Serous effusions: A practical algorithmic approach to diagnosis

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Educational objectives

• To revisit algorithms in lab handling of serous effusion samples
• To outline common diagnostic pitfalls and how to avoid them
• Algorithm for morphological evaluation and reporting terminology
• Algorithm to confirm the nature of a cellular infiltrate using ancillary tests
Serous effusions

• Abnormal accumulation of fluid in body cavities
• Presence of malignant cells implies advanced TNM Stage
• Pleural fluid
• Pericardial fluid
• Ascitic fluid
• (Peritoneal washings)

Synovial fluid and CSF not covered in this presentation
Macroscopic findings

- **Volume**: 75ml 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
Macroscopic findings

- **Appearance**
  - Straw coloured, blood-tinged
  - Heavily blood-stained (with clot)
  - Turbid
  - Milky (chyrous)
  - Viscous (hyaluronic acid rich)
Clear/chylous fluid sample

- Cytospins
  - MGG & Pap
- Liquid based preparations (ThinPrep, SurePath etc)
  - Pap
- Clot (if present)
  - H&E

Tiisue Pathways, RCPath www.rcpath.org
Turbid/heavily blood-stained fluid sample

- Direct smears from sediment (MGG & Pap)
- Cytospins & LBP after dilution
- Clot section
Classic algorithm rare in practice

- Fluid sample
  - Transudate
    - No cytology
  - Exudate
    - Cytology
Aliquoted at time of collection

Fluid sample

- Biochemistry
- Cytology
- Microbiology

Biochemistry to ascertain whether transudate or exudate and relevant tumour markers. Cytology for malignant cells or non-neoplastic conditions. Microbiology for diagnosis of suspected infections
Common diagnostic pitfalls

- **Small numbers of lesional cells and poor cellular preservation**: quality may be compromised at time of collection as well as during transport & processing
- **Adenocarcinoma vs mesothelial cells, reactive & neoplastic**
- **Cytoplasmic vacuolation** including signet ring-like cells
- **Single cell population**
- **Less frequently encountered tumours**: Poorly differentiated squamous cell carcinoma, small cell carcinoma, melanoma
Terminology and diagnostic categories

- C1: Non-diagnostic
- C2: No malignant cells seen
- C3: Atypia, equivocal for malignancy (or malignancy not excluded)
- C4: Suspicious for malignancy
- C5: Malignant cells seen
- Used mainly for breast FNA samples in the UK
- Thy 1-5 in thyroid cytology
- Not a reporting requirement for serous effusions but facilitates audit
Reporting exfoliative cytology samples by UK cytotechnologists

- In the UK, cytotechnologists (Biomedical scientists) with appropriate training & qualification (Diploma of Expert Practice in non-gynae cytology) may sign out negative urine, serous effusion and respiratory tract specimens
- With the Advanced Specialist Diploma in non-gynae cytology, they may sign out abnormal exfoliative cytology results
- [www.ibms.org.uk](http://www.ibms.org.uk)
Morphology & ancillary testing

Cytospins/LBP (+ clot/cell block)

- Non-diagnostic, C1
  - Repeat sample or clinical follow up

- No malignant cells seen, >75ml sample, C2
  - Clinical follow up

- Equivocal for malignancy, C3
  - Ancillary testing
  - Correlation with biopsy & clinical data

- Suspicious for malignancy, C4
  - Ancillary testing to establish primary site and predictive markers

- Malignant cells seen, C5
  - Ancillary testing
Ancillary testing: diagnostic & theranostic

Cytology atypical, suspicious (diagnostic) or malignant (theranostic)

- Clot/cell block/cytospins/smears for immunochemistry, molecular studies (paraffin curls) & cytogenetics (paraffin sections for FISH)
- Flow cytometry
- Biochemical analysis (mesothelin)

As material may be limited in amount, it should be used conservatively for immunochemistry so that sufficient material remains for molecular tests if necessary
Mesothelial cells

Present singly and in small groups with cells separated by spaces (windows)
Clasp-like cell junctions
Peripheral vacuolation (glycogen) and blebs (microvilli)
Two-zone cytoplasmic differentiation
Central vesicular nuclei with small nucleoli
Mesothelial proliferation
Morules or mulberry like clusters of mesothelial cells with inflammatory cells in the background
Lack large cytoplasmic vacuoles and relatively monomorphic nuclei although large and hyperchromatic
Some cells may be gigantic
MGG cytospin of the above case with identifiable mesothelial cell type, confirmed on calretinin immunostain
Ancillary testing of mesothelial proliferations

Confirm mesothelial origin
- WT1, Calretinin, D2-40/Podoplanin
- CK5/6, Thrombomodulin, mesothelin, HBME1

Exclude adenocarcinoma
- BerEP4, MOC31, TTF1

Reactive
- Desmin: cytoplasmic

Neoplastic
- EMA: thick membranous; p53: positive
- Claudin-4: no membranous staining
- GLUT-1: positive FISH; p16 deletion
- IMP3, CD146, Ki67

Particularly useful for confirming uncommon subtypes of mesothelioma such as small cell, clear cell, lymphohistiocytic, signet ring etc.

Limitations of different immunostains are mentioned in the handout and also in the reference list.
Mesothelial proliferation

• Atypical mesothelial proliferation: recommended term for equivocal cases (Intl Mesothelioma Panel)

• Correlation with biopsy and clinicoradiological findings
Epithelioid (epithelial) cells in effusions

Low power view of cytospin MGG showing a cellular infiltrate of lymphocytes, mesothelial cells and macrophages and 3 dimensional clusters of malignant cells
MGG & Pap (x20) cohesive groups without windows or gaps between the malignant cells (unlike mesothelial cells), abundant cytoplasm with vacuolation and nuclear pleomorphism
Papillary structures in effusions are indicative of adenocarcinoma from primaries in the lung, breast, thyroid, GI tract, pancreas, kidney, ovary & uterus
Algorithm for epithelioid malignant cells

Atypical, suspicious or malignant epithelioid cells

Clinical data:
Thoracic / abdominal
Female / male

Thoracic:
Breast: GATA-3
Lung: TTF1 Napsin A
Thyroid: thyroglobulin
Mesothelial: EMA, WT1

Abdominal:
Gynae: PAX8 CA125
GI: CDX2 CK20 CK7
Renal: PAX8
Bladder: GATA-3
Prostate: PSA PSMA NKX3.1

Other malignancies:
Small cell carcinoma, melanoma, sarcoma, histiocyte markers

Check clinical data including previous cytology or other relevant samples to avoid repeating tests already performed.
Theranostics

Ancillary tests

Breast & gastric cancer: Her2

Lung: Molecular tests:
 Next generation sequencing or
  EGFR, KRAS, BRAF
  Cytogenetics: ALK, ROS1, FGFR1

Head & neck squamous cell carcinoma: HPV ISH

Mesothelioma: p16 deletion
Other epithelioid cells (not site specific)

- **Small cell carcinoma**
  - CD56, Chromogranin, Synaptophysin, CAM5.2

- **Melanoma**
  - HMB45, S100, MART, Melan A

- **Sarcomas**
  - Specific chromosomal translocations
Pitfall: Cytoplasmic vacuolation

- Cytoplasmic vacuolation may be seen in adenocarcinoma but also in inflammatory cells and mesothelial cells
Cytoplasmic vacuolation in malignant cells. Large cell size, large vacuole with targetoid mucin, vacuoles with indistinct outlines or vacuoles filling up the cell indenting the nucleus which may be bulging or pushed up against the cell membrane. Epithelial marker BerEp4 confirms the epithelial nature of the vacuolated cells.
Macrophage accompanied a few lymphocytes and red blood cells with a degenerative vacuole (upper).
A malignant cell with a large cytoplasmic vacuole (lower). Note the large size of the malignant cells compared to the inflammatory cells in the background.
Cytoplasmic vacuolation in reactive mesothelial cells
Hyaluronic acid rich mesothelial cells may show false positive staining with CEA (40%), BerEp4 (20%), LeuM1 & B72.3 (10%). False positive staining lost after pretreatment with hyaluronidase
Pitfall: Single cell population

• A mixed population of cells (mesothelial, macrophages and inflammatory cells) provide a contrast against which malignant cells stand out

• Malignant cells when present as a single cell population may be mistaken for macrophages or reactive mesothelial cells
Cytospin (Pap) and cell block (HE stain) showing numerous dispersed signet ring cells and glandular structures from an adenocarcinoma, typically gastric but other sites eg pancreas, lung, breast, bladder, colorectal need to be excluded.
Dispersed rather than morular population of mesothelial cells may be seen in mesothelioma and may be confirmed on immunochemistry.
Lymphocytic proliferation
Eosinophils (interspersed between lymphocytes and macrophages) indicating previous aspirations of effusion
Macrophages, multinucleated giant cells and granular debris in a rheumatoid effusion
Lymphoma

Non-Hodgkin lymphoma (NHL)

- CLL
  - CD5, CD19, CD20, CD23
- DLBCL
  - CD19, CD20
- Follicular lymphoma
  - CD10, Bcl2
Peritoneal washings

Bland epithelial cells

Surgery for benign disease
- Report as benign, C2

Surgery for malignant disease
- Report with proviso that these may represent either well differentiated malignancy or peritoneal implants of a borderline malignant ovarian tumour, C3

This algorithm holds good for morphologically bland cells; malignant cells should be reported as malignant, C5.

Presence of bland cells in the setting of well differentiated ovarian carcinoma presents a diagnostic dilemma as benign mullerian cell rests and metaplasia are common in the pelvic peritoneum. When the features are clearly those of ciliated tubal type epithelium these should be reported as benign. However, if there non-ciliated endometrial type cells present, distinction between endometriosis and endometrioid carcinoma may be difficult. Correlation with the histopathology specimen including omental or peritoneal biopsies is essential.
FIGO Ovarian carcinoma staging 2014

• Stage I: Tumour limited to one or both ovaries
• IC1: Surgical spill
• IC2: Capsule rupture before surgery or tumour on ovarian surface
• IC3: Malignant cells in ascitic fluid or peritoneal washings
• Stage IVA: Pleural effusion with malignant cells
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
Peritoneal washing containing single cells and groups of malignant cells from an adenocarcinoma
Pitfall: Tumours seen less commonly in effusions

- Poorly differentiated squamous cell carcinoma
- Small cell carcinoma
- Melanoma
- Sarcoma
Fixation artefact may induce orangeophilia in mesothelial cells and being mistaken for keratinisation in squamous cell carcinoma
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. Intercellular bridges, keratinisation starting at the periphery of the cell.
Small cell carcinoma
Small cell carcinoma in effusions

- May show some degenerative changes and cytoplasmic vacuolation resembling adenocarcinoma, which might be TTF1. If neuroendocrine differentiation is not thought of on morphology, this important diagnosis may be missed
References


- Royal College of Pathologists. Tissue Pathways. www.rcpath.org


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References


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