Practical Effusion Cytology

A Community Pathologist’s Approach to Immunocytochemistry in Body Fluid Cytology

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Overview

• Prudent use of immunocytochemistry (ICC)
• Applications of ICC in body fluid cytology
• Pertinent case reviews
Pre-Analytical

• Form differential diagnosis
  – Morphology of conventional preparations
  – Clinical scenario

• Develop question to be answered by ICC

• Ensure sample used contains cells in question

• Adequate fixation
  – Alcohol fixed preparation; Thin Layer; Cell Blocks
Analytical

- Positive and negative controls
- Ideal if cytology sample used for control
  - Most labs use tissue control for convenience
- Notice the pattern of staining in positive controls
  - Membranous, cytoplasmic or nuclear.
- Expect heterogeneity of immunostaining within a sample
- Normal cells may have capability to react with ICC
ICC
Common Causes of False-Positive Results

• Non-specific antibody binding
• Misinterpretation of population as neoplastic
• Necrotic cells
• Inappropriate fixation
• Antigen diffusion
• Antibody concentrations too high

ICC
Common Causes of False-Negative Results

• Sample lacks neoplastic population
• Antigen expression below sensitivity of antibody
• Antibody concentration is too low
• Poor fixation
• Antigen diffusion
  – S100 and GCDFP-15 in alcohol fixatives
• Insufficient antigen retrieval
• Papanicolaou decolorization

## Body Fluid Cytology
### WBH-Troy 2003

<table>
<thead>
<tr>
<th>Fluid Type</th>
<th>Total</th>
<th>Positive for Malignancy</th>
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</thead>
<tbody>
<tr>
<td>CSF</td>
<td>88</td>
<td>5</td>
</tr>
<tr>
<td>Pericardial</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Pleural</td>
<td>201</td>
<td>41</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>89</td>
<td>31</td>
</tr>
<tr>
<td>Peritoneal wash</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>452</td>
<td>83 (18%)</td>
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## Immunocytochemical Analysis
Is it useful?

<table>
<thead>
<tr>
<th></th>
<th>Portion of workload</th>
<th>Diagnostically useful</th>
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</thead>
<tbody>
<tr>
<td>WBH-Troy</td>
<td>0.9%</td>
<td>70.4%</td>
</tr>
<tr>
<td>Shield et al.</td>
<td>1.6 %</td>
<td>75.8 %</td>
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<td>Royal Brisbane</td>
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<tr>
<td>Hospital</td>
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</tbody>
</table>
Immunocytochemical Analysis

When is it useful?

- Poorly differentiated malignancy
  - Subclassification
  - Primary site determination
- Discrimination of mesothelial cells and metastatic malignancy
- Mesothelioma vs. adenocarcinoma
Case 1

- 76 year old woman
- New onset ascites
- Previous history of breast cancer status post mastectomy and radiation therapy
Case 1
Case 1
H &E Cell Block
Case 1

BerEp4
Case 1
CK 7
Case 1
WT-1
Case 1

• Positive ICC:
  – CK 7 (cytoplasmic)
  – BerEp4 (membranous)
  – WT-1 (nuclear)

• Negative ICC:
  – CK 20
  – TTF-1 and Surfactant protein A
  – Calretinin and thrombomodulin
CK7/CK20 ICC Profile

• CK7+/CK20-
  – Non-small cell carcinoma of lung
  – Breast carcinoma (ductal and lobular)
  – Non-mucinous ovarian carcinoma
  – Endometrial adenocarcinoma
  – Mesothelioma
CK7/CK20 ICC Profile

• CK7+/CK20+
  – Urothelial carcinoma
  – Pancreatic carcinoma
  – Ovarian mucinous carcinoma
  – Merkel cell carcinoma

• CK7-/CK20+
  – Colorectal adenocarcinoma
CK7/CK20 ICC Profile

• CK7-/CK20-
  – Small cell carcinoma of lung
  – Squamous cell carcinoma of lung
  – Prostate adenocarcinoma
  – Renal cell carcinoma
  – Hepatoma
BerEp4

- Monoclonal antibody to two glycopeptides on human epithelial cells
- Usually stains in cell membrane distribution
  - Adenocarcinomas of various sites (86.7%)*
  - Epithelial mesothelioma (0.86%)*

*Sheibani et al. AJSP. 1991;15: 779-784*
WT-1
Antibody to Wilms Tumor Suppressor Gene Products

• Gene resides on 11p13
  – Inactivation causes susceptibility to Wilms tumor
  – Tissues of mesodermal origin
WT-1
Antibody to Wilms Tumor Suppressor Gene Products

• Nuclear immunostaining
  – Mesothelioma (92.9%)
  – Papillary carcinoma of ovary (100%)
  – Renal cell carcinoma (100%)

• Negative staining
  – Adenocarcinoma of lung
  – Squamous cell carcinoma of lung
  – Metastatic breast carcinoma
  – Metastatic colon carcinoma

Diagnosis:

- Metastatic adenocarcinoma consistent with serous surface or ovarian origin.
- Left ovarian mass later confirmed on pelvic CT scan.
Case 2

- 73 year old woman with new onset ascites
Case 2
Case 2

WT-1
Case 2
Cytokeratin AE 1/3
Case 2
Calretinin
Cytoplasmic and nuclear staining
Case 2
CK 5/6
Cytoplasmic staining
Case 2

• Positive ICC
  – Calretinin
  – Cytokeratin AE 1/3
  – Cytokeratin 5/6
  – WT-1 (nuclear)

• Negative ICC
  – B72.3
  – LeuM1 (CD15)
  – CEA
Case 2
Diagnosis

- Malignant mesothelioma
Epithelial Mesothelioma
Positive ICC markers

Cytokeratins

• AE 1/3
  – Good screening antibody
  – Broad spectrum

• CAM 5.2 (keratins 8/13)

• CK 5/6
  – High molecular weight keratin
  – Epithelial mesotheliomas strongly positive
  – Pulmonary adenocarcinoma negative to weak positivity
  – Squamous cell carcinoma +
  – Urothelial carcinoma (50%) +
  – Reactive mesothelial cells +
Epithelial Mesothelioma
Positive ICC markers
Calretinin

- Calcium binding protein similar to S-100
- Cytoplasmic and nuclear staining
- One of the most specific and reproducible positive markers
Epithelial Mesothelioma
Positive ICC markers
Epithelial Membrane Antigen
Human milk fat globule protein-2 (HMFG-2)

- Cell membrane staining pattern
- Adenocarcinomas show cytoplasmic staining
- May see membranous staining in non-mucinous BAC/ papillary RCC
- Benign mesothelial cells usually negative
Epithelial Mesothelioma
Positive ICC markers
Thrombomodulin

- Plasma membrane related glycoprotein with anticoagulant activity
- Thick membranous staining in malignant mesothelioma
- Thin membranous staining in reactive mesothelial cells
- Cytoplasmic staining may be seen in adenocarcinoma
**Epithelial Mesothelioma**

**Positive ICC markers**

- **N-cadherin**
  - Strong positivity with malignant mesothelioma
  - Focal weak in pulmonary adenocarcinoma

- **WT-1**
  - Nuclear staining of malignant mesothelioma, papillary ovarian tumors, and RCC
Epithelial Mesothelioma

Negative ICC markers

• Carcinoembryonic antigen (CEA)
  – 85-100% of pulmonary adenocarcinoma positive
• Leu-M1 (CD 15)
  – 50-100% of pulmonary adenocarcinoma positive
• B72.3
  – 84-96% of pulmonary adenocarcinoma positive
  – 10% epithelial mesothelioma positive
• BerEP4
Desmin reactivity of mesothelial cells

• Strong cytoplasmic reactivity in 22 of 24 cases (92%) of reactive mesothelial cells
• All cases of malignant mesothelioma and metastatic adenocarcinoma were negative
• Archival paraffin cell block material from body fluids
• ICC to determine malignancy
  – Exercise caution
  – More data before widespread use

Case 3

- 55yo man with new onset right pleural effusion
- History of smoking-40 pack years
Case 3
EMA
Diffuse cytoplasmic staining
Case 3
CD 15
Cytoplasmic staining
Case 3
Surfactant Protein A
Cytoplasmic staining
Case 3
ICC

- EMA+ (cytoplasmic)
- CD 15 (Leu-M1) +
- SPA+
- Calretinin-
- Thrombomodulin-
Diagnosis

• Metastatic lung adenocarcinoma
Surfactant Protein A (SPA)

- Antibodies to surfactant apoproteins
- Granular cytoplasmic positivity in primary lung adenocarcinoma
Thyroid Transcription Factor 1 (TTF-1)

- Member of the NKx2 family of transcription factors

- Positive nuclear staining
  - Thyroid tumors
  - Lung tumors
    - Adenocarcinoma
    - Small cell carcinoma

- Cytoplasmic staining
  - Hepatocellular carcinoma*

Summary
CAVEATS FOR ICC

- Know the antigenic profiles of the tumors in your differential diagnosis.
- Pay attention to controls.
- Identify which cells are staining.
- Scrutinize the positive cells for their pattern of reactivity.
Immunohistochemistry
Web site

• www.immunocentral.com
• www.immunoquery.com