Effusion Cytology: Diagnostic Challenges

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Outside Consult Case

• 45 year old woman, presented with nausea, dyspnea, emesis and productive cough
• Chest X-ray: left pleural effusion, LLL consolidation and atelectasis
• CT: Mediastinal lymphadenopathy
• Never smoker, no significant PMH
Left pleural fluid
Additional IHC
• P63 - , WT1 - , D240 -
• TTF1 –
• Mammaglobin -
• CEA +

Outside Dx: Atypical, Favor reactive mesothelial cells
Pleural fluid - Outside Consult
- WT1 -,
- D240 -,
- calretinin +,
- CK5/6 +
- P63 -,
- P40 -
- Moc 31+,
- BerEp4+,
- CEA+,
- B72.3 -
- Napsin A -,
- TTF1 -
- ER/PR -
- PAX8 +,
- WT1 -
- Mamaglobin -,
- GATA3 +,
- GCFP +

Dx: Met adenocarcinoma, breast or mullerian origin
CAP Inter-laboratory Comparison Program in Body Cavity Fluids

- Data bank of 10,396 lab responses, 1997-2001
- Assessed characteristics of specimens that performed well and performed poor
- Poor performers in hypocellular and hypercellular malignant specimens

*Moriarty et al 2004*
Hypocellular: Location errors

- Rare malignant cells, had diagnostic features but were overlooked.
- Adeno ca, squamous ca, small cell ca, melanoma and lymphoma
- This emphasizes importance of careful methodical screening

Moriarty et al 2004
Carcinoid tumor in peritoneal fluid- false negative
Hypercellular: Interpretation errors

*Cellularity not a factor*

- Poor carcinoma performers
  - Single cells
  - 3-D clusters
- Poor mesothelioma and lymphoma performers

*Moriarty et al 2004*
Common Challenges in Fluids

- Atypical cells, suspicious but not diagnostic of malignancy
- Obviously malignant cells, but uncertain of classification or site of origin
Outline

• Benign effusions
• Work up of malignant effusions
  – Cytomorphologic features
  – Specific histologic types
• Immunohistochemistry
• Diagnostic challenges and potential pitfalls
Benign Effusions

- 70-80% of all effusions
- Mesothelial cells, histiocytes, inflammatory cells
- Common etiologies:
  - Infectious: viral, bacterial, TB, fungal, parasitic
  - Congestive heart failure
  - Pulmonary embolism
  - Myocardial infarction
  - Cirrhosis
  - Nephritic syndrome
  - Collagen vascular disease
  - Pancreatitis
  - Trauma
Benign Effusions

- Variable cellularity, mostly single cells
- Occasional small clusters, < 10-12 cells
  - Exception: washings
- Windows
• Bi-nucleation and multi-nucleation common
• Dense cytoplasm, clear outer rim (lacy skirt)
• Oval-round nuclei, pale chromatin
• Small nucleoli, may be prominent
Atypia in Reactive Mesothelial Cells

- Nuclear hyperchromasia
- High N/C ratio
- Prominent nucleoli
Malignant effusions

- Rarely present as an occult malignancy (7-14%)
- Most patients have established history of malignancy
- Poor prognostic sign
- Lung, breast, ovary, GI tract - most common
Work-up of Malignant Effusions

- **Morphology**
  - Low power architectural pattern
  - High power cytologic detail
  - Specific histologic types

- **Ancillary studies**
  - IHC
  - Flow cytometry
General Features of Malignancy

- **Second (foreign) population**
  - Distinctly different from mesothelial cells
  - Cohesive clusters, > 10-12 cells
General Features of Malignancy

Malignant Nuclear Features
- High N/C ratio
- Nuclear hyperchromasias
- Nuclear irregularity
- Irregular distribution of chromatin
- Macro nucleoli
Architectural Patterns in Fluids

- Cell balls/cannonballs
- Papillary
- Acini
- Single cells (large or small)
- Linear/Indian file

- Signet ring
- Multinucleation
- Bizarre giant cells
- Clear cells
- Psammoma bodies
Cannonballs

- Tightly cohesive spherical cell clusters with an almost perfectly round contour and community border
• Uniform cells → suggest breast (more common)
• Pleomorphic cells → suggest ovary, lung
• Other sources include GI tract, mesothelial hyperplasia and mesothelioma
Papillary

- 3-D clusters that are longer than wider
- Ascites → ovary, uterus
- Pleural effusions → lung, breast
• Others: GU, pancreas, primary peritoneal, mesothelial hyperplasia, mesothelioma
• Thyroid PC is rarely associated with effusions
Acinar groups

- 3D groups with lumens
- Characteristic of adeno ca, but not specific
  - Lung, colon, ovary, stomach, breast, etc.
  - Mesothelial hyperplasia, mesothelioma
  - Small round cell tumors
Better appreciated on cell blocks
Indian/single files

- Breast
- Small cell ca
- Carcinoid tumor
- pancreas
- gastric
- mesothelioma
Single small cells

- Lymphoma/leukemia
- Small cell carcinoma
- Carcinoid
- Breast
- Stomach
- Small round cell tumor
Single large cells

- Squamous carcinoma
- Melanoma
- Poorly differentiated adenocarcinoma
- Renal, adrenal, hepatocellular ca, germ cell tumors
- Sarcoma
- Lymphoma
- Reactive mesothelial cells, mesothelioma
Lung non-small cell CA

Ber-EP4+
Bizarre giant cells

- Undifferentiated carcinoma
  - lung or pancreas
- Squamous ca
- Melanoma
- Mesothelioma
- Sarcoma
- R/O rheumatoid effusion, as it may show bizarre or giant cells
Cytoplasmic Vacuoles

Vacuoles without indentation

• Non-specific, often degenerative
  – Benign: degenerative
  – Malignant: nonspecific-ovary, lung, pancreas, etc.
Signet Ring Cells

Vacuoles indent the nucleus

- Lobular breast carcinoma
- Gastric carcinoma
- Colon
Diff DX: Degenerative Changes

- Degenerated mesothelial cells and histiocytes can have large vacuoles imparting a signet ring appearance
Multinucleated cells

- Reactive mesothelial cells, mesothelioma
- Giant cell carcinoma
- Hepatocellular carcinoma
- Melanoma
- Sarcoma
- Renal cell carcinoma
- Anaplastic large cell lymphoma
- Hodgkin disease
- Germ cell tumors
Clear cells

- Kidney
- Ovary/GYN
- Germ cell tumor
Clear cells in fluids may have an unexpectedly dense cytoplasm
Psammoma bodies

- Psammoma bodies have no diagnostic significance
- Ovary
- Thyroid
- Mesothelioma
- Mesothelial hyperplasia
- Endosalpingiosis

Mesothelial hyperplasia
Specific Histologic Types

- Cells tend to round up in fluids → a certain degree of uniformity among various cell types
  - Exaggerated in ThinPrep (personal experience)
- Mets in fluids, therefore, may not necessarily exactly resemble the primary tumor
- Familiar with variable presentations of specific histologic types
Histologic Types in Effusions

- Adeno CA: most common cancer
- Less common: squamous ca, small cell ca, lymphoma, melanoma
- Children: hematopoietic and SBCT
Adenocarcinoma

- Large clusters or isolated cells
- **Foreign population**
- Cannonballs: more common in breast cancer
- Signet ring: stomach and breast
Columnar cells

• Mucinous tumors, colon, ovary, endometriosis
• Gastric and colo-rectal cancers:
  – may lose tall columnar configuration in fluids
A second population of benign mesothelial cells may be absent

Tumor cells resemble histiocytes
86 yo woman, peritoneal fluid
• **Dx: Adenocarcinoma**
  – Tumor cells resemble histiocytes
• **Follow-up: Signet ring CA of stomach**
Potential Pitfalls

• Breast
  – Relatively common: predominance of intermediate cells with bland features, hidden among mesothelial cells ➔ potential false negative diagnosis
- Tumor cells may resemble mesothelial cells
- Lobular CA, lung, ovary, melanoma
- IHC helpful in these situations
Mesothelioma

- High cellularity, large clusters 30-200 cells
- Clusters have irregular knobby borders
• Mesothelial appearance of cells
• Spectrum: benign → atypical → malignant (No second foreign population)
• Single cells may predominate
• Pleural fluid, 66 yo man
Dx: Mesothelioma
Challenges in DX of Mesothelioma

- Sparsely cellular specimens-account for most of false Neg. cases
Variable atypia: Moderate → Severe
Carcinoma vs. Mesothelioma

- Severe atypia - difficult to distinguish MM from CA
• Subtle atypia- indistinguishable from benign reactive cells
• Mesothelioma should be considered when numerous clusters with > 15 cells, even if no significant atypia
<table>
<thead>
<tr>
<th></th>
<th>Mesothelioma</th>
<th>RMC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groups</strong></td>
<td>Large clusters, many Knobby borders</td>
<td>Small clusters, few flat sheets</td>
</tr>
<tr>
<td></td>
<td>Papillary clusters</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>Cell in cell and chains</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>Cells</strong></td>
<td>Large, more variable Multinucleation</td>
<td>Less variable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rare</td>
</tr>
<tr>
<td><strong>Cell borders</strong></td>
<td>Lacy skirts and blebs</td>
<td>Less prominent</td>
</tr>
<tr>
<td><strong>Nucleus</strong></td>
<td>-Severe irregularity</td>
<td>-Mild</td>
</tr>
<tr>
<td></td>
<td>-Irregular chromatin distribution</td>
<td>-Finely granular chromatin</td>
</tr>
<tr>
<td></td>
<td>-Macro nucleoli</td>
<td>-Small nucleoli</td>
</tr>
</tbody>
</table>
• Degenerated reactive mesothelial cells and histiocytes can mimic malignancy
• Do not issue a definitive DX → additional specimens
Diagnosis of Mesothelioma

- Adequate cellularity: Dx established in 2/3 cases
- Uncertain cases → “Atypical mesothelial proliferation, recommend biopsy”
- Pleural Bx alone → 40-60%
- Combined cytology and Bx → 80%
- P16 deletion (FISH)-homozygous deletion of 9p21 in malignant mesothelioma
  - 60% sensitivity, 100% specificity in fluids
- Definitive DX: histology to document invasion
  (AFIP fascicle 2006)

Monaco 2011
< 1% of effusions contain squamous ca
Primary tumor is known in most cases
Most commonly from lung, larynx and GYN
Single cells or clusters, dense cytoplasm
Keratinization is rarely seen (10%)
• Squamous Cells tend to round up in fluids and may form balls
• Maybe difficult to distinguish squamous from adeno ca
• Squamous CA frequently vacuolated
• Cytoplasmic vacuoles are nonspecific in malignancy
- Cell block: adeno CA may resemble squamous CA
- IHC and Pap cytology are more reliable

Pleural fluid from a 73 yo man
Small Cell Carcinoma

- Isolated cells, clusters and chains
- Round or angular nuclei, molding
- May show elongation and spindling
“Vertebral Column” arrangement

- Nuclear molding + Indian file
- **Differential diagnosis:**
  - Small cell CA
  - Lymphoma
  - Breast, GI tract
  - Pediatric tumors
Melanoma

Diagnosis can be very subtle in effusions
- Resemble mesothelial cells
  - isolated round cells
  - prominent nucleoli
  - clusters are uncommon
- May show single bizarre cells
- Rarely pigment or intra-nuclear inclusions
- ICC for S100, HMB 45, Melan A
Lymphoma

• Specific classification seldom needed
  – Most patients have Hx of lymphoma
  – May subclassify based on size of cells and nuclear irregularity
  – FCM and/or IHC essential for DX
Nuclear knobs suggest lymphoma, if single cells
• Karyorrhexis and apoptosis suggest lymphoma, when extensive
• Mesothelial cells are conspicuously absent
CAP inter-laboratory Comparison Program

Lymphoma

– Good performers with
  • History of malignancy
  • Monomorphous hypercellular population

Chronic inflammation (negative/reactive)

– Performed well when polymorphic: lymphs, plasma cells, neutrophils, reactive mesothelial cells

– Poor performer, if lymphocytes predominated \(\Rightarrow\) mistaken for lymphoma

Moriarty et al 2004
Immunohistochemistry

- PD malignancy ➔ specific cell lineage
- Determine primary site
  - Differential cytokeratins and non-keratins
  - Organ specific markers
- Adeno CA vs. reactive mesothelial cells or mesothelioma
### Practical IHC Panel

<table>
<thead>
<tr>
<th></th>
<th>Adeno CA</th>
<th>Mesothelial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ber-EP4, MOC-31, B72.3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Calretinin, WT1, CK5/6, D2-40</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TTF-1, Napsin A (lung)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>mCEA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Leu-M1 (CD 15)*</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>EMA**</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

*CD15: good marker in tissue, BUT in fluids, also stains inflammatory cells → interpretation compromised*
- EMA + in most adenocarcinoma and mesotheliomas
  - Thick membrane + in mesothelioma
  - Diffuse cyto + in adenocarcinoma
- Neg in reactive mesothelial cells (+ in 4% of cases)
- Antibody dependent and Lab dependent
EMA: Antibody Dependent

(Clone E 29 (Dako) has best S&S for mesothelioma)

(Clone Mc5 is not reliable to differentiate MM from RMC


Table 3  Sensitivity, specificity and false-positive rate of anti-EMA antibodies assessed by immunocytology on confirmed mesothelioma effusions and cases of benign reactive effusions

<table>
<thead>
<tr>
<th>Anti-EMA clone</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>False positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>VU2G7</td>
<td>5/20 (25%)</td>
<td>16/16 (100%)</td>
<td>0%</td>
</tr>
<tr>
<td>VU4H5</td>
<td>5/20 (25%)</td>
<td>16/16 (100%)</td>
<td>0%</td>
</tr>
<tr>
<td>CBL263 (VU3C6)</td>
<td>6/19 (32%)</td>
<td>15/15 (100%)</td>
<td>0%</td>
</tr>
<tr>
<td>MA552</td>
<td>9/18 (50%)</td>
<td>12/13 (92%)</td>
<td>0%</td>
</tr>
<tr>
<td>MA695</td>
<td>14/20 (70%)</td>
<td>5/15 (33%)</td>
<td>0%</td>
</tr>
<tr>
<td>E29</td>
<td>16/19 (84%)</td>
<td>14/15 (93%)</td>
<td>0%</td>
</tr>
<tr>
<td>Mc5</td>
<td>20/20 (100%)</td>
<td>0/14 (0%)</td>
<td>8/14 (57%)</td>
</tr>
</tbody>
</table>

(Creaney 2008)
Case Study: Pleural fluid from 52 yo man
• Diff DX: Reactive mesothelial cells vs. Adenocarcinoma
DX:
Reactive mesothelial cells
• Benign Follow-up
Many neoplasms show overlapping immunoreactivity

- **Calretinin +** → 10-30% adenocarcinoma
- **D2-40 +** → 15% serous carcinoma
- **MOC 31 +** → 10% mesothelioma
- **Ber-EP4 +** → 0-30% mesothelioma
Squamous CA vs. Mesothelioma

- Overlapping Reactivity in IHC

<table>
<thead>
<tr>
<th></th>
<th>Mesothelioma</th>
<th>Squamous CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calretinin</td>
<td>+ 100%</td>
<td>+ 30 %</td>
</tr>
<tr>
<td>D2-40</td>
<td>+ &gt; 90%</td>
<td>+ 50 %</td>
</tr>
<tr>
<td>CK 5/6</td>
<td>+ &gt; 90%</td>
<td>+ 100 %</td>
</tr>
<tr>
<td>WT1*</td>
<td>(+) &gt; 90%</td>
<td>(-) 0 %</td>
</tr>
<tr>
<td>P63*</td>
<td>(-) 0 %</td>
<td>(+) 100 %</td>
</tr>
<tr>
<td>MOC31,CEA,B72.3</td>
<td>(-) [+ &lt; 10%, focal]</td>
<td>(+) 50-95 %</td>
</tr>
</tbody>
</table>
IHC issues

• Calretinin is sensitive for mesothelioma, but not specific
• Epithelial markers are useful in distinguishing mesothelioma from carcinoma, but not squamous ca from adeno ca
• IHC panel should include positive and negative markers for each possible DX
  • Optimum 2 positive and 2 negative markers
Summary

• Careful methodical screening to eliminate location errors
• Appreciation of low power architectural patterns in addition to high power cytologic details
• Awareness of diagnostic challenges and potential pitfalls
• IHC is a powerful tool, but need to be familiar with overlapping reactivity patterns
Thank You!