Cytology of Serous Effusions
From basics to challenges

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• Objectives
  – Basic principles in the evaluation of serous effusions
  – Differential diagnosis between reactive mesothelial proliferations, metastatic carcinomas, and malignant mesotheliomas
  – Role of immunohistochemistry for correct interpretation
  – Use of other ancillary tests when needed.

Serous effusion
• Systemic / local disease
• Common
• Frequently reactive
• Any type of tumor may cause malignant effusion

Serous effusion
Transudates
CHF
Cirrhosis
Nephrotic syndrome
Malnutrition
Vena cava obst.
Meigs' syndrome
Exudates
Infections
Collagen vascular disease
Emboli
Infarction
Uremia
Pancreatitis
Hemorrhage, fistulas
Malignancies

Gross appearance is important
Increased viscosity suggests mesothelioma
Large amount is suspicious for malignancy
If stands, thick bottom layer develops, rich in cells
At least 20-50 ml for optimal assessment

Benign effusions

<table>
<thead>
<tr>
<th>Predominant cell type</th>
<th>Underlying disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte</td>
<td>CHF, Renal failure, Cirrhosis, Infections / TB, viral, Collagen vascular diseases, Malignancy</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>Infections/ empyema, pneumonia, Emboli, infarction, GII rupture, Collagen vascular diseases</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>Idiopathic, Air / repeated asp, pneumothorax, Infections/ parasitic, fungal, Hypersensitivity/ asthma, drugs, Emboli, infarction, Asbestosis, Malignancy</td>
</tr>
</tbody>
</table>

Plasma ultrafiltrate / hypercellular-rich in protein / lipid-rich
Clear, yellowish / blurred, bloody / white, milky

Rheumatoid Arthritis
Lupus Erythematosus

Courtesy of Koray Ceyhan and Claire Michael
Main question is….

• Is it malignant?
  A malignant effusion may be the manifestation of a known malignancy
  ✔ Determines the stage of the disease and the appropriate therapy
  ✔ Not always malignant in cancer patients!

Initial presentation of an unknown malignancy
  ✔ Primary site?

Clinical features of malignant effusions

- Most common histologic type is adenocarcinoma
- In children hematopoietic and small round cells tumors

Clinical features of malignant effusions

- Most common primary sites are:
  - Pleural
    - Male- Lung, lymphoma/leukemia, GI tract
    - Female- Breast, lung, genital tract, lymphoma/leukemia, GI tract
  - Peritoneal
    - Male- GI tract, lymphoma/leukemia, pancreas, lung
    - Female- Ovary, uterus, breast, GI tract, lymphoma/leukemia
  - Pericardial
    - Breast, lung, lymphoma/leukemia

Differential diagnosis in the land of mesothelial cells

- Mesothelial hyperplasia
- Mesothelioma
- Metastatic carcinoma

The Diagnosis of Malignancy in Effusion Cytology: A Pattern Recognition Approach

<table>
<thead>
<tr>
<th>Reactive Mesothelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
</tr>
<tr>
<td>Clusters</td>
</tr>
<tr>
<td>Clusters border</td>
</tr>
<tr>
<td>Windows</td>
</tr>
<tr>
<td>Cytoplasm</td>
</tr>
<tr>
<td>“Lacy skirt”</td>
</tr>
<tr>
<td>Sjoint ratio</td>
</tr>
<tr>
<td>Mitotic features</td>
</tr>
<tr>
<td>N/C ratio</td>
</tr>
<tr>
<td>Nucleus position</td>
</tr>
<tr>
<td>Nuclei shape</td>
</tr>
<tr>
<td>Nuclear membrane</td>
</tr>
<tr>
<td>Multinucleation</td>
</tr>
<tr>
<td>Chromatin</td>
</tr>
<tr>
<td>Nucleoli</td>
</tr>
</tbody>
</table>

(Cyt Diag Tech. 1990; 8:173-181)
Mesothelial cells

Vacuolation in mesothelial cells

• Degeneration
• Vacuoles in cytoplasms
• Vacuoles overlap the nucleus w/o pushing or distorting it
• No mucin
• Same nuclear morphology in both vacuolated and non-vacuolated cells
• No malignant nuclear features
• Larger vacuoles in chronic effusions
Adenocarcinoma

BerEp4

mucicarmin


Atypia in mesothelial cells

- Pulmonary infarct, uremia, pancreatitis, radiation, chemotherapy, cirrhosis, heart failure …,

Differential diagnosis in the land of mesothelial cells

- Mesothelial hyperplasia
- Mesothelioma
- Metastatic carcinoma

Mesothelial hyperplasia

Metastatic carcinoma

Pattern / Dual cell population

Unless all the cells are neoplastic

The Diagnosis of Malignancy in Effusion Cytology: A Pattern Recognition Approach

James E. Pavlov, MD* *Bryan C. Sease, MD PhD† †Seth F. Tepper, MD PhD‡ ‡ and James C. Pavlov, MD§

Mesothelial Adenocarcinoma

<table>
<thead>
<tr>
<th>Population</th>
<th>Usually dual population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clusters</td>
<td>Varying from few to many</td>
</tr>
<tr>
<td>Cluster size</td>
<td>Large, &gt; 12 cells</td>
</tr>
<tr>
<td>Cluster border</td>
<td>Solid</td>
</tr>
<tr>
<td>Windows</td>
<td>Unusual</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Delicate, homogeneous, uniform staining</td>
</tr>
<tr>
<td>&quot;Lacy skirt&quot;</td>
<td>Unusual</td>
</tr>
<tr>
<td>Nuclei</td>
<td>May be present, the vacuole pushes and distorts the nucleus</td>
</tr>
<tr>
<td>Mitotic features</td>
<td>May be present</td>
</tr>
<tr>
<td>N/C ratio</td>
<td>Mostly high</td>
</tr>
<tr>
<td>Nuclear position</td>
<td>Usually eccentric</td>
</tr>
<tr>
<td>Nuclear shape</td>
<td>Irregular</td>
</tr>
<tr>
<td>Nuclear membrane</td>
<td>Thick</td>
</tr>
<tr>
<td>Multinucleation</td>
<td>Common</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Course</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Large</td>
</tr>
</tbody>
</table>

Not all the foreigners are tumor cells

- Endometrial cells
- Endosalpingiosis
- Tuba epithelium
- Megakaryocytes
- Colonic mucosa
- Hepatocytes
- Lung parenchyma
- Striated muscle
- Skin
- Cartilage

Pattern / Tight 3-D clusters

Metastatic carcinoma

Intracytoplasic mucin

Psammoma bodies

- Most often in peritoneal effusions
  ~ 2/3 is malignant
  ~ 1/3 is benign
- Alarming if it is in pleura or pericardium

Mucin in the background
16.06.2016

**Metastatic carcinoma**

**Cellular features**

- Mesothelial cells may mimic malignant cells
- Some carcinomas may look rather bland
- Golden standard...?

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**Accuracy in effusion cytology**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metzgeroth, 2007</td>
<td>84</td>
<td>92</td>
</tr>
<tr>
<td>Grefte, 2008</td>
<td>78</td>
<td>73</td>
</tr>
</tbody>
</table>


---

**Non-mesothelial markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Mesothelial cell</th>
<th>Lung adenocarcinoma</th>
<th>Ovarian carcinoma</th>
<th>Breast carcinoma</th>
<th>SqCC</th>
<th>RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOC-31</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ber-EP4</td>
<td>-</td>
<td>+</td>
<td></td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CEA</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+/+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B72-3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TTF-1</td>
<td>-</td>
<td>+</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pax8</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Claudin4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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**Metastatic carcinoma**

- **Pattern**
  - Any discrete cell population?
  - 3-D tight, crowded groups?
- **Cellular features**
  - Nuclear?
  - Cytoplasmic?
- **Cell block- IHC**

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**Accuracy in effusion cytology**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Metzgeroth, 2007</td>
<td>84 - 94</td>
<td>92 - 100</td>
</tr>
<tr>
<td>Grefte, 2008</td>
<td>78 - 96</td>
<td>73 - 91</td>
</tr>
</tbody>
</table>

Serous effusion

- If it is malignant where is the primary?

  • Cell balls
    - Breast
    - Lung
    - Ovary
    - Mesothelioma

  • Papillary groups
    - Ovary
    - Lung
    - GIS
    - Mesothelioma...

  • Vacuolization
    - Ovary
    - Pancreas
    - Lung
    - Renal cell

  • Giant cells
    - Pancreas
    - Lung
    - Ovary
    - Mesothelioma...

81y. M
Followed-up with prostate carcinoma
Mediastinal LAP,
Nodules in the lung,
Pleural effusion

PSA
• Single cells
  - GI tract
  - Breast
  - Lung
  - Mesothelioma
  - Lymphoma/leukemia...

• Indian files
  - Breast
  - Small cell
  - GI tract

• GIS

• Breast

• M. Myeloma

Single small cells:
- Lymphoma/leukemia
- Small cell carcinoma
- Breast
- Stomach
- Small round cell tumors

Single large cells:
- Squamous cell carcinoma
- Melanoma
- Poorly diff adenocarcinoma
- Germ cell tumors

Squamous cell carcinoma

Urothelial carcinoma
Differential diagnosis in the land of mesothelial cells

Main difficulty!

Mesothelial hyperplasia

Mesothelioma

Metastatic carcinoma

**Cytology of malignant mesothelioma**

- Absence of a discrete cell population
- Morphologic continuum between native mesothelial cells and malignant cells

**IHC**

**Mesothelioma**

- Single population
- Many clusters
- Very large, >50 cells
- Scallop, knobby
- Common
- Two-tone staining: endoplasmic reticulum
- Present
- May be present in degenerated cells, but the nucleus is not distorted
- May be present
- Varies from low to high
- Unusually central or tangential
- Less pleomorphic than adenocarcinoma
- Thick
- Common
- Hyperchromatic
- Large
Cytology of malignant mesothelioma

- If sufficiently well differentiated to be easily recognized as mesothelial, difficult to call them malignant

<table>
<thead>
<tr>
<th></th>
<th>MM</th>
<th>Adenocarcinoma</th>
<th>Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural fluid (n=18)</td>
<td>10/25 (40.0%)</td>
<td>0/25 (0%)</td>
<td>0/25 (0%)</td>
</tr>
<tr>
<td>Peritoneal fluid (n=44)</td>
<td>5/15 (33.3%)</td>
<td>1/16 (6.3%)</td>
<td>5/23 (21.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>10/30 (33.3%)</td>
<td>1/14 (7.1%)</td>
<td>5/28 (17.8%)</td>
</tr>
</tbody>
</table>

Abbreviations: MM, malignant mesothelioma; SOSLC, small orangeophilic squamous-like cells.
### Positive mesothelial markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Useful</th>
<th>Not useful</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calretinin</td>
<td>Lung adenocarcinoma</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Keratin 5/6</td>
<td>Lung adenocarcinoma</td>
<td>Serous carcinoma (?)</td>
</tr>
<tr>
<td>Podoplanin/DP2-40</td>
<td>Lung adenocarcinoma</td>
<td>Serous carcinoma (limited)</td>
</tr>
<tr>
<td>WT1</td>
<td>Lung adenocarcinoma</td>
<td>Serous carcinoma</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>Lung adenocarcinoma</td>
<td>Serous carcinoma</td>
</tr>
<tr>
<td>Mesothelin</td>
<td>Renal cell carcinoma</td>
<td>Negative staining strongly indicates against a mesothelium</td>
</tr>
</tbody>
</table>

Modified from Ordonez NG, Human Pathology 2013; 44: 1-19

### Positive carcinoma markers

<table>
<thead>
<tr>
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<th>Not useful</th>
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<td>MO-31</td>
<td>Adenocarcinoma</td>
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<td>Lung adenocarcinoma</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>CEA</td>
<td>Lung adenocarcinoma</td>
<td>Serous carcinoma</td>
</tr>
<tr>
<td>TAG-72</td>
<td>Lung adenocarcinoma</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>BG-8</td>
<td>Lung adenocarcinoma</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>CD15</td>
<td>Renal cell carcinoma</td>
<td>Squamous cell carcinoma</td>
</tr>
</tbody>
</table>

Modified from Ordonez NG, Human Pathology 2013; 44: 1-19

### Claudin-4 Immunohistochemistry Is Highly Effective in Distinguishing Adenocarcinoma From Malignant Mesothelioma in Effusion Cytology

84 AdenoCa, 75 MM Membranous staining 99% AdenoCa 0% MM

---

### Organ specific markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF-1</td>
<td>Lung adenocarcinoma (75-85%)</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>Napsin A</td>
<td>Lung adenocarcinoma (80-90%)</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>PAX 8</td>
<td>Renal cell carcinoma</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>PAX 2</td>
<td>Renal cell carcinoma</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>Breast carcinoma (70%)</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>Mammaglobin</td>
<td>Breast carcinoma (50-85%)</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>CDX2</td>
<td>Gastrointestinal Pancreaticobiliary</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>P63</td>
<td>Squamous cell carcinoma (80-100%)</td>
<td>Mesothelioma</td>
</tr>
</tbody>
</table>

Modified from Ordonez NG, Human Pathology 2013; 44: 1-19
Expected immun pattern in malignant mesothelioma

**The Diagnostic Utility of p16 FISH and GLUT-1 Immunohistochemical Analysis in Mesothelial Proliferations**

Sara N. Monaco, MD, Yogesh Shrestha, MS, Mona Barad, MD, Alyssa M. Krasinskas, MD, and Sanja Dacic, MD, PhD

<table>
<thead>
<tr>
<th>p16 FISH</th>
<th>GLUT-1</th>
<th>Both</th>
<th>Neither</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>-</td>
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</tr>
</tbody>
</table>

**Guidelines for the Cytopathologic Diagnosis of Epithelioid and Mixed-Type Malignant Mesothelioma**

Scott L. Boerner, MD, FRCPath

".....morphology is our first line of defense, and if the case passes through this, we have already lost the battle"
“.....morphology is our first line of defense, and if the case passes through this, we have already lost the battle”

Scott L. Boerner, MD, FRCP(C)

“.....immunocytochemistry adds great value to our interpretation, and may be the only tool to save the patient ”