Indications: Evaluation of a Mass

- **US**
  - Rapid localization
  - Flexible imaging
  - Flexible patient positioning
  - No radiation

- **CT**
  - Resolution of small lesions
  - Best identifies the needle tip
  - Best determines tissue components and vascularity
  - No transmission of impediments (drains, bone, gas)
  - Precise anatomic relationships

- **EUS**
  - Resolution of 0.5 cm, better than CT
  - Short trajectory through stomach wall
  - Limited to left lobe of the liver

Guidance Systems

**US**
- Rapid localization
- Flexible imaging
- Flexible patient positioning
- No radiation

**CT**
- Resolution of small lesions
- Best identifies the needle tip
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**EUS**
- Resolution of 0.5 cm, better than CT
- Short trajectory through stomach wall
- Limited to left lobe of the liver
Tissue Management: No ROSE
FNA using 22-23 g needle

Direct smears fixed in alcohol  Liquid–based processing

Core Needle Biopsy
18 g trucut biopsy gun

Tissue core fixed in formalin

---

Tissue Management
FNA  Core

Results of FNAB and CNB in the Diagnosis of Malignancy in 49 Focal Liver Lesions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FNAB</th>
<th>CNB</th>
<th>FNAB + CNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (n)</td>
<td>32</td>
<td>31</td>
<td>36</td>
</tr>
<tr>
<td>TN (n)</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>FP (n)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FN (n)</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Nondx’ic (n)</td>
<td>8</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>92</td>
<td>89</td>
<td>95</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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Core Biopsy Supplements
Aspiration Smears

---
Most Common Differential Diagnosis of Mass Lesions

Cirrhotic liver
- Macropigmented nodule/Low-grade dysplastic nodule
- Small cell/High-grade dysplastic nodule
- Hepatocellular carcinoma (HCC)
- Cholangiocarcinoma

Non-cirrhotic liver
- Hepatocellular adenoma (HCA)
- Focal nodular hyperplasia (FNH)
- HCC
- Metastasis
- Cholangiocarcinoma

Hepatocellular Carcinoma

- Small HCC < 2cm
  - Early HCC- extremely well-differentiated; almost impossible to distinguish from small cell dysplasia
  - Progressed HCC- recognizable thickened hepatic plates

- Most nodules in a cirrhotic liver > 2cm = HCC
  - Characteristic imaging features with arterial hypervascularity and venous washout on CT/MRI
  - Ablated or resected without biopsy

Most Common Differential Diagnosis of Mass Lesions

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Non-cirrhotic liver
- Hepatocellular adenoma
- Focal nodular hyperplasia
- HCC
- Metastasis
- Cholangiocarcinoma

Hepatocellular lesions: Benign versus Malignant
MRN/ILow-grade dysplasia

- Well defined nodule 0.5-1.5 cm with more than one portal tract
- No increased cell density
- 1-2 cell thick trabeculae
- Large cell change focal or diffuse

Small cell/High-grade dysplasia

- Small cell dysplasia: 1-2 cm nodule without portal tracts
- Increased cell density
- Increased N/C ratio
- Increased hepatic plate thickness
- Increased proliferation rate
- Focal changes in larger nodules

Focal Nodular Hyperplasia

- Non-cirrhotic liver; F>M
- Tumor-like malformation from abnormal blood flow
- Hyperplastic nodules of hepatocytes, fibrous septae
- Stellate scar
- Abnormal vessels (eccentric thickening)
- Ductular reaction; thick, muscular vessels
- Preserved reticulin
- Focal capillarization of sinusoids
Hepatocellular Adenoma

- Non-cirrhotic liver: F>>M
- Oral Contraceptive use
- Surgically excised to reduce risk of hemorrhagic rupture
- Cords and sheets without acini
- Enlarged hepatocytes due to glycogen and/or fat; bland nuclei without nucleoli
- Thin-walled unpaired arterioles
- Retained reticulin; focal CD34+ sinusoids

Hepatocellular Carcinoma?

- HCC
**Well-differentiated Hepatocellular Carcinoma**

**Differential Diagnosis**
Hepatocellular nodule in a cirrhotic liver

**Differential Diagnosis**
Hepatocellular nodule in non-cirrhotic liver
Benign versus HCC

Components of the Benign Aspirate

**hepatocytes**: monolayered sheets, small groups without endothelial wrapping or central proliferation, and thin (1-2 cell) trabeculae; cells are polygonal with low N:C ratio, 1-2 central round nuclei, often prominent nucleoli, and abundant, granular cytoplasm. Lipofuscin pigment is common; bile occasionally.

Components of the Benign Aspirate

**bile duct epithelial cells**: smaller than hepatocytes and commonly present in flat sheets with honeycomb glandular appearance, occasionally on edge and in acinar arrangements.
Diagnostic Challenges

- Recognizing well-differentiated hepatocellular carcinoma

Differentiating benign from malignant hepatocytes

Architecture
- Cell group shape/arrangement/trabecular thickness
- Vascularity (endothelial cells)

Cytomorphology
- Nuclear:cytoplasmic ratio
- Nucleation; nucleoli
- Cytoplasmic features and contents

Background
- Presence/absence of bile duct epithelium
- Stripped naked nuclei

Physical Characteristics of the smear pattern

(Yang GC, Yang GL, and Tao LC. Distinguishing well-differentiated hepatocellular carcinoma from benign liver by the physical features of fine-needle aspirates. Mod Pathol 2004; 17(7): 798-802)
Architecture

Benign
- Jagged irregular shaped groups; thin trabeculae

Malignant
- Smooth edged clusters; nests and thick trabeculae

Benign
- Thin (<3 cells) trabeculae

Malignant
- Thick (>3 cells) trabeculae

Benign
- No endothelial wrapping

Malignant
- Endothelial wrapping
What is peripheral endothelial wrapping?

- It is the elongated endothelial cell nuclei wrapping around multicellular groups and rounded nests of hepatocytes.
- It is NOT the presence of normal endothelial cells lining the 1-2 cell layer thick hepatic plate architecture.

**Architecture**

<table>
<thead>
<tr>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare, focal endothelial cells</td>
<td>Frequent, prominent proliferating endothelial cells</td>
</tr>
</tbody>
</table>

**Cytomorphology**

<table>
<thead>
<tr>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleomorphism</td>
<td>Monomorphism</td>
</tr>
<tr>
<td>Cytomorphology</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>Benign</strong></td>
<td><strong>Malignant</strong></td>
</tr>
<tr>
<td>- low N:C ratio; some prominent nucleoli</td>
<td>- increased N:C ratio; macronucleoli; hyaline globules</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fibrolamellar Variant</th>
<th></th>
</tr>
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<tr>
<td><strong>Benign</strong></td>
<td><strong>Malignant</strong></td>
</tr>
<tr>
<td>- bile duct epithelium; few naked nuclei and not atypical</td>
<td>- No bile duct epithelium; atypical naked nuclei</td>
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<table>
<thead>
<tr>
<th>Background</th>
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</table>
**Cell Block**

**Core Biopsy**

HCC  LCA

**Ancillary Tests: Benign vs. Malignant**

- Reticulin Stain
- Immunocytochemistry
Ancillary Studies: Benign or Malignant
Reticulin (silver)

Benign

Malignant

Cirrhosis

Reticulin Stain Pitfall: Marked steatosis

False negative
Markers for Well-differentiated HCC
(differential diagnosis: benign versus malignant)

- AFP
- CD34
- Glypican 3

Alpha Fetoprotein

- Alpha Fetoprotein (AFP) is an oncofetal antigen of 70 kD
- AFP is expressed in fetal liver but is not present under normal circumstances in healthy adult tissues.
- It is expressed in some hepatocellular carcinomas, germ cell tumors, with high frequency in yolk sac tumors.

Ancillary Studies: Benign or Malignant
Immunocytochemistry

Alpha-fetoprotein
- helpful if positive, but only 35-40% positive
- negative stain does not rule out tumor
CD34

- Intercellular adhesion protein and cell surface glycoprotein; ligand is CD62L (L-selectin)
- Many uses in histology
- Liver: highlights capillarization of the sinusoids


Stain to highlight abnormal vascular pattern: capillarization of the sinusoids

CD34: Adenomas and FNH can have patchy staining pattern; SCD, eHCC similar focal staining
Glypican 3

- Membrane-anchored heparin sulfate proteoglycan, and oncofetal protein
- Staining can be membranous, canalicular, or cytoplasmic
- Useful to distinguish benign from malignant hepatocellular proliferations
- Stains variants of HCC including fibrolamellar and scirrhouos
- Well differentiated HCC can be negative
- Can be positive in nonseminomatous germ cell tumor, melanoma, rarely colonic adenocarcinoma
- Stains some high grade dysplastic nodules, so cannot be used to distinguish HGDN from WDHCC reliably (especially as a single stain)

**Other Markers**

- **Heat Shock Protein 70 (HSP70)**
  - Highly conserved protein involved in cell cycle regulation and apoptosis
  - Upregulated in HCC
- **Beta catenin**
  - Key component of the Wnt pathway in cell adhesion and proliferation
  - Mutations lead to nuclear translocation and thus nuclear staining with IHC
  - Positive in 20-40% of HCC; but some LCA as well; not FNH
- **Glutamine Synthetase (GS)**
  - Upregulated as a consequence of nuclear translocation of beta catenin, so tumors with beta catenin mutations show strong cytoplasmic staining

**Limitations of these stains with small biopsies due to overlapping and focal staining patterns**

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- HCC
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**Hepatocellular lesions: Primary versus Metastatic**
Differential Diagnosis of Primary Hepatic versus Metastatic Epithelial Malignancy

- Hepatocellular carcinoma, high grade
- 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 Tumors

- Accounts for 70-80% of malignant liver tumors
- Most Common
  - Colon
  - Pancreas
  - Breast
  - Lungs
- Less Common
  - Malignant melanoma
  - Squamous cell carcinoma
  - Prostate
  - Renal cell carcinoma
  - Neuroendocrine tumors: carcinoid and islet cell tumors
  - Small cell undifferentiated carcinoma
  - Lymphoma
  - Sarcomas

Computed Tomography
Gross Morphology of Hepatocellular Carcinoma

Metastatic Colon Carcinoma

Dirty necrosis

Metastatic Colon Cancer

- Confirmatory IHC
  - CDX2+ (nuclear)
- Up to 30% of patients can be cured or have > 5 yr. survival if completely resected
- Chemotherapy: Primary
  - Oxaliplatin plus FU and leucovorin (FOLFOX)
- Targeted Chemotherapy
  - Cetuximab (Erbitux®)
    - Works on KRAS neg. cancers
    - KRAS testing can be done on core bx. or CB
**Angiomyolipoma**

**Immunocytochemistry: Primary vs. Metastatic**

- **AFP**: Strong positive staining supports a diagnosis of HCC but negative staining does not rule it out.
- **HepPar-1**: anti-hepatocyte antibody- highly specific for hepatic differentiation but staining positivity decreases with decreasing differentiation.
- **Arginase-1**: Stains HCC and not other tumors with more sensitivity and specificity than HepPar1.
- **Glypican-3**: A specific (96%) marker for hepatic differentiation.
- **MOC-31**: adenocarcinoma including cholangiocca., bladder, renal and neuroendocrine tumors (80-90%); HCC neg to weakly positive.
- **CEA(polyclonal)**: Stains positively 24-80% HCC in a "canalicular" pattern, especially those tumors with trabecular or acinar morphology.

**Ancillary Studies: HCC vs. Metastasis**

- **Alpha-fetoprotein** - helpful if positive, but only 35-40% positive.
- **Negative stain does not rule out tumor**.
**Immunocytochemistry: HCC versus Metastasis**

**pCEA CD10**

**Adenoca.**

**HCC**

---

**Caveats:**
- Positivity of cells correlates with grade, e.g. neg. stain in PDCa does not rule out HCC
- Not 100% specific, e.g. other tumors, especially hepatoid tumors, also stain-gastric carcinomas +/- hepatoid differentiation

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**HepPar-1**

---

**Arginase-1**

- Arg-1 is more sensitive than Hep Par-1
  - Arginase
    - Well- 100%
    - Moderately- 96.2 %
    - Poorly differentiated HCCs- 85.7%
  - HepPar-1
    - Well- 100%
    - Moderately- 83.0%
    - Poorly differentiated- 46.4%
Immunocytochemistry: HCC versus Metastasis: MOC-31

Hep Par 1 and MOC-31

- Hep Par 1 diffusely positive; MOC-31 negative
  - Consistent with HCC
  - If morphology is not typical, add pCEA or GPC-3
- Hep Par 1 negative; MOC-31 diffusely positive
  - Highly unlikely to be HCC. Can confirm (if necessary) with arginase-1, pCEA and GPC-3.
  - Proceed to determination of primary
- Hep Par 1 positive, MOC-31 positive
  - Uncommonly HCC expresses MOC-31
  - Consider adenocarcinoma from stomach, esophagus, lung, etc. that can stain with Hep Par 1.

Hep Par 1 and MOC-31

- Hep Par 1 negative; MOC-31 negative
  - HCC lacking Hep Par 1, possibly poorly differentiated. Add glypican-3, arginase-1 and pCEA
  - Adenocarcinoma lacking MOC-31
    - Diffuse/strong keratin 7 and absent canalicular pCEA argue against HCC
    - Keratin 19 and CA19-9 support Cholangio.
    - Other markers to support ACA: B72.3, Ber-Ep4.
  - Polygonal cell tumors other than HCC, including renal cell carcinoma, adrenocortical carcinomas, and neuroendocrine tumors
A poorly differentiated hepatocellular carcinoma (A, H&E) that was negative for Arginase-1 and Hep Par-1 (not shown) is positive for albumin (B, in situ hybridization for albumin). Note the dot-like pattern of reactivity.


Carcinoma of Unknown Primary

<table>
<thead>
<tr>
<th>CK 7+ CK20+</th>
<th>CK 7+ CK20-</th>
<th>CK 7- CK20+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urothelial tumors</td>
<td>Lung Adenocarcinoma</td>
<td>Hepatocellular Ca</td>
</tr>
<tr>
<td>uroplakin III (50-60+)</td>
<td>TTF-1, Napsin A</td>
<td>AFP</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>Breast Carcinoma</td>
<td>HepPar-1</td>
</tr>
<tr>
<td>Ovarian Mucinous Ca</td>
<td>GCDFP-15</td>
<td>Arginase-1</td>
</tr>
<tr>
<td>Pancreatic Adenocarcina</td>
<td>Mammoglobin</td>
<td>Cam 5.2+/AE1-</td>
</tr>
<tr>
<td>CK 19</td>
<td>Thyroid Carcinoma</td>
<td>pCEA</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>TTR-1</td>
<td>CD10+</td>
</tr>
<tr>
<td>CK 19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Types of Cholangiocarcinoma

- Mass forming
- Peri-ductal
- Intraductal

FNA

FNA and Brush

Brush

Review: Gastroenterology 2013; 145:1215-1229
Core Biopsy of Liver Mass:

Adenocarcinoma consistent with cholangiocarcinoma

- Solitary liver mass (usually)
- Typical cribiforming morphology with sclerosis
- CK 19 and/or CA19-9 positive
- No other apparent primary

RNA-CISH

Albumin

Positive
- Hepatocellular carcinoma
- Intrahepatic cholangiocarcinoma
- Idioid tumor

Negative
- Metastatic adenocarcinoma
- Extrahepatic cholangiocarcinoma

TL Rice, et al. Chromogenic in situ hybridization for albumin distinguishes intrahepatic cholangiocarcinoma from non-hepatic neoplasms [2014 USCAP Poster]
Brush cytology during ERC(P)

- Method of choice for diagnosing biliary strictures
- Brush cytology has very high specificity (>95%) but very low sensitivity (17-40%)
- Low Sensitivity: Insufficient Quality and Quantity of cells in the sample

Brush cytology during ERCP

- Method of choice for diagnosing biliary strictures
- Brush cytology has very high specificity (>95%) but very low sensitivity (17-40%)
- Low Sensitivity: Insufficient Quality and Quantity of cells in the sample

High Specificity
(low false positive rate)
Low Sensitivity of Bile duct Brushing Cytology
(false negative result, e.g. not POSITIVE)

Insufficient Quality and Quantity of cells in the sample

Low Sensitivity of Bile duct Brushing Cytology

- Crush
- Blood
- Air drying
- Necrosis

ThinPrep® of Bile Duct Brushings

- Improves preservation
- May require modification of criteria
- Combined direct smears and TP improves sensitivity and accuracy

- Volmar, et.al., 2006
- Siddiqui, et.al., 2003
- Ylagan, et. Al. 2003
Indeterminate Interpretations Common

- High threshold for malignancy
- Underlying inflammatory disease
- Stent placement with reactive changes
- Overlapping features of repair, dysplasia and malignancy

Improving the accuracy of pancreaticobiliary tract cytology with fluorescence in situ hybridization

Identification of Malignant Cytologic Criteria in Pancreatobiliary Brushings with Corresponding Positive Fluorescence In Situ Hybridization Results
Fritcher, EB… and Clayton, AC. Am J Clin Pathol 2011. 136;442-449

- Evaluated cytomorphological criteria of FISH positive bile duct brushing specimens
  1) Single abnormal cells
  2) Irregular nuclear membranes
  3) Enlarged nuclei (> 3x normal or variation in a group)
Next-Generation Sequencing and Fluorescence in situ Hybridization have Comparable Performance Characteristics in the Analysis of Pancreaticobiliary Brushings for Malignancy


Proposed Terminology from PSC

I. Nondiagnostic
II. Negative
III. Atypical
IV. Neoplastic
   -Benign
   -Other
V. Suspicious
VI. Positive/Malignant

Take Home Points

- Diagnosis of HCC relies on architecture, cytology and background features
- Peripherally wrapping endothelial cells are virtually pathognomonic for HCC in my experience
- Arborizing vessels common in HCC, but also in RCC
- Abnormal hepatic plate architecture (>3 cells thick) supports HCC; is highlighted by reticulin stain; CD 34
- Glypican-3, HepPar-1 and Arginase-1 are helpful markers for the diagnosis of HCC
- Cholangiocarcinoma diagnosed by FNA is usually by clinical exclusion of other adenocarcinomas plus CK19/CA19-9+
- Bile duct brushing has high specificity but low sensitivity
  - Single cells, anisonucleosis 3x in a group; irregular nuclear membranes support malignancy
  - FISH holds promise for improvement in diagnosis
  - NGS may be easier, most cost-effective and just as accurate but needs more study