Immunohistochemistry on cytology specimens from pleural and peritoneal fluid

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Disclosures and Acknowledgements

I have no financial disclosures.

I would like to acknowledge the use of images from *Diagnostic Cytopathology* and Professor Mike Sheaff, my colleague and co-author.
Learning Objectives

• Problems with pathological assessment of serous effusion fluids

• Role of immunohistochemistry; antibodies/panels useful in routine practice

• Common sense approach for safe reporting
Introduction

• One of the most challenging areas in cytology reporting

• One of the areas most dependent on good quality immunohistochemistry:
  – technical quality
  – interpretation
Anatomy of the serous cavities

- Cranial cavity
- Dorsal body cavity
- Vertebral cavity
- Thoracic cavity: Superior mediastinum, Pleural cavity, Pericardial cavity within the mediastinum, Diaphragm
- Abdominal cavity
- Pelvic cavity
- Ventral body cavity (both thoracic and abdominopelvic cavities)

Lateral view

Anterior view
Structure and function of mesothelium

- Mesothelium: Single layer of epithelioid cells on a bed of vascular connective tissue
- Submesothelial cells - progenitors of surface epithelioid cells; possibly the cells of origin of malignant mesothelioma
- Stroma with network of capillaries and lymphatics
Structure and function of mesothelium

Normal serous fluid production:
• Blood $\rightarrow$ parietal stromal matrix $\rightarrow$ mesothelial layer $\rightarrow$ serous cavity

Normal serous fluid resorption:
• into capillaries of visceral serosa
• through stomata between mesothelial cells into lymphatics within the parietal serosa

Functions:
• Produce hyaluronate-rich fluid
• Facilitate transport of fluid and cells across serosal barrier

Also:
• Phagocytic function
• antigen presentation, inflammatory processes, coagulation, fibrinolysis, repair and tumour cell adhesion
Structure and function of mesothelium

Mesothelial lining cells

- Flat /cuboidal,
- Small to medium sized nuclei, small nucleoli
- Variable amounts of cytoplasm with luminal microvilli on the luminal
- Pinocytotic vesicles on both apical and basal sides of the cell
Histology of mesothelial cells - normal
Calretinin
## Effusions

<table>
<thead>
<tr>
<th>Transudate</th>
<th>Exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein low &lt;2.9g/dl</td>
<td>Protein high (&gt;3g/dl)</td>
</tr>
<tr>
<td>Non-fibrinous</td>
<td>Fibrin present</td>
</tr>
<tr>
<td>Cell content low</td>
<td>Inflammatory/tumour cells</td>
</tr>
<tr>
<td>LDH low</td>
<td>LDH high</td>
</tr>
<tr>
<td>Specific gravity low &lt;1.015</td>
<td>Specific gravity higher</td>
</tr>
<tr>
<td>Clear</td>
<td>Microorganisms or foreign material</td>
</tr>
<tr>
<td>Transudate</td>
<td>Exudate</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Common</strong></td>
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</tr>
</tbody>
</table>
| Left ventricular failure  
Liver cirrhosis  
Hypoalbuminaemia  
Peritoneal dialysis | Malignancy  
Parapneumonic effusions |
| **Less common** | **Less common** |
| Hypothyroidism  
Nephrotic syndrome  
Mitral stenosis  
Pulmonary embolism | Pulmonary infarction  
Rheumatoid arthritis  
Autoimmune diseases  
Benign asbestos effusion  
Pancreatitis  
Post-myocardial infarction syndrome |
Histology of mesothelial cells - reactive
Cytology of mesothelial cells

- round with indistinct cell membranes and fuzzy borders
- single or clumped with so-called ‘windows’ between cells
- large round or elliptical nucleus, often centrally placed, with a single prominent nucleolus
- may contain two or multiple nuclei
- cytoplasm is green with Papanicolaou and blue with MGG
- 2-zone cytoplasm
- vacuoles due to ‘degeneration’ or pinocytosis or glycogen
Other cells in effusion fluid

- Macrophages
- Lymphocytes: T>>B
- Neutrophils
- Eosinophils
- Plasma cells
- Mast cells
- Megakaryocytes
Challenges in effusion cytology in the presence of malignancy

• Multiple causes of effusions; may be due to a co-morbidity, not the cancer
• May be first manifestation of malignancy
• Even when due to cancer, this may be due to
  – lymphatic blockage
  – Irritation of mesothelium
  – ie NOT tumour cell seeding
• Difficulties in interpretation
Challenges in effusion cytology

• Interpretation challenges
  – Reactive mesothelial cells can show a range of atypia: bi-/multinucleation, nuclear atypia, mitotic activity, large cell aggregates, vacuolation, gland-like arrangement

  – Malignant cells are altered in effusions
    • Polygonal shape
    • 3-D aggregates
    • Degenerative changes

    • Do not resemble the primary
    • Look similar irrespective of origin

  – Some types deceptively bland

  – Number of cells present in the fluid is highly variable
Role of immunohistochemistry

1. MALIGNANT OR REACTIVE?

- Dual population but only subtle differences or overlapping features
- Single population of only malignant cells without obvious cytological features of malignancy
- Isolated malignant cells in a vast reactive population
# Malignant vs Reactive

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<th>Carcinoma</th>
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<tbody>
<tr>
<td>Calretinin</td>
<td>B72.3</td>
</tr>
<tr>
<td>D2-40</td>
<td>BerEP4</td>
</tr>
<tr>
<td>CK 5/6</td>
<td>CD15</td>
</tr>
<tr>
<td>Vimentin</td>
<td>CEAm</td>
</tr>
<tr>
<td></td>
<td>EMA (cytoplasmic)</td>
</tr>
<tr>
<td></td>
<td>MOC31</td>
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- Use a panel including +ve and –ve markers
- CARE when interpreting which cells are positive for what: SCIP
## Malignant vs Reactive Mesothelial

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<tr>
<td>EMA (negative/weak)</td>
<td>EMA (membranous)</td>
</tr>
<tr>
<td>Desmin (positive)</td>
<td>Desmin (negative)</td>
</tr>
</tbody>
</table>

Others: p53, bcl-2, Glut1
EMA: Strong membranous staining is typical in mesothelioma; Normal/reactive mesothelial cells: weak staining; Adenocarcinoma cells: diffuse strong cytoplasmic staining without membranous accentuation
Desmin: NEGATIVE in mesothelioma; positive in normal/reactive mesothelial cells
Role of immunohistochemistry

2. Determining primary site

General markers of malignancy and negative markers of mesothelial cells and macrophages

Specific markers for particular malignancies
## Antibodies useful for determining site of origin in effusions

<table>
<thead>
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<th>Cancer</th>
<th>Antibodies</th>
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<tbody>
<tr>
<td>Lung</td>
<td>TTF-1, CD56, CK19</td>
</tr>
<tr>
<td>Ovary</td>
<td>WT-1 (also positive in mesothelial cells), ER</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>CDX2, CK7/20, CA19-9, CEAm</td>
</tr>
<tr>
<td>Breast</td>
<td>ER, mammoglobin, CRx-A01,</td>
</tr>
<tr>
<td>Melanoma</td>
<td>S-100, HMB-45, melan A</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>CD3:CD20, other specific studies</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Vimentin, other specific</td>
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</table>
Common Examples
Adenocarcinoma of lung:
TTF1 +ve
Small cell carcinoma of lung: TTF1 and CD56 +ve
Gastric signet ring carcinoma: CEA positive
High grade serous tubo-ovarian carcinoma
Pitfalls in interpreting IHC in effusions

- MIXTURE of cells: take care to report expression in the correct population; particularly in presence of low cellularity
WT1 expressed in mesothelial cells

TTF1 expressed in malignant cells
Pitfalls in interpreting IHC in effusions

• PATTERN of expression where appropriate
EMA expression patterns

Reactive mesothelial cells: weak diffuse cytoplasmic

Mesothelioma: Cytoplasmic with STRONG membrane accentuation

Adenocarcinoma: Diffuse strong cytoplasmic expression, obscures cellular detail
Question 1

• Which of the following markers would not be useful in distinguishing reactive mesothelial cells from metastatic adenocarcinoma?

1. MOC31
2. BerEp4
3. Calretinin
4. CK7
5. CK 5/6
Question 2

• Which of the following markers would not be useful in the diagnosis of mesothelioma?

1. MOC31
2. Actin
3. Calretinin
4. Desmin
5. EMA
Case 1: Pleural fluid in known case of lung cancer: reactive or metastatic?
General approach to reporting malignancy in effusions

- Only report when CERTAIN: malignancy is easy to overcall and undercall: err on side of caution
- Always rule out malignancy if there is a +ve history
- Always rule out malignancy if there is doubt on morphology
- Look at all preparations before issuing final report
- Use a PANEL of markers, not just one
- Take care when interpreting IHC as there is a mixture of cells present; coordinate approach on serial sections
  - Due regard to expression by different cell types, eg WT1
  - Due regard to different patterns of expression, eg EMA
- Asking for another sample is not unreasonable