Video Microscopy Tutorial 5

Lool Alikes in Effusion Cytology: Review of Diagnostic Challenges

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There are no disclosures necessary.
Look-Alikes in Effusion Cytology: Review of Diagnostic Challenges

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Diagnostic Challenges
• Recognizing a reactive effusion
• Distinguishing reactive from malignant mesothelial cells
• Distinguish mesothelioma from adenocarcinoma
• Recognizing features of P.D. squamous cell carcinoma in effusions

Points to remember
• Fluid accumulation occurs with disease
• Fluids can be exudate or transudate
• Bloody fluid is indicative of malignancy or pulmonary infarction
• Malignant effusion is not always bloody
• Hemosiderin laden macrophages are indicative of chronic RBCs leak
**Quiescent mesothelium-1**
- Monolayer of flattened sheets with epithelial features
- Cells break off as single cells or in few groups
- Round to oval cells, 15-20 µm in diameter
- Cytoplasm moderate in amount, translucent, with peripheral glycogen vacuoles
- Long slender microvilli appear as a pale zone at the periphery causing a fuzzy or brush like appearance by LM
- Central portion of cytoplasm is denser and darker due to perinuclear intermediate filaments causing endo-ectoplasmic demarcation

**Quiescent mesothelium-2**
- Cells may be single or binucleated
- Nuclei are monotonous, centrally located, and oval to round, with evenly distributed chromatin
- Nucleoli are indistinct
- Occasional cells exhibit the characteristic “window” and “cellular clasping” appearance

**Normal Mesothelium**
Reactive/Hyperplastic Mesothelium

- Shed as doublets or triplets with windows between them
- Few papillary groups may be formed
- Connections by clasp-like articulations are more obvious
- Cells are round to oval, 20-40µm in diameter
- Abundant cytoplasm with endo-ectoplasmic demarcation and peripheral submembranous vacuoles
- Cytoplasmic protrusions distal to cellular connection

- Nuclei are round to oval with some variation in size and chromatin distribution
- Cell size vary slightly, however, within a small range and only few cells are out of proportion in size
- Nucleoli may become prominent
- Multinucleated cells increase
- Occasional intranuclear inclusions are noted

Mesothelial Cells
Causes of Mesothelial Hyperplasia

- Heart failure
- Infection (pneumonia, lung abscess)
- Infarction (may shed in sheets)
- Liver disease such as hepatitis or cirrhosis
- Collagen disease
- Renal disease/peritoneal dialysis
- Pancreatic disease
- Radiation and chemotherapy
- Traumatic irritation (surgery)
- Chronic inflammation (PID, pleuritis)
- Underlying neoplasm causing irritation (fibroid)
- Foreign substance (talc, asbestos)
Malignant Mesothelioma

Why is it important to diagnose in fluids?

1. Different therapeutic implications
2. Spare the patient additional procedures with higher morbidity and risk of tumor seeding
3. Medicolegal compensation for asbestos exposure

Malignant Mesothelioma

- Fluids are generally highly cellular
- Could be scant in cellularity
- Key cytologic feature: larger cells with some attaining gigantic size
Malignant Mesothelioma

- **Early stage**: Hundreds of tiny nodules on the serosal membrane.
- Pleural thickening and plaques when associated with asbestos exposure

- **Late stage**: Nodules become confluent and serosal membrane becomes thickened and gradually the parietal and visceral pleura fuse and fluid disappears.

Malignant Mesothelioma

- **Epithelial**: tubulopapillary, W.D, papillary, epithelioid, transitional, decidual, clear, microcystic, small cell
- **Sarcomatous**
- **Biphasic**
- **Anaplastic**

Malignant Mesothelioma

**Arriving at Diagnosis**

- **First**: Recognize mesothelial features

- **Second**: Recognize their malignant features
Malignant Mesothelioma
How to Recognize It-1

- Highly cellular smears
- All cells look alike, i.e. one cell population
- Cellular spheres with smooth borders (modules)
- Tight and loose clusters with scalloped borders
- High number of cells within clusters
- Cells vary in size and shape widely with some gigantic cells
- Large multinucleated cells approaching size of some morules
- Mesothelial cell features easily recognized/exaggerated

Malignant Mesothelioma
How to Recognize It-2

- Yellow glycogen is frequently detected
- Nuclei are usually bland or slightly atypical (nuclear irregularity, coarse chromatin, and hyperchromasia)
- Very prominent nucleoli
- Background of numerous lymphocytes or abundant blood
- Thick extracellular matrix (hyaluronic acid) causing the grossly recognized thick consistency described as “Tar-like” or “Honey-like” consistency.
Remember

- Not all fluids of mesothelioma are cellular
- Not all mesotheliomas associated with asbestos (1/3 of cases are not)
- Not all mesotheliomas contain morules, some present as single cells only
- Effusions are large, unilateral and recur fast and frequently
- A low cellularity on a repeated tap within a short interval does not exclude the diagnosis of mesothelioma

Differential Diagnosis:

Papillary Clusters or Cell Balls

- Adenocarcinoma
  - Breast
  - Lung
  - Ovary
  - Prostate (rare)
- Florid mesothelial hyperplasia
- Malignant mesothelioma
- P.D. squamous cell carcinoma

Differential diagnosis:

Large Single Cells

- Adenocarcinoma
  - Lung
  - Breast
  - Pancreatic
  - Renal cell
- Reactive mesothelium
- Malignant mesothelioma
- Malignant melanoma
- Large cell lymphoma
How To Approach The Diagnosis?

1. Is there one or two cell population?
2. Are the cells monotonous (look-alike) or pleomorphic (variable in shape and atypia)?
3. Is there a small size range or is there wide variation in size?
4. What cellular features you see? Two tone, vacuolated etc..
5. Are the nuclei highly atypical or not?

Adenocarcinoma: Breast

Adenocarcinoma: Ovary
Squamous Cell Carcinoma

Features Favoring P.D. Squamous Cell Carcinoma

- Third type cells with cyanophilic cytoplasm appearing singly or in large tight clusters
- Characteristic cell features:
  - Very dense and distinct cell border
  - Dense periphery or hyaline in appearance indicate attempt to keratinize
  - Refractile rings indicative of abnormal keratinization
  - Endo-ectoplasmic demarcation (ectoplasm is dense while endoplasm is more textured) because keratinization starts at the periphery
  - Ecto-endoplasmic border can be ruffled or thrown into linear folds “keratinizing fibrils” or “Herxheimer’s spirals”
  - Small clusters of cells arranged in whorls as they rap around each other recapitulating keratin pearl
  - Cells appearing as doublets or short cords (cell junctions)
Cell Junction Versus Window

Keratin Pearl

Unknown 1: Pleural Fluid
55 year old male with history of pneumonia
Unknown 2: Pleural Fluid
74 years old male with a 10 cm renal mass and ascites

Unknown 3: Peritoneal Fluid
66 years old female with possible adnexial mass

Unknown 3: Peritoneal Fluid
Features Favoring Reactive Mesothelium

- One cell population with monotonous appearance
- Atypia is not very pronounced
- Cellular clusters may be present but not as tight as spheres
- Little variation in size or shape of cells
- Classic features of mesothelium including cytoplasmic glycogen

Features Favoring Mesothelioma

- Monotonous population with mild to moderate atypia
- Morules and numerous discohesive cells
- Numerous multinucleated giant cells
- Markedly enlarged cells (5-10 times that of normal mesothelium)
- Background cells show a wide range of size
- Features indicative of mesothelial origin

Unknown 1: Pleural Fluid
Unknown 2: Pleural Fluid

Features favoring Adenocarcinoma

- Pleomorphic population of cells with obvious atypia
- Little variation in size of cells
- Two cell population (background of reactive mesothelium) may be detected
- Lack of two tone cytoplasm
- Cytoplasmic glycogen rarely seen (lung adenoca)
- True gland formation may be seen in some clusters

Unknown 3: Peritoneal Fluid
Reactive Versus Malignant

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Body cavity effusions are among the most commonly received specimens in the cytotology laboratory. They also are the most commonly referred specimens for consultation. Diagnostic difficulties in effusion cytology include the following:

1. Recognizing a reactive effusion
2. Distinguishing reactive from malignant mesothelial cells
3. Distinguishing mesothelioma from adenocarcinoma
4. Recognizing the features of poorly differentiated non-keratinizing squamous cell carcinoma in effusion

Points to remember

1. The body cavity is a virtual cavity with no fluid. Fluid accumulation occurs with diseases
2. Fluids can be transudate of exudates
3. Provided that a traumatic tap is ruled out, the presence of blood is indicative of either malignancy or pulmonary infarct.
4. A malignant effusion is not always bloody
5. The presence of hemosiderin laden macrophages is indicative of chronic RBCs

Benign mesothelium

Quiescent mesothelium:

1. A monolayer of flattened sheets with epithelial features (classic example are sheets seen in pelvic washes)
2. Cells break off as single cells or in few small groups
3. Round to oval cells, 15-20 µm in diameter
4. Cytoplasm is moderate in amount, translucent, and contain peripheral vacuoles containing glycogen
5. Long slender microvilli under light microscopy appear as a pale zone at the periphery causing a fuzzy or a brush like appearance.
6. The central portion of the cytoplasm is denser and darker due to the perinuclear intermediate filaments resulting in a two- tone or endo-ectoplasmic demarcation.
7. Cells may be single or binucleated
8. Nuclei are monotonous, centrally located, and oval to round, with evenly distributed chromatin.
9. Nucleoli are indistinct
10. Occasional cells exhibit the characteristic “window” and “cellular clasping” appearance
Reactive/hyperplastic/hypertrophic mesothelium

1. Shed as doublets or triplets with windows between them.
2. Few papillary groups may be formed
3. Connections by clasp-like articulations are more obvious
4. Cells are round to oval, 20-40µm in diameter
5. Abundant cytoplasm with endo-ectoplasmic demarcation and peripheral submembranous vacuoles
6. Cytoplasmic protrusions distal to cellular connection
7. Nuclei are round to oval with some variation in size and chromatin distribution. The cell size may vary slightly, however, within a small range and only few cells will appear out of proportion.
8. Nucleoli may become prominent
9. Multinucleated cells increase
10. Occasionally intranuclear inclusions are noted

Causes of mesothelial hyperplasia

1. Heart failure
2. Infection (pneumonia, lung abscess)
3. Infarction (may shed in sheets)
4. Liver disease such as hepatitis or cirrhosis (may cause pronounced hyperplasia)
5. Collagen disease
6. Renal disease such as uremia or peritoneal dialysis
7. Pancreatic disease
8. Radiation (split field, tandem ovoids)
9. Chemotherapy (bleomycin, cytoxan)
10. Traumatic irritation (hemodialysis, surgery)
11. Chronic inflammation (PID, pleuritis)
12. Underlying neoplasm causing irritation of the mesothelium (fibroid)
13. Foreign substance (talc, asbestos)

Note: Hyperplasia and hypertrophy with papillary formation may be so pronounced and may mimic malignancy (mesothelioma or adenocarcinoma).

Malignant mesothelioma

Malignant mesothelioma is a rare disease with an average incidence of 2 cases per 1 million people per year in the United States. However it is extremely important to recognize it since mesothelioma has different therapeutic implications from other tumors. In addition, its recognition will spare the patients repeated procedures such as core biopsies that frequently carries the risk of seeding along the needle tract.

Malignant mesothelioma may result into a very cellular fluid; however it is not unusual to obtain a fluid with low or scant cellularity. The increase in cell size is one of the most prominent features in mesothelioma (attain a gigantic size).
**Gross appearance:**
*Early stage:* appears as hundreds of tiny nodules on the serous membrane. Pleural thickening and plaques are noted when associated with asbestos exposure.
*Late stage:* nodules become confluent and the serosal membrane becomes thick and gradually the parietal and visceral membranes become fused together, with disappearance of any fluid.

**Types of mesothelioma:**
1. Epithelioid (tubulopapillary, well differential papillary, epithelioid, transitional, deciduoid, clear variant, microcystic, and small cell)
2. Sarcomatous
3. Biphasic
4. Anaplastic

Epithelioid variant is the most common type seen in effusion cytology particularly the first three patterns.

**The correct diagnosis involves two steps:**
*First, recognize the mesothelial origin*
*Second: recognize their malignant features*

**How to recognize a mesothelioma**

1. Highly cellular smears
2. All cells look alike, i.e. no evidence of two cell population
3. Cellular spheres with smooth borders (morules)
4. Tight and loose clusters with scalloped borders
5. High number of cells within the clusters
6. Individual cells show a wide variation in size and shape ranging from small to gigantic.
7. Large multinucleated cells with abundant cytoplasm, some of these cells approach the size of small morules
8. Mesothelial cell features are easily recognized and exaggerated
9. Yellow glycogen is frequently detected
10. Nuclei are usually bland or slightly atypical (nuclear irregularity, coarse chromatin and hyperchromasia).
11. Very prominent nucleoli
12. Background of numerous lymphocytes or abundant blood
13. Thick extracellular matrix (hyaluronic acid) is frequently present causing a grossly recognized thick consistency described as “Tar-like” or Honey-like”. This matrix can sometimes interfere with smear preparation particularly Liquid based Preps.

**Notes to remember**
1. Not all malignant mesothelioma fluids are cellular
2. Although highly associated with asbestos exposure, one third of the cases are not, and in many the history is not given to us.
3. Mesothelioma effusions are large, unilateral and recur fast and frequently.
4. A low cellularity on a repeated tap within a short interval does not exclude mesothelioma.
5. Some mesotheliomas manifest mainly as single large cells. These cells exhibit all the features described above.

**Mesothelioma versus Adenocarcinoma versus Squamous Cell Carcinoma**

Malignant mesothelioma need to be distinguished from a variety of adenocarcinomas and on rare occasions other neoplasms such as poorly differentiated squamous cell carcinoma.

*Differential diagnosis usually involves one of two patterns*:
1. Atypical cells mostly presenting as single cells with few groups.
2. Atypical cells mostly presenting as cellular spheres.

**Differential diagnosis of effusions with papillary clusters or balls:**
- Breast carcinoma
- Ovarian carcinoma
- Lung adenocarcinoma
- Prostatic adenocarcinoma (very rare)
- Poorly differentiated squamous cell carcinoma
- Malignant mesothelioma
- Florid reactive mesothelium

**Differential diagnosis of effusions with large single cells:**
- Lung adenocarcinoma
- Breast carcinoma
- Pancreatic adenocarcinoma
- Renal cell carcinoma
- Malignant melanoma
- Malignant mesothelioma
- Reactive mesothelium

**How to approach the diagnosis:**
1. Is there one or two cell population?
2. Are the cells monotonous in appearance (look-alike) or pleomorphic (wide variation in shape and atypia)?
3. Are the atypical cells within a small size range or is there a wide variation in size?
4. Do the cells exhibit a two tone cytoplasm, vacuolated, dense, etc.?
5. Are the nuclei highly atypical or not?

*Features favoring a reactive mesothelium:*
- One cell population with monotonous appearance
- Atypia is not very pronounced
- Cellular clusters may be present but not as tight as spheres
- Little variation in size or shape of cells
- Classic features of mesothelium including cytoplasmic glycogen

**Features favoring adenocarcinoma:**
- Pleomorphic population of cells with obvious atypia
- Little variation in size of cells
- Two cell population (background reactive mesothelium) may be detected
- Lack of two tone cytoplasm
- Cytoplasmic glycogen rarely seen (lung adenocarcinoma)
- True gland formation may be seen in some clusters

**Features favoring malignant mesothelioma:**
- Monotonous cell population with mild to moderate atypia
- Morules and numerous discohesive cells
- Numerous multinucleated giant mesothelial cells
- Markedly enlarged cells (5-10 times that of normal mesothelium)
- Background cells show a wide range of size
- Features indicative of mesothelial origin

**Features favoring poorly differentiated squamous cell carcinoma:**
- Third type cells with cyanophilic cytoplasm appearing singly or in large tight clusters
- Cells show characteristic endo-ectoplasmic demarcation
  - Very dense and distinct cell border
  - Dense periphery or hyaline in appearance (contrary to the fuzzy border of mesothelium) indicate attempt to keratinize
  - Refractile rings indicative of abnormal keratinization (layers of hyaline as if successive zones of keratinization process is occurring
  - Endo-ectoplasmic demarcation (ectoplasm is dense while endoplasm is more textured) this is the result of keratinization from the periphery inwards
  - Endo-ectoplasmic border can be ruffled or thrown into linear folds and if viewed in stretched cells appear as “Keratinizing fibrils” also known as “Herxheimer’s spirals”
  - Small clusters of cells arranged in whorls as they rap around each other recapitulating a keratin pearl
Immunostaining in effusion cytology:

Positive markers for mesothelium

Calretinin: Regarded as the most sensitive and one of the most specific of the positive mesothelial markers. Contrary to other markers, Calretinin is frequently expressed in most histologic types of mesothelioma. The diagnosis of mesothelioma in the absence of staining with Calretinin should be considered cautiously. Staining pattern is strong and diffuse, and occurs in both the nuclei and cytoplasm. Adenocarcinoma (ADC) staining has been reported between 0-38% but the staining is usually focal although occasionally strong and diffuse (lung 6-10%, ovary 31-38%, renal 0-4%). Squamous cell carcinoma could be immunoreactive in 23-39%

Wilms Tumor 1 Protein (WT1): Stains up to 93% of epithelioid mesotheliomas. It is also highly expressed in ovarian serous carcinoma. Lung ADC is reported to have 0% or very minimal expression thus making WT1 one of the best markers to distinguish between mesothelioma and lung ADC.

Keratins 5/6: Reported to stain 64-100% of mesotheliomas. Staining is cytoplasmic and can be very focal resulting in false negatives. Staining is positive and strong in most squamous cell carcinomas. Also could stain 22-35% of ovarian serous carcinoma and was reported to stain 0-19% of lung ADC. CK5/6 has no utility in distinguishing mesothelioma from squamous carcinoma.

Thrombomodulin: Reported to stain 34-100% of mesotheliomas (more around 75%), and between 5-77% of lung ADC (more around 14%). Mesothelioma tended to present with diffuse strong membranous pattern while adenocarcinoma showed focal week staining. Thrombomodulin is also expressed in angiosarcoma and squamous carcinoma.

D2-40: Newly developed antibody that reacts with oncofetal M2A antigen in fetal germ cells and germ cell tumors. D2-40 is reported to stain 86-96% of epithelioid component of mesothelioma with a membranous or luminal pattern. It is also reported to stain about 15% of ovarian ADC but none of the other adenocarcinomas.

Podoplanin: This is a membrane mucoprotein detected on podocytes of glomerular epithelial cells. It has similar sensitivity and specificity as D2-40.

Mesothelin: Reported to stain up to 100% of epithelioid mesotheliomas with diffuse strong membranous staining. However, positive staining in a variety of adenocarcinomas was also reported though it may be cytoplasmic or focal. Despite the low specificity of this marker, a negative staining argues against the diagnosis of mesothelioma.

Negative markers for mesothelium

MOC-31: Recognizes epithelial cell adhesion molecule. Currently considered to be the most sensitive and specific negative marker for mesothelioma although 5-10% are
reported to show focal staining. Positive strong and diffuse cytoplasmic staining is reported in almost all adenocarcinomas of lung and ovary. Up to 50% of renal cell carcinoma are positive.

**CD15 (Leu-M1):** This marker is currently believed to be negative in mesothelioma. 70-75% of lung ADC and 30-60 of ovarian ADC are immunoreactive. Majority of renal cell carcinomas are also positive.

**BG-8:** Recognizes Lewis blood group antigen. Expressed in up to 89% of adenocarcinomas of various origins with strong diffuse cytoplasmic staining. Reported to stain in 3-7% of epithelioid mesothelioma, however, the reaction is usually focal or scant. Renal cell carcinomas are usually negative.

**Ber-EP4:** Reported to stain 86-96% of a variety of adenocarcinomas. Staining is usually strong and diffuse. Almost all lung and ovarian ADC, and 35-50% of renal cell carcinoma are positive. It is believed that it may show positive focal reaction in up to 20% of mesotheliomas. Some propose that only lateral membranous staining should be regarded as truly positive and that focal staining is of limited value.

**Carcinoembryonic antigen (CEA):** Expressed in up to 80% of lung ADC. Can also be expressed in gastrointestinal ADC particularly colon. CEA is expressed in a minority of ovarian and is not expressed in renal adenocarcinoma. It is considered to be one of the best negative mesothelioma markers to distinguish between epithelioid mesothelioma and lung ADC.

**B72.3:** Reacts with a tumor-associated protein TAG-72. Expressed in up to 80% of ADC from a variety of organs. It is expressed in up to 81% of lung ADC, up to 87% of ovarian ADC, and less than 3% of epithelioid mesotheliomas. It is not expressed by renal cell carcinoma.

**CA 19-9:** Related to Lewis blood antigen. Has low sensitivity. Commonly expressed in tumors of gastrointestinal origin, pancreas, ovary, and lung. Although not expressed in mesothelioma, it is not useful to separate it from lung ADC as it is only expressed in 39-53% of those adenocarcinomas.

**Other immunostains**

**E-Cadherin and N-Cadherin:** Originally thought to be useful in the differentiation between adenocarcinoma and mesothelioma (E-Cadherin in adenocarcinoma and N-Cadherin in mesothelioma). Now believed to have no utility in the differential.

**Thyroid Transcription Factor-1 (TTF-1):** Stains 58-97% of lung adenocarcinoma. Squamous cell carcinoma is usually negative and so is mesothelioma.
**Renal cell carcinoma marker (RCC Ma):** Reported to stain up to 80% of renal cell carcinoma. Also may stain in few metastatic breast ADC. 8% of mesotheliomas were reported to show focal staining in few cells.

**CD10:** Stains the majority of renal cell carcinoma. Up to 48% of mesotheliomas may also have positive staining.

**Keratin 7 and 20:** Keratin 7 is strongly expressed in epithelioid mesothelioma, lung ADC and ovarian ADC. All the above are negative to keratin 20. Therefore these two stains are not useful in the differential of mesothelioma from adenocarcinoma. However, negative staining for both or strong staining to CK 20 is evidence against mesothelioma. Note that keratin 20 may rarely be expressed focally in mesothelioma but these cases also show strong staining to keratin 7.

**Desmin and EMA:** These two are usually used to separate malignant mesothelioma from reactive mesothelium. It is believed that benign mesothelium preferentially expresses desmin and loses such expression with the malignant transformation. The majority of malignant mesotheliomas, up to 80% are believed to express EMA. There is some controversy regarding the utility of these stains. Occasionally EMA is expressed focally in few cells in reactive mesothelium.

**PAX-8:** Useful in identifying malignant cells of Müllerian or renal cell origin.
References:


6. Li Q, Bavikatty N, Michael CW. The role of Immunohistochemistry in distinguishing squamous cell carcinoma from mesothelioma and adenocarcinoma in pleural effusion (in press, Seminars in Diagnostic Pathology).


