ASC Webinar: Practical Approach to Liver Cytology

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LIVER OUTLINE

- Background
- Cytology of benign liver and liver nodules
- Cytology of Primary Liver Cancers
  - Hepatocellular carcinoma
  - Cholangiocarcinoma
- Ancillary studies for key differential diagnoses
- Metastases

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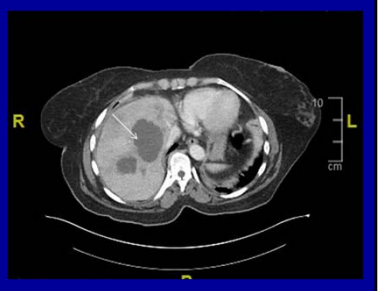
Indication: Evaluation of a Mass

- Nonneoplastic lesions
  - hemangioma
- Benign liver nodule
  - FNH
  - Adenoma
- Primary epithelial cancers
  - HCC
  - ICC
- Less common nonepithelial neoplasms and malignancies
- Metastases



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KEY DIAGNOSTIC ISSUES

- Distinction of benign or reactive hepatocytes in nonneoplastic or benign liver nodules from well-differentiated hepatocellular carcinoma
- Distinction of poorly differentiated hepatocellular carcinoma from cholangiocarcinoma or metastases
- Determination of primary site of origin of metastases
- Determination of histogenesis of poorly differentiated malignancie

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APPROACH TO THE DIAGNOSIS OF LIVER LESIONS

- Clinical history
  - Age and gender
    - Hepatoblastoma in infants
    - Adenoma in females
  - Underlying liver disease
    - HCV and Cirrhosis as a predisposing risk factor for HCC
  - Previous history of carcinoma
- Radiological imaging
  - Borders, possible vascular lesion
- Cytological findings
- Ancillary studies
- Correlate all findings

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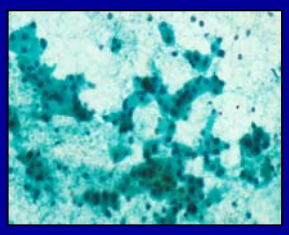
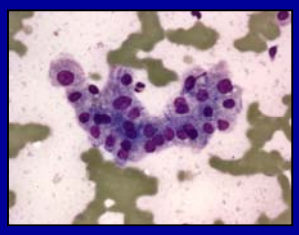
Hepatocytes

- **Monolayered sheets,thin trabeculae, single cells or small, loose groups**
- **No endothelial wrapping**
- **Polygonal cells with abundant granular cytoplasm**
- **Pigments and inclusions: bile, iron, lipofucsin,**

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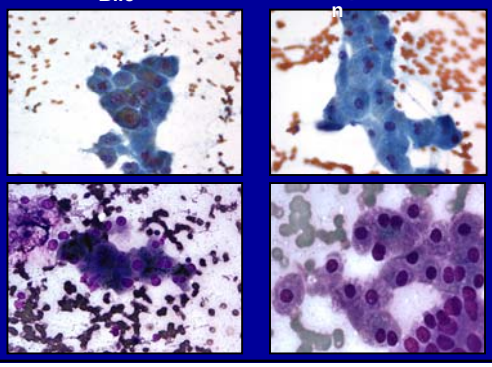
PIGMENTS

Bile

Lipofucsin



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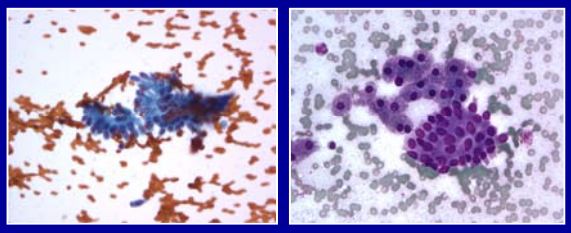
Bile Duct Cells



- **Smaller than hepatocytes**
- **Flat sheets with honeycomb pattern**
- **On edge and acinar formation**

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BENIGN LIVER NODULES

Differential diagnosis: well differentiated hepatocellular carcinoma

- Differential Diagnosis
  - Macro-regenerative nodules
  - Cirrhosis with dysplastic nodules
  - Focal nodular hyperplasia
  - Adenoma
- Common cytological Features
  - Benign or reactive hepatocytes in irregular sheets without peripheral endothelial wrapping,
  - Mixed cell population (except for adenomas)
  - Core biopsy and/or cellblock
  - Correlation with clinical and radiological findings needed for definitive diagnosis

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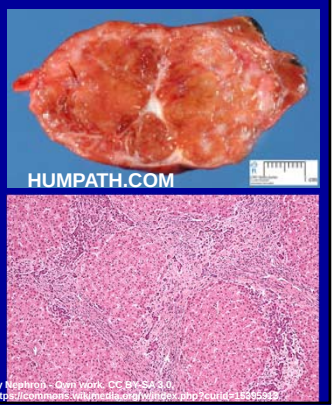
Focal Nodular Hyperplasia

- Clinical
  - Non-neoplastic response to altered
  - blood flow
  - Typically solitary, may be multifocal
  - More common in females
- Gross
  - Circumscribed lesion with central scar
- Histology
  - Abnormally thickened vessels
  - Bile duct proliferation



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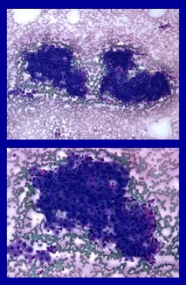
Benign Nodules
Reactive Hepatocytes

- Architecture
  - Groups with jagged, irregular borders
  - No peripheral endothelial cell wrapping
  - Transgressing endothelium
- Cytological features
  - Nuclear pleomorphism
  - Low N/C
  - Frequent binucleation
- Background
  - Bile duct cells
  - Inflammatory cells



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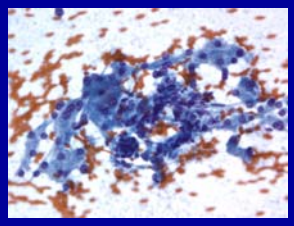
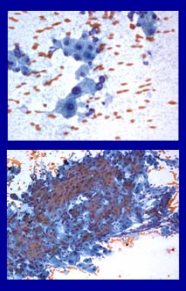
Reactive Hepatocytes





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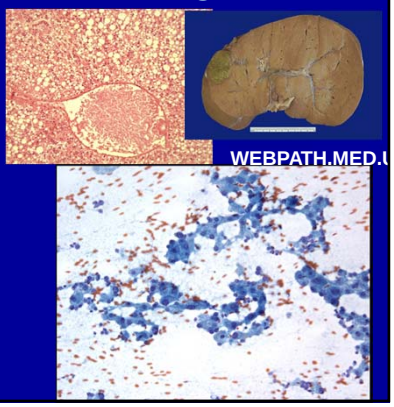
HEPATOCELLULAR ADENOMA

- Noncirrhotic liver
- Classified according to molecular alterations
- Benign hepatocytes, thin walled, unpaired arteries, normal architecture
- Cytology smears benign hepatocytes, no bile ducts



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Update on the New Classification of Hepatic Adenomas

Clinical, Molecular, and Pathologic Characteristics

Sadhna Dhingra, MD; M. Isabel Fiel, MD

Hepatic Adenoma Subtypes, Frequencies, Molecular Findings, Pathologic Features, and Immunohistochemistry

HA Subtype	Frequency Range, %	Molecular Findings	Pathologic Features	Immunohistochemistry
HA-H	35–40	Somatic mutations of (85%) *TCF1* (*HNF1A*) gene; heterozygous germline mutations (<5%) of *CYP1B1* gene	Steatosis, lack of inflammation, and cytologic atypia	Decreased or absent L-FABP in neoplastic hepatocytes, compared with nonneoplastic liver; patchy CD34 expression; focal, interspersed CK7 positivity in small hepatocytes
HA-B	15–19	β-catenin gene–activating mutations	Pseudoacinar pattern, cytologic atypia, steatosis, and/or lack of inflammation	Increased nuclear β-catenin protein expression; strong diffuse glutamine synthetase positivity
HA-I	30–35	Gain-of-function mutations of the *IL6ST* gene; 10% with coexisting β-catenin gene mutations	Pseudoportal tracts with thick-walled arteries and lack of bile ducts or veins; inflammatory infiltrate; ductular reaction; sinusoidal dilatation; and peliosis	Diffuse, strong serum amyloid-A and C-reactive protein expression; CD34 reactivity around pseudoportal tracts; diffuse glutamine synthetase positivity if associated with β-catenin mutation
HA-U	10	No specific mutations	No distinctive morphology	No specific protein expression

(*Arch Pathol Lab Med.* 2014;138:1090–1097; doi: 10.5858/arpa.2013-0183-RA)

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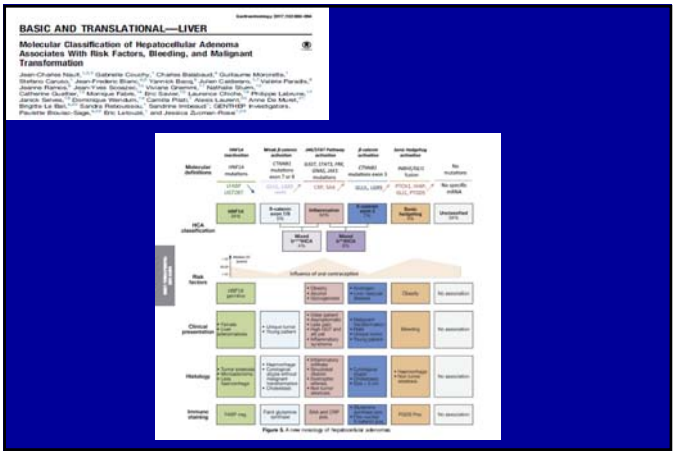

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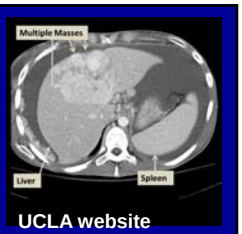
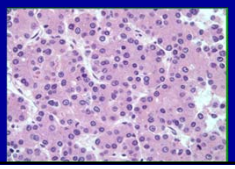
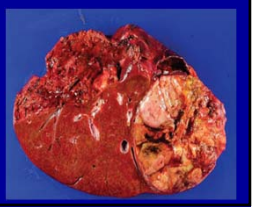
Hepatocellular carcinoma

- Most common primary cancer of the liver
- Age of onset depends on geographic location and underlying risk factors
- More common in men
- Risk factors: cirrhosis, most due to ETOH and HepB and C
- Patients with cirrhosis may present with increasing serum AFP



UCLA website





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Hepatocellular Carcinoma

Cytological Diagnosis

- Gross appearance or naked eye appearance of sample
- Background
  - Presence or absence of bile duct epithelium
  - Stripped, single atypical nuclei
- Architecture
  - Vascular pattern : Peripheral endothelial cell wrapping (PE) and transgressing endothelium (TE)
  - Cell group shape and arrangement, thickness of trabeculae, dispersed single cells, pseudoacini
- Cytological features
  - Nuclear to cytoplasmic ratio (N/C)
  - Nucleation, nucleoli
  - Cytoplasmic features and contents

•Tao et al 1984, Pedio et al1988, Cohen 1991, Sole et al 1993, Silverman et al 1996, Granados et al 2001

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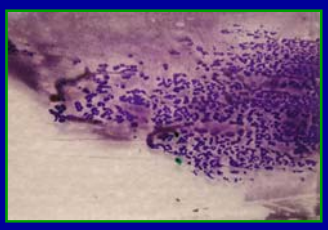
Naked Eye Inspection



- Benign hepatic parenchyma sticks together, and forms core like fragments (right). HCC disintegrates and forms a granular pattern (left).

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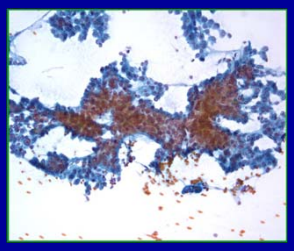
Hepatocellular Carcinoma

Classic Patterns

- Clean background, lacking inflammation and bile ducts
- Widened trabeculae
- Smooth edges due to peripheral endothelial cell wrapping
- Increased N:C, monotonous nuclei



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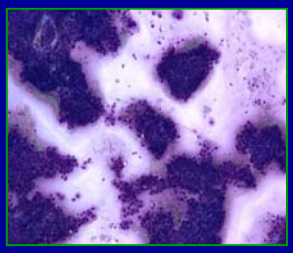
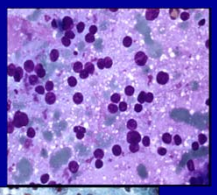
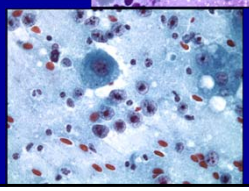
HEPATOCELLULAR CARCINOMA

Background







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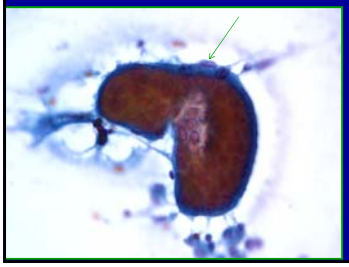
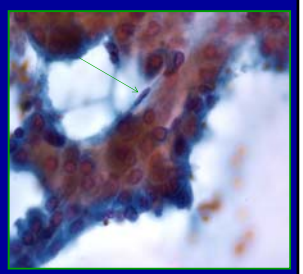
HEPATOCELLULAR CARCINOMA

Peripheral Endothelial Cell Wrapping Pattern





Endothelial cells wrapping around the neoplastic hepatocytes

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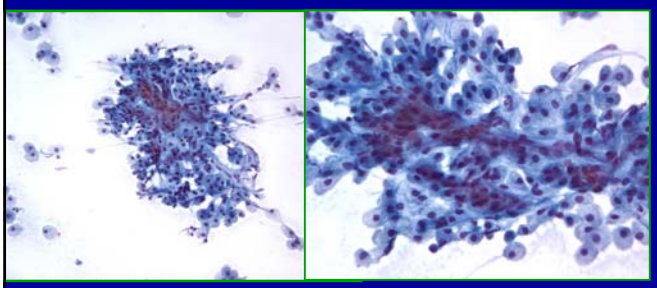
HEPATOCELLULAR CARCINOMA

Transgressing (Arborizing)Endothelial Cell Pattern



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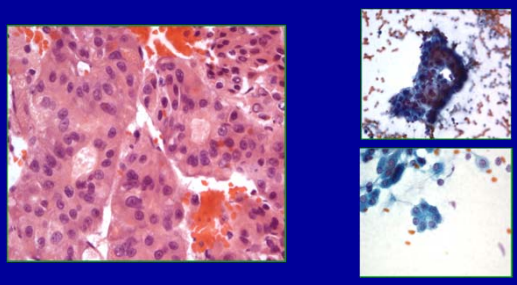
HEPATOCELLULAR CARCINOMA

Pseudoacinar Pattern



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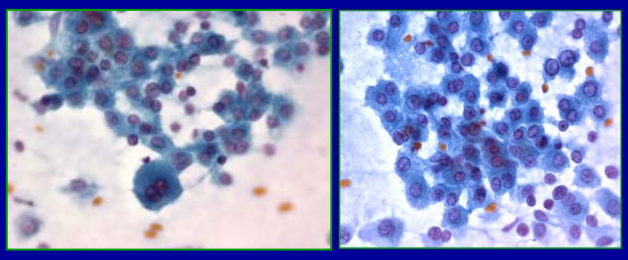
HEPATOCELLULAR CARCINOMA

Nuclear and Cytoplasmic features



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Benign vs Malignant Liver Nodules

Criteria	Benign Liver Nodules	Adenoma	WDHCC
Background	Bile ducts, inflammation, benign stripped nuclei	Lacks bile ducts	Lacks bile ducts, atypical stripped nuclei
Vascular pattern	TE focally	TE focally	TE and PE
Architecture	Trabeculae 1-2 cells thick Jagged, irregular borders	Trabeculae 1-2 cells thick Jagged, irregular borders	>3 cells thick Crowding Pseudoacini Smooth borders
N:C	Low	Low	High
Nuclear features	Pleomorphic Even chromatin Some nucleoli	Pleomorphic Even chromatin Some nucleoli	Monomorphic Prominent nucleoli

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ANCILLARY STUDIES
Benign Vs. Malignant Liver Nodules

- **Special stains for reticulin**
  - **Reticulin**
    - **Most helpful, smears or cell block**
  - **Iron stain**
    - **Iron absent in malignant hepatocytes**
    - **Patients with hemochromatosis**
- **Immunohistochemical studies for AFP**
  - **Only in 40% of tumors**
  - **May occur in reactive processes**
- **Immunohistochemical studies for CD 34**
  - **No better than reticulin and more expensive**
- **Other Immunohistochemical markers**
  - **B-catenin. -Glutamine synthetase**
  - **Glypican 3 - HSP70**

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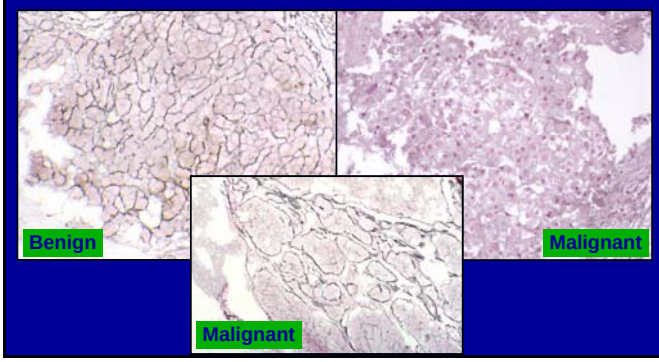
Ancillary Studies: Benign or Malignant
Reticulin (silver)



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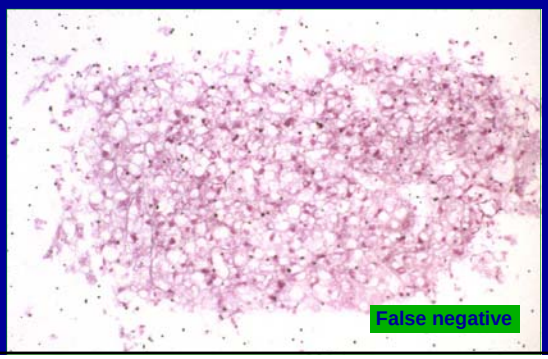
Reticulin Stain Pitfall: Marked steatosis



False negative

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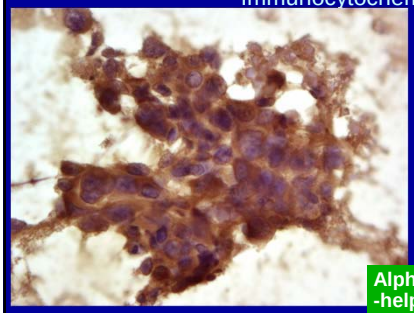
Ancillary Studies: Benign or Malignant

Immunocytochemistry



Alpha-fetoprotein
-helpful if positive, but only 35-40% positive
-Negative stain does not rule out tumor

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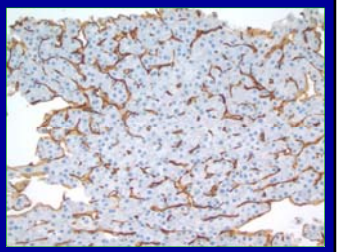
CD34

- Many uses in histology
- Liver: highlights capillarization of the sinusoids



Ruck P, Xiao JC, Kaiserling E. Immunoreactivity of sinusoids in hepatocellular carcinoma. An immunohistochemical study using lectin UEA-1 and antibodies against endothelial markers, including CD34. Arch Pathol Lab Med. 1995 Feb;119(2):173-8.

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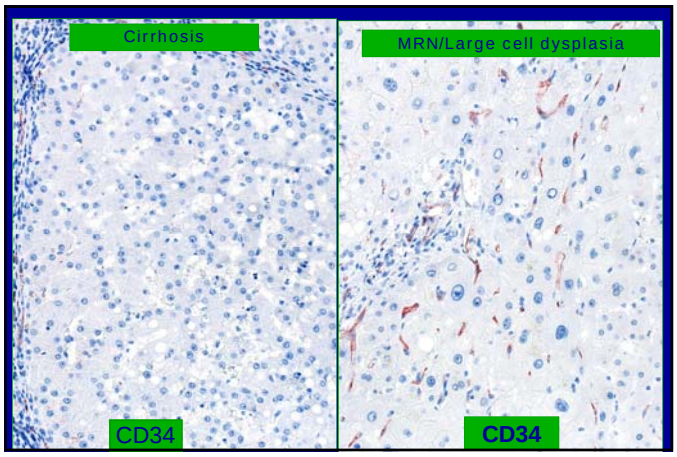

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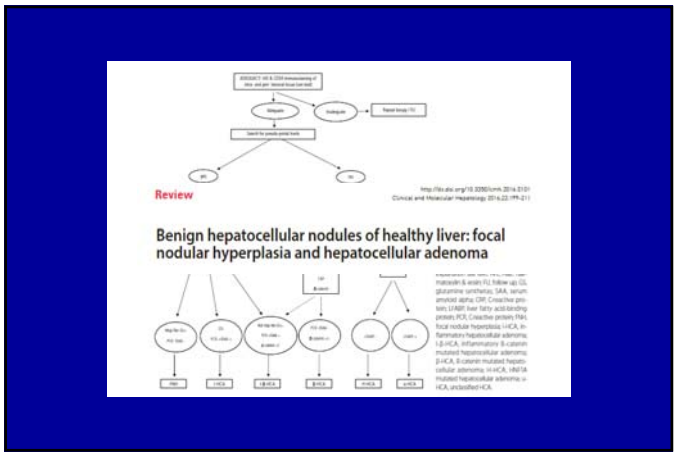

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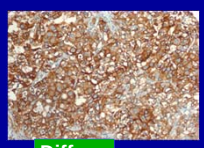
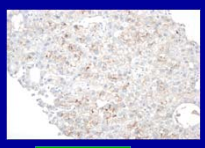
Glypican 3

- Upregulated in HCC
- Expressed most often in poorly differentiated HCC
  - Some false negatives in WDHCC
- Also reactive in high grade dysplastic nodules
- Negative in benign hepatocyte nodules
- Expressed in other malignancies
  - Wilm's tumor, melanoma, ovarian carcinomas, and other malignancies
  - Not specific for hepatocytic differentiation



Diffuse



Granular

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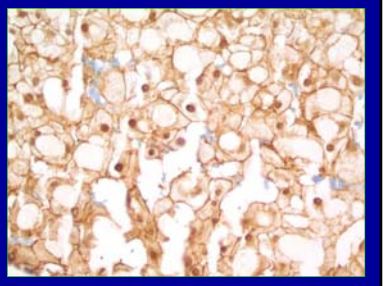
ß-Catenin

- Cytoplasmic staining is normal
- Nuclear staining is abnormal
  - supports diagnosis of ß-catenin activated LCA
  - In cirrhosis, nuclear staining supports HCC



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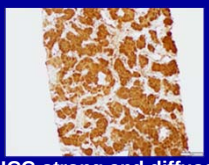
Glutamine synthetase

Condition	Staining Pattern
Normal	Zone 3/perivenular cuff
Cirrhosis	Patchy and weak periportal
FNH	Strong map-like pattern
B-catenin mutated adenoma	Strong, diffuse
Other adenomas	Weak/patchy
HCC	Strong/diffuse

Joseph NM, *et al. Modern Pathol. 2014; 27(1):62-72.*



HCC-strong and diffuse

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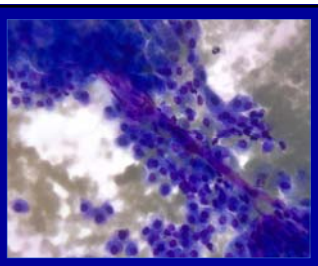
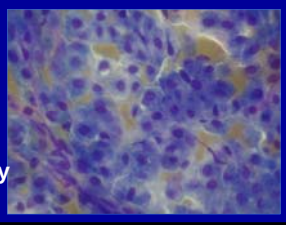


Indeterminate Aspirate



-Transgressing endothelium
-Increased N/C
-Monotonous pattern
-Focal on the smear
-Material insufficient for ancillary testing

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Ancillary studies
Benign vs Malignant

Marker	Benign	HCC
Reticulin	Normal	Loss or variable pattern
Iron	Present	Lost (hemochromatosis)
Glypican 3	Negative	Positive
HSP 70	Negative	Positive
GS	Centered on central veins	Diffuse
CD 34	None in nonlesional liver	Increased capillarization

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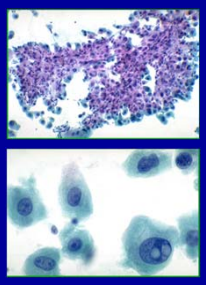
HEPATOCELLULAR CARCINOMA
Fibrolamellar Variant

- **1% of HCC**
- **Low median age**
- **No background liver disease**
- **Prognosis similar to HCC**
- **Cytology:**
  - **Low N/C ratio**
  - **Intranuclear inclusions**
  - **Prominent nucleoli**
  - **Single cells**
  - **Transgressing endothelial pattern**
  - **Paucicellular smears (fibrous area)**



Courtesy of Dr. Pitman

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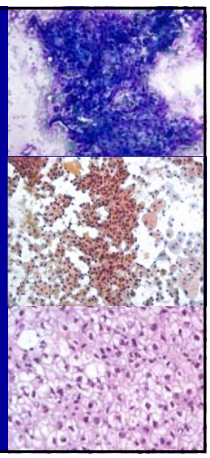
HEPATOCELLULAR CARCINOMA
Clear Cell Variant

- 3-7% of all HCC
- >80% clear cell morphology from glycogen or steatosis
- Better prognosis
- On cytology, differential diagnosis includes other clear cell neoplasms, such as renal cell carcinoma



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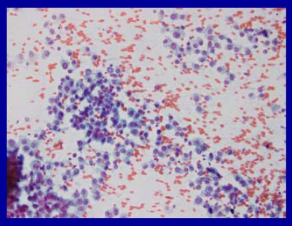
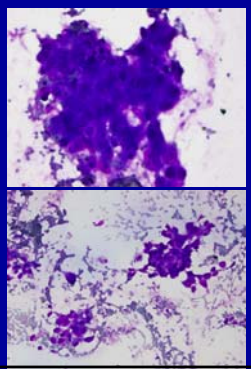
Poorly differentiated HCC

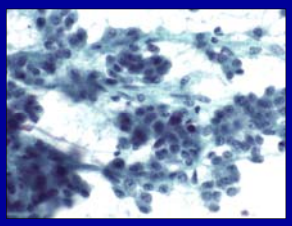




40

Hepatoblastoma

- Most frequent malignant liver tumor in children
  - 6 months-5 years
  - Prenatal, neonatal, older children, rarely adults
- The cytomorphology recapitulates the histopathology:
- Fetal type cell
  - resembles normal hepatocytes, but cells are smaller
  - Nuclear and cytoplasmic features are similar and the cytoplasm may contain fat and glycogen, just as normal hepatocytes.
- Embryonal cell
  - Higher N/C ratio, less cytoplasm
  - lacks the cytoplasmic contents of normal liver
  - Nuclei are hyperchromatic, the cytoplasm scant
  - Cells form rosettes and trabeculae

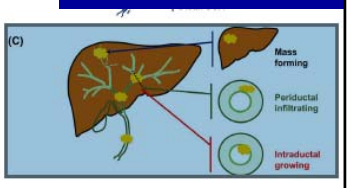


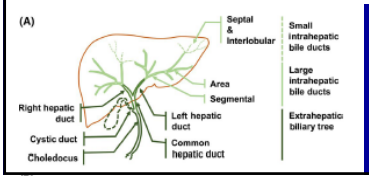
Fetal
Embryonal
Mixed epithelial
Mixed epithelial and mesenchymal

41

Anatomical, histomorphological and molecular classification of cholangiocarcinoma

Timothy Kendall[1*] | Joanne Verheij[2*] | Eugenio Gaudio[3] | Matthias Evert[4] | Maria Guido[5] | Benjamin Goeppert[6] | Guido Carpino[7]





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ICC Variants

- Small duct type
  - Cholangiolocarcinoma
  - Ductal Plate malformation Pattern
- Large Duct Type
  - Adenosquamous
  - Mucinous carcinoma
  - Signet ring cell
  - Clear cell carcinoma
  - Mucoepidermoid
  - Lymphoepithelioma like

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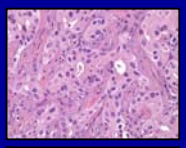
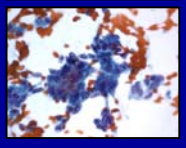
Intrahepatic Cholangiocarcinoma (ICC)

- Second most common malignancy of the liver
- Older, > 65 years
- Patients present at advanced stage
- Predisposing factors:
  - Includes HCV, BV, cirrhosis
  - Diseases causing biliary inflammation
    - Primary sclerosing cholangitis
    - Primary biliary cirrhosis





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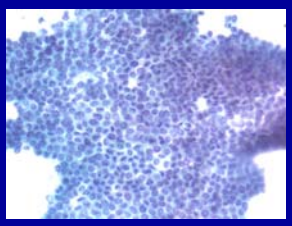
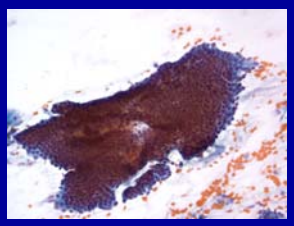
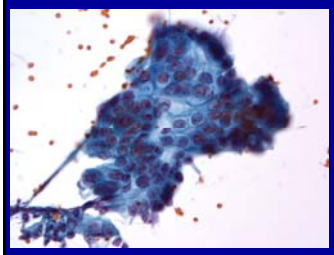
FNA Smear Pattern- Cholangiocarcinoma

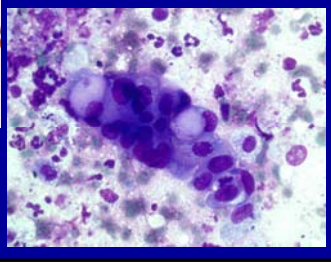




45

FNA Smear Pattern- Cholangiocarcinoma





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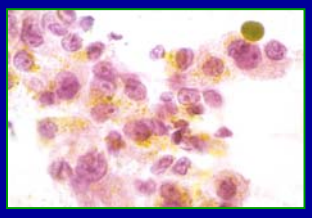
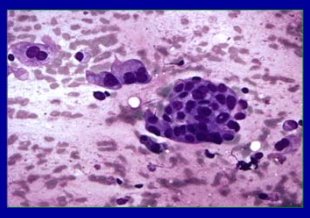
Challenge: HCC vs Adenocarcinoma (ICC)

Bile pigment in HCC



Mucin in ICC



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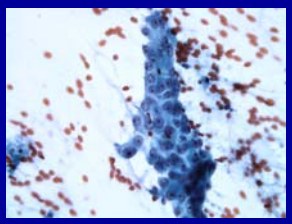
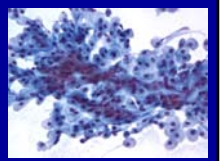
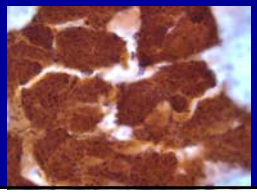
HCC VS ICC

- Gland formation in ICC



- HCC TE pattern or PE pattern





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HCC vs. ICC

Feature	HCC	ICC
Gland formation	-	+
PE or TE	+	-
Mucin	-	+
Bile	+	-
Stripped nuclei	+	-

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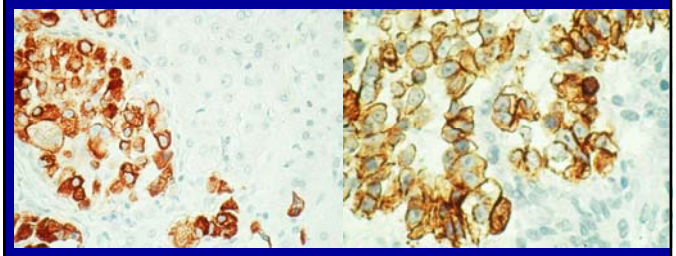
KERATIN IHC



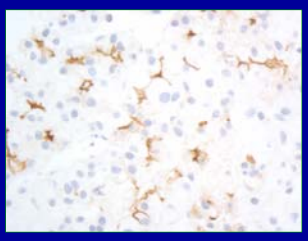
HMW CK in adenocarcinoma

LMW CK in HCC

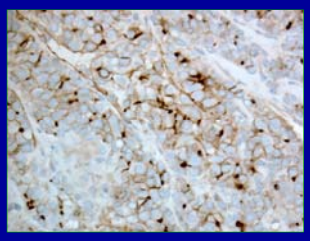
50

Canalicular Pattern Markers

CD10



pCEA



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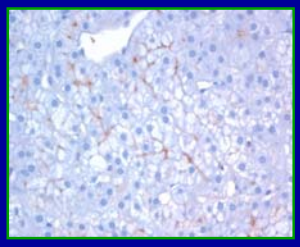
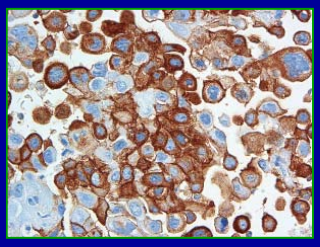
CEA (P)

HCC



Adenocarcinoma



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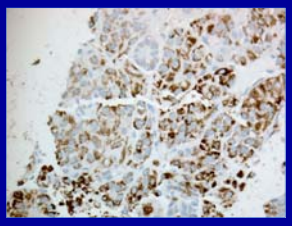
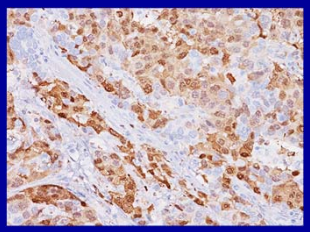
Markers of Hepatocytic Differentiation

HepPar



HepPar false positives

Arginase



More sensitive and specific

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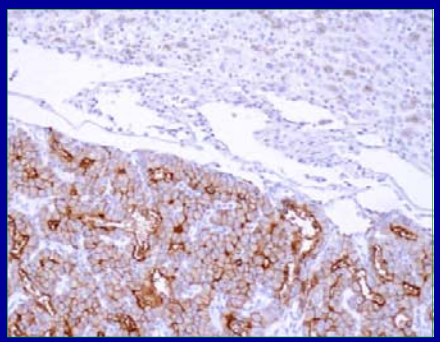
Markers of Glandular Differentiation

MOC31



Arch Pathol Lab Med. 2007;131:1648–1654

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Differential Diagnosis of Primary Hepatic Carcinomas versus Metastatic Epithelial Malignancy

- Hepatocellular carcinoma, poorly differentiated
- Cholangiocarcinoma (adenocarcinoma)

versus

- Metastatic adenocarcinoma
- Metastatic renal cell carcinoma
- Metastatic adrenal carcinoma
- Metastatic melanoma

Common morphological features: large poylgonal cells with abundant cytoplasm, large nucleoli and intranuclear inclusions

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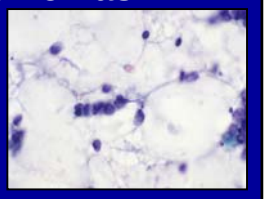
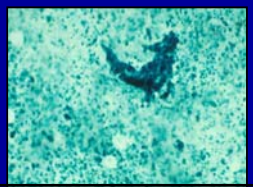
Metastatic Adenocarcinomas

- Very few adenocarcinomas have distinct morphological patterns.
- Top image is metastatic breast cancer.
- Bottom image is metastatic colorectal carcinoma.





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IHC For The Work-up Of Carcinoma Of Unknown Primary

- CK7/CK20
  - CK7-/CK20+ has predictive probability of 78% for colorectal carcinoma
  - CK7+/CK20- least specific
- CK17: pancreatobiliary, other adenocarcinomas
- DPC4: loss in pancreatic primaries
- CDX2, villin: gastrointestinal and colorectal
- TTF1, napsin: lung adenocarcinoma
- GATA3, BRST2, ER, mammoglobin: breast
- NKX3.1, PSA, PAP: prostate
- GATA3: urothelial carcinoma
- SATB2: colorectal, appendiceal, osteoblastic tumors
- PAX8: renal cell carcinomas, female genital tract, primaries, thyroid
- PAX 8, TTF1, thyoglobulin: thyroid primaries
- ISH high risk HPV: lower anogenital tract primaries, some oral cancers

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Ann Surg Oncol. 2016 January ; 23(1): 290–296. doi:10.1245/s10434-014-4247-8.

The Ability to Diagnose Intrahepatic Cholangiocarcinoma Definitively Using Novel Branched DNA-Enhanced Albumin RNA In Situ Hybridization Technology

Cristina R. Ferrone[1], David T. Ting[2], Mohammed Shahid[1,3], Ioannis T. Konstantinidis[1], Francesco Sabbatino[1], Lipika Goyal[2], Travis Rice-Stitt[3], Ayesha Mubeen[1], Kshitij Arora[1,3], Nabeel Bardeesey[2], John Miura[4], T. Clark Gamblin[4], Andrew X. Zhu[2], Darrell Borger[1], Keith D. Lillemoe[1], Miguel N. Rivera[3], and Vikram Deshpande[3]

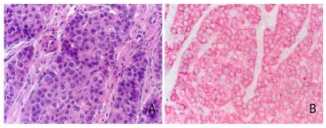

FIG. 2.
Hepatocellular carcinoma (HCC) diffuse and strongly positive for albumin. a Hematoxylin and eosin (H&E) stain (*left*). b In situ hybridization (ISH) for albumin (*right*)

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Albumin In Situ Hybridization Can Be Positive in Adenocarcinomas and Other Tumors From Diverse Sites

Aqsa Nasir, MBBS, Heidi D. Lehrke DO, Toufic Mounajjed, MD, Samar Said, MD, Lichi Zhang, MD, Saba Yasir, MD, Sejal S. Shah, MD, Vishal S. Chandan, MD, Thomas C. Smyrk, MD, Roger K. Moreira, MD, Jennifer M. Boland Froemming, MD, Loren P. Herrera Hernandez, MD, TeongTeh Wu, MD, PhD, and Randall P. Graham, MBBS

From the Division of Anatomic Pathology, Mayo Clinic, Rochester, MN.

Table 1
Tumor Types Evaluated With Albumin In Situ Hybridization (ISH)

Tumor Type	Age Range (Median), y	No. of Cases	No. (%) of Albumin ISH-Positive Cases	Score of Albumin ISH-Positive Cases				
				0	+1	+2	+3	+4
Conventional hepatocellular carcinoma	23-83 (59.5)	22	22 (100)					22
Acinar cell carcinoma	43-78 (60)	7	2 (29)	5	1	1		
Adrenocortical carcinoma	22-79 (60)	11	0	11				
Ampullary adenocarcinoma	20-82 (70)	16	0	16				
Breast invasive ductal carcinoma	35-95 (86)	11	2 (18)	9	1	1		
Chromophobe renal cell carcinoma	52-84 (71)	11	0	11				
Colorectal carcinoma	31-83 (59)	15	0	15				
Clear cell renal cell carcinoma	56-78 (67)	6	0	6				
Extrahepatic cholangiocarcinoma	52-82 (68.5)	14	0	14				
Fibrolamellar carcinoma	19-32 (25)	4	4 (100)					4
Gallbladder adenocarcinoma	39-70 (63)	13	5 (39)	8	2	3		
Gastroesophageal adenocarcinoma	52-88 (65)	10	2 (20)	8	1	1		
Hepatoid pancreatic adenocarcinoma	65-78 (75.5)	3	1 (33)	2				1
Intrahepatic cholangiocarcinoma	35-82 (63)	27	22 (81)	5	8	4	2	8
Lung adenocarcinoma	52-84 (71.5)	15	3 (20)	12	3			
Pancreas ductal adenocarcinoma	59-83 (76)	14	0	14				
Stomach adenocarcinoma	35-92 (72.5)	17	0	17				
Yolk sac tumor	11-68 (19.5)	8	2 (26)	6	2			

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HCC vs. Look-alikes

- Renal cell carcinoma
- Adrenal cortical carcinoma
- Angiomyolipoma
- Malignant melanoma
- Metastatic hepatoid carcinomas
- Hepatoid yolk sac tumor
- Thyroid follicular carcinoma, oncocytic type

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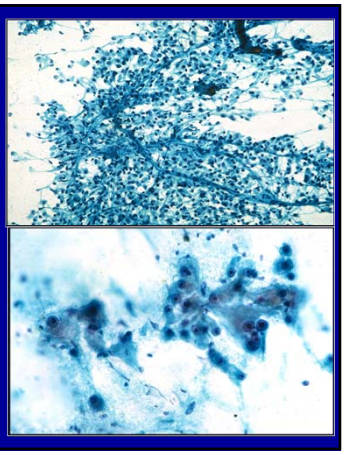
Renal Cell Carcinoma
Morphological features

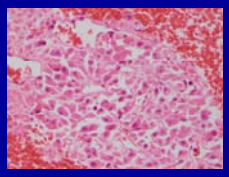
- Mostly single cells or loose clusters
- Cytoplasm clear or granular
- Atypical nuclei with prominent nucleoli
- Single naked nuclei
- Vascular neoplasm with transgressing endothelial pattern
- IHC: PAX 8, CD10 cytoplasmic, RCC

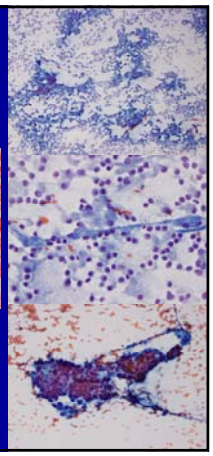


61

Adrenal Cortical Carcinoma

- Very vascular
- Can have peripheral
- endothelial cell wrapping pattern, particularly on cell block





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IHC: HCC vs. RCC vs. ACC

Antibody	HCC	RCC	ACC
LMW/HMW	+/-	+/+	-/-
pCEA	canalicular	-	-
vimentin	-	+	+
Synaptophysin/MART-1/ inhibin	-	-	+
CD 10	canalicular	cytoplasmic	-
HepPar/Arginase	+	-	-
PAX8/CAIX	-	+	-

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Summary

- Diagnosis of HCC relies on architecture, cytomorphology and background features
- Peripherally wrapping endothelial cells are pathognomonic for HCC.
- Arborizing vessels common in HCC, but also in RCC
- Abnormal hepatic plate architecture (>3 cells thick) supports HCC; highlighted by reticulin stain and CD 34
- Glypican-3, HepPar-1, Arginase-1, ß-catenin, HSP70 and GS are helpful markers for the diagnosis of HCC
- Intrahepatic cholangiocarcinoma is diagnosed usually by clinical exclusion of other adenocarcinomas
  - CISH for albumin sensitive and specific

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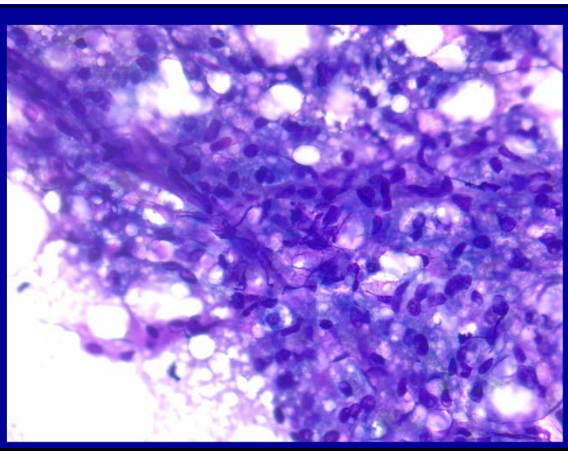
Case 1

- The patient was a 35 year old female on oral contraceptives. Imaging was performed because of abdominal pain and a round, well-defined mass was identified in the liver.

65



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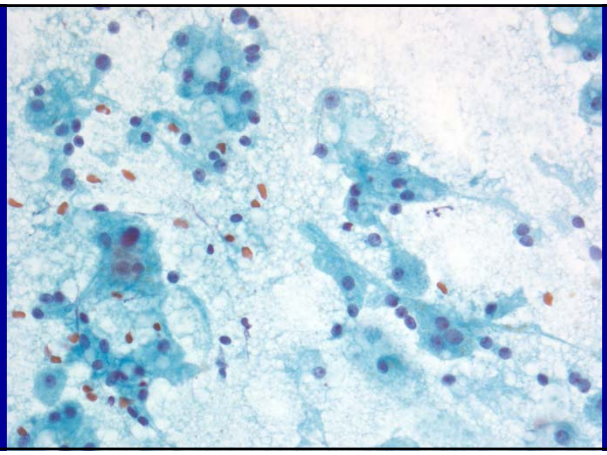

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Case 1: The findings represent?

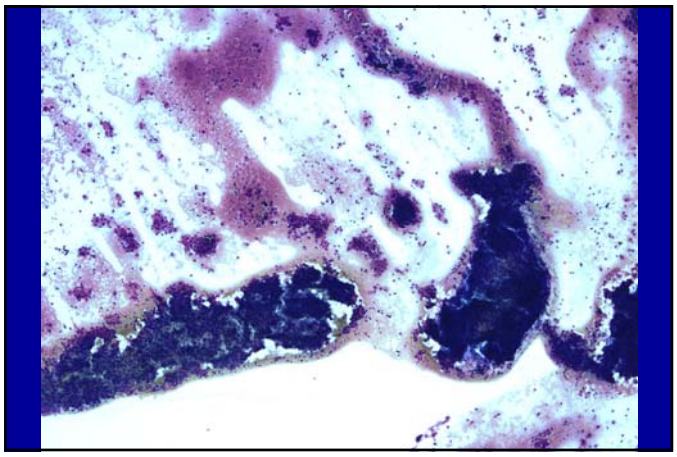
- A. Hepatoblastoma
- B. Angiomyolipoma
- C. Benign hepatocytes, consistent with adenoma
- D. Well differentiated hepatocellular carcinoma

68

Case 2

- The patient is a 66 yo male with a history of cirrhosis. He has a rising AFP. Imaging shows a liver mass. The patient has a core biopsy with touch imprint for on-site adequacy.

69



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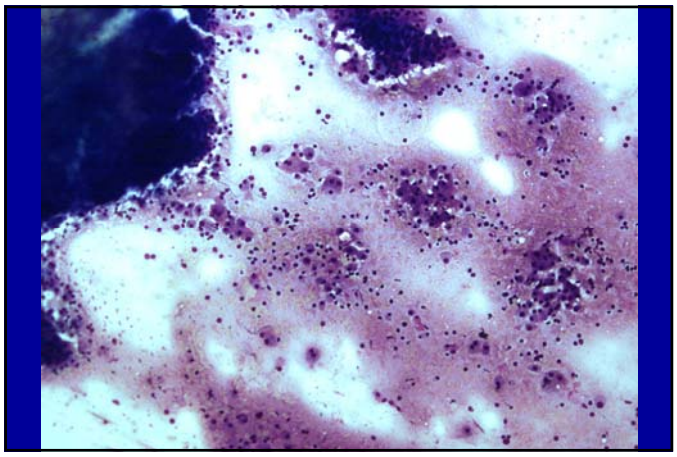

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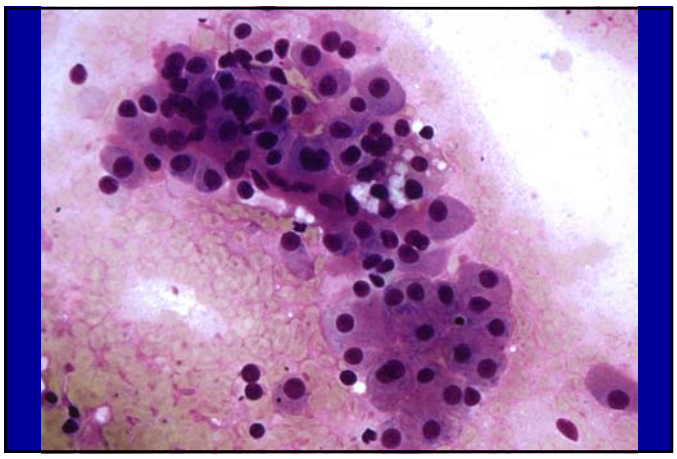

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Case 2: What is your assessment?

- A. Benign hepatocytes
- B. Liver adenoma
- C. Hepatocellular carcinoma
- D. Cholangiocarcinoma

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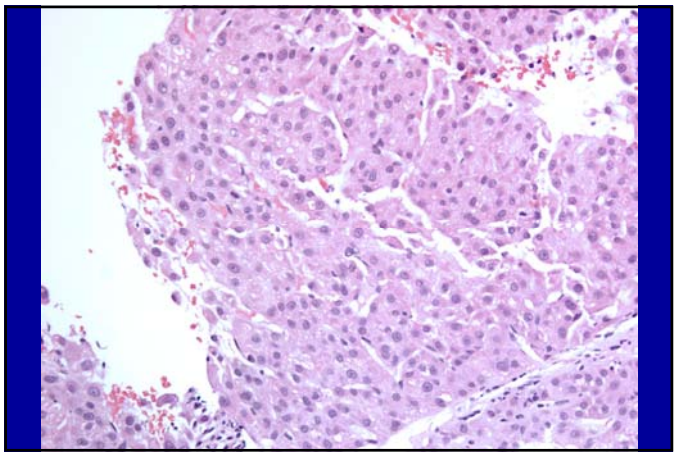

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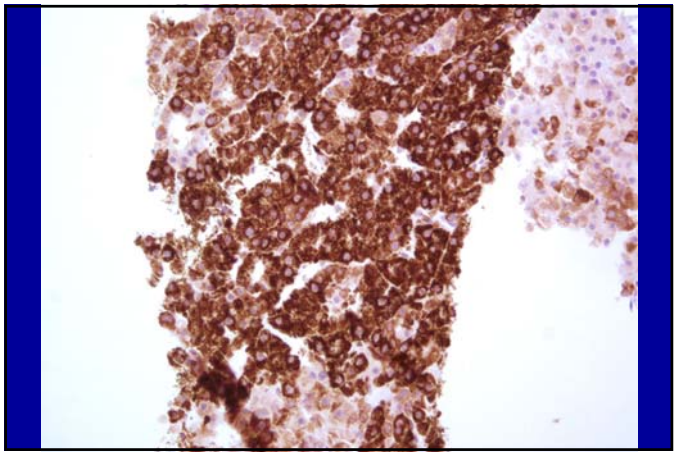

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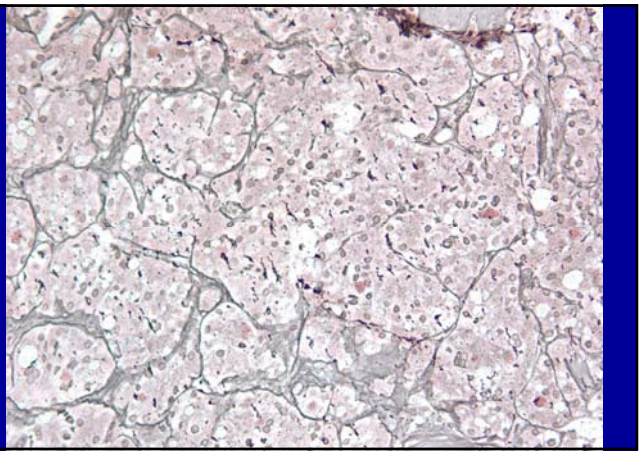

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Case 3

- The patient was a 67 year old male who presented with a kidney mass and a liver mass. The FNA is of the liver mass.

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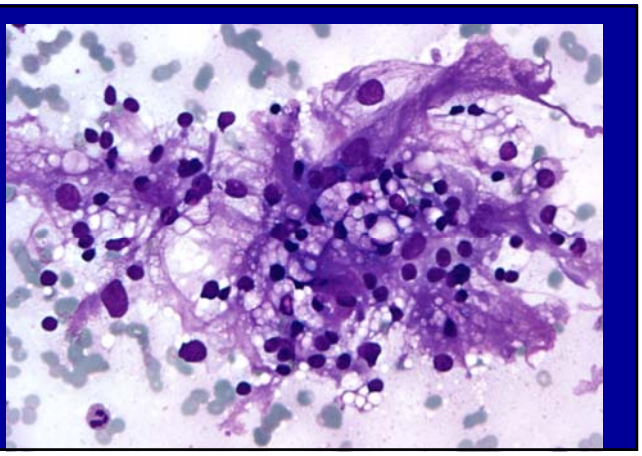

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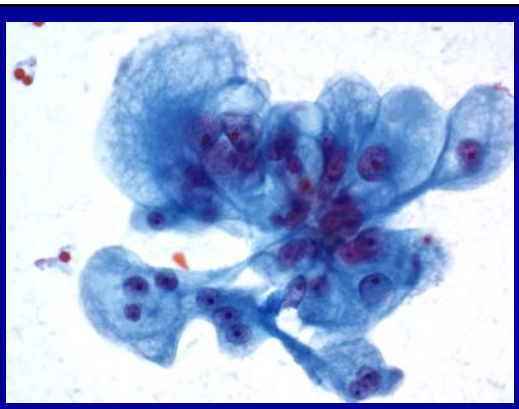

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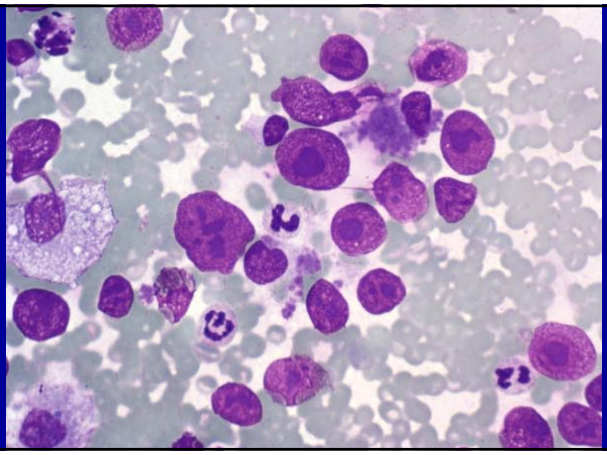

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Case 3: What is your assessment?

- A. Poorly differentiated adenocarcinoma
- B. Clear cell variant hepatocellular carcinoma
- C. Metastatic renal cell carcinoma
- D. Liposarcoma

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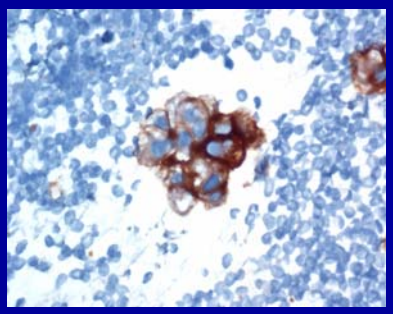
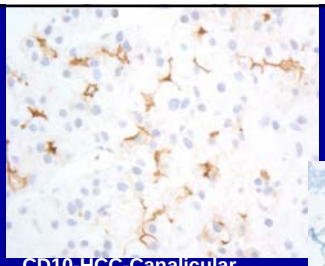
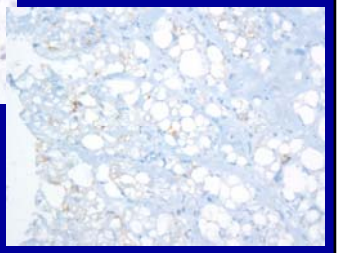
CD10



82



CD10-HCC-Canalicular pattern



HepPar granular and focal in clear cell HCC

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