Malignant Mesothelioma in cytology: How far can we go?

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Outline

- Effusion cytology
  - Practical approach to serous effusions
- Reactive vs malignant
- Distinguishing from other malignancies
- Fine needle aspiration and touch prep cytology
- Malignant mesothelioma in cytology – how far can we go? Or how far should we go?
Effusions

- Results of a pathologic process – **No normal effusions**
- Transudate vs. exudate
- Numerous etiological factors; malignancy is only one of them
- Fluid cytology – one of the most common non-gyn specimens
  - 1.5 million people are diagnosed/year (USA)
- The great majority (~80%) are benign
- Malignant effusions – poor outcome
Transudate vs. Exudate

- Ultrafiltrate of plasma – increased hydrostatic pressure (CHF) or decreased oncotic pressure (cirrhosis, nephrosis, malnutrition)
  - Watery, clear, low proteins (<3.0 g/dL), low sp. gravity (< 1.015)
  - Low cellularity
  - Usually benign

- Unfiltered plasma
  - Irritation of mesothelium, damaged vessels, change in permeability
  - Cloudy, yellow, bloody, high protein and high sp. gravity
  - Can be malignant
Fluids - causes

- Malignancy – minority of cases
- Benign Conditions
  - Cirrhosis
  - Congestive heart failure
  - Inflammation - bacterial, TB, fungal, viral, parasitic; abscess
  - Injuries, trauma,
  - Pulmonary embolism
  - Autoimmune diseases
  - Malnutrition
# Effusions at Loyola (2014-2018)

<table>
<thead>
<tr>
<th></th>
<th>Percentage</th>
<th>Pleural</th>
<th>Pericardial</th>
<th>Peritoneal</th>
<th>Pelvic</th>
<th>“Abdominal”</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total number</strong></td>
<td>100%</td>
<td>1639</td>
<td>193</td>
<td>983</td>
<td>1246</td>
<td>95</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>83.52%</td>
<td>80.38%</td>
<td>87.96%</td>
<td>78.27%</td>
<td>91.47%</td>
<td>78.95%</td>
</tr>
<tr>
<td><strong>Atypical</strong></td>
<td>2.41%</td>
<td>2.20%</td>
<td>2.09%</td>
<td>3.47%</td>
<td>1.69%</td>
<td>5.26%</td>
</tr>
<tr>
<td><strong>Suspicious</strong></td>
<td>0.53%</td>
<td>0.67%</td>
<td>0.52%</td>
<td>0.71%</td>
<td>0.24%</td>
<td>0.00%</td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>13.54%</td>
<td>16.75%</td>
<td>9.42%</td>
<td>17.55%</td>
<td>6.60%</td>
<td>15.79%</td>
</tr>
</tbody>
</table>
Pathologist-Related Factors Associated with Indeterminate Diagnoses ("Atypical" and "Suspicious") in Body Fluid Cytology

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Processing

Toluidine blue - “super positive”

ThinPrep – Pap stain

Cell Block – H&E stain (formalin fixed; if alcohol fixed – validation)

Cytospin – Wright Giemsa stain (hematology)
Cell Block – this is also cytology!

“Cytology” maybe the only material you’ll have
Effusions – 3D and single cells

- Spontaneous
- Cytology:
  - Reactive mesothelial cells in 3D clusters, small round groups and mostly single cells
LaPlace’s Law

- Cells in the fluid will conform to the lowest possible energy configuration - sphere
  - all cells appear epithelioid
  - all clusters are 3-dimensional
Washings - flat sheets

- Minimal reactive changes
- Flat sheets of polygonal cells

Cell block – Pitfall – pseudopapillary fragments
Practical Approach to Serous Effusions

- Immunocytochemical studies, clinical and radiographic correlations required
Mesothelial cells

- Mesodermal origin
- Single layer of flat cells lining body cavities
- Irritation – mesothelial hyperplasia
- Cytology –
  - 3D groups (berry-like)
  - Spaces between cells – windows (EM – long microvilli)
  - Grasping, clasping, hugging
  - Multinucleated giant cells
  - Endoplasm (dense) and ectoplasm (pale)
  - “Lacy” cytoplasmic borders – skirts
“Reactive” mesothelial cells

Pleomorphism
Degenerated mesothelial cells

Small orangiophilic squamous-like cells in malignant mesothelioma
Multinucleated mesothelial cells
Clasping, grasping, cannibalism
Histiocytes vs mesothelial cells
Histiocytes

CD68

Calretinin
Malignant effusions - two cell populations

Females
- Pleural
  - Breast
  - Lung
  - Lymphoma
- Peritoneal
  - Ovary
  - Breast
  - GI

Males
- Pleural
  - Lung
  - Lymphoma
  - GI
- Peritoneal
  - GI
  - Pancreas
  - Lymphoma
Adenocarcinoma

- The most common cause of malignant effusions

**Cytology:**
- Increased N/C ratio
- **Irregular nuclear membranes**
- Large nucleoli
- Secretory vacuoles
- 3D clusters with smooth community borders
Adenocarcinoma - patterns

- Cell balls, morulas, cannon balls – most commonly breast ca, also ovarian and lung ca
- Single cells – lobular ca, stomach (signet ring cells)
- Single cell files (“Indian files”) – breast, small cell ca (stock of coins, “vertebral columns”)
- Bizarre or giant cells – lung, pancreas, thyroid
- Clear cells – RCC, 3D clusters – ovarian ca
Classic “cannon balls” - Pleural fluid – breast ADC
Breast ca – “mesothelial” pattern
Lobular ca - classic
Signet ring carcinoma
Ovarian mucinous carcinoma
Ovarian papillary serous ca.
Small cell carcinoma

- Small cells resembling lymphocytes in small tight groups or singly
- Molding and Indian files
- Apoptosis
Squamous cell carcinoma

- Very rare in effusions
- **Cytology:**
  - Single cells with dense cytoplasm, small cell clusters
  - Non-keratinizing ca – mimics ADC
Lymphoma
Another less common...

- Wilm's tumor
- Angiosarcoma
- Melanoma
- LMP
Mesothelial cells vs. Adenocarcinoma vs. Mesothelioma
**Immunomarkers**

**Mesothelium**
- Calretinin
- D2-40
- WT-1
- CK 5/6

**Adenocarcinoma**
- Claudin-4
- MOC-31
- Ber-Ep4
- B72-3

**Specific Primary**
- TTF1 – Lung ADC
- Napsin A – Lung ADC
- Mammogobin – Breast ADC
- ER – Breast/GYN ca
- PAX 8 – Serous ca
- Cdx-2 – GI ca
- P40 - Sqcca

2 + 2
Use of “Pan-epithelial” immunostains

<table>
<thead>
<tr>
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<th>Carcinomas with pleural and peritoneal involvement or metastasis</th>
<th>Mesotheliomas (Pleural and Peritoneal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 4</td>
<td>All lung ADC &amp; 95% SQC; 90% RCC; 98% PSC</td>
<td>Negative</td>
</tr>
<tr>
<td>MOC31</td>
<td>95% lung ADC &amp; 97-100% SQC; 50% RCC; 98% PSC</td>
<td>Focal staining in up to 10% of cases</td>
</tr>
<tr>
<td>Ber-EP4</td>
<td>95-100% lung ADC &amp; 85-100% SQC; 40% RCC; 95% PSC; &gt;98% of pancreatic and gastric ADC</td>
<td>Focal staining in up to 20% of cases</td>
</tr>
<tr>
<td>pCEA and mCEA</td>
<td>80-100% lung ADC &amp; 5-50% lung SQC; 80% of non Gyn ADC; negative in RCC and 0-45% PSC</td>
<td>Negative or less than 5%, focal staining</td>
</tr>
<tr>
<td>B72.3</td>
<td>75-85% lung ADC &amp; 50-75% lung SQC; 80-98% of non Gyn ADC; negative in RCC and 65-100% PSC</td>
<td>Negative or less than 5%, focal staining</td>
</tr>
<tr>
<td>CD15</td>
<td>50-75% lung ADC &amp; 5-50% lung SQC; &gt;75% of RCC; 58% of PSC</td>
<td>Negative or less than 5%, focal staining</td>
</tr>
</tbody>
</table>

## “Mesothelial” markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Type of staining</th>
<th>Results in EMM</th>
<th>Pitfalls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calretinin</td>
<td>Nuclear and cytoplasmic</td>
<td>Nearly 100%</td>
<td>Focal + lung ADC (5-10%) and SQC (40%)</td>
</tr>
<tr>
<td>Podoplanin (D2-40)</td>
<td>Membranous</td>
<td>80-100%</td>
<td>Focal + lung ADC (&lt;15%) and SQC (50%)</td>
</tr>
<tr>
<td>WT-1</td>
<td>Nuclear</td>
<td>70-95%</td>
<td>- in lung ADC and SQC, + in ovarian pap serous</td>
</tr>
<tr>
<td>Keratin 5/6</td>
<td>Cytoplasmic</td>
<td>74-100%</td>
<td>Focal + lung ADC (2-20%) and SQC (100%)</td>
</tr>
</tbody>
</table>
Mesothelioma

- Malignant Mesothelioma (MM): malignant tumor of serosal cavities
- Two basic clinical types: Diffuse (common) or localized
- In the pleura:
  - Typically starts as small nodules along parietal pleura
  - Progression to confluent masses to form an encasing rind
Mesothelioma - Etiology

- Asbestos
  - Complex relationship b/w asbestos and MM
  - long latency period
  - 70-80% of MM in men associated with asbestos while only 20% of women have this association
  - Only 5% of asbestos workers develop MM
- Erionite
- Therapeutic radiation
- Germline mutations in BAP1
Inactivation of tumor suppressor genes is a key mechanism in pathogenesis of MM

- **Inactivation of cyclin-dependent kinase inhibitor 2A (CDKN2A) / alternative reading frame (ARF) gene, located on chromosome 9p21.3**
  - Homozygous deletions of the CDKN2A/ARF gene is noted in approximately 70-100% of MM
  - Can also be due to promoter hypermethylation or point mutation
  - Loss of p16INK4a, p15INK4b and MTAP

- **Neurofibromatosis type 2 (NF2) gene located on 22q12.1**
  - Seen in approximately 40-50% of MM
  - Loss of NF2 leads to activation of mTOR pathway and inactivation of the Hippo pathway

- **Inactivation of BRCA1-associated protein (BAP1) gene on 3p21.1**
  - Somatic mutations are seen in about 20-30% of MM
  - Germ line mutations are associated with familial tumors < 5%
Mesothelioma – histologic types

- Epithelioid
- Biphasic
- Sarcomatoid

Cytology
Mesothelioma - Cytology

- No obvious features of malignancy (usually mild atypia)
- Invasion cannot be assessed on cytology
- “there is no known criterion nor constellation of criteria which are universally diagnostic of malignancy”
Mesothelial cells vs. Mesothelioma
Mesothelioma – Cell aggregates

- Highly cellular specimens
- 3D, scalloped borders, collagen
- Different sizes (up to 100-200 cells)
- Different shapes – round, elongated, papillary
Mesothelioma – Nuclear atypia

- Monotonous
- Not that common
- Mild to moderate
- Mild nuclear irregularity
Mesothelioma – cell engulfing
Mesothelioma – Cellular enlargement

- Giant forms
- Cyto- and nuclear enlargement
Mesothelioma – Macronucleoli

- Numerous cells
Clue – small, orangiophilic squamous-like cells
Mesothelioma – a real life
Follow up
Mesothelioma – cytologic diagnosis

- Establish
  - Mesothelial phenotype
  - Malignancy
    - BRCA1-associated protein 1 (BAP1) mutations (loss)
    - Deletion of the 9p21 region/loss of p16 (CDKN2A)

- All mesothelioma biopsy/cytology pairs showed the same pattern of BAP1 or p16 retention or loss in the biopsy and cytology specimens (Am J Surg Pathol 2016;40:120–126)

Figure 1: BAP1 immunohistochemical (IHC) staining of malignant mesothelioma (MM) and reactive mesothelial cell (RMC) proliferations in cytology samples. (A) MM, epithelioid type [H&E, 40X objective]. (B) Absence of nuclear BAP1 IHC staining in malignant cells. Note internal control of RMCs and histiocytes. (C) MM, papillary type. (D) Presence of nuclear BAP1 IHC staining in MM. (E) RMCs in peritoneal fluid. (F) Presence of nuclear BAP1 IHC staining in mesothelial and inflammatory cells.

Diagn Cytopathol 2019 Mar;47(3):160-165
BAP1 – loss of nuclear staining

- Inactivation of BRCA1-associated protein \((BAP1)\) gene on 3p21.1
  - Somatic mutations are seen in about 20-30% of MM
  - Germ line mutations are associated with familial tumors < 5%
- Retained nuclear staining – non-diagnostic
- Cytoplasmic staining – irrelevant
p16 (FISH) homozygous deletion

- Inactivation of cyclin-dependent kinase inhibitor 2A (CDKN2A)/alternative reading frame (ARF) gene, located on chromosome 9p21.3
  - Can also be due to promoter hypermethylation or point mutation
  - Loss of p16INK4a, p15INK4b and MTAP
- Only loss is diagnostic
- About 30% of pleural mesotheliomas and at least 50% of peritoneal mesotheliomas do not show homozygous p16 deletion
- p16 loss can be seen in many types of malignancies
- IHC not recommended
MTAP

- *Methylthioadenosine phosphorylase*
- Located in the 9p21.3 locus and co-deleted with *p16*

5-hmC (5-hydroxymethylcytosine)

- Nuclear loss in >50% of nuclei
- Sensitivity 92%, specificity 100%
- 5-hmC + BAP1: Sensitivity 98%, specificity 100%

Chapel DB et al. IHC evaluation of nuclear 5-hmC accurately distinguishes malignant pleural mesothelioma from benign mesothelial proliferations. Mod Pathol 2019;32(3):376-86
Guidelines for the Cytopathologic Diagnosis of Epithelioid and Mixed-Type Malignant Mesothelioma

General Recommendations

The cytological diagnosis of MM in effusions should fulfil one of the following criteria:
- Indisputable malignant cells on cytomorphological criteria which demonstrate a mesothelial phenotype, which should be verified by ancillary techniques;
- Cytomorphological features which are not unequivocally malignant, but ancillary techniques confirm malignancy and a mesothelial phenotype.

When evaluated in clinical practice, these two options can make the specific diagnosis of MM with a high degree of sensitivity and accuracy [5], while noting that the diagnosis of sarcomatoid MM can rarely be established by effusion cytology.
Mesothelioma - FNA

- Rare (mostly case reports)
- Cytology FNA ≠ Cytology effusions
- Primary dx (localized nodules) vs. metastatic disease
- Mesothelial cell lesions of pleura – solitary fibrous tumor, nodular pleural plaque, adenomatoid tumor, simple mesothelial cyst, multicystic mesothelioma, well-differentiated papillary mesothelioma, localized malignant mesothelioma
- FNA and TP – similar cytologic features
Mesothelioma on FNA and TP

- Cellular aspirate
- Clusters, flat sheets, single cells
Mesothelioma on FNA and TP

- Papillary groups (core)
Mesothelioma on FNA and TP

- Intercellular spaces (rare)
Mesothelioma on FNA and TP

- Mild-moderate pleomorphism
- Multi-, binucleated cells
Mesothelioma on FNA and TP

- Cells – plasmacytoid, polygonal, spindle (sarcomatoid, biphasic)
Malignant mesothelioma in cytology – how far should we go?

Never make a dx on cytology

Follow the guidelines, common sense, clinical/imaging findings, and remember, behind every glass slide there is a human being

Dx of mesothelioma on cytology – piece of cake! No problem!

And soon…
Question...

- Pleural fluid – highly cellular, Mesothelial phenotype, BAP1 – Negative
- **No** pleural thickening, **No** mass
- Diagnosis????
- Mesothelioma in situ?????
Malignant mesothelioma in situ

- Churg et. al. Histopathology. 2018 May; 72(6):1033-1038. PMID: 29350783
- 2 cases of surface mesothelial proliferation (one pleural, one peritoneal)
- Both with loss of BAP1 and p16 deletion
In memoriam of my great friend Bogdan
1942 - 2018