There Are No Magic Bullets: When Immunostains Can Get You into Trouble in Hepatic & Gastrointestinal Pathology

John Hart, M.D.
Sections of Surgical Pathology & Hepatology
University of Chicago Medical Center
john.hart@uchospitals.edu

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D. John Hart has nothing to disclose.

I LOVE Immunostains
WE'VE TRIED NOTHING
AND WE'RE ALL OUT OF IDEAS

I LOVE Immunostains
Rectal Syphilis
H&E
Warthin-Starry

Korean J Gastroenterol 2016; 68: 218-20

Two Types of Pitfalls
The Clinician Helps You Blow It
You Blow It All By Yourself
Topics of Discussion

1. Hepatoid adenocarcinoma metastatic to the liver
2. Hepatic epithelioid hemangioendothelioma
3. Combined HCC-cholangiocarcinoma

Clinical History
Case Courtesy of Dr. J. Gonzalez, Alexian Brothers Medical Center

- 72 y.o. M with shortness of breath & abdominal pain
- s/p distal gastrectomy in 1959 for peptic ulcer disease
- TB = 0.7, AST = 41, ALT = 55, alk phos = 201
- CT scan reveals multiple masses in the liver
- HBV and HCV negative
- Serum AFP = 3,455

Clinical History

- CT scan reveals multiple masses in the liver
- HBV and HCV negative
- Serum AFP = 3,455

Clinical History

- CT scan reveals multiple masses in the liver
- HBV and HCV negative
- Serum AFP = 3,455

Final Dx: Metastatic Hepatoid Adenocarcinoma

Upper endoscopy revealed a gastric tumor

Hepatoid Adenocarcinoma

<table>
<thead>
<tr>
<th>Primary gastric</th>
<th>Arginase-1</th>
<th>CDX2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary gastric</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
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<tr>
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<tr>
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<td>negative</td>
</tr>
<tr>
<td>Hepatic metastasis of gastric primary</td>
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</table>
**Immunoreactivity Panel (rough estimates)**

<table>
<thead>
<tr>
<th></th>
<th>HCC</th>
<th>Foregut AdenoCa</th>
<th>Hepatoid AdenoCa</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM5.2</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>HepPar-1</td>
<td>80%</td>
<td>40%</td>
<td>90%</td>
</tr>
<tr>
<td>Gypican-3</td>
<td>80%</td>
<td>10%</td>
<td>90%</td>
</tr>
<tr>
<td>CD10</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>CK7/CK20</td>
<td>20%</td>
<td>90%</td>
<td>25%</td>
</tr>
<tr>
<td>Arginase-1</td>
<td>90%</td>
<td>&lt; 5%</td>
<td>0%</td>
</tr>
<tr>
<td>CDX2</td>
<td>0%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>MOC31</td>
<td>5%</td>
<td>&gt; 90%</td>
<td>&gt; 90%</td>
</tr>
</tbody>
</table>

**Table 2. Results of Immunohistochemistry in Surgical Cases**

<table>
<thead>
<tr>
<th></th>
<th>ARG1</th>
<th>HepPar-1</th>
<th>BSEP</th>
<th>MDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCCs (N = 54)</td>
<td>52 (96)</td>
<td>30 (56)</td>
<td>49 (91)</td>
<td>45 (83)</td>
</tr>
<tr>
<td>Well to moderately differentiated (N = 33)</td>
<td>31 (94)</td>
<td>32 (97)</td>
<td>33 (100)</td>
<td>31 (94)</td>
</tr>
<tr>
<td>Poorly differentiated (N = 21)</td>
<td>21 (100)</td>
<td>17 (81)</td>
<td>16 (76)</td>
<td>14 (67)*</td>
</tr>
<tr>
<td>ICCs (N = 34)</td>
<td>3 (9)</td>
<td>2 (6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatoid carcinomas (N = 27)</td>
<td>6 (22)</td>
<td>12 (44)</td>
<td>1 (4)†</td>
<td>0*†</td>
</tr>
</tbody>
</table>

**Figure 1: BSEP and MDR3**

**Figure 2: Differential proteomic and literal expression analyses identify volatile diagnostic biomarkers of hepatocellular differentiation and hepatoid adenocarcinomas**

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**Figure 3: Altered CDX2 expression in Hepatocellular Carcinomas: An Important Diagnostic Pitfall.**

Sejal S. Shah, M.D., Tzung-Teh Wu, M.D., Ph.D., Michael S. Torbenson, M.D., and Vishal S. Chaudhri, M.D.
Hepatoid Gastric Carcinoma

Hepatoid Gastric Carcinoma vs HCC

Bottom Line
- HepPar-1, AFP, Gypican-3, pCEA, CD-10 – pitfalls
- Arginase-1 and CDX2 – limited utility
- BHMP and FABP1 – worthy of study (with arginase-1)
- BSEP and MDR3 – worthy of study


Clinical History
Case Courtesy of Dr. G Kunz, Norton HealthCare, Louisville, KY
- 61 y.o. F with a 1.5 cm mass in the liver

CK7
CD31
Epithelioid Hemangioendothelioma
A Vascular Tumor Often Mistaken for a Carcinoma
SHARON W. WEISS, MD, AND F. M. ENDINGER, MD

Hepatic Vascular Lesions
- Angiosarcoma
- Epithelioid hemangioendothelioma
- Hepatic small vessel neoplasm
- Anastomosing hemangioma
- Hemangioma

Distribution of Keratins in Normal Endothelial Cells and a Spectrum of Vascular Tumors: Implications in Tumor Diagnosis
Hпор Hol. 31:1062-1067, 2000
MARKU MIEITINEN, MD, AND JOHN F. FETSCH, MD

Hepatic Angiosarcoma

48-year-old healthy male who developed abdominal pain and diarrhea while on a family vacation in Mexico
- Upon return to the U.S., he developed jaundice
- TB-2.4, AST=544, ALT=204, ALP prior = 223
- CT scan—massive infiltration of the liver
- Random liver biopsy performed

Robert Wadlow – WORLD’S TALLEST MAN (4th from left)
• 61 y.o. F presents with abdominal pain & bloating
• CT and MRI results – favor HCC:
  • 16 cm hypervascular liver mass
  • Innumerable small satellite lesions
  • 7 small lesions in spleen
  • No lymphadenopathy
  • No masses in other organs
• PET scan – only a 2 cm focus of hypermetabolic activity within the right liver lobe
• Serum AFP = 4.2
• TB = 0.5, ALP = 75, ALT = 75, alk phos = 177
• No known history of liver disease

Case Courtesy of Dr. Rashna Madan
University of Kansas Hospital

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Clinical History

• 67 year old asymptomatic male discovered to have a high serum PSA in December, 2004
• In April, 2005 the patient underwent radical prostatectomy for prostate cancer
• A post-operative CT scan revealed a non-specific subcentimeter liver mass

Clinical History

• In February, 2006 the patient underwent right hemicolecctomy for a T2, NO cecal adenocarcinoma
• Nine months after surgery the serum CEA began to rise and repeat CT scan revealed two lesions consistent with metastatic colonic adenocarcinoma

Clinical History

• The patient received FOLFOX plus bevacizumab with a substantial treatment response as seen in a follow-up CT scan

Clinical History

• The patient underwent resection of two liver lesions on November 28, 2007
• Gross examination:
  • One lesion was irregular and exhibited gross fibrosis and necrosis
  • The other lesion was well circumscribed and uniform
  • The surgical margin was free of tumor
Treated colon cancer metastasis

Second grossly circumscribed lesion

TFE3
Follow-up

- The patient developed pulmonary metastatic colonic adenocarcinoma which has responded to treatment with FOLFIRI
- FISH performed on the EHE reveals WWTR1/CAMTA1 gene fusion

Fig. 3 Genomic breakpoints demonstrated by PCR and FISH: Incidence of the WWTR1/CAMTA1 gene fusion in vascular neoplasms.


39 year old woman with incidentally found liver mass

Maria Westerhoff, M.D., University of Washington

Nuclear Expression of CAMTA1 Distinguishes Epithelioid Hemangioendothelioma From Histologic Mimics


<table>
<thead>
<tr>
<th>TFE3</th>
<th>CAMTA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic EHE</td>
<td>CD31</td>
</tr>
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</table>

TABLE 1. Summary of IHC Staining for CAMTA1 in EHE and Other Epithelioid Neoplasms.

<table>
<thead>
<tr>
<th>Marker</th>
<th>EHE</th>
<th>Other Epithelioid Neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERG</td>
<td>100% (21/21)</td>
<td>50% (10/20)</td>
</tr>
<tr>
<td>CD34</td>
<td>82% (32/39)</td>
<td>60% (24/40)</td>
</tr>
<tr>
<td>Pankeratin</td>
<td>31% (11/35)</td>
<td>50% (20/40)</td>
</tr>
<tr>
<td>CKB/18</td>
<td>30% (6/20)</td>
<td>40% (8/20)</td>
</tr>
<tr>
<td>TFE3</td>
<td>88% (21/24)</td>
<td>50% (12/24)</td>
</tr>
<tr>
<td>WWTR1-CAMTA1</td>
<td>94% (33/35)</td>
<td>60% (21/35)</td>
</tr>
<tr>
<td>YAPI-TFE3</td>
<td>6% (2/35)</td>
<td>20% (5/25)</td>
</tr>
</tbody>
</table>

Diagnostic Pathology 2014, 9:131

Novel YAPI-TFE3 Fusion Defines a Distinct Subset of Epithelioid Hemangioendothelioma

Cristina R. Alfonso, F.M. Guzman, A. Alfonso, J. Wu, W. Tse, K. K. Shih, and D. M. Fletcher

Diagnostic Pathology 2014, 9:131
Hepatic Epithelioid Hemangioendothelioma

Bottom Line

- Morphologic overlap with carcinoma
- Cytokeratin expression in 30% of cases
- TFE3 and CAMT1 immunostains helpful for diagnosis
- FISH testing for YAP1/TFE3 fusion and WWTR1/CAMTA1 fusion available

Clinical History

- 57 year old female with a 10 year history of chronic HCV hepatitis, genotype 2 (grade 2, stage 3)
- Serum AFP = 5321 IU/mL
- CT scan reveals a 3.5 cm mass in the liver
**Diagnosis**

Combined HCC-CholangioCa with progenitor cell features

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**Mutational Analysis**

- TP53 splice, site 920-2 A > G
- ATRX C1094*
- NOTCH2 W1710*

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**Constitutive Notch2 Signaling Induces Hepatic Tumors in Mice**

Michael T. Dill, Luigi Tocchini, Thoerion P. Fritsma, Luigi Terracciano, David Seneta, Bernhard Butzer, Markus H. Heim, and Jan S. Tschoni

*HEPATOLOGY 2013; 57:1607-1619.*

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**Hepatocellular Carcinoma**

**NOTCH-2**

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**Embryonic development of SOX9 positive bile ducts**

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**Intrahepatic Bile Ducts Develop According to a New Mode of Tubulogenesis Regulated by the Transcription Factor SOX9**

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**NOTCH**

- **Sox9**
  - Transcription factor
- **Hes1**
- **Cholangiocyte**
- **Hepatocyte**

---

**Cholangiocarcinoma**
What Defines Combined HCC-CholangioCa?

**HCC component**
- Typical morphology:
  - Polygonal cells
  - Bile production
- Immunohistochem:
  - Arginase-1
  - HepPar-1
  - Glypican-3
  - CD10/pCEA

**CholangioCa component**
- Typical morphology:
  - Gland formation
  - Mucin production
- Immunohistochem:
  - MOC-31
  - CK19
  - SOX9 (?)

Immunohistologic Pitfalls

- Misdiagnosis of CholangioCa:
  - MOC-31 reactivity in 5% of HCC
  - CK19 reactivity in 10% of HCC
- Misdiagnosis of HCC:
  - Sox9?

Combined HCC-CholangioCA Bottom Line

- Definitional issues still need to be resolved
- Lineage markers still need to be further delineated