Ascitic Fluid and Use of Immunocytochemistry

Mercè Jordà, University of Miami
Is It Malignant?

Yes  ?  No
Ascitic Fluid
Cytomorphologic Useful Findings

- Tight clusters with smooth borders
- Cellular and nuclear molding
- Large papillary groups
- Two-cell types
- Signet ring cells in groups
- Abnormal cell morphology
Ascitic Fluid
Cytomorphologic Useless Findings

Cytoplasmic Vacuoles
“Signet Ring Cells” individual
Psammoma Bodies
“Cell within Cell”
Prominent Nucleoli
Mitosis
Multinucleation
Reactive Mesothelium
“Signet Ring Cells”

Look for them in Groups!
Malignant
Benign
By cytomorphology

Cellular Pattern
Cellular Morphology
Malignant Ascitic Fluid

Cellular Pattern

- Cells in Clusters
- Isolated Cells
## Malignant Ascitic Fluid: Cells in Clusters

<table>
<thead>
<tr>
<th>Cells</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tight and compact</td>
<td>No cells</td>
</tr>
<tr>
<td>Smooth borders</td>
<td>Reactive mesothelial cells</td>
</tr>
</tbody>
</table>

**Diagnosis:** Cytomorphology
Malignant Effusions: Cells in clusters

Differential Diagnosis

- Carcinoma
- Malignant Mesothelioma

Diagnosis:
Cytomorphology & Immunocytochemistry
Malignant Effusions: Isolated Cells

- Abnormal single cells
- May be overlooked in low power
- Look for small clusters
Malignant Effusions: Isolated Cells

Abnormal Cell Morphology

• Pleomorphism
• High N/C ratio
• Hyperchromasia
• Abnormal nucleoli
• Clumped, irregular chromatin
• Intraluminal mucin

Diagnosis:
Cytomorphology & Immunocytochemistry
Tight cell balls in breast Ca.
Psammoma bodies in serous Papillary Ca.
“Indian filling” in breast, gastric and pancreatic Ca.
“Signet ring cells” in breast, gastric and ovarian Ca.
Keratin pearls in squamous cell Ca.
Melanin in malignant melanoma.
Intranuclear inclusions in Adenocarcinoma of lung lipidic, papillary thyroid carcinoma and melanomas
“Knobby clusters” in mesotheliomas
Cell balls in Breast Ca.
Psammoma Bodies
Cellular Chain
Keratin Pearl
Knobby cluster in Mesothelioma
Malignant Effusions
Specific Type and Site of Origin

Diagnosis:
Cytomorphology & Immunocytochemistry
Is It Malignant?

Yes

Mesothelioma

Adenocarcinoma

No

Small Cell Ca
Lymphoma
Squamous cell Ca
Others

Others

Site of Origin
When to Use Immunocytochemistry in Ascitic fluid Cytology
Alamo 1917
Immunocytochemistry in Cytology
University of Miami Experience

The Potential Value of Immunoperoxidase Techniques in Diagnostic Cytology

Mehrdad Nadji, M.D., M.I.A.C.

Acta Cytol 1980; 24: 442-447
IHC Applications

University of Miami

- Diagnosis/Classification 65%
- Prognosticators/Predictors 18%
- Target therapy, Others 17%
How Often?
University of Miami

“Percent of our Total Cases”

- Surgical Pathology: 5.9%
- Cytopathology: 4.9%
- Autopsy Pathology: 18%
Type of Specimen
ICC in Cytology

- FNA: 55%
- Effusion: 41%
- Others: 4%
Diagnostic IHC Facts

- IHC is an important diagnostic tool in tumor pathology.
- Traditionally used on histologic material and cytologic cell blocks.
- The technique is not widely used in diagnostic cytology.
Why IHC is Not Widely Used in Cytology?

• Limited cytologic material
• Problems in interpretation
• Lack of specific markers to differentiate benign from malignant cells
Technical Considerations

- Use cell block if possible (Cellular)
- Use alcohol fixation (95% isopropyl)
- Alcohol-fixed, Pap-stained archival slides can be used
- No de-staining is necessary
- Most cytology samples can be used
Immunoxytochemistry Not good in:

- Air-dried slides
- Diff-Quick-stained slides
- De-stained slides (cellular antigens maybe removed)
- Slides with plastic coverslip
Immunocytochemistry

Not good in:

• Filter preparation
• Serous fluid specimens with excess blood and proteins

Wash specimen or use Saccomanno solution
Immunocytochemistry

Fixation

• 95% isopropyl alcohol
• Buffered formalin
• Formol-acetone
• Mixture of ethanol & formalin
Prolonged fixation (wks/months) in formalin may result in antigenic loss.

Prolonged fixation in alcohol-based fixatives is not a major problem.
Easy 3-Step Procedure

1. Use a diamond pen to mark the cells on the back of the slide
2. Remove the coverslip
3. Start your routine IHC/ICC procedure
Immunocytochemistry Using Archival Slides

- Removal of coverslip may be difficult
- When diagnostic slides are limited, ICC can be performed on a previously negative slide
IHC=ICC
Technique

No technical alterations needed for cytologic specimens
True Positive

EMA

Calretinin
You Should Know your Antibody
ICC in Diagnostic Cytology
Applications

- Tumor Diagnosis/Classification
- Prognostic/Predictor Markers
- Target Therapy
ICC in Diagnostic Cytology

Applications

- Tumor Diagnosis/Classification
- Prognostic/Predictor Markers
- Target Therapy
Selection of Markers

- Cytomorphology
- Clinical Information
- Working Diagnosis
- Differential Diagnosis
- Selection of ICC Markers
- Final Interpretation
ICC in Diagnostic Cytology
Selection of Markers
“tailor-made” Approach

• When the differential diagnosis is narrowed down, usually not more than 2-3 markers are needed (“tailor-made”)
• In many occasions only one marker is used to confirm the working diagnosis
Diagnosis/Classification

Our 3-Step Approach

1. Define a specific differential Dx
2. Select a small panel of ICC markers
3. Combine cytomorphology and ICC
Is It Malignant?

- Yes
- ?
- No

**Site of Origin**

- Mesothelioma
- Adenocarcinoma
- Small Cell Ca
- Lymphoma
- Squamous cell Ca
- Others

**Others**

**ICC**

- ICC
- ICC
- ICC
Is It Malignant?

Yes

Mesothelioma

Adenocarcinoma

Site of Origin

Others

No

ICC

Small Cell Ca

Lymphoma

Squamous cell Ca

Others
First Step…..

Reactive Mesothelial cells

versus

Malignant Process
• The reactive mesothelial cells may group.
• If so, the grouping usually presents as loose clusters, without nuclear overlapping.
Differential Diagnosis of Atypical cells in Ascitic Fluid

<table>
<thead>
<tr>
<th>Reactive Mesothelial Cells</th>
<th>Malignant Morphology</th>
<th>Resemble Mesothelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Malignant Mesothelioma</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>
Commonly Used Markers In Effusions

EMA: Malignant: adenocarcinoma, malignant mesothelioma

CEA: Malignant: adenocarcinoma

Ber-EP4: Malignant: adenocarcinoma

LeuM1: Malignant: adenocarcinoma

Desmin: Benign: reactive mesothelium
In our laboratory, EMA (clone E29) is the most frequently used antibody in defining “atypical cells” in effusions.
Reactive Mesothelium vs. Adenocarcinoma and Mesothelioma

EMA

<table>
<thead>
<tr>
<th>Reactive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>Positive (Cytoplasm)</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>Positive (membrane)</td>
</tr>
</tbody>
</table>
In our experience, the most useful limited panel of ICC includes:

- EMA
- Calretinin

Nuclear and intracytoplasmic positivity for calretinin and negativity for EMA confirms a reactive mesothelial proliferation.

Acta Cytol 2000; 44 : 854
Diag Cytopathol 2008, 34:
Reactive Mesothelium
Calretinin
EMA Positivity
Strong, Intracytoplasmic & Easily seen on Low Power
EMA Positive in MM: Strong Membrane/Cytoplasmic
EMA Positive in MM

Strong Cytoplasmic

Strong Membrane/Cytoplasmic
Positive EMA in Serous Effusions

 Represents adenocarcinoma, if:

  – Easily seen on low power
  – Is strong and intracytoplasmic
Ascitic Fluid

Is It Malignant?

Yes

Mesothelioma

ICC

Adenocarcinoma

Others

Small Cell Ca
Lymphoma
Squamous cell Ca
Others

No
Malignant Mesotheliomas in Effusions

Low Power

- Small or large 3D groups
- “Knobby clusters”
Resemblance to Mesothelial Cells
Malignant Mesothelioma in Effusions

Differential Diagnosis of Mesothelioma

- Cytomorphology
- Electron microscopy
- Cytochemistry
- Immunocytochemistry (ICC)
When Malignant Mesothelioma Mimics Adenocarcinomas

Use ICC
In our experience, the most useful limited panel of ICC includes:

- EMA
- Calretinin

Nuclear and intracytoplasmic positivity for calretinin and Positivity for EMA confirms a Malignant Mesothelioma

Acta Cytol 2000; 44: 854
Diag Cytopathol 2008, 34:
Malignant Mesothelioma

Calretinin
## Ascitic Fluid

<table>
<thead>
<tr>
<th></th>
<th>Malignant Mesothelioma</th>
<th>Lung Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calret</td>
<td>MM Pos</td>
<td>D2-40 Pos</td>
</tr>
<tr>
<td>TTF-1</td>
<td>MM Neg</td>
<td>CEA Neg</td>
</tr>
<tr>
<td>CEA</td>
<td>LA Neg</td>
<td>D2-40 Pos</td>
</tr>
<tr>
<td>D2-40</td>
<td>LA Pos</td>
<td>CEA Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MM Neg</td>
</tr>
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Is It Malignant?

Yes

Mesothelioma

Adenocarcinoma

ICC

No

Others

Small Cell Ca
Lymphoma
Squamous cell Ca
Others

Site of Origin
Adenocarcinoma in Ascitic Fluid
Primary Sites in Adult Male

- Adenocarcinoma
  - GI tract-
  - Pancreas
  - GU
  - Lung
Adenocarcinoma in Ascitic Fluid

Primary Sites in Adult Female

- Adenocarcinoma
  - Ovary
  - Breast
  - GI Tract-Pancreas
  - Lung
Breast/GYN adenocarcinoma
ER-1D5

In Fluids
Remember!

- Be careful with the use of ER in peritoneal effusions of female patients
- Benign epithelial inclusions may cause false positive results
- **First** establish the malignant nature of the cells by cytomorphology
<table>
<thead>
<tr>
<th></th>
<th>TTF-1</th>
<th>CK20</th>
<th>CK7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma of Lung</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Colonic Carcinoma</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ICC Markers for Colon Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• CK 7</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• CK 20</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• CDX-2</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• CEA</td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Hepatocellular Carcinoma vs Metastatic Adenocarcinoma

<table>
<thead>
<tr>
<th></th>
<th>Hepatocellular Ca</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CK7</strong></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>HCA</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular Carcinoma</td>
<td>Renal Cell Carcinoma</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td><strong>HCA</strong></td>
<td>Hepatocellular Ca. +</td>
<td>Renal Cell Ca. -</td>
</tr>
<tr>
<td></td>
<td>Renal Cell Ca. -</td>
<td>EMA + +</td>
</tr>
</tbody>
</table>
TTF-1 in Lung Adenocarcinoma.

• TTF-1 is useful for diagnosis of lung adenocarcinomas in effusions

• Only nuclear staining must be considered positive

Cancer Cytopathol 96: 289-93, 2002
TTF-1; Neg.
Ascitic Fluid

Is It Malignant?

Yes

Mesothelioma

Adenocarcinoma

Site of Origin

Others

No

Small Cell Ca
Lymphoma
Squamous cell Ca
Others

ICC
Small Cell Carcinoma in Ascitic Fluid

**Low Power**
- Tight cell balls
- Indian file/chain
- Isolated cells may be overlooked

**High Power**
- Nuclear molding
- Coarse chromatin
- Wrinkled nuclear membrane
- Occasional cells with nucleoli
## Lung Carcinoma

**Non-Small vs Small Cell**

<table>
<thead>
<tr>
<th></th>
<th>CK</th>
<th>SYN</th>
<th>CHR</th>
<th>TTF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Small</td>
<td>+</td>
<td>-/+</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Small Cell</td>
<td>+(dot)</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
</tbody>
</table>
Small Cell Carcinoma in Ascitic Fluid

Differential Diagnosis

- Malignant lymphoma
- “Small blue cell tumors”
# ICC in Differential Diagnosis of Small Cell Malignancies

<table>
<thead>
<tr>
<th></th>
<th>LCA</th>
<th>KER</th>
<th>CHR</th>
<th>DES</th>
<th>NB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small Cell Ca</strong></td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lymphoma</strong></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Rhabdomyosarcoma</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Neuroblastoma</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Malignant Lymphoma in Ascitic Fluid

Low Power

Isolated Cells
Malignant Lymphoma in Ascitic Fluid

High Power

• Nuclear variation in size and shape
• Nuclear indentation/convolution
• Vesicular nuclei with prominent nucleoli
• Individual cell necrosis (apoptosis)
• Scant, basophilic cytoplasm, rarely well preserved
Malignant Lymphoma in Ascitic Fluid

HHV8 associated lymphoma
Lymphoma vs. Carcinoma vs. Germinoma vs. Melanoma

Favor Lymphoma
- Only isolated cells
- Nuclear clefts
- Apoptotic cells

Immunocytochemistry
- LCA (+)
- Keratin (-)
- PLAP (-)
- S100 (-)
Small “Mature-Looking” Lymphocytes in Effusions

Differential Diagnosis

• Chronic pleuritis (TB)
• Small cell lymphomas
• Chronic lymphocytic leukemia
• Waldenstrom’s macroglobulinemia
# Lymphocytes in Effusions

<table>
<thead>
<tr>
<th>Effusion Type</th>
<th>CD45 (LCA)</th>
<th>CD20 (B-cell)</th>
<th>CD3 (T-cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Malignant</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
It is an Artifact
Ancillary Techniques to Rule Out Malignant Lymphoma

- Flow cytometry
- Gene rearrangement
Squamous Cell Carcinomas are Rare in Effusions

Site of Origin

- Lung
- Cervix
- Skin
- Esophagus

Diagnostic Difficulties

- Tumor cells do not shed
- May be mistaken for poorly differentiated adenocarcinomas or mesotheliomas
Squamous cell ca
Squamous cell ca
Remember!

- Squamous carcinoma cells are usually overlooked in body cavity fluid cytology - Only few cells shed
- They might be confused with necrotic /degenerative mesothelial cells
- \textit{p63} and \textit{p40} are very helpful to detect squamous cells

Cancer Cytopathol 2009; 117: 46-50
## Carcinoma vs Melanoma

<table>
<thead>
<tr>
<th></th>
<th>CK</th>
<th>S100</th>
<th>HMB45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>+</td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td>Melanoma</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Melanoma Markers

- S100 Protein: +++
- HMB-45: +++
- Melan-A: ++
- Tyrosinase: ++
ICC in Diagnostic Cytology

Applications

- Tumor Diagnosis/Classification
- Prognostic/Predictor Markers
- Target Therapy
Detection of HER2 in cytology
ICC, FISH, CISH
Predictive Value

NOT standard of Care for Breast CA

Diagn Cytopathol 1994; 11:262-265
ICC in Diagnostic Cytology

Applications

• Tumor Diagnosis/Classification

• Prognostic/Predictor Markers

• Target Therapy
NSCLC: Target Therapy

– tyrosine kinase inhibitors (TKI) first-line therapy in patients with advanced lung adenocarcinoma with **EGFR mutations**
– adenocarcinomas with **ALK rearrangements** are responsive to crizotinib (ALK inhibitor).
– Patients with **KRAS or BRAF mutation** do not respond to TKI, ALKI
NSCLC: Target Therapy

– patients with adenocarcinoma or NSCLC, not otherwise specified (NSCLC-NOS), are more responsive to pemetrexed than those squamous cell carcinoma

– squamous cell carcinoma is associated with life-threatening hemorrhage in patients treated with bevacizumab; therefore, it is contraindicated in lung cancer patients with this histology.

Arch Pathol Lab Med 2013, 137:668-684
Squamous Cell Carcinoma

p63
ICC Limitations

• Large 3D cellular clusters in cytospin samples
• Histiocytes, macrophages, cells in mitosis, tumor giant cells

Look for single cells or smaller 2D groups

ICC Limitations

• Lack of internal control
• Negative results in ICC are not as meaningful as positive reactions

Diag Cytopathol 1986; 81-2, 1986
Final Words....

• Use our 3-step approach:
  – Define a specific differential Dx
  – Select a small panel of ICC markers
  – Combine Cytomorphology and ICC
Final Words…. 

• ICC can be used on previously alcohol-fixed Pap-stained slides without de-staining 

• The technique does not require any modification of the routine ICC staining protocol
Springer, 2007

Demos, 2011
ASCP Workshops

Diagnostic problems in body cavity fluid cytology; a practical approach.

Immunocytochemistry in Diagnostic Cytology: Values and Limitations

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Sylvester Comprehensive Cancer Center