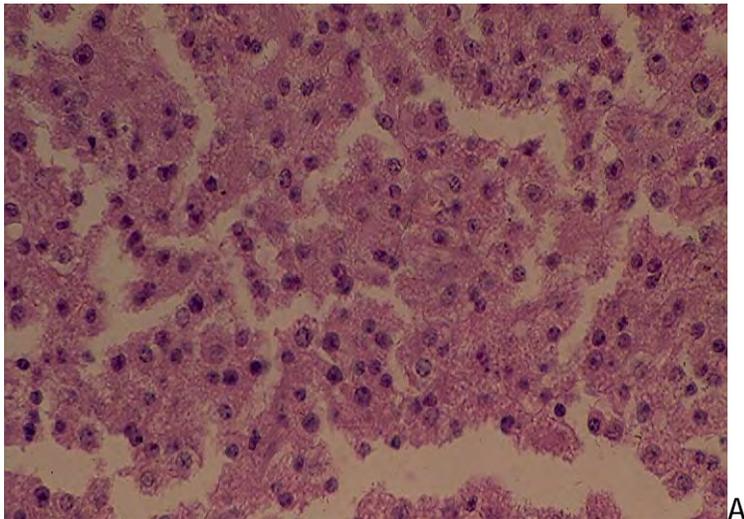
Figures 4.3. A. Histology of an adrenal cortical adenoma (HE, x 250)  
B. FNA of the tumor showing numerous naked tumor cell nuclei and a few tumor cells with foamy, clear cytoplasm. (Pap, x 400).

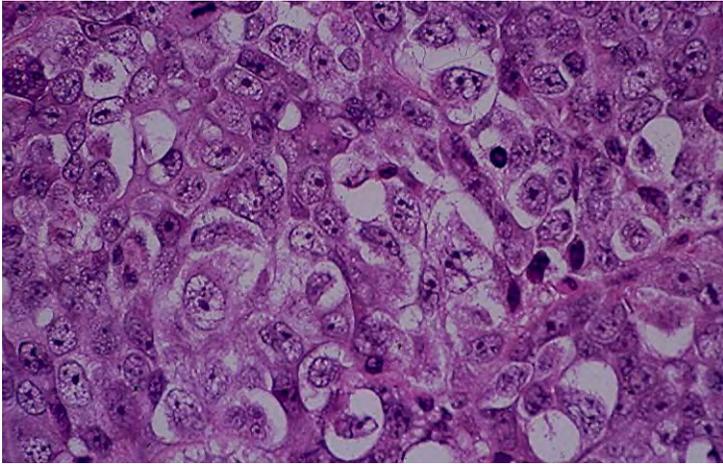
### **Adrenal Cortical Carcinoma**

Adrenal cortical carcinoma (ACC) is a very rare tumor. Its prevalence in the general population is estimated to be about 1 in 250,000 and it affects both male and female patients at all age groups. An ACC may be non-functional, associated with virilization or Cushing syndrome, or both syndromes. The tumor is usually larger than 6 cm in

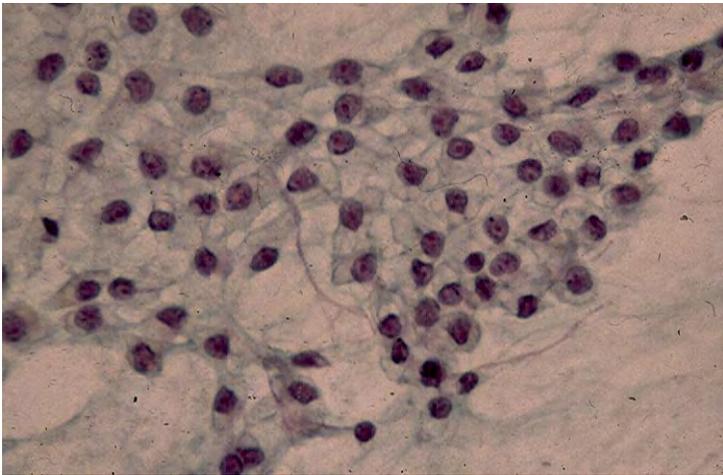
greatest dimension, and a palpable adrenal cortical tumor is malignant in practically every instance. However, smaller ACCs have also been documented.

Histologically, an ACC may be composed of benign-appearing cells as seen in an ACA, or it may consist of bizarre malignant cells. As a result, an ACC may yield in FNA cells similar to those seen in a needle aspirate from an ACA or malignant epithelial cells with clear, vacuolated or granular cytoplasm present singly and in sheets. (Figures 4.4 and 4.5). Depending on the tumor grade the nuclei of ACC cells may be small, monomorphic with inconspicuous nucleoli or large, pleomorphic with prominent nucleoli. These cells are EMA, CEA and B72.3 negative and react positively to adrenal 4 binding protein, A103 (melan A), keratin and vimentin antibodies. Recent molecular studies on tumor cells removed from the smears by microdissection in 10 cases of ACC have demonstrated that at least a loss of heterozygosity at p53 locus on chromosome 17 and/or the putative neuroblastoma tumor suppressor locus on chromosome 1 and/or the von Hippel-Lindau locus on chromosome 3 were present. This finding was not observed in any benign adrenal cortical lesions or in normal adrenal tissue; and it may be helpful in distinguishing an ACC from an ACA. However, more case studies are needed to validate this interesting finding.

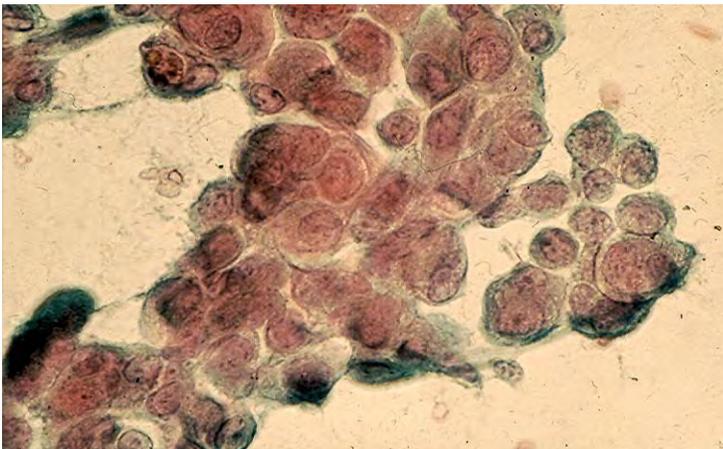




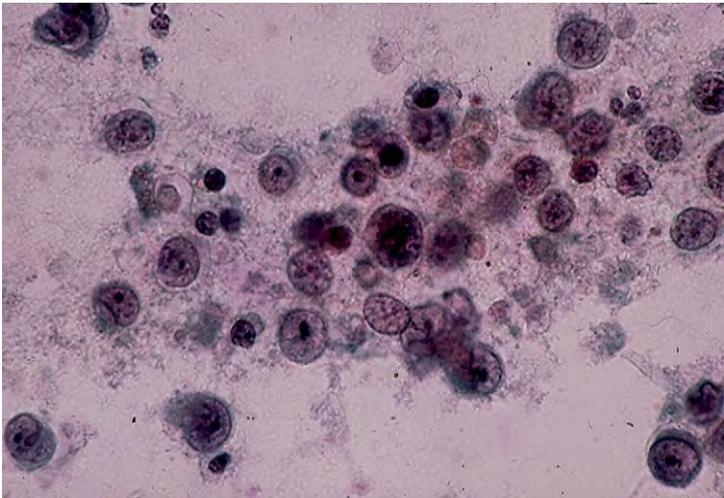
Figures 4.4. Histology of an adrenal cortical carcinoma showing:  
A. Tumor cells with granular cytoplasm and small oval nuclei in solid pattern.(HE,x 250).  
B. Large pleomorphic malignant cells with prominent nucleoli and clear or granular cytoplasm.(HE, x 250).



A



B



Figures 4.5. FNA of 3 adrenal cortical carcinomas showing in FNA:  
 A. A large monolayered sheets of benign appearing cells with oval nuclei, clear cytoplasm in honeycomb pattern.  
 B. Loosely clustered large, pleomorphic malignant cells with granular cytoplasm.  
 C. Pleomorphic malignant cells with clear, ill-defined cytoplasm and large nuclei with prominent nucleoli. (Pap, A-C x 400).

## Pheochromocytoma

This tumor arises from the paraganglionic system and is classically known as a 10-percent tumor: 10% are malignant, 10% bilateral, and 10% extra-adrenal. About 20% of all pheochromocytomas are asymptomatic or non-functioning, and 80% of the patients have hypertension secondary to catecholamine secretion by the tumor. They may be associated with multiple endocrine neoplasia syndrome of type IIA or IIB. The majority of pheochromocytomas can be confidentially diagnosed by clinical findings, abdominal diagnostic imaging methods and measuring urine catecholamines and their metabolites, principally metanephrines and vanillylmandelic acid. However, cases without clinical and biochemical characteristic features of pheochromocytoma exist. When a pheochromocytoma is confidentially diagnosed FNA of the tumor for cytologic confirmation is contraindicated as it may cause fatal catecholamine storm.

Histologically, a pheochromocytoma is characterised by irregular nests of tumor cells separated by fibrovascular septae. The tumor cells have an ill-defined, granular cytoplasm and oval, elongated or pleomorphic nuclei. Intracytoplasmic melanin pigment may be present in a small number of cases. The tumor cell cytoplasm stains positively with neuron-specific enolase, synaptophysin and chromogranin antibodies and shows numerous intracytoplasmic pleomorphic neurosecretory granules by ultrastructural study.

FNA from a non-functioning and unsuspected pheochromocytoma reveals 3 types of cells in different proportions. The most common cells are small-to medium-sized cells with ill-defined and fibrillary cytoplasm in syncytial clusters or sheets, with oval or slightly pleomorphic, hyperchromatic nuclei and small nucleoli. Spindle and large myoid-like cells with eccentrically located nuclei are present but in small number. (Figure 4. 6). Staining of a tumor cell block sections with neuron-specific enolase, chromogranin and synaptophysin antibodies will reveal a strong immuno-positive reaction of the tumor cell cytoplasm with these antibodies. (Figures 4.7 to 4.9).

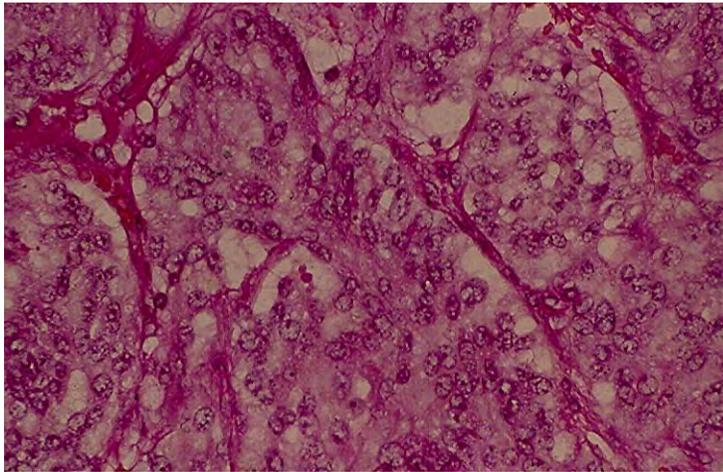
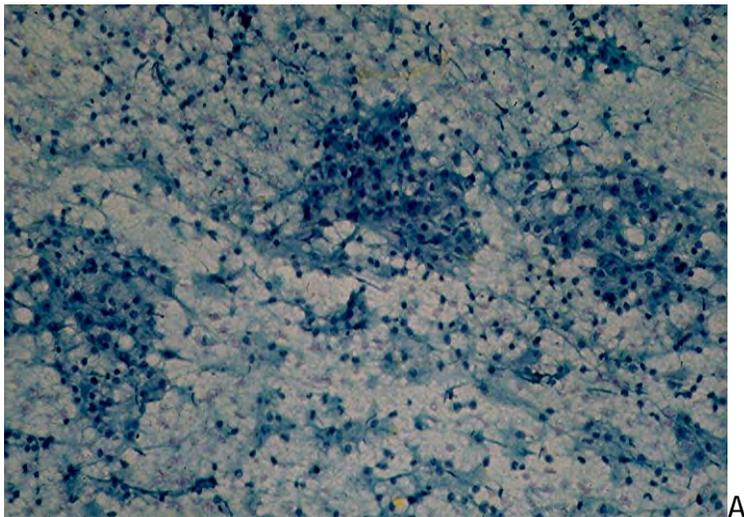
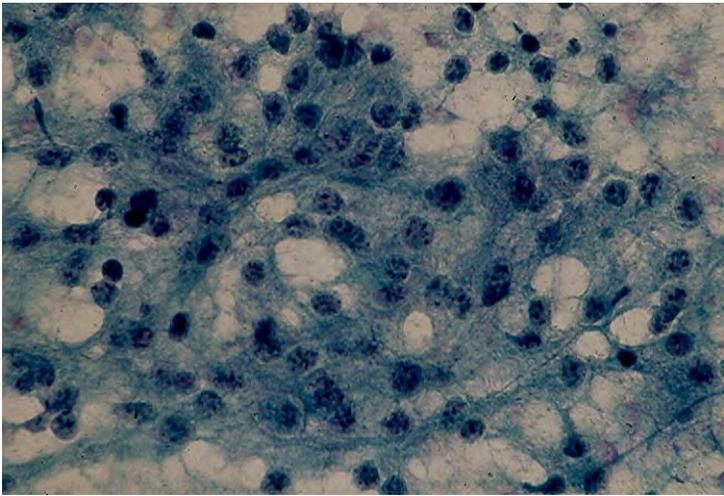
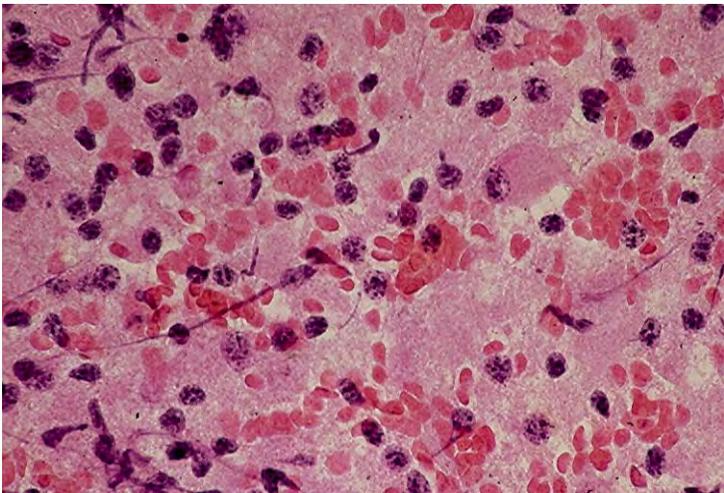


Figure 4.6. Histology of an adrenal pheochromocytoma (HE, x 250).





B



C

Figures 4.7. FNA of an adrenal pheochromocytoma showing:  
A. Tumor cells present singly and in large sheets.  
B. A large sheet of tumor cells showing ill-defined, granular cytoplasm and oval nuclei.  
C. Single tumor cells with plasma cell configuration showing granular cytoplasm and eccentrically located round nuclei.  
(Pap, A x 100, B x 400; HE, C x 400).

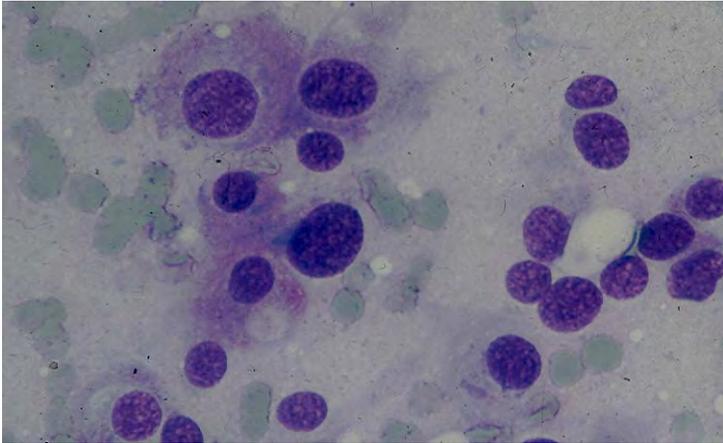
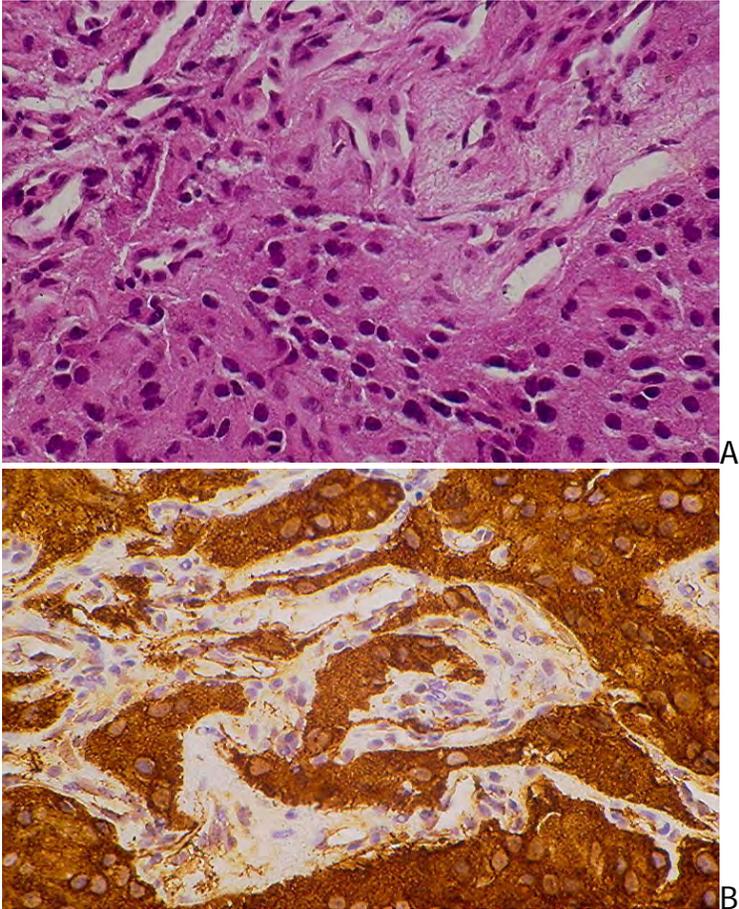
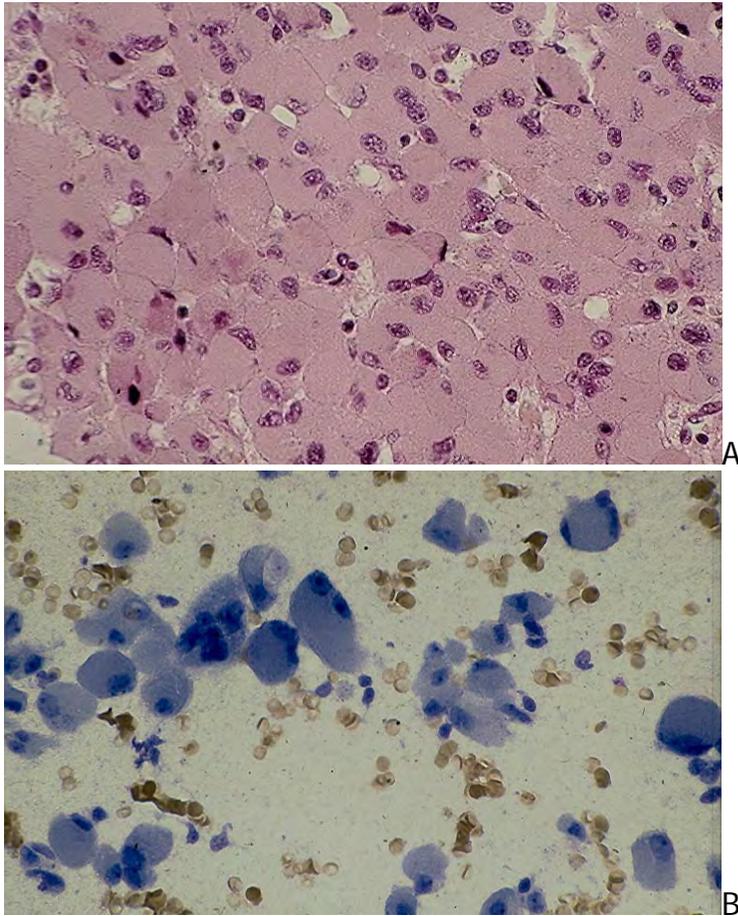


Figure 4.8. FNA from an adrenal pheochromocytoma showing a few polygonal tumor cells with variable abundant, ill-defined cytoplasm, eccentrically located nuclei and intracytoplasmic azurophilic granules and naked tumor cell nuclei. (Diff-Quik, x 500).



Figures 4.9. A and B. FNA cell block from a pheochromocytoma showing a fragment of tumor tissue consisting of polygonal cells with granular cytoplasm that stains positively with chromogranin antibody (A, HE, x 250; B, ABC x 250).

**Adrenal Oncocytic Tumor** is a highly uncommon neoplasm and can be benign or malignant. FNA from an adrenal oncocytic tumor reveals single and clustered polygonal cells with abundant, granular cytoplasm and centrally or eccentrically located oval nuclei (Figures 4.10). The tumor cells show by electron microscopy abundant intracytoplasmic smooth endoplasmic reticulum and mitochondria with tubulovesicular crystals and react positively with adrenal 4 binding protein and mitochondria antibodies.



Figures 4.10. Adrenal oncocytic tumor.

A. Histology of an adrenal oncocytoma (HE, x 250).

B. Single tumor cells with abundant granular cytoplasm and small nucleoli seen in FNA of an adrenal oncocytoma (Diff Quik, x 250).

### Metastatic Cancers

Adrenals are common sites for metastases of cancers arising from other anatomic sites. The involvement is usually bilateral and rarely cause adrenal insufficiency. Bronchogenic carcinomas have a strong affinity to adrenals, and metastases from carcinomas of the

breast, stomach, colon, pancreas, kidney and cutaneous melanomas are also common. Cytodiagnosis of metastatic cancers to adrenals by FNA is usually straightforward. A special staining of aspirated tumor cells with mucicarmine, periodic acid-Schiff or IM studies with S-100 protein, HMB-45, chromogranin, neuron-specific enolase, carcinoembryonic antigen, renal cell carcinoma, inhibin, TTF-1, CK7, WT-1 and HepPar antibodies may provide valuable information for a more accurate tumor typing.

A renal cell carcinoma may invade the adjacent adrenal gland and yields in FNA cells similar to those of an ACC. IM staining of the tumor cells with renal cell carcinoma, inhibin and HepPar antibodies will be helpful to differentiate an ACC from other tumors having a clear cytoplasm change such as renal cell carcinoma, hepatocellular carcinoma, bronchogenic carcinoma, ovarian carcinoma and epithelial mesothelioma. Electron microscopic study of aspirated tumor cells may also reveal useful information for tumor typing, as an ACC shows abundant intracytoplasmic smooth endoplasmic reticulum and mitochondria with tubulovesicular crystals while cells derived from a renal cell carcinoma are rich in glycogen and lipid and show short microvilli on their free borders. (Figures 4.11 and 4.12).

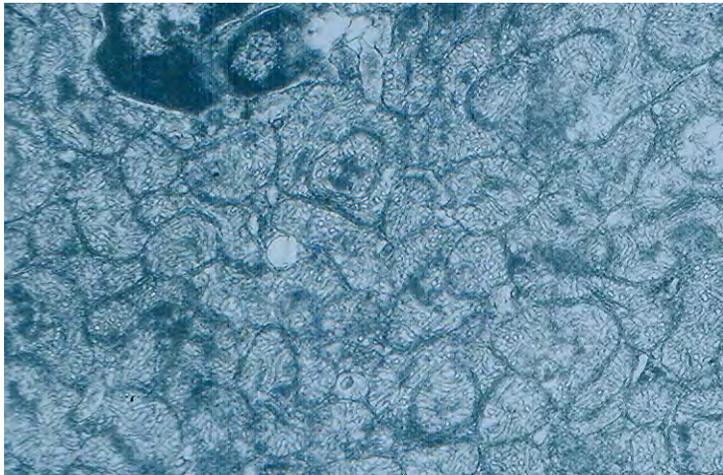


Figure 4.11. Ultrathin section of a minute tissue fragment from an adrenal cortical carcinoma showing abundant intracytoplasmic smooth endoplasmic reticulum and intramitochondrial tubulovesicular crystals (Uranyl acetate and lead citrate x 30,000).

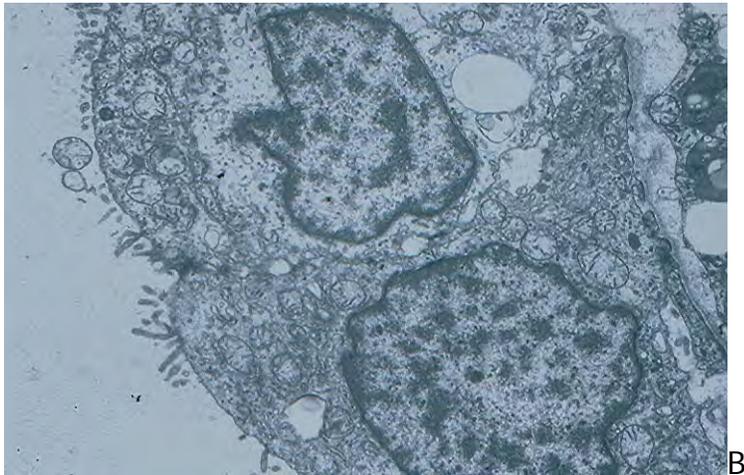


Figure 4.12. Ultrathin section of a minute tissue fragment from a renal cell carcinoma invading the adrenal showing epithelial cells with well-formed cell junctions, intracytoplasmic fat droplets and glycogen and a few short and irregular microvilli on the free tumor cell surface (Uranyl acetate and lead citrate x 24,000).

## OTHER ADRENAL MASS LESIONS

*An adrenal endothelial cyst* is also a rare lesion that yields in FNA fragments of endothelium with reniform nuclei. *Adrenal hyperplasia* in adults is usually bilateral and can be nodular or diffuse. The nodular form has no clinical significance, but the diffuse form can be associated with aldosteronism and Cushing syndrome. FNA of a hyperplastic adrenal reveals cells similar to those of an adrenal adenoma. A few cases of fungal infection of the adrenal with *histoplasmosis* or *cryptococcosis* correctly diagnosed by FNA have been reported.

## SUMMARY

Fine needle aspiration is a reliable procedure to identify adrenal tumors. Most adrenal neoplasms, such as adrenal myelolipoma, adrenal cortical adenoma, pheochromocytoma, some cases of adrenal cortical carcinoma and metastatic carcinomas have specific cytologic manifestations, permitting a correct diagnosis in the majority of cases. The tumor size has been used as a criterion of malignancy for primary adrenal epithelial neoplasms: tumors larger than 6 cm in greatest dimension are practically malignant regardless of their benign appearing cytologic and histologic features. Immunohistochemical and/or ultrastructural studies of aspirated tumor cells may yield important information for tumor typing in more problematic cases.

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## Chapter 5

# OTHER MASS LESIONS

In this chapter, cytologic aspects of important retroperitoneal tumors and a few intraabdominal neoplasms are illustrated and briefly discussed. Cell block and core tissue biopsy should be obtained for histologic and immunohistochemical studies that will provide more valuable information for tumor typing. A comprehensive discussion on tumor immunohistochemistry of tumor core tissue is not attempted as it is beyond the scope of this monograph. The readers are referred to standard Surgical pathology textbooks for tumor immunohistochemistry.

Retroperitoneal spaces in the past were not easily accessible by roentgenograms, and exploratory laparotomy with tumor tissue biopsy was the only means available for excluding or confirming a malignant retroperitoneal tumor in many cases. With the event of US and CT scans, magnetic resonance imaging, guided FNA cytology and core-needle biopsy for histologic evaluations, almost all retroperitoneal mass lesions can be correctly identified preoperatively. Therefore, appropriate treatments can be tailored for individual cases.

In one large series consisting of 308 cases of intraabdominal and retroperitoneal tumors investigated by US-guided TFNA reported by Droese and associates, the overall diagnostic accuracy rate was 88.9% with a sensitivity of 84.4% and specificity of 98%. The predictive values of positive and negative results were 98.9% and 74.6%, respectively. Juul et al. reported 96 cases of retroperitoneal mass lesions examined by US-guided TFNA. An adequate cytologic material was obtained in 91 cases (95%) and a correct cytodagnosis was made in 88% of the patients, with no false-positive reports. Suen reviewed 255 cases of retroperitoneal FNA and found an overall diagnostic accuracy of 90.5%, a sensitivity of 96%, a specificity of 94%, a predictive value of a positive result of 99% and a predictive value of a negative result of 79%.

Excluding mass lesions arising from the duodenum, pancreas, adrenal gland, kidney, urinary excretory system, 4 major groups of malignant tumor are encountered in retroperitoneum: soft-tissue tumors, lymphomas, metastatic cancers and primary germ cell tumors.

## SOFT TISSUE TUMORS

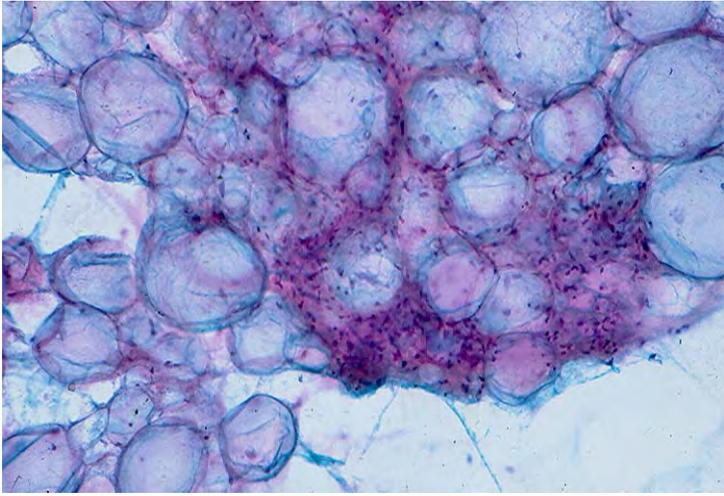
Almost all types of soft tissue neoplasm can arise in the retroperitoneum and benign tumors are rare and outnumbered by sarcomas. Of the sarcomas, liposarcoma, malignant fibrous histiocytoma and leiomyosarcoma are the most common ones. Rhabdomyosarcoma occurs mainly in children and is often of embryonal type. Fibrosarcoma and neurogenic sarcoma are rare in this location. The soft tissue neoplasms are complex and their typing is difficult and full of pitfalls, even with the availability of numerous commercial antibodies and electron microscopy. When FNA cytology is combined with multiple core needle biopsies much more information will be obtained for tumor typing. Recently, with the adjunctive utility of molecular genetic techniques, classification of soft tissue sarcomas has become more feasible. For additional information on the utility of molecular techniques in cytodagnosis of soft tissue neoplasms the readers are referred the article by Kilpatrick and Geisinger, listed in the bibliography section.

Due to the limited amount of cytologic material obtained by FNA, it is not always possible to classify soft-tissue sarcomas according to histogenesis. For a practical diagnostic approach, it is possible to separate soft tissue sarcomas into 5 main cytologic groups:

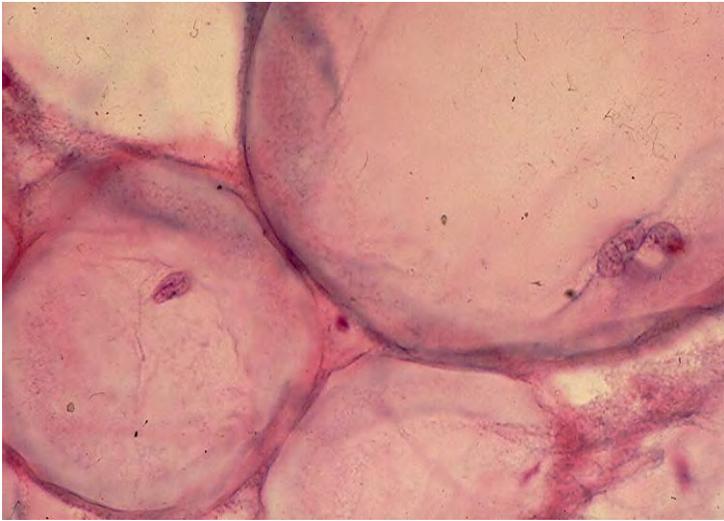
1. Fat cell (round cell, well-differentiated and pleomorphic liposarcomas)
2. Spindle cell (leiomyosarcoma, hemangiopericytoma, myxoid liposarcoma, fibrosarcoma, malignant nerve sheath tumor, spindle cell synovial sarcoma, angiosarcoma ...)
3. Pleomorphic cell (malignant fibrous histiocytoma, pleomorphic rhabdomyosarcoma, biphasic synovial sarcoma...)
4. Round cell (embryonal and alveolar rhabdomyosarcomas, Ewing sarcoma, neuroblastoma, desmoplastic small round cell tumor...)
5. Epithelioid/polygonal cell (epithelioid sarcoma, epithelioid schwannoma, alveolar soft part sarcoma, malignant melanoma of soft part, epithelioid angiosarcoma...)

### 1. Fat cell tumors

These neoplasms are typified by a round cell, a well-differentiated and a pleomorphic liposarcomas. FNA from a well-differentiated liposarcoma reveals clustered mature fat cells displaying enlarged nuclei (Figures 5.1). A pleomorphic liposarcoma yields in FNA single and loosely clustered bizarre malignant lipoblasts with pleomorphic nuclei and vacuolated cytoplasm. (Figure 5.2).



A



B

Figures 5.1. FNA of a retroperitoneal well-differentiated liposarcoma showing tumor cells with slightly enlarged nuclei and inconspicuous nucleoli. (Pap, A x 100, B x 400).

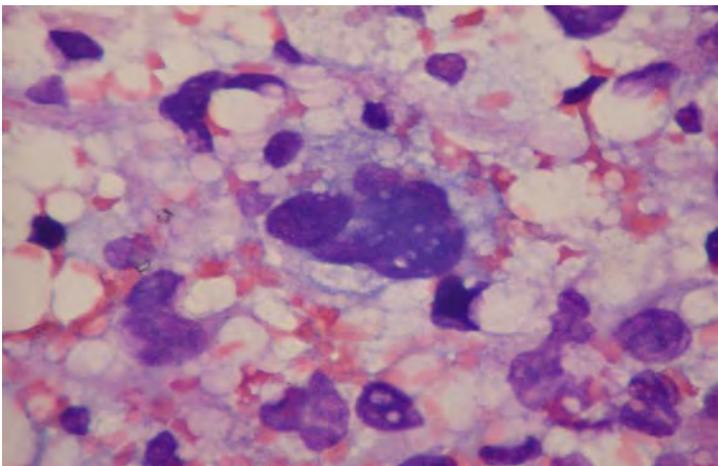
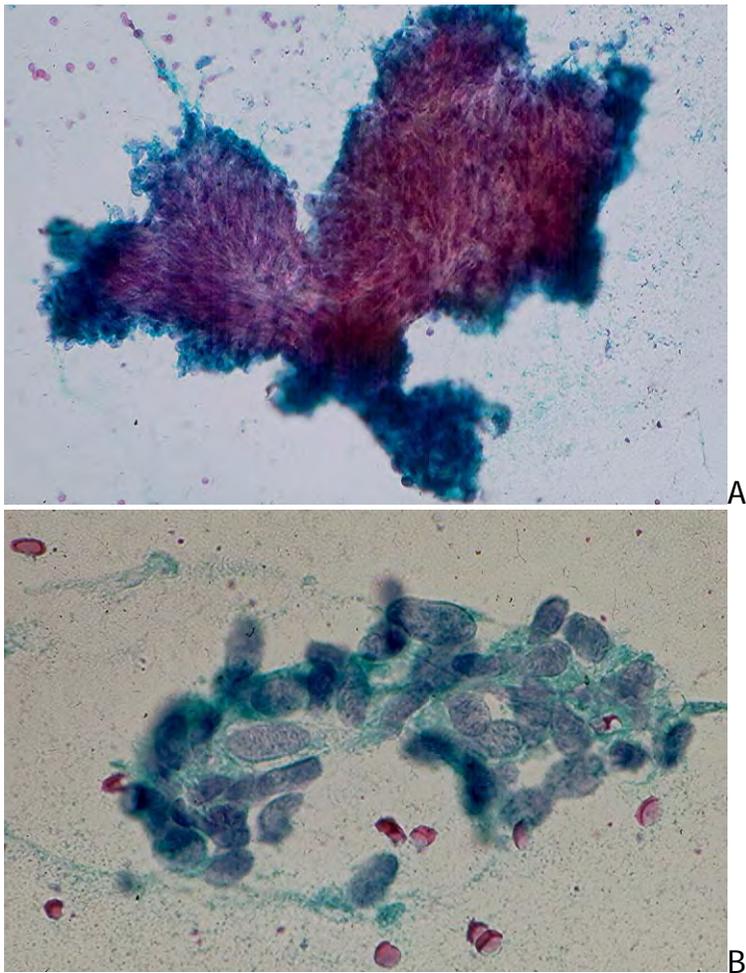


Figure 5.2. FNA of a pleomorphic liposarcoma showing single bizarre tumor cells with single or multiple nuclei, prominent nucleoli and vacuolated cytoplasm. (Diff-Quik,x400).

## 2. Spindle cell tumors

*A low-grade leiomyosarcoma* yields in FNA spindle-shaped malignant smooth muscle cells singly and in minute tissue fragments with tumor cells arranged in parallel fashion. (Figures 5.3).



Figures 5.3. FNA of a retroperitoneal well-differentiated leiomyosarcoma showing in:  
A. A large and irregular fragment of spindle tumor cells.  
B. A cluster of smooth muscle cells with cigar-shaped and blunt-ended nuclei.  
(Pap, A x 40; B x 400).

FNA from a *hemangiopericytoma* reveals dyshesive and cohesive clusters of spindle cells with bland nuclei and ill-defined cytoplasm. Tumor cells arranged around a space may be observed. (Figure 5.4). A *malignant schwannoma* yields in needle aspirate dyshesive, malignant spindle cells with elongated or oval nuclei. (Figure 5.5).

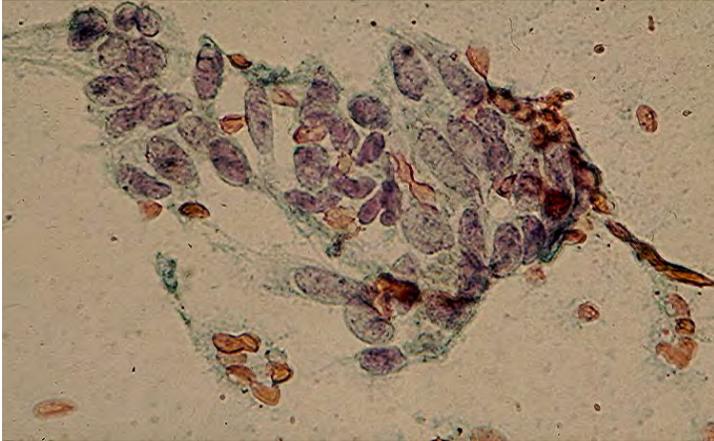


Figure 5.4. FNA of a retroperitoneal hemangiopericytoma reveals a loosely clustered spindle tumor cells with elongated, blunt-end and hypochromatic nuclei (Pap, x 400).

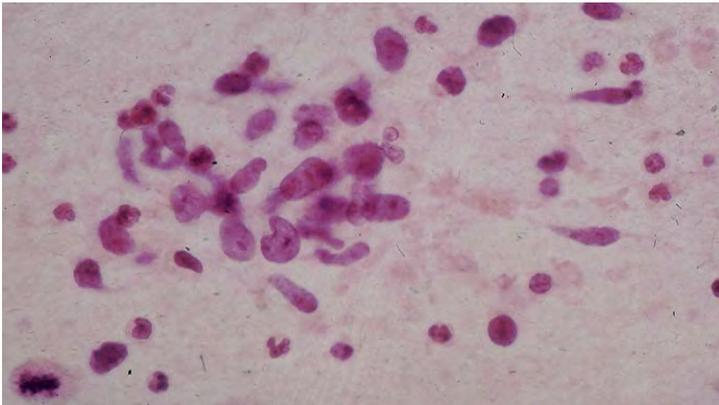
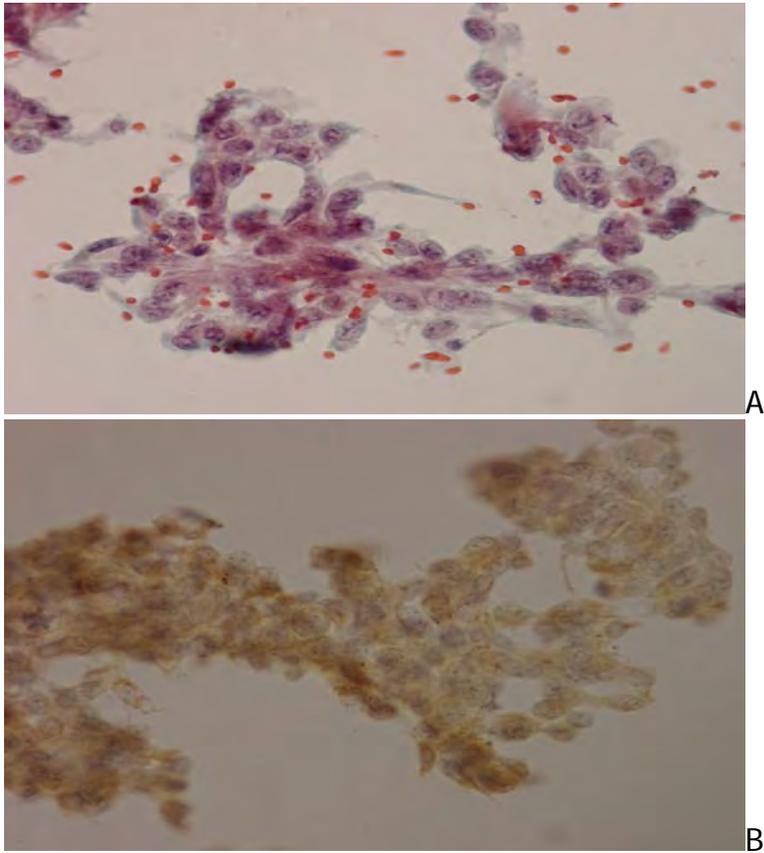


Figure 5.5. A malignant Schwannoma shows in FNA an aggregate of spindle and slightly pleomorphic nuclei. (HE, x 400).

An *angiosarcoma* shows in FNA single and clustered malignant spindle cells forming lumens (vasoformative) that express Factor VIII related antigen. (Figures 5.6).



Figures 5.6. FNA from an angiosarcoma showing in:  
 A. Spindle malignant cells with vasoformative arrangement.  
 B. Tumor cells expressing Factor-VIII related antigen.  
 (A, Pap, x 400; B, ABC x 400).

### 3. Pleomorphic cell tumors

FNA from a retroperitoneal *malignant fibrous histiocyoma* reveals bundles of spindle cells admixed with polygonal cells and large multinucleated cells. A *pleomorphic rhabdomyosarcoma* yields in needle aspirates pleomorphic malignant cells with enlarged, hyperchromatic and eccentrically located nuclei and globular, eosinophilic cytoplasm. A *biphasic synovial sarcoma* displays in FNA clustered spindle cells and polygonal epithelioid cells. (Figures 5.7 -5.9).

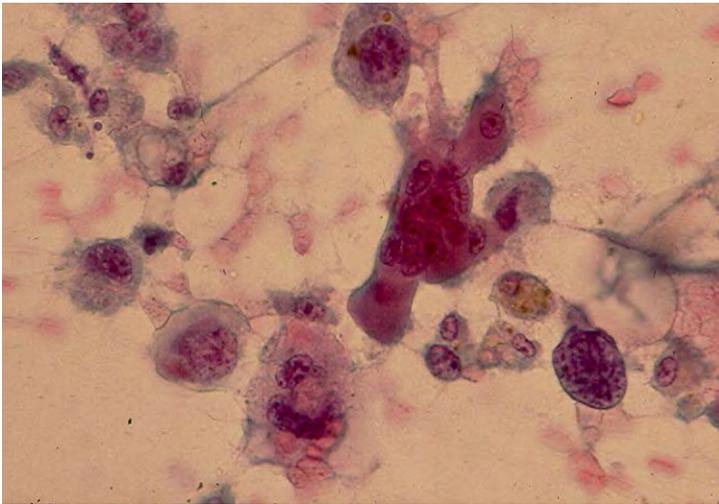


Figure 5.7. FNA of a malignant fibrous histiocytoma shows isolated, pleomorphic malignant cells and a multinucleated giant cell. (Pap, x 400).

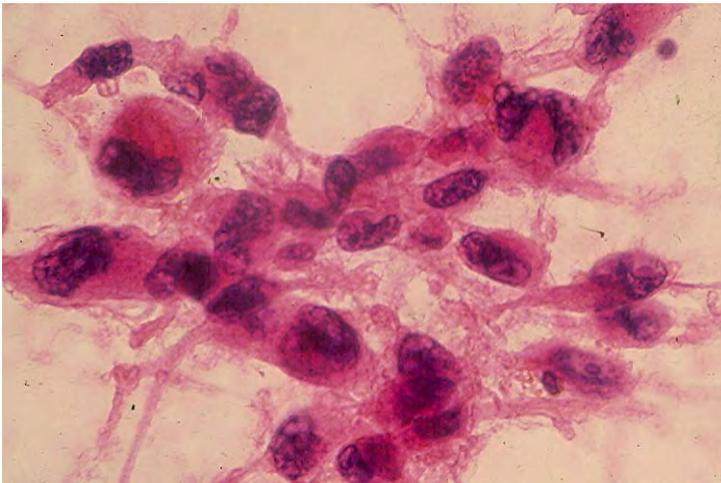
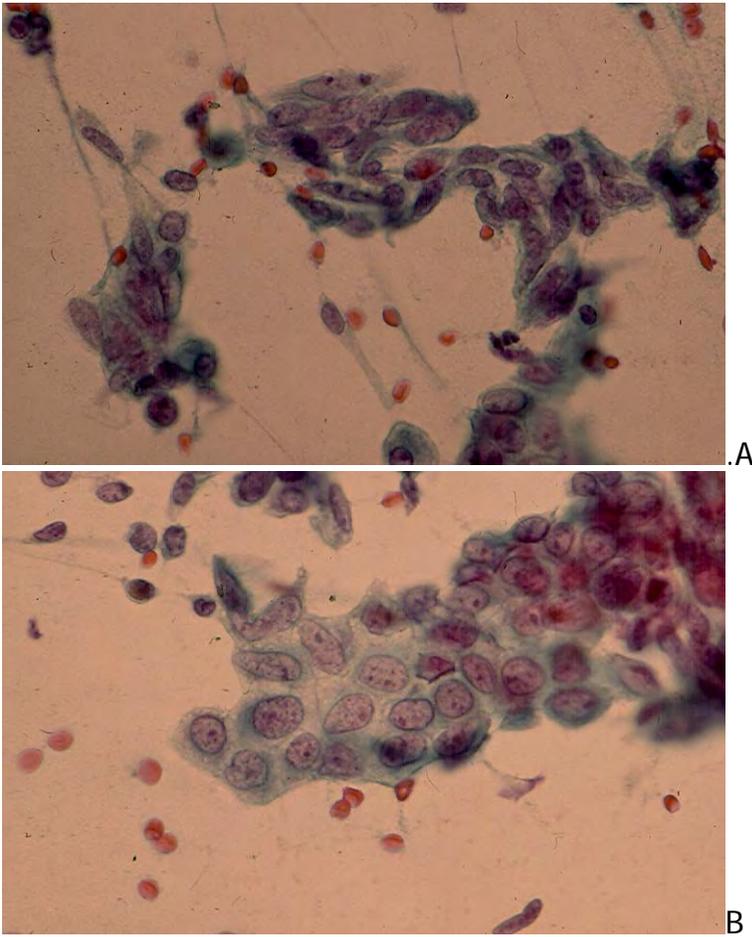


Figure 5.8. A pleomorphic rhabdomyosarcoma showing in FNA a loosely clustered pleomorphic malignant cells. (HE, x 400).



Figures 5.9. A biphasic synovial sarcoma showing in FNA:  
 A. Clusters of spindle tumor cells.  
 B. A sheet of epithelioid tumor cells with oval nuclei and inconspicuous nucleoli.  
 (Pap, A and B x 400).

#### 4. Round cell tumors

FNA from an *embryonal rhabdomyosarcoma* reveals abundant round malignant cells with hyperchromatic nuclei. An extraskeletal *Ewing sarcoma* displays in needle aspirates abundant round malignant cells singly and in clusters. The tumor cell cytoplasm has a high glycogen content and stains positively with PAS. Pseudo-rosette formation may be observed. FNA from a *neuroblastoma* yields abundant malignant small round cells showing focal, vague rosette formation. The tumor cells react positively with chromogranin and neuron-specific enolase antibodies. (Figures 5.10 and 5.11).

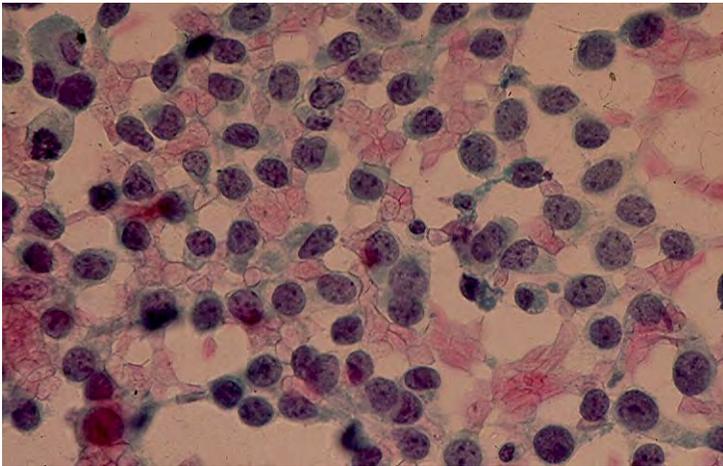


Figure 5.10. FNA of an Ewing sarcoma showing dyshesive round tumor cells with scant cytoplasm and round or oval nuclei (Pap, x 400).

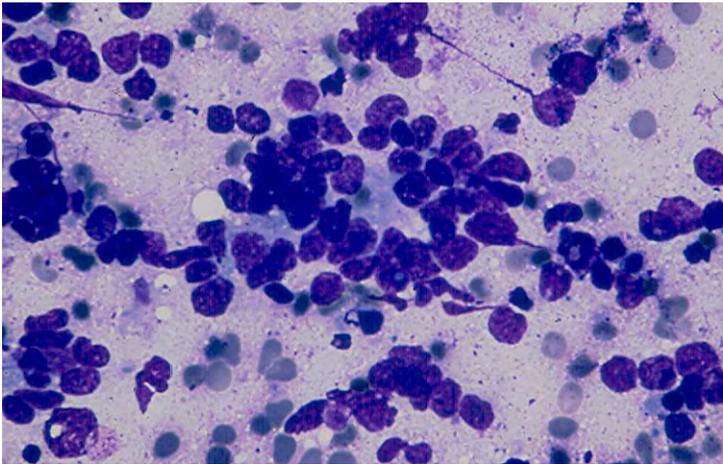


Figure 5.11. Aspirated cells from an extraadrenal neuroblastoma showing numerous small round cells with scant cytoplasm. Tumor cells forming a rosette are seen at the center of the figure (Diff-Quik, x 400).

## 5. Epithelioid cell tumors

FNA from a *malignant melanoma of soft parts* shows dyshesive and loosely clustered polygonal malignant epithelioid cells. (Figure 5.12).

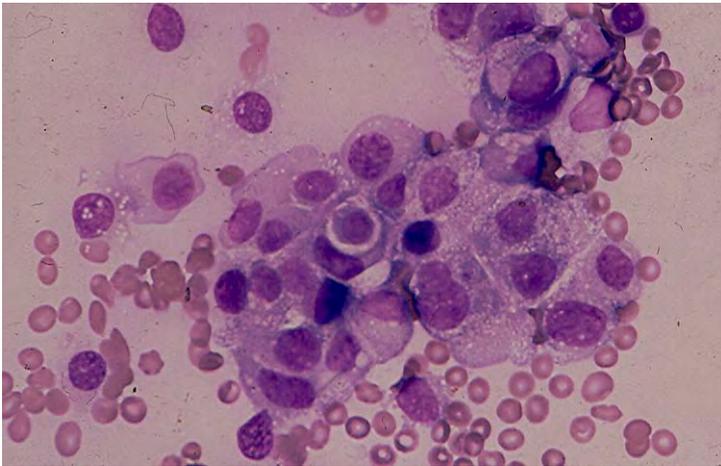
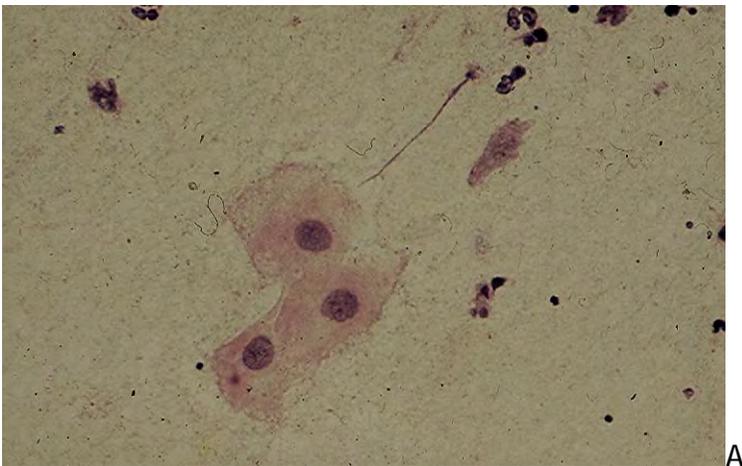


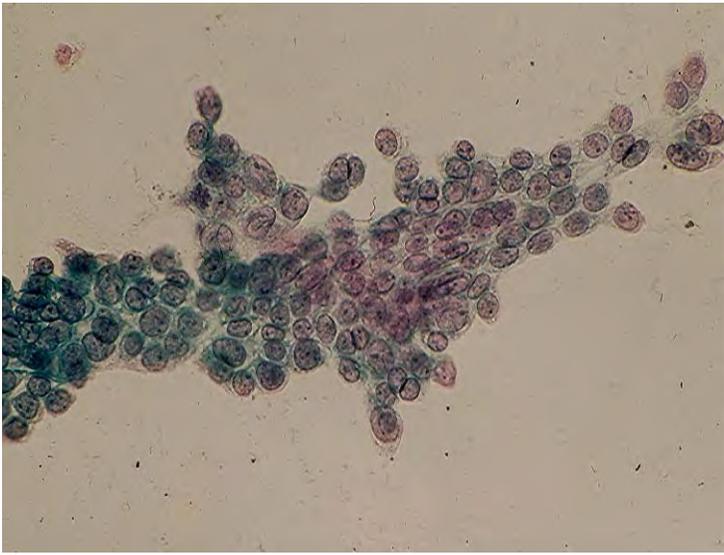
Figure 5.12. A cohesive cluster of large, polygonal malignant cells from a melanoma of soft parts. (Diff-Quik,x 400).

## GERM CELL TUMORS

Primary germ cell tumors may occur in the retroperitoneum. These neoplasms are located in the midline, paravertebral or prevertebral areas and include teratoma, dysgerminoma, embryonal carcinoma, yolk sac tumor and choriocarcinoma. These neoplasms may occur in pure forms or in combination with other histologic types.

A *mature teratoma* yields in FNA single and loosely clustered benign-appearing squamous cells and sheets of glandular epithelial cells. FNA from a *dysgerminoma* reveals dyshesive large polygonal cells with prominent nucleoli. An *embryonal carcinoma* is characterized by cohesive clusters of poorly differentiated epithelial cells with indistinct cell borders, large, pleomorphic nuclei and prominent nucleoli. (Figures 5.13 and 5.14).





Figures.5.13. A benign retroperitoneal mature teratoma showing in FNA (A) benign squamous cells and in (B) a sheet of benign glandular cells. (Pap, A and B x 400).

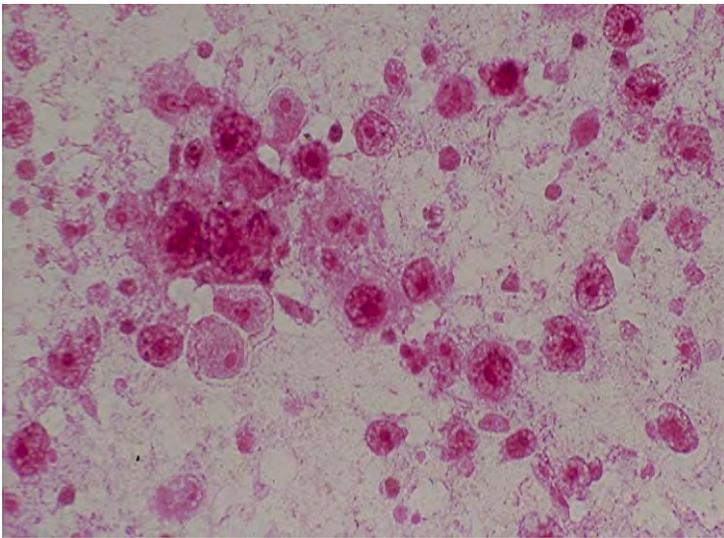


Figure 5.14. A retroperitoneal dysgerminoma showing in FNA tumor cells with ill-defined cytoplasm and large, round nuclei with prominent nucleoli. (HE, x 400).

A *yolk sac tumor* (endodermal sinus tumor) shows in needle aspirate single and clustered of malignant cells with some tumor cells showing intracytoplasmic hyaline globules that may be seen free in the smear background. Tumor cells forming Schiller-Duval bodies (glomeruloid structures formed by invagination of a tuft of malignant cells into an empty space) may be observed.

## LYMPHADENOPATHY

FNA of enlarged mesenteric or retroperitoneal lymph nodes is performed by under ultrasonographic or CT guidance. Multiple samples should be taken to secure an adequate cell sample for routine cytologic evaluation, cell marker studies and culture for identification of a suspected bacterial or fungal infection. A few tissue cores should be obtained by an 18-gauge cutting needle for supplemental histologic and immunohistochemical studies, as some antibodies work better with formalin-fixed or fresh and frozen tissue. A smear should be prepared and stained by the Diff-Quik technique to evaluate the sample adequacy. If lymphoma is suspected several samples will be obtained and fixed in RPMI solution for cell marker, molecular and cytogenetics studies. For a more accurate typing of a Non-Hodgkin lymphoma (NHL) 12 to 15 antibodies are needed. The most commonly used markers are: CD3 and CD5 for T-cell tumors; CD19, CD20, lambda and kappa for B-cell tumors; CD30 for Reed-Sternberg cells and anaplastic large cell lymphoma; and Tdt (terminal deoxynucleotidyl transferase) for lymphoblastic lymphoma. Molecular genetic studies are helpful when immunophenotypic methods are inconclusive in establishing clonality and/or cell lineage. A T-cell clonality can only be established by molecular genetic methods. Three molecular techniques may be used: Southern blot hybridization, polymerase chain reaction and fluorescence in situ hybridization.

### Reactive hyperplasia

This condition may affect intraabdominal or retroperitoneal lymph nodes. It is characterized cytologically by a polymorphous lymphocytic population consisting of lymphoid cells at various stages of transformation and tangible-body macrophages. If small lymphocytes predominate the picture, a small cell NHL should be ruled out by cell marker studies.

### Lymphoma

FNA is an established procedure for diagnosing metastatic tumors and recurrent lymphoma. Its value in the primary diagnosis of malignant lymphoma is still controversial, although there are recent papers reporting a high-diagnostic accuracy of NHL by combining cytomorphology with ancillary techniques (cell marker and molecular genetics studies).

**1. Hodgkin lymphoma** accounts for about 30% of all lymphomas. It has a bimodal curve with a peak incidence at 15-35 years of age and in late adult life. The tumor cells are large Reed-Sternberg (RS) cells and their mononuclear variants. RS cells are monoclonal B cells and are positive for CD30 and fascin. Classic Hodgkin lymphoma has 4 subtypes: nodular sclerosis, lymphocytic-rich, mixed cellularity and lymphocyte depleted. Most patients are male and are 30-50 years of age. Hodgkin lymphoma is

characterized by the presence of RS cells. These are large cells with one large, often folded or multilobulate nucleus with prominent nucleoli. Hodgkin lymphoma yields in FNA small lymphocytes, eosinophils and RS cells that usually represent 0.1 to 10% of the total cell population. (Figure 5.15).

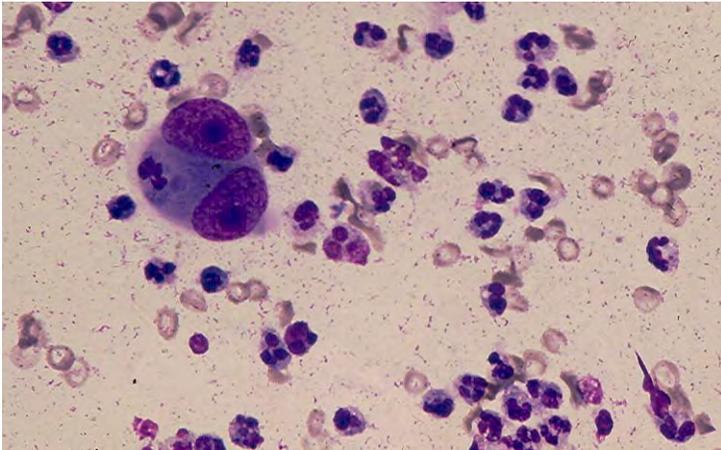


Figure 5.15. A classic Reed-Sternberg cell in a Hodgkin lymphoma (Diff-Quik, x 500).

## 2. Non-Hodgkin Lymphomas

NonHodgkin lymphomas (NHL) are classified into B- and T-cell tumors, according to the recent WHO classification. In the United States about 90% of NHLs are B-cell neoplasms and the remaining 10% are T-cell tumors. Null-cell NHLs are very rare.

**2.1. Precursor T- and B-cell lymphoblastic lymphoma** accounts from 30-50% of childhood lymphoma and is an aggressive disease. About 90% of the tumors are of T-cell type and the other 10% are B-cell tumors. It is most commonly located in the anterior mediastinum. The tumor yields in FNA abundant lymphoblasts with round or convoluted nuclei, finely granular chromatin, inconspicuous nucleoli and scant cytoplasm. (Figure 5.16). Lymphoblastic lymphoma cells are Tdt (+).

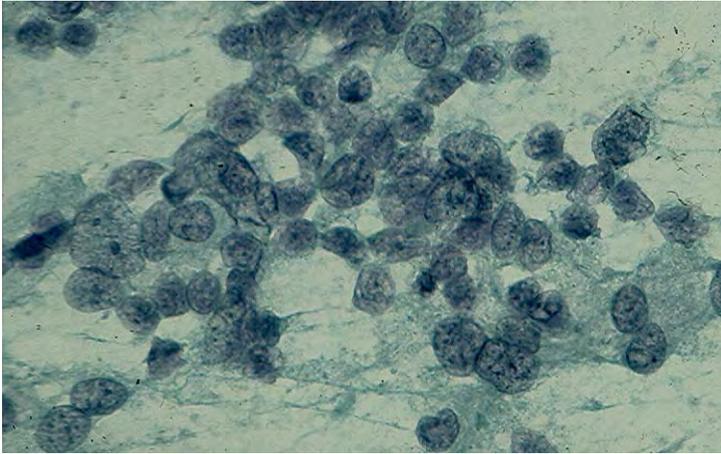


Figure 5.16. Lymphoblastic lymphoma showing large tumor cells with round or convoluted nuclei, finely granular chromatin, inconspicuous nucleoli and scant cytoplasm. Nuclear molding is noted. (Pap, x 500).

## 2.2. B Cell Lymphoma consists of several subtypes:

1. *Small lymphocytic lymphoma* (SCL) accounts for about 6% of all NHLs. It occurs in older patients, is indolent and incurable. It is widespread at the time of diagnosis with blood and bone marrow involvement. In FNA a monotonous population of small lymphocytes with clumped chromatin, smooth or minimally irregular nuclear contour, small nucleoli and scant cytoplasm is observed. (Figure 5.17). SCL cells are CD5 (+) and CD23 (+).

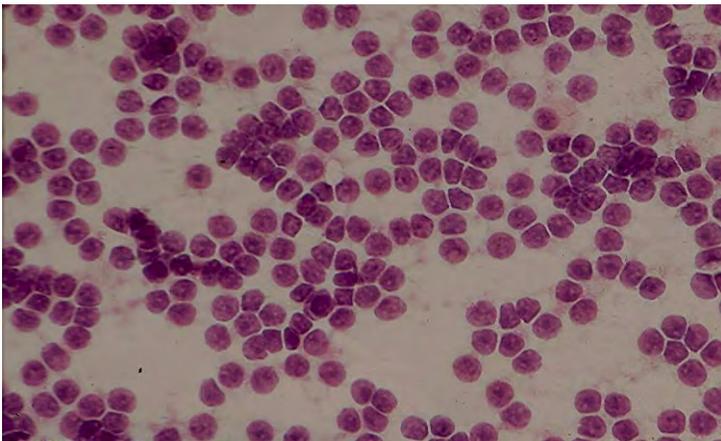


Figure 5.17. Monotonous small lymphoid cells from a small lymphocytic lymphoma. (Pap, x 500).

2. *Mantle cell lymphoma* accounts for 6-8% of all NHLs. The tumor is aggressive and incurable. It occurs more commonly in men than in women over 50 years of age. Most patients have disseminated disease at initial diagnosis and have a median survival of 2-

5 years. A t(11;14)(q13;32) translocation is present in 75% of patients. It shows in FNA abundant monomorphous small lymphoid cells with fine nuclear chromatin, irregular nuclear contours, small nucleoli and scant cytoplasm. (Figure 5.18). The tumor cells are CD5 (+) and CD23 (-).

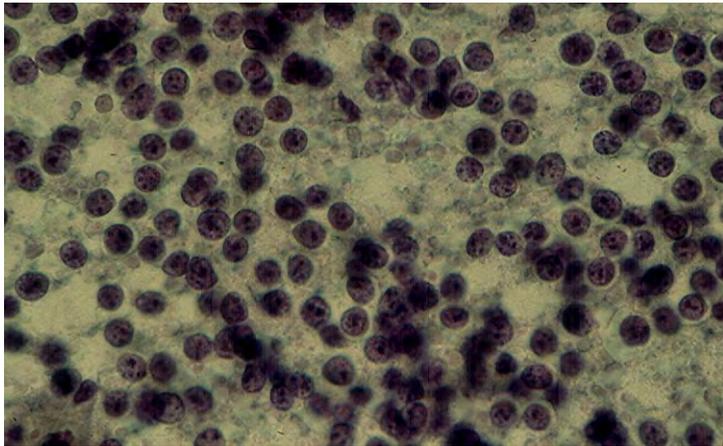


Figure 5.18. Monomorphous population of small lymphoid cells with small round nuclei in FNA of a mantle cell lymphoma (Pap, x 500).

3. **Follicular lymphoma** accounts for about 35% of adult NHLs. The patients are generally over 50 years of age and over 80% of them have disseminated disease at the time of diagnosis. In 25-35% of patients with follicular lymphoma, transformation to a large B-cell lymphoma occurs. The tumor is divided into low-grade and high-grade, depending on the number of centroblasts. In over 75% of cases a t(14:18) translocation with bcl-2 gene rearrangement is present. In FNA material abundant small irregular/cleaved lymphocytes admixed with a smaller number of large cleaved/non-cleaved lymphocytes are seen. (Figure 5.19). Follicular lymphoma cells are CD5 (-) and CD10 (+).

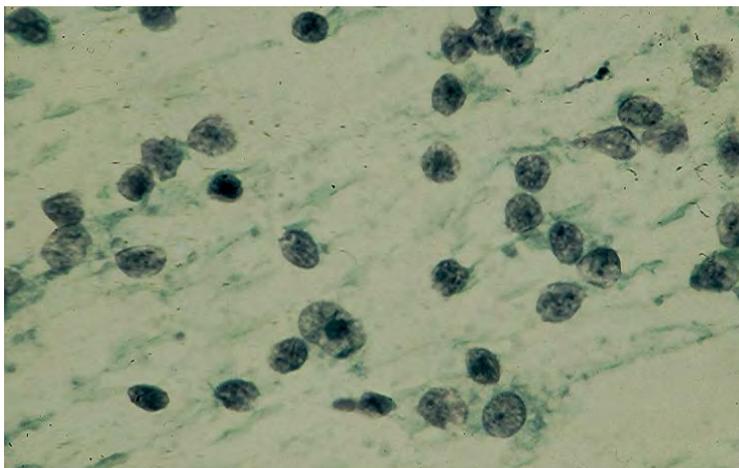


Figure 5.19. Monomorphous tumor cells with nuclear indentation and protrusion in FNA of a follicular lymphoma. A centroblast with prominent nucleolus is noted. (Pap, x 500).

4. **Marginal zone lymphoma (MZL)** is a low-grade lymphoma that is divided into nodal and extranodal types. The extranodal type is called mucosa-associated lymphoid tissue lymphoma (MALT) and is commoner than the nodal type. MALT lymphoma accounts for 7-8% of all B-cell lymphomas and is usually associated with autoimmune diseases. MZL patients usually have stage I or II disease and are potentially curable by surgery or regional radiotherapy. MZL cells are CD5 (-), CD10 (-), CD23 (-) and cyclinD1 (-). The tumor cells are cytoplasmic immunoglobulin (+) and may express CD43. In FNA a polymorphous population of lymphoid cells consisting of small- and intermediate-size lymphocytes with round or irregular nuclei admixed with monocytoid cells. (Figure 5.20). A reactive lymphoid hyperplasia should be ruled out by cell marker study.

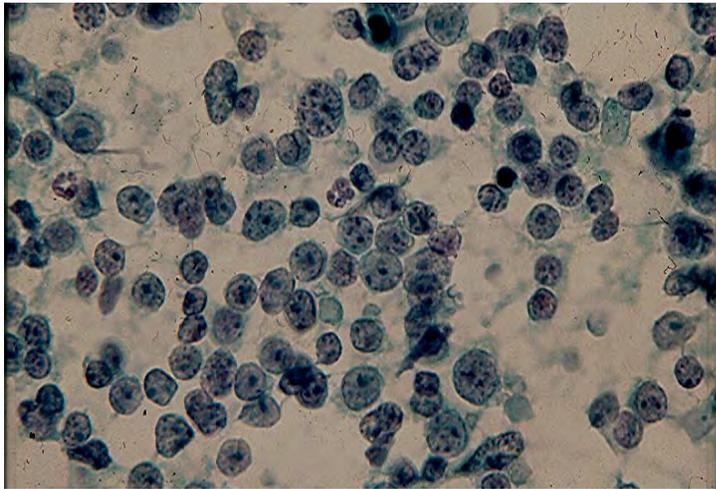


Figure 5. 20. A polymorphous cell population consisting of small and intermediate-sized lymphoid cells from a MZL. (Pap, x 500).

5. **Diffuse large B-cell lymphoma** is an aggressive but potentially curable disease. It accounts for about 35% of adult lymphoma. About 40% of patients present with extranodal disease. The bcl-2 gene rearrangement is present in 30% of cases. It is characterized cytologically by a predominance of large lymphoid cells. (Figure 5.21).

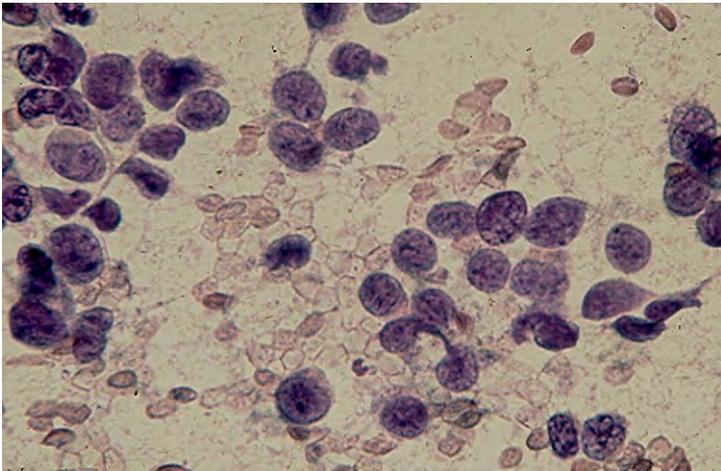


Figure 5.21. A monomorphic tumor cells with large and vesicular nuclei, conspicuous nucleoli from a diffuse large B-cell lymphoma (Pap, x 500).

6. **Burkitt lymphoma** is a highly aggressive but potentially curable by chemotherapy. The disease occurs in 3 clinical settings: endemic form (Africa and Asia), sporadic form (the United States and other countries) and an immunodeficiency-associated form. The endemic form is almost always associated with EBV latent infection and is commoner in children, while the sporadic and immunodeficiency-associated forms are rarely EBV associated. Burkitt lymphoma is characterized in FNA by abundant uniform intermediate-sized lymphoid admixed with abundant tangible-body macrophages. The tumor cells are CD19 (+) and CD20 (+). Three types of translocation are seen: t(8;14),80%; t(2;8),15% and t(8;22),5%.

### 2.3. T-cell Lymphoma

Most T-cell lymphomas have no specific immunophenotypic profiles. The diagnosis is suggested by an aberrant T-cell immunophenotype, such as loss of 1 or more T-cell markers. Molecular genetic studies to demonstrate a rearrangement of T-cell receptor genes are often required to confirm the clonality of a T-cell proliferation.

1. **Peripheral T-cell lymphoma**, unspecified type is commoner in Asia. It is characterized cytologically by the presence of monomorphic small or large lymphoid cells or a mixture of small and large lymphoid cells with irregular nuclei. Histiocytes, plasma cells, eosinophils and Reed-Sternberg-like cells may be seen.

2. **Anaplastic large cell lymphoma** accounts for about 3% of adult NHLs and 10-30% of childhood NHLs. It is commonly associated with t(2;5)(p23;q35) translocation and ALK expression is present in 60-85% of cases. The lesion is characterized by abundant intermediate and large cells with irregular nuclei, large nucleoli, Reed-

Sternberg-like cells, histiocytes and macrophages. (Figure 5.22). The tumor cells are CD30 positive.

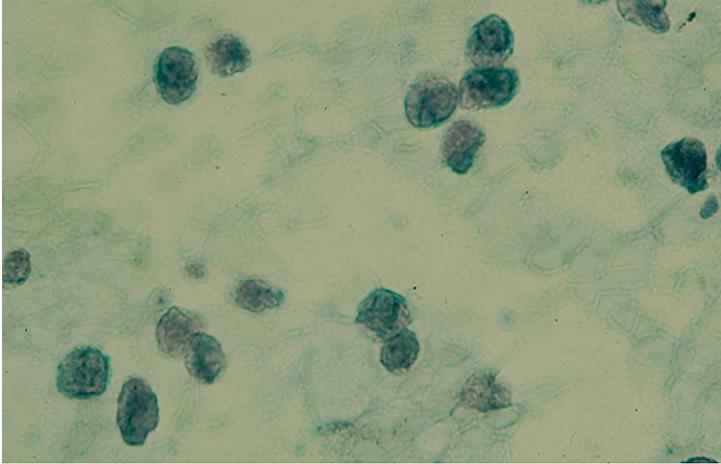


Figure 5.22. Large tumor cells with markedly variable nuclear shapes in FNA of an anaplastic large cell lymphoma. (Pap, x 250).

The cytodiagnostic accuracy of Hodgkin lymphoma by FNA is high, approaching 90% but for NHL it is much lower. However, an accuracy rate greater than 80% have been reported in some recent series.

## **METASTATIC CANCERS**

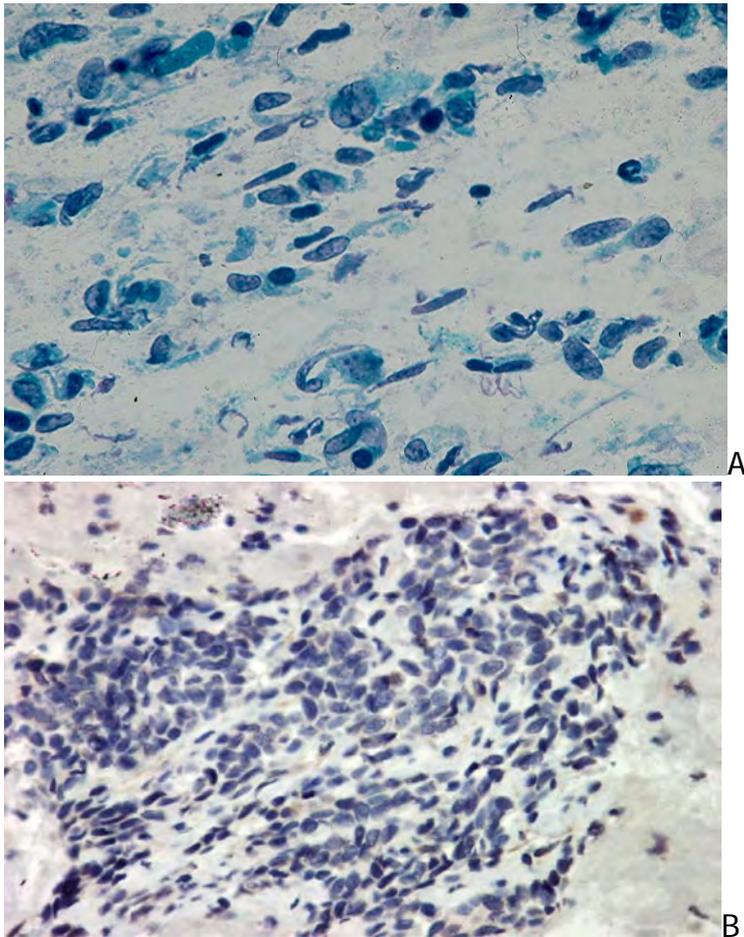
Among these tumors carcinomas are the most common ones that often arise from different anatomic sites including the female and male genital tracts, urinary tract, gastrointestinal tract, pancreas, lung, breast and cutaneous melanoma. In most instances, cytodiagnosis of metastatic tumor by FNA is straightforward as differentiating "alien" cells from lymphocytes is relatively easy. Epithelial cells also are usually arranged in clusters or sheets, as opposed to the dissociated pattern of the lymphoid cells. The readers are referred to Chapter 5 in the author's monograph on Essentials of Head and Neck Cytology for illustrations. Metastatic germ cell tumors in LNs are cytologically similar to those of retroperitoneal germ cell tumors previously discussed.

## **MISCELLANEOUS TUMORS**

### **Gastrointestinal tumors**

With the introduction of EUS-FNA in clinical practice mural tumors and extramural tumors (mesentery, lymph node, biliary tree, pancreas and liver...) of the

gastrointestinal tract may be diagnosed cytologically. An gastric well-differentiated **adenocarcinoma** shows in FNA clustered malignant glandular cells. A signet-ring cell carcinoma of the stomach shows in needle aspirate mucus material containing single malignant cells with large intracytoplasmic vacuoles. A moderately differentiated colonic adenocarcinoma yields in FNA clusters malignant columnar cells or epithelial fragments showing elongated nuclei in palisade. A **gastrointestinal stromal tumor (GIST)** of spindle cell variant shows in FNA spindle tumor cells. (Figures 5.23). A a GIST of epithelioid variant displays dyshesive or cohesive polygonal cells. (Figure 5.24). A positive staining of the tumor cell cytoplasm with CD117 and CD34 antibodies will confirm the diagnosis.



Figures 5.23. FNA of a mesenteric spindle cell GIST showing in:  
A. Isolated spindle tumor cells.  
B. Clusters of tumor cells in a cell block section.  
(Pap, A x 400; B x 250).

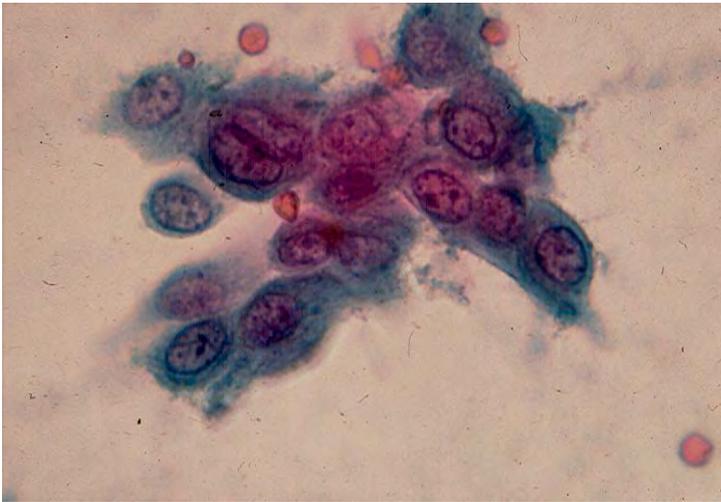
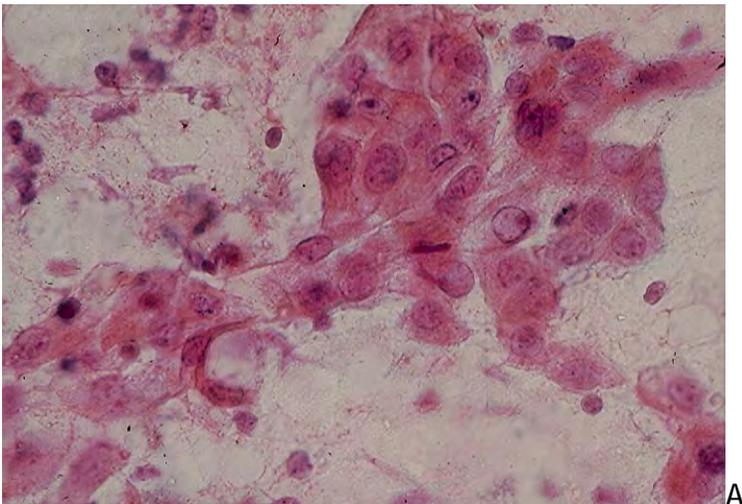
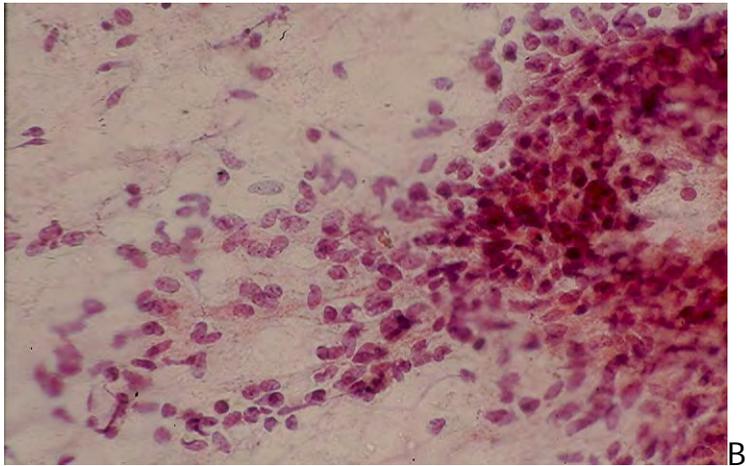


Figure 5.24. A cohesive cluster of polygonal epithelioid cells with atypical oval nuclei are seen in EUS-FNA of a GIST, epithelioid variant. (Pap, x 500).

### Other tumors

*Intra-abdominal desmoplastic round cell tumor* is a highly aggressive cancer and characterized cytologically by the presence of abundant small round cells with scant cytoplasm that expresses pancytokeratin, neuron-specific enolase and WT1. *Cystic lymphangioma* of the retroperitoneum shows in FNA abundant benign lymphoid cells. A mesenteric *mixed mesodermal tumor*, homologous type yields in FNA malignant round cells admixed with fragments of glandular epithelium. (Figures 5.24).





Figures 5.24. FNA from a mesenteric mesodermal tumor reveals in:  
 A. A cluster of malignant epithelial cells.  
 B. Single and clustered malignant round and spindle cells.  
 (Pap, x 400).

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**THE END**