Effusion fluid cytology
- Reference materials from the literature

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Serous borderline tumour and mucinous tumours of the ovary

References:


Serous borderline tumour

**FIGURE 2.** A serous neoplasm is shown. (A) A complex branching architecture of atypical epithelial cells is demonstrated. (B) Many cell groups with complex papillary branching architecture are shown. Note the background of mesothelial cells. (C) A cell block section demonstrating complex papillary architecture of atypical epithelial cells is shown.
Pseudomyxoma peritonei

Reference:


Pseudomyxoma peritonei. (Left) The case shown in the left image presented with only thick copious mucin in the peritoneal cavity (DQ stain, ×200). (Right) The case shown on the right had rare well differentiated groups of adenocarcinoma floating in abundant background mucin (Pap stain, ×200)
Figure 1. Peritoneal fluid smears. (a, b) Acellular smears with lakes of mucinous material in the background (MGG, 200×). (c) Small epithelial cell clusters with mild to moderate nuclear pleomorphism (MGG, 200×). (d) Tight papillaroid clusters of columnar cells in a background containing thick mucin and scattered mesothelial cells (MGG, 200×). (e) High-power view showing prominent nucleoli and marked nuclear pleomorphism in the case of PMCA (MGG, 400×). (f, g) Fibroblast-like, spindle-shaped cells with entrapped mucin (MGG, 200×). (h) Monolayer sheet of mesothelial cells (MGG, 200×).
Figure 2. SurePath® liquid-based cytology smears. (a) Thick mucinous material [PAP, 200×]. (b, c) Cellular smear with small nests of epithelial cells floating in pools of mucin with mild to moderate nuclear pleomorphism (PAP, 200×). (d) Hypercellular smear with many hyperchromatic crowded clusters of malignant epithelial cells in an inflammatory background (PAP, 200×). (e) High-power view highlighting prominent nucleoli and intracytoplasmic mucin vacuoles (PAP, 400×). (f) Tight papillaroid clusters of epithelial cells with mild pleomorphism and prominent nucleoli with scattered mesothelial cells in the background (PAP, 200×). (g) Signet ring cells (PAP, 400×). (h) Atypical mitotic figure (PAP, 400×).
Figure 3. Histopathology of peritoneal biopsy. (a, b) Pools of mucinous material [haematoxylin and eosin (H&E), 200×]. (c) Periodic acid–Schiff stain highlighting the same (PAS-AB, 200×). (d, e) Disseminated peritoneal adenomucinosis: mucinous material together with a few scattered CDX2 strips of mucinous tall columnar epithelium (H&E, 200×). (f) Peritoneal mucinous carcinomatosis: many clusters of malignant epithelial cells admixed with mucin (H&E, 200×).
Malignant mesothelioma

References:

  • The section on “Cytologic diagnosis of malignant mesothelioma” (p.650-652) is a concise summary of the recognized cytologic features of mesothelioma.

• The most useful cytologic features of **epithelioid malignant mesotheliomas** are as follows:
  • The presence of numerous relatively large (>50 cells) balls of cells with berrylike external contours is characteristic of MM. Most cells are much larger than the average mesothelial cells. This includes enlargement of cytoplasm, nucleus, and nucleolus.
  • The presence of macronucleoli. However, prominent nucleoli can be present in reactive mesothelial cells and not all MM cells have macronucleoli.
  • Nuclear atypia, if present.

• One also has to recognize that not all mesotheliomas yield effusions and the sarcomatoid mesotheliomas are virtually never diagnosed on effusion cytology. In such cases, fine-needle aspiration, combined with core biopsy (or larger tissue samples), are necessary to establish the diagnosis.

• Many of the cytologic features (scalloped borders of cell clumps, intercellular windows, with lighter dense cytoplasm edges, and low nuclear/cytoplasmic ratios) are shared between reactive and malignant epithelioid mesothelial cells. Usually, the malignant cells in sarcomatoid MM are not shed into the effusion fluid, which may contain the overlying reactive epithelioid mesothelial cells that may mislead the pathologist.

Guidelines for Pathologic Diagnosis of Malignant Mesothelioma
2012 Update of the Consensus Statement from the International Mesothelioma Interest Group

Figure 10. A through D, Cytologic features of malignant mesothelioma (MM). A, Numerous large clumps of cells are present in effusion of MM. B, The clumps have a berrylike external contour. C, Multiple binucleated cells are seen. D, Cell block also shows frequent clumps and can be very useful in performing special stains (Papanicolaou, original magnifications ×40 [A], ×200 [B], and ×400 [C]; hematoxylin-eosin, original magnification ×200 [D]).

Key cytologic features of adenocarcinoma are as follows:

- Clumps of cells usually have smooth rather than berrylike borders.
- The nuclear to cytoplasmic ratio is usually higher than in MM.
- Nuclear variability in shape and size is much more common.
- Cytoplasmic vacuoles often contain epithelial mucin in contrast to mesothelial cells, which contain hyaluronic acid.
- Cytoplasm is less dense than in mesothelial cells, and “windows” are rarely present.
- Psammoma bodies (when present) are more likely to be a feature of adenocarcinoma than MM, but they do occur in MM rarely.
Serous effusions from patients with MM at any location can be divided into three groups:

- Those cases that are malignant on cytomorphological criteria;
- Those cases where the cytological material requires some form of ancillary testing to establish malignancy;
- Those cases that cannot be diagnosed by cytology. This category includes cases with minimal cell shedding, typically almost all sarcomatoid and desmoplastic MM, and also those epithelioid MM that do not have malignant cytomorphology or diagnostic positive ancillary tests.

In addition to cytomorphology, the first two categories generally require ancillary techniques such as ICC or IHC and in rare cases EM, to confirm the phenotype as of mesothelial origin. The main differential diagnosis in this setting is usually between MM and metastatic adenocarcinoma. The third category will require some form of histological material.
Guidelines for cytopathologic diagnosis of epithelioid and mixed type malignant mesothelioma. Complementary statement from the International Mesothelioma Interest Group, also endorsed by the International Academy of Cytology and the Papanicolaou Society of Cytopathology

- Overtly malignant cells with diagnostic nuclear malignant features may be seen in various anaplastic tumors, and in this setting establishing phenotype may be difficult. Such cells are less common in MM than in adenocarcinoma and other metastatic malignancies. Attempts to establish a malignant diagnosis should be undertaken, when the sample contains exceptionally large numbers of mesothelial cells even when the nuclear atypia is less apparent. Fluorescent in situ hybridization (FISH) and/or ICC or IHC will often confirm the presence of malignant cells.
• Summary of cytomorphological criteria indicating malignant mesothelioma

Highly cellular sample, often including large and small tissue fragments
Mesothelial cells which are significantly larger than normal mesothelial cells either singly or in tissue fragments, each of the components of the whole cell is enlarged: Cytoplasm, nucleus and nucleolus
Papillary tissue fragments forming spheres with a smooth surface or berry-like tissue fragments with a scalloped surface, sometimes with clear spaces or “windows” between the cells
Acidophilic extracellular matrix cores also known as collagen or basement membrane cores within the tissue fragments and an extracellular granular acidophilic background indicating large amounts of hyaluronan
The presence of macronucleoli
Protrusions from the cell membrane or blebbing
Prominent degree of cell-within-cell arrangements
Background with multinucleated giant cells and small pyknotic eosinophilic or orangophilic cells
Vacuoles overlapping the nuclei of MGG-stained cells

MGG: May-Grünewald-Giemsa
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Effusions with epithelioid mesothelioma. The specimen is often highly cellular, containing large and small tissue fragments (left Papanicolaou PAP, right May-Grünewald-Giemsa; bar = 50 μm).

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Effusions with epithelioid mesothelioma. The specimen is often highly cellular, containing large and small tissue fragments (left Papanicolaou PCytomorphology of epithelioid mesothelioma cells and tissue fragments in effusions. The tissue fragments sometimes show gaps or windows, (a and b) occur both as spheres with smooth surfaces (c and d) and in berry-like clusters with scalloped surface (e and f). The tissue fragments may contain acidophilic extracellular matrix cores also known as collagen or basement membrane cores (g and h) which with May-Grünewald-Giemsa (MGG) becomes strongly acidophilic, similar to the extracellular granular material that can be seen in the background [Figures [Figures11 and and2f],2f], indicating large amounts of hyaluronan (left PAP stain, right MGG stain; bar = 50 μm).

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Cytomorphology of the epithelioid mesothelioma cells in effusions. The malignant mesothelioma cells often show macronucleoli. (a and b) There are prominent degree of cell-within-cell arrangements (c and d) and the tumor cells are sometimes multinucleated. (e and f) Some cells may be squamoid with eosinophilic or orangophilic cytoplasm and pyknotic nuclei (g), while others show rounded protrusions or “blebbing” from the cell membrane. (h) The tumor cells may develop cytoplasmic vacuoles which punch holes in the nucleus in May-Grünwald-Giemsa (MGG)-stained slides, (i) as well as a reddish haze at the periphery of the cells, (j) corresponding to the location of hyaluronan synthesis. (a, c, e and f PAP stain; b, d, f, h, i, j MGG stain; bar = 25 μm).

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- The two main differential diagnoses of MM are adenocarcinoma and benign, reactive mesothelial cells. The distinction of MM from metastatic carcinoma is in most cases made with the help of ICC or IHC. Metastatic adenocarcinomas react with BerEp4, B72.3, CEA and/or CD15. MMs are positive to calretinin (stronger than to BerEp4), podoplanin (D2-40), mesothelin, EMA accentuated on the cell membrane and WT1, although the last three are common also in ovarian carcinomas.

- An often more challenging task is to differentiate between reactive, benign mesothelial cells and MM. ICC and IHC are often helpful. The most often recommended epitope is desmin, which is lost early during the malignant transformation of mesothelial cells, but some reactivity may remain in the malignant cell population and the effusion may be a mixture of malignant and benign mesothelial cells. Reactivity to EMA in most cases overrides the weaker reactivity to desmin in the malignant cells, and the use of desmin/EMA double stains is recommended. In some cases, there is also a general reactivity to p53 indicating a malignant condition. The use of ploidy analysis by Urovysion® FISH will in most cases indicate malignancy.
Malignant mesothelioma – Parakeratotic-like cells as a clue

References:


Parakeratotic-like cells in effusions — A clue to diagnosis of malignant mesothelioma

- Parakeratotic-like cell. Parakeratotic-like cells are small, degenerated orangeophilic cells with pyknotic nuclei that look like parakeratotic cells in the Pap test (Pap stain, ×400)

• Mimickers of PK-like cells. Cells that had orangeophilic cytoplasm, but lacked degenerated pyknotic nuclei or cells with eosinophilic cytoplasm (regardless of nuclear structure) were not counted as PK-like cells. These mimicker cells typically had a rim of blue staining cytoplasm (Pap stain, x400)
Small orangiophilic squamous-like cells: An underrecognized and useful morphological feature for the diagnosis of malignant mesothelioma in pleural effusion cytology

**FIGURE 1.** (A) Malignant mesothelioma (MM) from a pleural fluid specimen (ThinPrep) from a 66-year-old man in the current study demonstrated rare small orangiophilic squamous-like cells (arrow; Papanicolaou stain, \times 60\). (B) The above pleural fluid cytology specimen (ThinPrep) also showed papillary groups of mesothelial-like cells with knobby outlines (Papanicolaou stain, \times 40\). The cytology diagnosis was “suspicious for MM.” (C) Cell block from the above case demonstrated papillar groups of atypical cells (H & E, \times 40\). (D) Immunocytochemical stain of calretinin on the above cell block demonstrated both nuclear and cytoplasmic staining of the atypical mesothelial cells (\times 40\). (E) Immunocytochemical staining of cytokeratin 5/6 on the above cell block showed strong staining of the atypical mesothelial cells (\times 40\). (F) Pleural biopsy of the above case indicated definitive invasion of MM into the pleural space (H & E, \times 20\).
Small orangiophilic squamous-like cells: An underrecognized and useful morphological feature for the diagnosis of malignant mesothelioma in pleural effusion cytology

Figure 2. (A and B) Malignant mesothelioma (MM) of pleural fluid cytology (smear) of another patient from the current study (a man aged 78 years) demonstrated several small orangiophilic squamous-like cells (arrows; Papanicolaou stain, × 60). (C) The above pleural fluid cytology (smear) demonstrated bland-appearing mesothelial-like cells. There were no papillary groups noted. The arrow indicates the feature of “windows” (clear spaces between adjacent cells) (Papanicolaou stain, × 60). The cytology diagnosis for this case was “atypical mesothelial cells, favor reactive.” (D) Pleural biopsy of the above case showed definitive invasion of MM into the pleural space (H & E, × 10).
Small cell carcinoma

Reference:

Small-cell carcinoma with a predominance of large clusters in pleural fluid. The cells have wrapped themselves around one another forming large cell clusters (left, Pap stain, ×400). Cell block (right, H and E, ×200) shows a lacuna around large clusters with hollow cores and pseudo-lumen caused by tumor cell necrosis. Synaptophysin immunostain (right lower, ×400) confirms neuroendocrine differentiation in these tumor cells.
The cytomorphologic spectrum of small-cell carcinoma and large-cell neuroendocrine carcinoma in body cavity effusions: A study of 68 cases

Small-cell carcinoma with a predominant single-cell pattern mimicking lymphoma in pleural fluid. The nuclei show a finely distributed granular chromatin texture and small nucleoli, left and middle (Pap stain, left ×200 and middle ×400). Chains of small cells with nuclear molding (middle, center of the image) and karyorrhexis (left) are seen. In cell block sections (right, H and E stain, ×400) tumor cells display a discohesive pattern.
The cytomorphologic spectrum of small-cell carcinoma and large-cell neuroendocrine carcinoma in body cavity effusions: A study of 68 cases

- Small-cell carcinoma with marked apoptosis and small nucleoli seen in the Thin Prep (left and middle, Pap stain, ×200 and ×400, respectively) and cell block (H&E stain, ×400)

Khalbuss et al. Cytojournal. 2011;8:18
Primary effusion lymphoma

Reference:

Primary effusion lymphoma: a series of 4 cases and review of the literature with emphasis on cytomorphologic and immunocytochemical differential diagnosis

**FIGURE 1.** Case 3. Cytology of the pleural effusion. Note (A) the large cell size and coarse chromatin, (B) the moderately abundant basophilic cytoplasm and the eccentric location of nuclei in some cells, and (C) the irregular nuclear outline and prominent nucleoli (cytospin preparations, Papanicolaou stain, Panel A: ×400 and Panels B and C, ×600).

**FIGURE 2.** Case 3. Cell block of the pleural effusion. Note the prominent nucleoli, the occasional perinuclear hof, and the large amount of apoptotic bodies and nuclear debris present (H & E, ×600).