Video Microscopy Tutorial #20

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Disclosure information

“The speaker has no relationship that represents a possible conflict of interest with respect to the content of this presentation.”
SEROUS EFFUSION CYTOLOGY- A PRACTICAL APPROACH

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Introduction

Accumulation of fluid in the pleural, pericardial or peritoneal cavities is referred to as a serous effusion and is always pathological. The pathology may be either neoplastic or non-neoplastic. Cytology is very helpful in confirming the presence of malignant neoplastic cells in serous effusions. It is also performed for effusions secondary to inflammatory and reactive conditions. In these conditions, cytology is useful when taken in conjunction with biochemical and microbiological investigations on the fluid.

Malignant effusions may be the initial presentation in 10-15% of patients with malignancy. The diagnostic accuracy of the cytologic examination of malignant pleural effusion ranges from 40 to 87%.

Recommended sample volume

For diagnostic purposes, variable amounts of fluid are received by the cytology laboratory. The BSCC code of practice document suggested that 20ml of representative sample may be adequate for diagnostic purposes while the British Thoracic Society recommends 50ml of fluid for cytology. However, this does not mean that samples of smaller volumes are unacceptable for processing. All samples including those that are heavily blood-stained or clotted should be processed and examined.

Macroscopic examination

The volume, colour and presence of clots should be recorded in the lab. Chylous (milky) fluid may indicate lymph in the serous cavity and fistula formation either pathologically or following surgery. A high hyaluronic acid content of the fluid associated with mesothelioma makes it viscous in consistency and should be noted.

Processing and staining

The usual method involves cytocentrifugation and examination of one Pap and one Giemsa stained preparation. Liquid based cytology (LBC) may be used but some methods allow the use of Pap stain only. Processing of clots and cell block preparations are particularly helpful in blood-stained samples as cytological material may be trapped among the red blood cells. Immunostains and special stains may be performed on any of the above preparations.

Pre-screening and reporting by scientists

In the UK, Biomedical Scientists (BMS) or cytotechnologists may be trained to screen and even sign out negative serous effusion samples. Adequate training and experience is required for this and there is also a Diploma in Expert Practice in Non-gynae Cytology offered by the Institute of Biomedical Scientists (IBMS). Reporting of serous effusions,
urine and respiratory cytology samples by BMS may be practised under the guidance of the lead cytopathologist.

**Cytological interpretation**

This should be done in the light of the clinical information and a provisional report may be issued to alert the clinician when malignancy is suspected but awaiting confirmation or further characterisation on immunostains. For reporting purposes, cytological interpretation of serous fluids fall under the following categories:

1. **Unsatisfactory.** These are usually small volume samples composed entirely of blood, probably reflecting an unsatisfactory procedure (unless hemothorax is suspected clinically). Occasionally, poor cellular preservation due to delay in transport to the lab may compromise the specimen quality, resulting in difficulty in interpretation. Effusions are never acellular and even small numbers of macrophages or inflammatory cells should be reported. The presence of mesothelial cells helps in confirming that the procedure was successful even if paucicellular.

2. **Benign.** These mostly represent a non-specific reactive picture with a heterogeneous population of macrophages, mesothelial cells and variable numbers and types of inflammatory cells. A more specific diagnosis such as empyema may be suggested if the inflammatory cells are predominantly neutrophils. A chronic infection such as tuberculosis may be associated with a dominant lymphocytic population.

3. **Atypical cells present, probably degenerated mesothelial cells or macrophages.** The atypical cells in this category usually represent degenerated mesothelial cells or macrophages but immunostains are deemed necessary to exclude the possibility of malignancy, particularly when clinical findings are suspicious. If the representative cells are present on the immunostains, it may be possible to resolve this dilemma, however, this is often not the case when only one or two small groups of these cells are present on the cytopsins. These cases may benefit from a multi-headed discussion, combining the collective interpretive skill and experience of pathologists and scientists. In a small number of cases, an inconclusive report may have to be issued, requesting a further sample if possible and seeking any useful clinical clues.

4. **Atypical cells present, suspicious for malignancy.** This may be provisionally reported to the clinician under a number of circumstances, usually before immunostains have been requested. Many of these cases will be diagnostic once the immunostains become available.
   i) small numbers of single, dispersed atypical cells that are suspicious but not diagnostic of malignancy
   ii) atypical cells obscured by inflammatory or other cells in a thick, hypercellular preparation
   iii) difficulty in distinction between reactive mesothelial cells, malignant mesothelial cells and adenocarcinoma.
5. **Malignant cells present.** A confident diagnosis of malignancy is made, usually metastatic adenocarcinoma and is consistent with the available clinical information. Immunostains may help in characterising the malignant cells further as glandular, squamous, neuroendocrine or lymphoid in type.

Other ancillary investigations such as flow cytometry, PCR, FISH, ploidy etc may be useful for detecting signature molecules for specific malignancies. These are useful in the light of clinical and cytomorphological findings, however, their relevance in therapeutic intervention in the absence of identifiable malignant cells remains uncertain at present.

For malignant glandular cells (ie. adenocarcinoma), a panel of immunostains to confirm the primary site may be necessary. The choice of stains should be guided by clinical and radiological information. In metastases from known previous primaries eg breast or lung, therapeutically relevant markers such as Her-2, EGFR/K-RAS may be required.

**Choice of immunostains**

The choice of immunostains will depend on the clinical question to be answered in a particular scenario.

In the category ‘**atypical cells present, probably degenerated mesothelial cells or macrophages**’, the distinction between degenerated mesothelial cells from epithelial cells may be made by using a panel incorporating 2 tried and tested markers for each of the two cell types. However, as pointed out earlier, in this category the atypical cells are few in number and may not be represented on the cell block or immunostains. A discussion with the clinician may help gauge the suspicion of malignancy and the need for a repeat sample. If there is no residual fluid to send for cytology, follow up of these patients with a view to early detection of a recurrent effusion may provide a second chance for cytological diagnosis.

In the category ‘**atypical cells present suspicious of malignancy**’, confirmation of metastatic carcinoma would rely on the use of at least 2 generic epithelial markers and if the clinical history points to a particular site or sites, specific markers should be included. Once again, the number of cells may be small (4i above) not allowing certainty of the diagnosis on cytomorphology or immunostains. In densely cellular preparations (4ii), dilution of the sample may unmask the malignant cells. It may also be easier to characterise these cells better in a cell block or clot with confirmation on immunostains. A further sample may be required and is usually available from a clinically suspicious or malignant effusion. Mesothelial markers must always be included for cases in 4iii as degenerate mesothelial cells may mimic malignancy and furthermore, mesothelioma may be missed if these are not performed.

In the category ‘**malignant cells present**’, the diagnosis is made with confidence either supported by the knowledge of a known primary or based purely upon the unmistakable
cytological features. The purpose of immunostains in this category is to ascertain the primary site of malignancy. The choice of immunostains would be guided by the clinical information but also influenced by the patient’s gender and site of the effusion (Table 1). Metastatic adenocarcinoma is the commonest metastatic malignancy in effusions but mesothelioma, poorly differentiated squamous cell carcinoma and other cell lineages must all be considered, particularly if the effusion is the first manifestation of malignancy or when the clinical primary is unknown.

There is no value in routinely performing immunostains on cytologically benign effusions or on recurrent effusions where a diagnosis has previously been confirmed by immunostains. Drainage of a recurrent effusion may be for therapeutic purposes only although these samples are reflexly dispatched to the cytology lab.

**Choice of markers**

**Mesothelial cells**

Reactive mesothelial cells: EMA (thin membranous staining), Desmin +, diploid
Mesothelioma: EMA (thick membranous staining), p53/Ki67 ++, aneuploid

Calretinin highly sensitive marker for the mesothelium, however, it is also expressed in squamous cell carcinoma and ovarian serous adenocarcinoma. Good for distinguishing mesothelioma from lung adenocarcinoma and RCC.

WT-1 distinguishes between mesothelioma and squamous cell carcinoma but is expressed in ovarian serous adenocarcinoma.

D2-40 and Podoplanin: Highly sensitive for mesothelial cells, negative in lung adenocarcinoma but squamous cell carcinoma (30%) and ovarian serous adenoca (15%) may be positive

Mesothelin: strong cytoplasmic positivity in mesothelioma but focal membranous staining may be seen with lung adenoca and squamous cell ca

Tenascin-X: positive in mesothelioma, negative in reactive mesothelial cells and ovarian/peritoneal carcinoma

**Epithelial markers**

BerEP4: adenocarcinoma and squamous ca lung, but also 10-15% mesotheliomas

MOC-31: adenoca and squamous ca lung, <10% mesotheliomas

Monoclonal CEA: distinguishes lung adenoca from epithelioid mesothelioma (polyclonal CEA may be positive in a third of cases)
PAX-8 (more sensitive) and PAX-2: Mullerian epithelium, thyroid follicular cells and renal tubular cells

p63: positive in squamous cell carcinoma, negative in mesothelioma

B72.3: expressed in high percentage of adenocarcinomas, but negative for mesothelial cells

**Melanoma**

HMB45, Melan A, S-100, MART-1

**Neuroendocrine markers**

CD56, NSE, Chromogranin A, Synaptophysin

**Germ cell tumours**

PLAP, CD117 (seminoma), CD30 (embryonal ca), AFP (yolk sac tumour), HCG (chorioca)

**Renal cell carcinoma**

RCC antigen, CD10, PAX-2

CK7+ CD15- (Chromophobe RCC)

CK7+ AMACR+ (Papillary RCC Type 1)

**Macrophages**

CD68 (PGM1)

**Lymphoid markers**

LCA, CD3, CD20, kappa and lambda light chains

**New markers**

Claudin-4: reported to be highly sensitive and specific ‘single shot’ marker for metastatic carcinoma, always negative in mesothelioma

IMP3: negative in reactive mesothelial cells, positive in carcinomas

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
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### Table 1. Likely primary sites based on gender and body cavity involved

<table>
<thead>
<tr>
<th>Body Cavity</th>
<th>Primary Sites</th>
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<tbody>
<tr>
<td><strong>Pleural</strong></td>
<td>Lung, GIT &amp; pancreas, lymphoma</td>
</tr>
<tr>
<td><strong>Peritoneal</strong></td>
<td>GIT, pancreas, lymphoma, lung, genito-urinary tract</td>
</tr>
<tr>
<td>Breast, lung, ovary, GIT, lymphoma</td>
<td></td>
</tr>
<tr>
<td>Ovary, breast, endometrium, stomach, lymphoma, GIT, pancreas</td>
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### Table 2. Typical pattern of CK7 and CK20 at various primary sites

<table>
<thead>
<tr>
<th>Primary Site</th>
<th>Typical Antibodies</th>
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<tbody>
<tr>
<td><strong>Lung</strong></td>
<td>TTF1 (adenoca), surfactants, p63 (squamous), CK5/6 (squamous)</td>
</tr>
<tr>
<td><strong>GIT</strong></td>
<td>CDX2, mCEA, villin</td>
</tr>
<tr>
<td><strong>Breast</strong></td>
<td>GCDFP, mammoglobin, ER</td>
</tr>
<tr>
<td><strong>Ovary</strong></td>
<td>WT-1 (serous), ER, CA125, mesothelin (serous), PAX-8, PAX-2</td>
</tr>
<tr>
<td><strong>Pancreas</strong></td>
<td>CA19.9, CA125, mesothelin</td>
</tr>
<tr>
<td><strong>Prostate</strong></td>
<td>PSA, PRAP, PSMA, AMACR</td>
</tr>
<tr>
<td><strong>Thyroid</strong></td>
<td>Thyroglobulin, TTF1, calcitonin (medullary ca)</td>
</tr>
<tr>
<td><strong>Bladder</strong></td>
<td>HMW-CK, uroplakin-3, p63</td>
</tr>
<tr>
<td><strong>Serous cavities (mesothelium)</strong></td>
<td>Calretinin (nuclear), WT-1 (nuclear), D2-40, podoplanin, mesothelin, thrombomodulin, EMA (membranous)</td>
</tr>
<tr>
<td><strong>Endometrial mucinous ca</strong></td>
<td>MUC-1+, ER+, PR+, p16-</td>
</tr>
<tr>
<td><strong>Endocervical adenocca</strong></td>
<td>MUC-1-, ER-, PR-, p16+</td>
</tr>
</tbody>
</table>

### Table 3. Typical antibody expression by organ type
References


Prospective Study To Determine the Volume of Pleural Fluid Required To Diagnose Malignancy. Chest January 2010 137:1 68-73.


