Serous effusions

- Abnormal accumulation of fluid in body cavities
- Systemic/local disease
- Frequently reactive
- Any type of tumour may cause malignant effusion
- Presence of malignant cells implies advanced TNM Stage
- Pleural fluid, Pericardial fluid, Ascitic fluid
- (Peritoneal washings)

The need for a reporting terminology for serous fluid cytology

- Common specimen type in a cytology service
- No internationally accepted terminology
- Questions about adequacy in terms of sample volume and cell content

IAC-ASC collaboration

- Porto, Feb 2018. First announcement of the proposal at the IAC tutorial
- Madrid, June 2018. First meeting of the co-editors representing the IAC & ASC and launch of the terminology

Co-editors

- Professor Fernando Schmitt
- Dr Barbara Crothers
- Dr Dan Kurtycz
- Dr Ashish Chandra
AIMS

• Basic principles in laboratory handling of serous effusion samples
• Diagnostic pitfalls and how to avoid them
• Morphological evaluation and reporting terminology
• Role of ancillary tests

Macroscopic findings

• Volume: 75 ml eliminates the influence of specimen volume on diagnostic adequacy (Rooper et al, Cancer Cytopath 2014)
• Practical implication: 75ml of fluid needed to say that a benign effusion is truly benign
• Smaller volumes acceptable for processing

Macrosopic findings

• Appearance
  • Straw coloured, blood-tinged
  • Heavily blood-stained (with clot)
  • Turbid
  • Milky (chylous)
  • Viscous (hyaluronic acid rich)

Reporting terminology for serous fluid cytology

Proposed diagnostic categories

• Non-diagnostic (ND)
• Negative for malignancy (NFM)
• Atypia of uncertain significance (AUS)
• Suspicious for malignancy (SFM)
• Malignant (M) – specify type

• Not very different to current reporting practices
• Need to determine criteria and risk of malignancy for each category
• Follow in the footsteps of the pioneering Bethesda systems

Categories (Provisional) For The International System for Reporting Serous Fluid Cytopathology

Non diagnostic (ND)

• A specimen should be considered ND if it provides no diagnostic information in the appropriate clinical context.
• Specimens with a volume inferior to 50 ml should be regarded with caution and an exploratory note could be added advising of its limited sensitivity.

• INCIDENCE: rate 0.2 - 1%
• MAIN CAUSES: low volume, bad preservation of cells, technical aspects.
• ADEQUACY CRITERION
   no study has addressed so far the issue of a minimal cell count for sample adequacy, being general knowledge that any observation consistent with the clinical presumption should be considered diagnostic.
   there’s relatively strong evidence in the literature that a minimal range volume of around 50-75 ml should be adopted, in order to diminish potential false negatives and optimize the test sensitivity.
   Any atypia should be reported as such and put under the atypical or “suspicious” category.
• MANAGEMENT: Samples reported as non-diagnostic should be regarded as non-contributory and a repeat aspirate could be performed.
Non-diagnostic (ND)

- Haemorrhage, artefacts or contaminants
  - Consider representing on additional preparations and cell block
  - Sample rendered diagnostic
  - Sample remains ND. Advise repeat sample

A specimen containing cells whose nature are uncertain (macrophages, mesothelial cells, cells from cytologically bland malignant tumor - usually carcinomas) due to qualitative (poor preservation) and/or quantitative factors (small number).

- INCIDENCE: rate 0.6%
- RISK OF MALIGNANCY: 40-75% (less in peritoneal than pleural fluid)
- MAIN CAUSES: degenerative changes in mesothelial cells/macrophages (especially in cases with low expectation of finding metastatic malignancy).
- WHAT TO DO:
  - Immunostains to ascertain the cell lineage.
  - If representative cells are seen on immunostains and demonstrated to be macrophages or mesothelial cells, down grade from AUS to NFM on preliminary report to NFM on final report.
  - If representative cells are not seen on immunostains, final report remains AUS.
- MANAGEMENT: clinical follow-up or repeat cytology if effusion persists.

A specimen obtained from peritoneal, pleural or pericardial cavities may be considered negative for malignancy when the specimen is composed of only benign or reactive cellular components, without malignant tumor cells, or cells concerning for malignancy. These components include mesothelial cells without apparent neoplastic phenotypic changes and/or immunohistochemical or molecular alterations associated with malignancy. Varying ratios and types of inflammatory cells may be present depending on the condition responsible for the effusion, and this category also includes effusions due to infectious causes.

- INCIDENCE: rate 70-80%
- RISK OF MALIGNANCY: 20-30%
- MAIN CAUSES: many systemic and localized diseases (infections, circulatory diseases, auto-immune diseases, etc.)
- MANAGEMENT: clinical follow-up.

A specimen containing cells whose nature are uncertain (macrophages, mesothelial cells, cells from cytologically bland malignant tumor – usually carcinomas) due to qualitative (poor preservation) and/or quantitative factors (small number).

- INCIDENCE: rate 0.6%
- RISK OF MALIGNANCY: 40-75% (less in peritoneal than pleural fluid)
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  - If representative cells are not seen on immunostains, final report remains AUS.
- MANAGEMENT: clinical follow-up or repeat cytology if effusion persists.

**Negative for Malignancy (NFM)**

- Consider reprocessing or additional cytospins and cell block
- A serous effusion specimen obtained from peritoneal, pleural or pericardial cavities may be considered negative for malignancy when the specimen is composed of only benign or reactive cellular components, without malignant tumor cells, or cells concerning for malignancy. These components include mesothelial cells without apparent neoplastic phenotypic changes and/or immunohistochemical or molecular alterations associated with malignancy. Varying ratios and types of inflammatory cells may be present depending on the condition responsible for the effusion, and this category also includes effusions due to infectious causes.

**Atypia and suspicious categories — current practice**

- Holding categories while awaiting cell block/clot and immunostains
- Two step reporting process with a preliminary and a final report
- In the absence of diagnostic criteria, atypia and suspicious used almost interchangeably

**Atypia and suspicious categories — why two different categories?**

- The justification for diagnostic categories is best supported by a significant difference in the ROM from adjacent categories.
- The proportion of cases should be small in both categories and ancillary testing may be used to move cases into a more definitive diagnostic category (benign or malignant).
- Interobserver is usually high in these categories due to heterogeneity of diagnostic criteria and also dependent on the experience of the observer.
Atypia of uncertain significance (AUS)

- Preliminary report: AUS
  - Small numbers of cells, favour degenerate macrophage/mesothelial cells
- Macrophage/mesothelial markers positive. Final report: NFM
- Epithelial markers positive. Final report: Malignant - metastatic
- No residual material on cell block. Final report: AUS

Suspicious for malignancy (SFM)

- Preliminary report: SFM
  - Small numbers of cells, favour epithelial or other malignancy
- CB+ IHC: Confirms malignancy Final report: Malignant
  - Metastatic adenocarcinoma etc
- CB+ IHC: Insufficient material for confirmation IHC equivocal Final report: SFM

Categories (Provisional) For The International System for Reporting Serous Fluid Cytopathology

Suspicious for Malignancy (SFM)

- The term suspicious is defined as the presence of some cytological features which are usually found in malignant lesions, but with insufficient features either in number or quality to make a definitive diagnosis of malignancy. The type of malignancy suspected should always be stated (epithelial/mesothelial, lymphoid...)
- INCIDENCE: rate 2.3%
- RISK OF MALIGNANCY: 75-85% (less in pleural than peritoneal fluid)
- MAIN CAUSES: quantitative factor
- WHAT TO DO:
  - Immunostains to confirm the cell lineage. If epithelial, for example, confirms metastatic carcinoma.
  - Maybe upgraded from SFM on preliminary report to Malignant on final report if immunostainings indicate metastatic carcinoma or mesothelioma (see later).
  - If representative cells are not seen on immunostainings, final report remains SFM.
- MANAGEMENT: ancillary testing, correlation with clinical data and biopsy.

Malignant Effusions

- A malignant effusion may be the manifestation of a known malignancy
- Initial presentation of an unknown malignancy
- Most common histologic type is adenocarcinoma
- In children: hematopoietic and small round cell tumors

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

Categories (Provisional) For The International System for Reporting Serous Fluid Cytopathology

Malignant

- Serous effusion specimens classified as “malignant” must contain cytomorphological features that, either alone or in combination with ancillary studies, are diagnostic of a secondary (metastatic disease) or primary malignancy (mesothelioma). The cell type of origin should be identified as epithelial, neuroendocrine, hematolymphoid, melanocytic, mesenchymal, germ cell tumors or mesothelial.
- INCIDENCE: rate 20-25%
- RISK OF MALIGNANCY: 98-100%
- MAIN CAUSES: a malignant effusion may be the manifestation of a known malignancy or initial presentation of an unknown malignancy. Most common histologic type is adenocarcinoma and in children hematopoietic and small round cell tumors.
- MANAGEMENT: ancillary testing to establish primary site and study of prognostic and predictive markers (especially in secondary tumors) and correlation with clinical data and biopsy (especially in primary tumors).
Metastatic carcinoma

(Where is the primary?)

<table>
<thead>
<tr>
<th>Primary Tissue</th>
<th>Metastatic Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic</td>
<td>Breast</td>
</tr>
<tr>
<td>Breast</td>
<td>Lung</td>
</tr>
<tr>
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</tr>
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</tr>
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</tr>
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</tr>
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</tr>
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</tr>
<tr>
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<td>Prostate</td>
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<td>Other malignancies</td>
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<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Malignant Mesothelioma</th>
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<tr>
<td>Thoracic / abdominal</td>
<td>Female/male</td>
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Check clinical data including previous cytology or other relevant samples to avoid repeating tests already performed.

Malignant Mesothelioma

(Morphological pattern)

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

Malignant Mesothelioma

(Morphological pattern)

- Small parakeratotic cells seen in mesothelioma. Their presence does not always indicate squamous cell carcinoma
- WT1 is helpful in distinguishing between mesothelioma and poorly differentiated squamous cell carcinoma

**TABLE 1. Reactive Mesothelial Cells Versus Metastatic Adenocarcinoma**

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Some General Principles Regarding IHC of Mesotheliomas

- Use of 2 mesothelial and 2 carcinoma markers recommended.
- A broad spectrum carcinoma marker is a good idea unless you have a reason to select an organ specific marker.
- However, some broad spectrum carcinoma markers can stain mesotheliomas:
  - Claudin 4: not reported to stain mesotheliomas
  - MOC 31: stains mesotheliomas in some labs
  - BerEP4: can stain 20% of mesotheliomas
  - CEA, CD15 and B72.3: low sensitivity and cross-reactivity

Some General Principles Regarding IHC of Mesotheliomas

- Virtually all epithelial mesotheliomas stain for calretinin. Be cautious if staining is negative.
- However, calretinin also stains a variety of other tumours so by itself can be misleading.
- High grade serous carcinoma can stain any “mesothelial” marker.
  - A reliable broad spectrum carcinoma marker should be used in this setting.
  - BAP1 is almost never lost in high grade serous carcinoma.
  - Mesotheliomas can sometimes stain with organ specific lineage markers such as PAX-8.

Recommended Antibodies for Separating Mesothelioma from Adenocarcinoma

- **Mesothelioma Markers**
  - Calretinin
  - Cytokeratin 5/6*
  - WT-1**
  - D2-40
  - Mesothelin

- **Carcinoma Markers**
  - Claudin-4
  - MOC-31 (can stain mesotheliomas)
  - TTF1 (check clone)
  - P40 (for squamous ca)
  - PAX-8 (for gynae tumours – can stain mesotheliomas)

Use of AUS & SFM in mesothelial proliferations – current practices

- Clinical context essential for reporting and interpretation
- **AUS**: mesothelial proliferation with inconclusive immunostains. Clinically no/low suspicion of mesothelioma
- **SFM**: mesothelial proliferation with inconclusive immunostains.
  - Clinical suspicion of mesothelioma
  - Upgrade to Malignant if immunostains conclusive and clinical & radiological suspicion of mesothelioma
  - Majority of survey respondents already make diagnosis of mesothelioma on cytology.

Ancillary testing of mesothelial proliferations

- **Confirmed mesothelial origin**
  - WT1, Calretinin, D2-40, Podoplanin, Thrombomodulin, Mesothelin, Vimentin

- **Reactive**
  - Desmin, smooth muscle

- **Neoplastic**
  - EMA: thick membranous
  - p53: positive
  - BAP 1 deletion
  - GLUT-1: positive
  - FISH: p16 deletion

- **Exclude adenocarcinoma**
  - BerEP4, MOC31, TTF1, Claudin-4

Role of BAP-1

- Immunohistochemical findings of BAP1, IDH1 and IDH2 in epithelial mesotheliomas and reactive mesothelial hyperplasia.
Function and Detection of Genes Mutated or Deleted in Mesotheliomas

- **BAP1**: controls DNA repair and genes related to cell proliferation, cell cycle, cell death. Function as a tumour suppressor gene.
  - Wild type BAP1 protein is detectable by IHC.
  - Deletions or mutations in BAP1 lead to loss of IHC-detectable nuclear protein (protein either lost or sequestered in cytoplasm)
  - Loss of nuclear BAP1 always a marker of malignancy

**Practical Utility of BAP1 IHC**

- Reactive proliferations never lose nuclear staining.
- Loss of nuclear BAP1 by IHC 100% specific for mesothelioma
- **Epithelial mesotheliomas**
  - Pleural: sensitivity 60 to 70%
  - Peritoneal: sensitivity 60 to 70%
- **Sarcomatous mesotheliomas**
  - Sensitivity very low (<20%)
  - Works well on effusion cytology specimens

Churg, Arch Pathol 2016; Hwang et al AJSP 2016

**BAP1 IHC - Cautions**

- Loss of staining is diagnostic of mesotheliomas; however:
  - Only nuclear staining is of interest
  - A significant proportion (30-40%) of epithelial mesotheliomas will not show BAP1 loss
  - Most sarcomatous/desmoplastic mesotheliomas will not show BAP1 loss
  - Crucial that sections show a positive internal control (inflammatory or stromal cells)
  - Other tumours (ocular and cutaneous melanomas, some renal cell carcinomas and others) can have BAP1 loss in the BAP1 cancer syndrome and as occasional somatic mutations.
  - Confirm that process is mesothelial before using BAP1

Function and Detection of Genes Mutated or Deleted in Mesotheliomas

- **P16 INK4a (CDKN2A)**: prevents cell cycle progression. Tumour suppressor gene.
  - Loss of p16 (9p21 locus) can be detected by FISH.
  - P16 IHC does not give same information as p16 FISH
  - Reactive conditions can have a p16 methylation and hence no staining
  - When p16 is expressed in mesotheliomas, the IHC is patchy

**Practical Utility of p16 (FISH)**

- Specificity of homozygous loss for malignancy: 100%.
- Reactive proliferations never show p16 (CDKN2A) loss
- **Epithelial mesotheliomas**
  - Pleural: sensitivity 60 to 70%
  - Peritoneal: sensitivity <50%
- **Sarcomatous mesotheliomas**
  - Pleural: sensitivity 70-80%
  - Peritoneal: sensitivity unknown
- Works well on effusion cytology specimens

Churg, Arch Pathol 2016; Hwang et al AJSP 2016

**P16 FISH - Cautions**

- For differentiate mesothelioma and reactive proliferation FISH is always required. IHC does not give the same information!
- Picking out the cells of interest by fluorescence microscopy can be difficult.
- Homozygous losses must be above the truncation background.
- Roughly 30% of epithelial pleural mesotheliomas and at least 50% of epithelial peritoneal mesotheliomas do not show homozygous p16 deletion.
- P16 loss can be seen in many types of malignancies, so first be sure that you are dealing with a mesothelial proliferation.
MTAP IHC - Cautions

- Gives essentially the same information as p16 FISH.
- Quicker, cheaper to run, easier to interpret than p16 FISH.
- Only loss of MTAP significant:
  - 100% loss allows an unequivocal diagnosis of mesothelioma
  - Significance of partial loss unclear at this point but probably malignant.
- Positive internal control required for interpretation.
- Combined with BAP1 IHC, sensitivity in the pleural cavity for epithelial mesotheliomas probably around 80 to 90%.

Diagnosis of Mesotheliomas in Effusions

- CT & Clinical information is very helpful in evaluating effusion cytology specimens of potential mesotheliomas:
  - Be cautious if there is no clinical or radiological evidence of tumour
  - Provided a cell block is available, separation of mesothelial cells from carcinoma cells is not a problem.
  - Morphology can be helpful but many mesotheliomas have deceptively bland cytology.
  - Combinations of BAP1 IHC, MTAP IHC and p16 FISH can boost sensitivity to 80 to 90%.

Ancillary testing

- Frequently effusions are the first manifestation of a neoplasia and cytology is the first approach to search neoplastic cells.
- Ancillary techniques are used for:
  - diagnosis of mesothelioma
  - characterization of metastasis
  - study of therapeutic targets.
Theranostics

Breast & gastric cancer

Lung

Cytogenetics: ALK, ROS1, FGFR1

Head & neck squamous cell carcinoma: HPV ISH

Mesothelioma: p16 deletion

Peritoneal washings

NORMAL MESOTHELIAL CELLS

Peritoneal washings

SBL

ENDOMETRIOSIS

HIGH GRADE SEROUS CARCINOMA

PERITONEAL WASHINGS

NORMAL MESOTHELIAL CELLS

SBL

ENDOMETRIOSIS

HIGH GRADE SEROUS CARCINOMA

GCT

Carl Exner bodies

Approach for Reporting Peritoneal Washings

• In the majority of cases, peritoneal washings or lavage are performed to screen for the presence of tumor cells in the peritoneal cavity and are performed in association with a surgical procedure.
• The goal of this assessment is not a primary cytologic diagnosis, but to confirm the presence or absence of tumor cells at that site, which makes comparison with the surgical specimen.
• The cytology interpretation should be specific when the tumor type is known from histology review.
• Tumor cells suspected to be, or confirmed to be from borderline tumors of low malignant potential are categorized as atypia of undetermined significance (AUS), while those suspected to be from malignant processes are categorized as either suspicious for malignancy (SFM) or malignant (M) once comparison with the surgical specimen confirms their nature.

Peritoneal washings

SBL

ENDOMETRIOSIS

HIGH GRADE SEROUS CARCINOMA

GCT

Carl Exner bodies
Peritoneal washing showing artefact from the fluid used for irrigating the peritoneal cavity.
- This material may be mistaken for mucin (MGG) or psammoma bodies (Pap) in peritoneal washings.

### Diagnostic categories & clinical management

- **Cytomorphological diagnosis on cytospins/LBP + clot/cell block**
- **Non-diagnostic**: Repeat sample
- **Negative for Malignancy**: Discharge or clinical follow up. Specific diagnosis eg empyema or features of auto-immune disorders for further investigation
- **Atypia of Undetermined Significance**: Ancillary testing to exclude malignancy
- **Suspicious for Malignancy**: Ancillary testing to confirm malignancy
- **Malignant – Primary (mesothelioma)**: Correlate with clinical findings and biopsy
- **Malignant – Secondary (metastasis/lymphoma)**: Ascertaining the primary and prognostic/predictive markers

### The International System for Reporting Serous Fluid Cytopathology