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

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
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 malignanec

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

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, lipofuscin, cholestasis
**PIGMENTS**

- Bile
- Lipofuscin

**Bile Duct Cells**

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

**BENIGN LIVER NODULES**

- 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
**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**
HEPATOCELLULAR ADENOMA

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

Update on the New Classification of Hepatic Adenomas
Clinical, Molecular, and Pathologic Characteristics

<table>
<thead>
<tr>
<th>Hepatic Adenomas</th>
<th>Subtypes</th>
<th>Clinical, Molecular, Pathologic, and Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA1a</td>
<td>33-40%</td>
<td>Benign hepatocytes, thin-walled arteries, normal architecture</td>
</tr>
<tr>
<td>HA1b</td>
<td>17-19%</td>
<td>Benign hepatocytes, thin-walled arteries, normal architecture</td>
</tr>
<tr>
<td>HA1c</td>
<td>30-33%</td>
<td>Benign hepatocytes, thin-walled arteries, normal architecture</td>
</tr>
<tr>
<td>HA1d</td>
<td>10%</td>
<td>Benign hepatocytes, thin-walled arteries, normal architecture</td>
</tr>
</tbody>
</table>

In addition to the above Table,请参阅图示内容。
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

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, pseudoacin
- Cytological features
  - Nuclear to cytoplasmic ratio (N/C)
  - Nucleation, nucleoli
  - Cytoplasmic features and contents

Naked Eye Inspection

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

HEPATOCELLULAR CARCINOMA
Background

HEPATOCELLULAR CARCINOMA
Peripheral Endothelial Cell Wrapping Pattern
### Benign vs Malignant Liver Nodules

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

### 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
Reticulin Stain Pitfall:
Marked steatosis

Ancillary Studies: Benign or Malignant
Immunocytochemistry

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

CD34

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

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
  - Wilms tumor, melanoma, ovarian carcinomas, and other malignancies
  - Not specific for hepatocytic differentiation
**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**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Staining Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Zone 3/perivenular cuff</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Patchy and weak perportal</td>
</tr>
<tr>
<td>FNH</td>
<td>Strong map-like pattern</td>
</tr>
<tr>
<td>ß-catenin mutated adenoma</td>
<td>Strong, diffuse</td>
</tr>
<tr>
<td>Other adenomas</td>
<td>Weak/patchy</td>
</tr>
<tr>
<td>HCC</td>
<td>Strong/diffuse</td>
</tr>
</tbody>
</table>


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**Indeterminate Aspirate**

- Transgressing endothelium
- Increased N/C
- Monotonous pattern
- Focal on the smear
- Material insufficient for ancillary testing
Ancillary studies
Benign vs Malignant

<table>
<thead>
<tr>
<th>Marker</th>
<th>Benign</th>
<th>HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulin</td>
<td>Normal</td>
<td>Loss or variable pattern</td>
</tr>
<tr>
<td>Iron</td>
<td>Present</td>
<td>Lost (hemochromatosis)</td>
</tr>
<tr>
<td>Glypican 3</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>HSP 70</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>GS</td>
<td>Centered on central veins</td>
<td>Diffuse</td>
</tr>
<tr>
<td>CD 34</td>
<td>None in nonlesional liver</td>
<td>Increased capillarization</td>
</tr>
</tbody>
</table>

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

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
Poorly differentiated HCC

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

Anatomical, histomorphological and molecular classification of cholangiocarcinoma
ICC Variants

- Small duct type
  - Cholangiolocarcinoma
  - Ductal Plate
    malformation Pattern

- Large Duct Type
  - Adenosquamous
  - Mucinous carcinoma
  - Signet ring cell
  - Clear cell carcinoma
  - Clear cell carcinoma
  - Mucoepidermoid
  - Lymphoepithelioma like

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

FNA Smear Pattern-Cholangiocarcinoma
FNA Smear Pattern - Cholangiocarcinoma

Challenge: HCC vs Adenocarcinoma (ICC)
- Bile pigment in HCC
- Mucin in ICC

HCC VS ICC
- Gland formation in ICC
- HCC TE pattern or PE pattern
HCC vs. ICC

<table>
<thead>
<tr>
<th>Feature</th>
<th>HCC</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gland formation</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PE or TE</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mucin</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Bile</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Stripped nuclei</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

KERATIN IHC

HMW CK in adenocarcinoma
LMW CK in HCC

Canalicular Pattern Markers

CD10
pCEA
CEA (P)

HCC

Adenocarcinoma

Markers of Hepatocytic Differentiation

HepPar

Arginase

HepPar false positives

More sensitive and specific

Markers of Glandular Differentiation

MOC31

Arch Pathol Lab Med. 2007;131:1648–1654
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 polygonal cells with abundant cytoplasm, large nucleoli and intranuclear inclusions

Metastatic Adenocarcinomas

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

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
- GP14: 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, thyroglobulin: thyroid primaries
- ISH high risk HPV: lower anogenital tract primaries, some oral cancers
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
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

Adrenal Cortical Carcinoma

• Very vascular
• Can have peripheral
• Endothelial cell wrapping pattern, particularly on cell block

IHC: HCC vs. RCC vs. ACC

<table>
<thead>
<tr>
<th>Antibody</th>
<th>HCC</th>
<th>RCC</th>
<th>ACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMW/HMW</td>
<td>+/-</td>
<td>+/+</td>
<td>-/-</td>
</tr>
<tr>
<td>pCEA</td>
<td>canicular</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>vimentin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Synaptophysin/MART-1/Inhibin</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD 10</td>
<td>canicular</td>
<td>cytoplasmic</td>
<td>-</td>
</tr>
<tr>
<td>HepPar/Arginase</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PAX8/CAIX</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
**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.

**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.
Case 1: The findings represent?

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

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.
Case 2: What is your assessment?

• A. Benign hepatocytes
• B. Liver adenoma
• C. Hepatocellular carcinoma
• D. Cholangiocarcinoma
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.
Case 3: What is your assessment?

- A. Poorly differentiated adenocarcinoma
- B. Clear cell variant hepatocellular carcinoma
- C. Metastatic renal cell carcinoma
- D. Liposarcoma
CD10-HCC-Canalicular pattern

HepPar granular and focal in clear cell HCC