The 2020 International System for reporting Serous Fluid Cytopathology

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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
Announcement: The International System for Reporting Serous Fluid Cytopathology

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The International System for Serous Fluid Cytopathology

This book is the culmination of an international effort to bring consistency and diagnostic efficiency to effusion cytology for the sake of patient care. The authors recognize special challenges in serous fluid cytopathology, such as the presence of mesothelial cells in peritoneal fluids. What is an appropriate serous fluid volume to ensure adequacy? How should mesothelial proliferations be reported, and as it appropriate to make an interpretation of malignant mesothelial lesions? How specific should a report be regarding the origin and subtyping of tumors found in serous fluids? What are the appropriate quality measures for this specimen type? Special chapters on considerations for peritoneal washings, cytopreparatory techniques, staining methods, and quality management are included to address these issues. The text contains literature reviews that elucidate existing evidence in support of current practices and recommendations. Expert opinions on where evidence was lacking, the most common practices were adopted by consensus, and where there was no commonality, are employed.

Written by experts in the field, The International System for Serous Fluid Cytopathology serves as a collaborative effort between the International Academy of Cytology and the American Society for Cytopathology and calls upon participation of the international cytopathology community to contribute to the development of a truly international system for reporting serous fluid cytology.

Ashish Chandra
Barbara Crothers
Daniel Kurtycz
Fernando Schmitt
Editors

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We need answers to practical clinical questions!

- Evaluating evidence of adequacy (volume & cellularity)
- Defining what is a true negative sample
- The use of atypia and suspicious categories
- Mesothelioma: revisiting the value of cytology in diagnosis
- Peritoneal washings: how to report the presence of epithelial cells
The five categories of international system for reporting serous fluid cytopathology

1. Non-diagnostic (ND)
2. Negative For Malignancy (NFM)
3. Atypia of Undetermined Significance (AUS)
4. Suspicious For Malignancy (SFM)
5. Malignant, Primary (MAL-P)
   - Malignant, Secondary (MAL-S)
Three parts of a sample report

1. Adequacy statement
2. Diagnostic category
3. Clinical comment
Factors involved in adequacy

- Sample volume -

• Is there a recommended volume for fluid samples?
  • 75ml optimal volume (Rooper et al 2014) for cytological assessment
  • 60ml for pericardial fluid (Rooper et al, 2016)
  • Smaller volume samples should not be rejected but commented upon.
  • Aliquot for investigations other than cytology ideally at time of collection
Factors involved in adequacy
- Cellular content -

- Do we need to see mesothelial cells?

- **Acceptable to find only lymphocytes** (TB, chylous effusion) or neutrophils (acute bacterial infections) in benign effusions without mesothelial cells

- Diagnosis of malignancies with a one cell population **may be made without mesothelial cells**
Factors involved in adequacy
-Cellular preservation-

• Can a sample be non-diagnostic in spite of being cellular?

• Loss of quality due to degenerative changes due to delay in reaching the lab, bacterial overgrowth, technical artefacts and contaminants
CASE 1

54-year-old male with left sided pleural effusion. Smoker, cough and chest pain for one week.

Macro: 2ml of heavily blood-stained fluid received. One ThinPrep and one DQ cytospin prepared.
Sample report for non-diagnostic category

- Evaluation limited by heavy blood-staining, likely non-representative sample.
- NON-DIAGNOSTIC
- Repeat sampling advised (75ml volume if possible).
CASE 2

64-year-old male with liver cirrhosis and ascites.

Macro: 60ml of straw colored fluid. Two cytospins, Pap and Giemsa, prepared.
Sample report for negative for malignancy category

- Satisfactory for evaluation.
- Neutrophils, mesothelial cells and a few lymphocytes are present.
- NEGATIVE FOR MALIGNANCY
- A high proportion of neutrophils is present and may represent spontaneous bacterial peritonitis (SBP). Please correlate with clinical findings.
Negative for malignancy (NFM)

<table>
<thead>
<tr>
<th>Normal (expected) cell populations in variable numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td>Mesothelial cells</td>
</tr>
<tr>
<td>Neutrophils</td>
</tr>
<tr>
<td>Eosinophils</td>
</tr>
</tbody>
</table>
Patterns of reactive effusions

- If specific pattern of reactive effusion present such as eosinophilic or lymphocytic, suggest possible causes in the clinical comment.

- **Eosinophilic effusion**: Recent pleural fluid aspiration, allergic conditions including hypereosinophilic syndrome etc

- **Lymphocytic effusion**: Viral infections, TB

- **Neutrophilic effusion**: Empyema (purulent fluid) usually indicative of bacterial infection, occasionally malignant eg. lung squamous cell carcinoma rupturing into pleural cavity
CASE 3

46-year-old female with history of breast carcinoma 6 years ago. Now, cough and small pleural effusion.

Macro: 20ml straw-colored fluid. Cytospins, Pap, MGG
Atypia of undetermined significance (AUS)

- Occasional poorly preserved cells with nuclear enlargement and mild hyperchromasia but no obvious chromatin or nuclear membrane abnormalities
- Likely degenerated macrophages or mesothelial cells
- Cell block made and IHC performed to detect any epithelial cells (BerEP4, MOC31, Claudin-4) (Epithelial cell adhesion molecules)
- Downgraded to NFM as epithelial markers negative
Cell block
Atypia of undetermined significance (AUS)

- Uncommonly used as a diagnostic category in effusions
- Some experienced cytologists don’t use it at all
- Can we do without it completely?
- Survey respondents tell us that they use it, albeit variably but should be included in the terminology

**Two-step process** – preliminary report (optional) and final report
AUS Algorithm

Small number of atypical cells
macrophages? mesothelial cells? epithelial cells?

Preliminary assessment: AUS

ICC demonstrates atypical cells to be macrophages or mesothelial cells
Final report: NFM

ICC demonstrates atypical cells to be epithelial
Final report: SFM or Malignant (secondary)

Insufficient representative cells or ICC equivocal
Final report: AUS
CASE 4

68-year-old man with ascitic fluid. History of lung carcinoma.

Macro: 30ml of blood-tinged fluid.
Giemsa
Small number of cells on cytospins (and clot/cell block). Features favor epithelial or other malignancy

Preliminary assessment: SFM

ICC confirms malignancy. Final report: Malignant (secondary)

Insufficient representative cells or ICC equivocal. Final report: SFM
Cell block
ICC:TTF-I and Napsin A were positive

SFM upgraded to:

Malignant (secondary)-
lung adenocarcinoma
Ancillary testing of lung adenocarcinoma is critical for future treatment.

- Insufficient cells for PD-L1, ALK, ROS1 (IHC)
- Insufficient cells for mutation analysis (NGS or just EGFR, KRAS)
- Further sample may be needed for targeted chemotherapy
- **Restrict use of IHC (TTF1, Napsin A, P40) to a minimum to conserve material for molecular testing.**
Comparison of AUS and SFM categories: the international system for reporting serous fluid cytopathology

<table>
<thead>
<tr>
<th></th>
<th>AUS</th>
<th>SFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytological features</td>
<td>Only mild cytological abnormalities such as nuclear enlargement and hyperchromasia present usually as small numbers of dispersed cells and occasional small groups</td>
<td>Greater degree of cytological abnormalities present usually as small numbers of cells, including architectural features such as occasional 3 dimensional groups</td>
</tr>
<tr>
<td>Cell lineage</td>
<td>Benign cell type favored, but epithelial or other malignant cell of origin not excluded</td>
<td>Epithelial or other malignant cell of origin strongly favored</td>
</tr>
<tr>
<td>Immunochemistry</td>
<td>Outcomes may be benign, SFM/malignant or inconclusive</td>
<td>Outcomes usually malignant or inconclusive.</td>
</tr>
<tr>
<td>Suggested Risk of Malignancy</td>
<td>~20%</td>
<td>~80%</td>
</tr>
</tbody>
</table>
CASE 5


MACRO: 80ml of blood-stained fluid with a clot. Cytospins, MGG, Pap, HE
Cytology of normal mesothelial cell

- central round nuclei
- moderate amount of light purple cytoplasm
- Binucleated and multinucleated may be seen if the cells are reactive.
- "skirt" or "halo" at pale outer rim of cell
- Two or more mesothelial cells are often separated by "window". 
Ancillary tests: mesothelioma vs carcinoma

- Good panel to start with: 2 mesothelial, 2 epithelial markers and a macrophage marker:
  
  WT1, Calretinin, MOC31, BerEP4, CD68

- Good adenocarcinoma markers: MUC4, Claudin-4 can be included in panel above if available

- Others: PAS-D, CEA

- Site specific markers: TTF-1, Napsin A, BAP1+ (lung); CDX2, SATB2, CK20 (GI); PAX8 (ovarian/renal); GATA3 (breast, urothelial); PSA, PSMA, NKX3.1 (prostate); Thyroglobulin

- WT1+ carcinomas: gynae tract, breast (basal phenotype), someSqCC

- GATA3 stains can be positive in 50% mesotheliomas

- SqCC & Mesothelioma: Both CK5/6+ and maybe calretinin and D2-40+; p63/p40+ (SqCC) and WT1+ (Meso) useful in this situation
Ancillary testing of mesothelial proliferations

<table>
<thead>
<tr>
<th></th>
<th>NFM</th>
<th>MESOTHELIOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmin (cytoplasmic)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>EMA (membranous)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>HEG1 (membranous)</td>
<td>-</td>
<td>+ (epithelioid, not sarcomatoid)</td>
</tr>
<tr>
<td>BAPI (nuclear)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MTAP (IHC nuclear / FISH)</td>
<td>IHC + / FISH: No deletion</td>
<td>IHC - / FISH: Deletion detected</td>
</tr>
<tr>
<td>5-hmC (IHC nuclear)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>P16/CDKN2A (FISH)</td>
<td>No deletion</td>
<td>Deletion detected</td>
</tr>
</tbody>
</table>

*MTAP loss by IHC was 78% sensitive and 96% specific for CDKN2A homozygous deletion.*
Algorithm for establishing a diagnosis of malignant mesothelioma.

- **Pleural lesion**
  - Claudin-4+, MOC-31+, Ber-EP4+
  - WT1+ C2-40+ calretinin+ CK5/6+ HES1+

- **Overt malignant morphology**
  - Meiohelial proliferation
  - Diagnostically challenging morphology

- **Benign mesothelial proliferation**

- **Overt benign morphology**

- **Nuclear BAP1 lost**
  - >60% cells with loss of nuclear 5-mC
  - MTAP lost
  - CDKN2A homozygous deletion

- **5-hmC immunohistochemistry**
  - MTAP immunohistochemistry
  - MTAP retained
  - CDKN2A fluorescence in situ hybridization
  - No CDKN2A homozygous deletion

- **Malignant mesothelioma**

- **Altypical mesothelial proliferation, cannot rule out malignant mesothelioma**

**Mod Pathol 2019;32:376-86.**
Sample reports for a mesothelial proliferation

- Satisfactory for evaluation.
- Small spherical groups and dispersed mesothelial cells with mild nuclear pleomorphism are present suspicious for mesothelioma.
- Immunostains requested for confirmation (on cell block or biopsy).
- If immunostains confirmatory- MALIGNANT (PRIMARY): MESOTHELIOMA. Clinical correlation essential.
- If morphology classic but immunostains not confirmatory: SUSPICIOUS FOR MESOTHELIOMA
- If morphology not classic and immunostains not confirmatory: ATYPICAL MESOTHELIAL PROLIFERATION. Further investigation advised.
Recognisable abnormal cell population present and **adequate for robust diagnosis** on which clinical management may be based.

Malignant cell type should be specified on morphology alone or supported by immunochemistry

**Malignant- Primary:** Mesothelioma

**Malignant- Secondary:**

Metastatic carcinoma – adenocarcinoma, small cell carcinoma, squamous cell carcinoma

Lymphoma, Melanoma, Other malignancies e.g. sarcoma, leukemias

Primary organ site may need to be investigated for adenocarcinomas
CASE 6

45-year-old female. Ascites.

MACRO: 35ml of blood-stained fluid. MGG, Pap
Cell block
Ascertaining the primary origin

<table>
<thead>
<tr>
<th>Site specific markers:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung: TTF1, Napsin A, (BAP1+)</td>
</tr>
<tr>
<td>Breast: GATA3, mammaglobin, GCDFP15</td>
</tr>
<tr>
<td>Thyroid: Thyroglobulin, PAX8</td>
</tr>
<tr>
<td>GI: CK20, CDX2</td>
</tr>
<tr>
<td>Ovarian: PAX8, WT1, CA125</td>
</tr>
<tr>
<td>Kidney (CCRCC): PAX8, CAIX, RCC antigen, Vimentin</td>
</tr>
<tr>
<td>Urothelial: GATA3, Uroplakins, p63, p40, 34BE12</td>
</tr>
<tr>
<td>Prostate: PSA, PRAP, PSMA, NKX3.1</td>
</tr>
</tbody>
</table>
Sample report for MALIGNANT (SECONDARY)

- Satisfactory for evaluation.
- Spherical groups of tightly cohesive large cells with vacuolated cytoplasm and nuclear pleomorphism are present. Dispersed single cells are also present.
- MALIGNANT (SECONDARY)
- Immunostains requested to ascertain the primary, gynae and GI tracts being the most likely sites.
Diagnostic categories & clinical management

Cytospins/LBP (+/- clot/cell block)

Non-diagnostic
- Repeat sample

Negative for malignancy
- Discharge or clinical follow up

Atypia of Undetermined Significance

Suspicious for malignancy
- Ancillary testing
  - Correlation with biopsy & clinical data

Malignant
- Ancillary testing to establish primary site & prognostic/predictive markers
International System for Reporting Serous Fluid Cytopathology: Implied Risk of Malignancy (ROM)

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>% ROM (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic (ND)</td>
<td>17% (± 8.9%)</td>
</tr>
<tr>
<td>Negative for Malignancy (NFM)</td>
<td>21% (± 0.3%)</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance (AUS)</td>
<td>66% (± 10.6%)</td>
</tr>
<tr>
<td>Suspicious for Malignancy (SFM)</td>
<td>82% (± 4.8%)</td>
</tr>
<tr>
<td>Malignant (MAL)</td>
<td>99% (± 0.1%)</td>
</tr>
</tbody>
</table>
Practical Approach to Serous Effusions

Effusions

Inadequate

Adequate

Expected cellular findings (mesothelial cells, some inflammatory cells)

In regard to volume and distribution:

Mostly mesothelial cells arranged singly and/or in small clusters. No cellular atypia. Some histiocytes, lymphocytes, neutrophils

Dx. Negative for malignancy (NFM)

Increased volume and/or cell distribution:

Predominantly mesothelial cells (single and/or numerous clusters)

Dx. NFM

Dx. Mesothelioma

Predominantly histiocytes (often appearing like a "second cell population")

Dx. NFM

Predominantly lymphocytes

Dx. NFM

Dx. Lymphoma

Predominantly or increased eosinophils

Dx. NFM

Predominantly neutrophils

Dx. NFM

Unexpected cellular and non-cellular findings

Second (malignant) cell population

Single cells

Dx. Melanoma, lymphoma, breast (lobular) ca, sarcoma

Small clusters

Dx. AdenoCa, breast, lung, small cell carcinoma

Large clusters

Dx. AdenoCa, ovarian, pancreatic

Psammoma bodies

Dx. NFM

Collagen balls

Dx. NFM

Asbestos bodies

Dx. NFM

LE cells

Dx. NFM

Necrosis, spindle and giant cells

Dx. NFM

Detached ciliary tufts

Dx. NFM

Infectious organisms

Dx. NFM

Immunoocytochemical studies, clinical and radiographic correlations required. Slide courtesy Dr Eva Wojcik

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REFERENCES