Malignant Mesothelioma in cytology: How far can we go?

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In Memoriam – My friend Bogdan (1942-2018)

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>Percentage</th>
<th>Pleural</th>
<th>Pericardial</th>
<th>Peritoneal</th>
<th>Pelvic</th>
<th>&quot;Abdominal&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>100%</td>
<td>1639</td>
<td>193</td>
<td>983</td>
<td>1246</td>
</tr>
<tr>
<td>Negative</td>
<td>83.52%</td>
<td>80.38%</td>
<td>87.96%</td>
<td>78.27%</td>
<td>91.47%</td>
</tr>
<tr>
<td>Atypical</td>
<td>2.41%</td>
<td>2.28%</td>
<td>2.09%</td>
<td>3.47%</td>
<td>3.69%</td>
</tr>
<tr>
<td>Suspicious</td>
<td>0.53%</td>
<td>0.67%</td>
<td>0.52%</td>
<td>0.71%</td>
<td>0.24%</td>
</tr>
<tr>
<td>Positive</td>
<td>13.54%</td>
<td>16.75%</td>
<td>9.42%</td>
<td>17.55%</td>
<td>6.60%</td>
</tr>
</tbody>
</table>

Pathological/Related Factors Associated with Intraperitoneal Diagnosis
- Toluidine blue - "super positive"
- ThinPrep – Pap stain
- Cell Block – H&E stain (formalin fixed; if alcohol fixed – validation)
- Cytospin – Wright Giemsa stain (hematology)

Processing
Thin Prep - Cell Block

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

Squamous cell carcinomas

Cell block – Pitfall – pseudopapillary fragments
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

Degenerated mesothelial cells

Small orangophilic squamous-like cells in malignant mesothelioma
Multinucleated mesothelial cells
Clasping, grasping, cannibalism

Histiocytes vs mesothelial cells

Histiocytes
Malignant effusions - two cell populations

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

Mela-
noma

LMP

Mesothelial cells vs. Adenocarcinoma vs. Mesothelioma

Adenocarcinoma

ImmunoMarkers

Mesothelioma
  - Calretinin
  - D2-40
  - WT-1
  - CK 5/6

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

Specific Primary
  - TTF-1 – Lung ADC
  - Napsin A – Lung ADC
  - Mammoglobin – 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>
<th>Marker</th>
<th>Type of staining</th>
<th>Results in EMM</th>
<th>Pitfalls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 4</td>
<td>All lung ADC &amp; 95% SQC; 95% RCC; 98% PSC</td>
<td>Negative</td>
<td>Focal staining may be 10% of cases</td>
</tr>
<tr>
<td>MOC31</td>
<td>86% lung ADC &amp; 97-100% SQC; 50% RCC; 96% PSC</td>
<td>Focal staining may be 20% of cases</td>
<td></td>
</tr>
<tr>
<td>BerEP4</td>
<td>85-100% lung ADC &amp; 95-100% SQC; 95% RCC; 98% PSC</td>
<td>Negative or less than 5% focal staining</td>
<td></td>
</tr>
<tr>
<td>pCEA and mCEA</td>
<td>60-100% lung ADC &amp; 80-90% lung SQC; 80% of non-Gyn ADC; negative in RCC and 7-40% PSC</td>
<td>Negative or less than 5% focal staining</td>
<td></td>
</tr>
<tr>
<td>B72.3</td>
<td>75-90% lung ADC &amp; 60-70% lung SQC; 86-96% of non-Gyn ADC; negative in RCC and 60-100% PSC</td>
<td>Negative or less than 5% focal staining</td>
<td></td>
</tr>
<tr>
<td>CD165</td>
<td>50-70% lung ADC &amp; 5-80% lung SQC; &lt;10% of RCC; 58% of PSC</td>
<td>Negative or less than 5% focal staining</td>
<td></td>
</tr>
</tbody>
</table>

“Mesothelial” markers

<table>
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<th>Results in EMM</th>
<th>Pitfalls</th>
</tr>
</thead>
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<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>Neg in lung ADC and SQC; Pos 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
- Germine 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

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

- 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

Criteria for mesothelial differentiation

- Single cell population, lack of "foreign" cells
- Structure of cell aggregates – rounded berry-like external counters, "flower palites"
- Cytoplasmic characteristics – abundant, dense, basophilic (like matatplastic squamous cell); in Pap – tintorial gradation, from redish to green at the periphery
- Cell-to-cell relationship – brush-like border of microvilli – windows; cell engulfing – "embracement", "pincer-like grip"; multinucleation
- Vacuoles – multiple small vacuoles at the periphery (macrophage-like appearance) or solitary, large, hard-edged vacuoles, even signet ring-like vacuoles – hyaluronic acid
- Collagen basement material – collagenous core
- Glycogen – can be seen – cytoplasmic yellow discoloration
- Squamous-like cells – occasional small red-orangiophilic 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 (MM) and reactive mesothelial (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.

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

Hida T et al. Immunohistochemical detection of MTAP and BAP1 protein loss for mesothelioma diagnosis: Comparison with 9p21 FISH and BAP1 IHC. Lung Cancer 104:98-103, 2017
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):376-85

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, multilocular 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.

Do 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