215 Problematic Cases in Pulmonary Cytopathology

Stan Eilers MD
Paula LaPolice CT(ASCP)
Adebowale Adeniran MD, FASCP
Ajay Shah MD
Indra Balachandran PhD, SCT(ASCP), CFIAC
Umesh Kapur MD, FASCP

2011 Annual Meeting – Las Vegas, NV

AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
33 W. Monroe, Ste. 1600
Chicago, IL 60603
215 Problematic Cases in Pulmonary Cytopathology

This course will present images from selected pulmonary cytology cases which proved to be challenging for participants in the ASCP NonGYN Assessment Program. The audience will participate with an interactive audience response system. The cytomorphologic criteria for the differential diagnoses will be discussed with additional examples including material from the ASCP Asset Warehouse. This session will also place special emphasis on the role of cytopathology in the personalized medicine of treating pulmonary malignancies through appropriate choice and use of ancillary and molecular technologies. Participants should expect information that is timely and germane to the practicing pathologist seeking to employ the best combination of microscopic review skills and ancillary and molecular testing methods to provide the best diagnosis and course of treatment for patients with pulmonary malignancies.

- Sharpen diagnostic skills for cytotechnologists and cytopathologists by emphasizing and illustrating diagnostic cytologic criteria in the following specific settings: the differential diagnosis of reactive atypia versus adenocarcinoma; the differential diagnosis of small cell carcinoma versus non-small cell carcinoma; and the diagnosis of pulmonary malignancies from extra pulmonary sites, specifically hepatic aspirates and pleural fluid. In each of the above mentioned scenarios, the precise diagnosis is necessary to provide the correct treatment since each entity has significantly different treatment protocols.
- Emphasize and inform the participants on the role of ancillary studies in the diagnosis and treatment of pulmonary malignancies. Specifically, the use of immunohistochemical stains in the differential diagnosis of squamous cell carcinoma, adenocarcinoma and small cell carcinoma. In addition, the role of molecular studies, primarily in adenocarcinoma, will be discussed. The specific cell type and molecular genotype is critical in developing a specific treatment plan in this era of personalized medicine. For some participants this may represent a review, but for others this represents new more detailed information.
- Increase awareness of ASCP Non-GYN Assessment Products that can be used to enhance pathologist and cytotechnologist practice-based professional work.

FACULTY:

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Umesh Kapur MD, FASCP

Practicing Pathologists
Cytopathology
Cytopathology (Non-Gynecologic)
1.0 CME/CMLE Credit

Accreditation Statement: The American Society for Clinical Pathology (ASCP) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education (CME) for physicians. This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME).

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Problematic Cases in Pulmonary Cytopathology

As Identified from the ASCP NonGYN Assessment Program

Stan Eilers, MD
ASCP NonGYN Assessment Committee
Chair
Mercy Medical Center, Cedar Rapids, IA
Mercy Medical Center, Clinton, IA
Weland Laboratories, Cedar Rapids, IA

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ASCP Non-GYN Committee

2010-2011 Members
Pulmonary Course Faculty Italicized

- Stanley Eilers, MD, FASCP, Chair
- Paula LaPolice, CT(ASCP), Vice-Chair
- Perkins Mulinowadza, MD, FASCP, Medical Member
- Umesh Kapur, MD, FASCP, Medical Member
- Ajay Shah, MD, FASCP, Medical Member
- Husain Salath, MD, MBA, Medical Member
- Adebowale Adeniran, MD, FASCP, Medical Member
- Amberly Nunez, MD, Resident Member
- Amy J. Wendel Spiczka, MS, SCT, HTL(ASCP), Cytotechnologist Member
- Indra Balachandran, PhD, SCT(ASCP), Cytotechnologist Member
- ASCP Staff: Jennifer J. Clark, SCT(ASCP), Product Development Manager

ASCP Non-GYN Committee Programs

NonGYN/FNB Glass Slide Programs (10)
Assessment Programs, which include detailed Educational Case Studies:
1. NonGYN Assessment – mixture of NonGYN/FNB cases
2. FNB Site-Specific Assessment / Breast
3. FNB Site-Specific Assessment / Thyroid
4. FNB Site-Specific Assessment / Lymph Node
5. FNB Site-Specific Assessment / Salivary Gland

Review Programs, Target Answers and Peer-comparison Statistics only
1. NonGYN Review – mixture of NonGYN/FNB cases
2. FNB Site-Specific Review / Breast
3. FNB Site-Specific Review / Thyroid
4. FNB Site-Specific Review / Lymph Node
5. FNB Site-Specific Review / Salivary Gland

Importance of Pulmonary Cytology

- 220, 520 new cases of lung cancer annually
- 157,300 annual deaths from lung cancer
- 70% of all lung cancer patients will have only cytology/small biopsy specimen
- Cell type is critical for appropriate treatment and ancillary tests
- EBUS and related procedures are increasing the number of pulmonary cytologic specimens

ASCP Non-GYN Committee Programs

NonGYN Digital Image Program
A printed assessment product with digital images of 20 NonGYN/FNB cases

Cytology eLearning Modules in 6 areas:
1. GYN
2. NonGYN
3. FNB
4. Technical/Ancillary Studies
5. Laboratory Mgt & Admin
6. Safety
What Is This Presentation About?

The cases were selected from the ASCP Non-GYN Assessment Glass Slide Program and have been reviewed by hundreds of cytologists. They represent “everyday” cases, but presented challenges to the participants. Discussion of key morphologic features and ancillary tests helpful to accurate diagnosis will be the focus of our expert faculty.

Format

- Non-GYN Assessment Committee Members will review topic areas of interest with statistics and images from selected cases for:
  - Reactive/Reparative Processes
  - Adenocarcinoma vs. Squamous cell carcinoma
  - Small cell vs NSCLC
  - Small cell carcinoma presenting as metastatic disease
  - Lung cancer in fluid cytology
- Discussion will include a review of cytomorphologic criteria, molecular diagnostics, and therapeutic treatments
- Additional images from the ASCP Asset Warehouse will be used as supporting material
- At program end, a brief interactive morphology quiz using the Audience Response System will be given comparing results to program participant statistics.
Problematic Cases in Pulmonary Cytopathology

As identified from the ASCP NonGYN Assessment Program

Paula LaPolice CT(ASCP)
ASCP NonGYN Assessment Committee
Vice Chair
Baystate Medical Center
Springfield, Ma
Educational Coordinator
Cytology Services

ASCPO CME Disclosure of Relevant Financial Relationships

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Reactive Cases in Pulmonary Specimens

How do we differentiate:

Reactive/Repair from Neoplastic processes

Clinical Disease Features

• Cough
• Wheezing
• Increased mucus production
• Shortness of breath
• Chest pain
• Hemoptysis

Causes of a Reactive Process

• Pneumonia
• Bronchitis
• Bronchiectasis
• Bronchial asthma
• Toxin exposure
• Radiation
• Chemotherapeutic agents
• Instrumentation

Statistical Performance of Problematic Cases

Cases from the ASCP NonGYN Assessment Program

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
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<tr>
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Reactive/Reparative Features

- Cohesive orderly flat sheets
- Abundant cytoplasm
- Nuclei-oval to round, enlarged
- Nuclear size variation
- Inflammatory background

Reactive/Reparative Features

- Chromatin bland, finely granular and evenly distributed
- Prominent nucleoli, uniform in size and shape
- Mitosis common
- Patient history

Reactive Bronchial Epithelium

Reactive Squamous Metaplasia

Reactive Case #1

A 42-year-old man presented with bilateral pneumonia, pleural effusions, shortness of breath and mediastinal adenopathy. A fine needle aspiration was performed.
Reactive Case #1

Reactive/Repair

Squamous Cell Ca

Reactive Case #2

A 74-year-old man presented with long standing cough and a history of multiple myeloma for which he had recently received chemotherapy. A bronchial brushing was performed.

Reactive Case #2

Reactive/Repair

Adenocarcinoma
Common Differential Diagnoses

- Adenocarcinoma
- Squamous cell Carcinoma

Molecular/Ancillary Testing Protocols

Unfortunately there are none!

Therapeutic Options

Treat the cause of the reactive process to heal the injury

Key Take Away Points

- **Key Cytologic Features**
  - Patient history
  - Cohesive cellular sheets
  - Smooth nuclear membranes
  - Prominent nucleoli
  - Abundant cytoplasm
  - Inflammatory background

- **Key Ancillary Tests**
  - None

- **Therapeutic Implications**
  - Treat the cause of the injury

References

- Erozan YS., Ramzy I. Pulmonary Cytopathology. Springer 2009: 29-45, 103-131
- Koss L, Melamed M. Koss Diagnostic Cytopathology and its Histopathologic Bases. Lippincott Williams & Wilkins 2008: 595-596

Thank You
Problematic Cases in Pulmonary Cytopathology

As Identified from the ASCP NonGYN Assessment Program

Umesh Kapur, MD
ASCP NonGYN Assessment Committee
Medical Member

Assistant Professor, Department of Pathology
Loyola University Medical Center
Maywood, IL

ASCP CME Disclosure of Relevant Financial Relationships

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Statistical Performance of Problematic Cases
Cases from the ASCP NonGYN Assessment Program

Pulmonary, NonGYN, Positive for Malignancy, Adenocarcinoma
Cumulative Statistics from 8 Reference Cases

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<th>Diagnostic Category</th>
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Statistical Performance of Problematic Cases
Cases from the ASCP NonGYN Assessment Program

Lung, FNA, Positive for Malignancy, Adenocarcinoma
Cumulative Statistics from 5 Reference Cases

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Reference Interpretation Choices

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| Total                           | 1328  | 100.0%

Adenocarcinoma-Cytologic Features

• Cellularity
• Flat honeycomb sheets, rosettes, acinar structures or three dimensional groups
• Medium to large size cells with abundant delicate cytoplasm
• Intracytoplasmic mucin
• Round to oval eccentric nuclei with fine chromatin and prominent nucleoli
Adenocarcinoma-Cytologic Features

- Look what is inside….
- Targetoid mucin

Squamous cell carcinoma-Cytologic Features

- Cellularity
- Large sheets, cohesive or dyshesive
- Polymorphic cell shapes, rounded, elongated (tadpole)
- Dense cytoplasm, keratinization, orangeophilia
- Pyknotic nucleus, nucleolus (sometimes)
- Anucleate squames
Squamous cell carcinoma - Cytologic Features

What about these?

Non-small cell lung carcinomas - NSCLC

Difficulties in accurate sub-classification

- Poorly differentiated tumors
- Non-keratinizing squamous carcinoma
- Mixed /combined tumors

Immunoperoxidase stains

- Several studies have evaluated immunoperoxidase stain algorithms for sub-typing non-small cell carcinoma
- Excellent accuracy in predicting tumor sub-type using a panel comprising of TTF-1, P63 and CK5/6 (Rekhtman et al. & Khayyata et al.)
- Katzenstein et al. showed accurate sub-typing utilizing a novel antibody napsin A along with TTF-1, P63 and CK5/6 in small lung biopsies

Immunoperoxidase Testing Algorithm (Rekhtman et al.)

<table>
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<tr>
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<th>P63 (negative)</th>
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<td>ADC</td>
<td>ADC</td>
<td>ADC</td>
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<tr>
<td>TTF-1 (focal)</td>
<td>ADC</td>
<td>ADC</td>
<td>SCC</td>
</tr>
<tr>
<td>TTF-1 (negative)</td>
<td>IND</td>
<td>IND (CK5/6)</td>
<td>SCC</td>
</tr>
</tbody>
</table>
Significance of sub-classifying

Distinction between adenocarcinoma and squamous carcinoma impacts treatment and patient outcome

- Bevacizumab therapy is contraindicated in patients with squamous cell carcinoma because of potential fatal hemorrhage
- Adenocarcinoma histology is a strong predictor for outcome to pemetrexed therapy in advanced-stage patients
- EGFR mutation is a validated predictive marker for response and progression-free survival with EGFR-TKIs in the first-line therapy in advanced lung adenocarcinoma
- KRAS, ERCC1, RRM1, TS, EML4-Alk

Key Take Away Points

- Diagnostic cytologic features present
  - Squamous cell carcinoma
  - Adenocarcinoma
- Poorly differentiated Non-small cell carcinoma. Proceed to IHC (TTF-1, P63, CK5/6)
  - Non-small cell carcinoma, favor adenocarcinoma
  - Non-small cell carcinoma, favor squamous carcinoma
- IHC all negative or non-contributory
  - Non-small cell carcinoma
- Do not use non-squamous cell carcinoma
- Most important is diagnosis, conserve tissue for IHC and molecular studies

References

- Rekhtman N, Ang DC, Sima CS et al. Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimen. Mod Pathol 27 May 2011. Advance online publication

References

Small cell carcinoma (SCCa) vs non small cell carcinoma (NSCCa)

Important tenets in lung cytology

- Lung carcinoma in cytology is categorized into two broad histologic categories into small cell and non-small cell carcinoma
- Important clinical, therapeutic and prognostic significance
- Clinical management of lung cancer is based mainly on stage at diagnosis and also the histologic subtype
- Most SCCa are highly disseminated with possible metastasis at the time of diagnosis
- SCCa highly aggressive tumors with high mitotic rate
- SCCa remarkable sensitivity to chemotherapeutic agents and also radiation and therefore first in line of treatment
- Except for a rare Stage I SCCa, surgery is not considered

Clinical Disease Features

- Hemoptysis
- Dyspnea
- Chest pain
- Wheezing
- Post-obstructive pneumonia
- Malaise
- Distended neck veins (Superior Vena Cava Syndrome) or with recurrent laryngeal nerve paralysis
- Ectopic hormone production by the tumor producing symptoms like Cushing’s syndrome
- Large, bulky central tumors with paratracheal, mediastinal or other central node involvement diagnosed by EBUS-TBNA
- Chest X-ray often with peripheral mass or mediastinal widening
- Increased propensity for metastasis to brain diagnosed in cerebrospinal fluid cytology, and liver, pleura and other sites by FNA
- Long term survival is approximately 10% at 2 years

Etiology/Pathogenesis

SCCa and other neuroendocrine tumors of the lung

- Related to cigarette smoking with a male preponderance
- All lung tumors are derived from a common entodermal stem cell
- The current WHO classification based on divergent differentiation
- Wherein certain group of tumors acquire specific neuroendocrine markers such as chromogranin, synaptophysin and neural cell adhesion molecule (CD56) and are therefore classified as neuroendocrine tumors such as typical carcinoid, atypical carcinoid, small cell carcinoma and large cell neuroendocrine carcinoma
- Divergent differentiation also explains large cell neuroendocrine carcinoma and combined small cell carcinoma with non-small cell carcinoma (CSCLCs)
Etiology/Pathogenesis

SCCs and other neuroendocrine tumors of the lung

- CSCLCs arise when a subset of non-small cell carcinoma not considered morphologically neuroendocrine express neuroendocrine differentiation with neuroendocrine markers due to random acquisition of genetic or epigenetic alterations.
- WHO classification of CSCLCs as a subset of small cell carcinoma is based on the same clonal origin with neuroendocrine differentiation concept.
- This theory also explains the presence of rare cases of mixed small cell and large cell neuroendocrine carcinoma.

Cytologic Features

SCCs in sputum
- Malignant cells 1.5 times larger than mature lymphocytes
- Degenerate and appear as aggregates of cell variety along streaks of mucus
- Renalophagia and cytoplasm
- High nuc/cyto ratio
- Nuclear molding
-斑斓 nuclear molding
- Nucleus with nucleoli
- Single file arrangement

Cytologic Features

In bronchial wash/brush samples
- Better preserved cellular samples than sputum
- Malignant cells 1.5 times the size of lymphocytes
- High nuclear/cytoplasmic ratios
- Areas of crush artifact
- Congenital, basophilic cytoplasm
- Absent nucleoli
- Increased presence of atypical cells
- Increased presence of atypical cells
- Increased presence of atypical cells
- Increased presence of atypical cells

Statistical Performance of Problematic Cases

Cases from the ASCP NonGYN Assessment Program

Lung, FNA, Small Cell Carcinoma

Cumulative Statistics from Reference Cases

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
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<tbody>
<tr>
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<tr>
<td>Infectious/Inflammatory Process</td>
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<td>Benign Neoplasm</td>
<td>4</td>
<td>1.0</td>
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<tr>
<td>Lesion of Uncertain Biologic Potential</td>
<td>7</td>
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<tr>
<td>Positive for Malignancy</td>
<td>159</td>
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Total: 383 100.0%

Reference Interpretation Choices

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Statistical Performance of Problematic Cases

Cases from the ASCP NonGYN Assessment Program

Pulmonary, NonGYN, Small Cell Carcinoma

Cumulative Statistics from Reference Cases

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<tr>
<td>TOTAL</td>
<td>424</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Cytologic Features

General characteristics of SCCa

- SCCa can present as classic oat cell carcinoma (lymphocytelike), intermediate, spindle cell type or combined type (CSCLCs) with SCCa may have a squamous or adenocarcinoma component.
- Morphology of SCCa vary also based on the type of cytology specimen under consideration and the type of cytopreparation.
- Bronchial brush and fine needle aspiration specimens provide a direct sample of the tumor and provide a tumor with well preserved cells with all the classic SCCa criteria.
- The current WHO classification does not separate the various types of SCCa based on morphology since all the three types are similar in clinical presentation and response to chemotherapy.
- Combines all these subcategories into one as SCCa with CSCLCs as a subset.
- The subcategories are a morphologic continuum and also show similar response to neuroendocrine markers such as chromogranin, synaptophysin, and CD56.
- All show dense core neurosecretory granules in the cytoplasm in transmission electron microscopy.
Cytologic Features

SCCa in fine needle aspirations
- Numerous tumor cells arranged singly and in irregular syncyial aggregates
- Tumor cells may be small or intermediate in cell size and may be round to pleomorphic spindle shaped cells
- Cytoplasm is delicate and scanty
- N/C ratio of tumor cells is very high
- Marked apoptosis and necrosis in the background
- Nuclear molding seen within aggregates
- Nucleoli are absent or rarely seen as micronucleoli
- Chromatin is finely granular and shows salt and pepper consistency
- Occasionally some of the cells may be larger due to direct sampling of the tumor

SCCa in ThinPrep®

Common Differential Diagnoses
- Most common differential diagnoses include basal cell or reserve cell hyperplasia, lymphoma, and small cell type of squamous carcinoma

<table>
<thead>
<tr>
<th>Small cell carcinoma</th>
<th>Reserve cell hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular aggregates of cells showing anisokaryosis and anisocytosis</td>
<td>Sheets of small uniform cells and no single cells</td>
</tr>
<tr>
<td>Nuclear molding</td>
<td>No nuclear molding</td>
</tr>
<tr>
<td>Apoptosis and necrosis</td>
<td>No apoptosis and necrosis</td>
</tr>
<tr>
<td>Salt and pepper chromatin</td>
<td>Finely granular evenly distributed chromatin</td>
</tr>
</tbody>
</table>

Differential diagnosis
- Reserve cell or basal cell hyperplasia
- Small cell carcinoma

Common Differential Diagnoses
- Most common differential diagnoses include basal cell or reserve cell hyperplasia, lymphoma, and small cell type of squamous carcinoma

<table>
<thead>
<tr>
<th>Small cell carcinoma</th>
<th>Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular aggregates of cells showing anisokaryosis and anisocytosis</td>
<td>Monoclonal proliferation of single malignant cells</td>
</tr>
<tr>
<td>Nuclear molding</td>
<td>No nuclear molding</td>
</tr>
<tr>
<td>Apoptosis and necrosis</td>
<td>Lymphoglandular bodies may be present, nuclear debris may be seen</td>
</tr>
<tr>
<td>Salt and pepper chromatin</td>
<td>Coarsely granular evenly distributed chromatin with prominent nuclei</td>
</tr>
<tr>
<td>TTF1, Chromogranin, synaptophysin and CD56+</td>
<td>TTF1, Chromogranin, synaptophysin and CD56- but flow cytometry illustrates immunophenotyping with various lymphoid markers</td>
</tr>
</tbody>
</table>

Differential diagnosis
- Small cell carcinoma
- Large cell lymphoma
**Common Differential Diagnoses**

- Most common differential diagnoses include basal cell or reserve cell hyperplasia, lymphoma, and small cell type of squamous carcinoma

<table>
<thead>
<tr>
<th>Small cell carcinoma</th>
<th>Small cell variant of squamous carcinoma *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular aggregates of cells showing anisokaryosis and anisocytosis</td>
<td>aggregates of uniform population of small and/or large cells with focal intercellular bridges and isolated cell keratinization</td>
</tr>
<tr>
<td>Nuclear molding, Mosaic and apoptosis prominent</td>
<td>No nuclear molding, Mosaic and apoptosis not prominent</td>
</tr>
<tr>
<td>Salt and pepper chromatin</td>
<td>No prominent nuclei</td>
</tr>
<tr>
<td>TTF1 (chromogranin, synaptophysin, CD56+), CK5/6- EM shows cytoplasmic dense core granules</td>
<td>TTF1, chromogranin, synaptophysin, CD56- CK5/6+ EM shows cytoplasmic intermediate keratin filaments</td>
</tr>
</tbody>
</table>

**Histologic Features**

- nests, rosettes or ribbons of small cells
- peripheral palisading of the nests
- necrosis
- crush artifact
- Azzopardi effect may be seen
- cells round to fusiform
- scant cytoplasm
- finely granular chromatin with absent or inconspicuous nucleoli
- nuclear molding difficult visualize
- high mitotic rate exceeding 10/single HPF is characteristic

**Molecular/Ancillary Testing Protocols**

**Immunohistochemistry**

- TTF1 shows nuclear+
- Synaptophysin and CD56 cytoplasmic+
- Chromogranin positivity depends upon the number of neurosecretory granules and can be negative often
- Apoptosis related proteins such as bcl-2 and p53 are positive
- Large cell neuroendocrine carcinoma -membrane positivity to CK7, CK18, E-cadherin and B-catenin and differentiate from SCCa
- CSCLCs with areas showing squamous differentiation CK5/6+, TTF1-
- CSCLCs with areas showing adenocarcinoma will exhibit CK7+ positivity, TTF1+ will be positive in the SCCa component as well as adenocarcinoma component

**Molecular profile of SCCa**

- Chromosomal abnormalities (mainly deletions in the short arm) on chromosome 3 very common
- N-myc and L-myc oncogenes are most commonly amplified than C-myc
- Extent of deletions may vary between cell lines
- In deletions in the short arm on chromosome 3, the minimum common region of overlap was assigned to bands 3p23-3p24
- Loss of heterozygosity (LOH)22q13 common

**Therapeutic Options**

<table>
<thead>
<tr>
<th>Limited - stage disease (LD)</th>
<th>Extensive stage disease (ED)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the time of diagnosis, disease is confined to hemithorax of origin, the mediastinum or the suprACLAVICULAR nodes</td>
<td>Tumors spread beyond the suprACLAVICULAR nodes</td>
</tr>
<tr>
<td>Surgery may be considered as an option</td>
<td>Surgery not considered</td>
</tr>
<tr>
<td>Best results with thoracic radiation therapy combined with chemotherapy</td>
<td>Thoracic irradiation combined with chemotherapy does not significantly improve clinical outcome. Palliative radiation in minimizing symptoms of primary tumor, extensive disease especially brain, epidural or bone metastases</td>
</tr>
<tr>
<td>Chemotherapy usually a two drug combination of platinum and etoposide</td>
<td>Combination chemotherapy with etoposide plus cisplatin or carboplatin</td>
</tr>
<tr>
<td>Prophylactic cranial irradiation to prevent CNS recurrence</td>
<td>Prophylactic cranial irradiation to prevent CNS recurrence may be considered in patients with an excellent response to chemotherapy early on</td>
</tr>
</tbody>
</table>
Key Take Away Points

**Key Cytologic Features**
- Small cells about 1.5 times the size of lymphocyte
- High n/c ratio with scant delicate cytoplasm, salt and pepper chromatin and rare to absent micronucleoli
- Nuclear molding
- Crush artifact, apoptosis and necrosis

**Key Ancillary Tests**
- Immunohistochemistry showing characteristic synaptophysin and CD56 positivity in the cytoplasm and TTF1 positivity in the nucleus
- Electron microscopy shows cytoplasmic dense core granules
- Chromosomal abnormalities in short arm of chr.3
- Loss of heterozygosity (LOH)22q13

**Therapeutic Implications**
- Treatment for Stage 1- surgery may be considered as an option
- For most SCCa chemotherapy with platinum analogues and podophyllotoxins with or without radiation
- Prophylactic brain irradiation to prevent meningeal carcinomatosis

References

10. Siddiqui MT, Fatma N. Pulmonary neuroendocrine tumors: A review of clinicopathologic and cytologic features. ASCP Check Sample 2011(C-11-9(C-443)
Metastatic Neuroendocrine Neoplasms from Lung
Prototype – Small Cell Carcinoma

Disease Features
- Carcinoids - Grade I – malignant because metastasizes, rarely. More common with Grade II carcinoids
- Small Cell & Large Cell – Grade III
- Neural Type NET – rare
- Mets from lung more common than other primary site relates to frequency

Prototype – Small Cell
- Male : Female 3-4:1 peak in 60's
- Smokers
- Central, rarely peripheral – BUT almost always disseminated at time of diagnosis
  -60 to 75% with hilar/mediastinal mets
  - 2nd ry deposits often larger than 1 ry
- Products: Neuroamines & Neuropeptides
- Non specific clinical features: ple effusion, cough, hemoptysis, pop, wt. loss, abd pain, etc.

Cytology – Small Cell
- Small cell 2-4x size of lymphocytes, isolated but more commonly in synctia
- Delicate cells – traumatic effects
- High N/C ratio; scant cytoplasm
- Nuclear Molding
- Salt & Pepper to fine chromatin
- Nucleoli inconspicuous
- Mitotic activity
Metastatic to Pleural Fluid

• 83 yr old male with pleural effusion

• 45 year old male with lung mass and pleural effusion

Metastatic to Pleural Fluid

• Case 3 - 54 year old with history of invasive breast carcinoma 10 years ago now presents with 2 cm lung mass and pleural effusion.

Statistical Performance of Problematic Cases

Cases from the ASCP Non-GYN Assessment Program

Body Fluid, NonGYN, Metastatic Small Cell Carcinoma

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Total</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Negative/Reactive/ Hyperplasia/Developmental</td>
<td>55</td>
<td>1.7%</td>
</tr>
<tr>
<td>Infectious/Inflammatory Process</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Benign Neoplasm</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Lesion of Uncertain Biologic Potential</td>
<td>18</td>
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<tr>
<td>Positive for Malignancy</td>
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<tr>
<td>Total</td>
<td>3201</td>
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Reference Interpretation Choices

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<tr>
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<th>Total</th>
<th>%</th>
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<tbody>
<tr>
<td>BENIGN/REACTIVE MESOTHELIAL CELLS</td>
<td>59</td>
<td>1.8%</td>
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<td>BRONCHOALVEOLAR CARCINOMA</td>
<td>48</td>
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<td>GERM CELL TUMOR</td>
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<td>0.1%</td>
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<td>LARGE CELL UNDIFFERENTIATED CARCINOMA</td>
<td>14</td>
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</tr>
<tr>
<td>LYPHOMA</td>
<td>13</td>
<td>0.4%</td>
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<tr>
<td>MESOTHELIOMA</td>
<td>9</td>
<td>0.3%</td>
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<tr>
<td>METASTATIC ADENOCARCINOMA</td>
<td>134</td>
<td>4.2%</td>
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<tr>
<td>METASTATIC MESOTHELIOMA</td>
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<tr>
<td>RHUMATOID EFFUSION</td>
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</tr>
<tr>
<td>Total</td>
<td>3199</td>
<td>100.0%</td>
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</table>

Metastatic to Liver

• 73 yr old male with abnormal sputum cytology and multiple liver nodules
### Statistical Performance of Problematic Cases
**Cases from the ASCP NonGYN Assessment Program**

#### Liver/Pancreas, FNA, Metastatic Small Cell Carcinoma

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Total</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Negative/Reactive/Hyperplasia/Developmental</td>
<td>25</td>
<td>2.8%</td>
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<tr>
<td>Infectious/Inflammatory Process</td>
<td>0</td>
<td>0.0%</td>
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<tr>
<td>Benign Neoplasm</td>
<td>2</td>
<td>0.2%</td>
</tr>
<tr>
<td>Lesion of Uncertain Biologic Potential</td>
<td>10</td>
<td>1.1%</td>
</tr>
<tr>
<td>Positive for Malignancy</td>
<td>858</td>
<td>95.9%</td>
</tr>
<tr>
<td>Total</td>
<td>895</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

#### Reference Interpretation Choices

<table>
<thead>
<tr>
<th>Choice</th>
<th>Total</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>CHOLANGIOCARCINOMA</td>
<td>22</td>
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<tr>
<td>HEPATOCELLULAR CARCINOMA</td>
<td>37</td>
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<tr>
<td>LYMPHOMA</td>
<td>7</td>
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<tr>
<td>NORMAL CELLULAR ELEMENTS</td>
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<tr>
<td>METASTATIC MELANOMA</td>
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<td>0.6%</td>
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<tr>
<td>METASTATIC SMALL CELL CARCINOMA</td>
<td>748</td>
<td>83.6%</td>
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<tr>
<td>Total</td>
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#### Pancreas, FNA, Metastatic Carcinoid

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<tr>
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<tr>
<td>Infectious/Inflammatory Process</td>
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<td>0.3%</td>
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<tr>
<td>Benign Neoplasm</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Lesion of Uncertain Biologic Potential</td>
<td>23</td>
<td>8.0%</td>
</tr>
<tr>
<td>Positive for Malignancy</td>
<td>258</td>
<td>89.6%</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>100.0%</td>
</tr>
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</table>

#### Reference Interpretation Choices

<table>
<thead>
<tr>
<th>Choice</th>
<th>Total</th>
<th>%</th>
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<tbody>
<tr>
<td>HEPATOCELLULAR CARCINOMA</td>
<td>51</td>
<td>17.7%</td>
</tr>
<tr>
<td>NORMAL LIVER/HEPATOCYTES</td>
<td>4</td>
<td>1.4%</td>
</tr>
<tr>
<td>METASTATIC ADENOCARCINOMA</td>
<td>8</td>
<td>2.8%</td>
</tr>
<tr>
<td>METASTATIC SMALL CELL CARCINOMA</td>
<td>212</td>
<td>75.6%</td>
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<tr>
<td>No Interpretation Selected</td>
<td>13</td>
<td>4.5%</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

### Differential Diagnosis

- **Site dependent**
  - Liver (met adenoca, HCC, Cholangioca)
  - Pancreas (islet cell, adenoca)
  - Lymph Node (met ca, lymphoma)
  - Pleura (met adenoca, mesothelioma)

- **Age dependent**
  - Pediatric (“neural” type more common) vs. Adult

### Molecular/Ancillary Studies

- **IHC**:
  - especially CD 56/NCAM, Synaptophysin, Chromogranin
  - others include TTF-1, NSE, CD57, PAX-5, b-sCK (CAM 5.2, MNF116 )

- **Molecular**:
  - p53, Rb, Ki-67
  - p53, k-ras-2, c-raf-1

- Morphology and Immunoprofile define NET

- Molecular genetic testing for dx is limited

55 yr old dx with metastatic prostate ca 2yrs ago, rx with Lupron, then radiation 6 mo ago for low back and hip pain, now presents with supraclavicular mass – ultrasound guided FNA preformed:
Therapy

- Limited success over past decade in survival
- 5 year survival in Grade III NEC vs. NSCLC (stage I resectable) 30% vs. 60%
- Presence of NE differentiation in any portion of the tumor is associated with poor prognosis – thus accurate typing particularly noting presence of any neuroendocrine morphology or differentiation is critical for Rx.
- No “targeted therapy” for NEC as yet.

Key Take Away Points

- Key Cytology Features:
  - salt & pepper to fine granular chromatin pattern,
  - nuclear molding, mitosis, high N/C scant cytoplasm.
- Key Ancillary Tests:
  - IHC – Synaptophysin, Chromogranin, CD56
- Therapeutic Implications:
  - Presence of NE morphology or differentiation in any portion of tumor impacts on Rx and outcome therefore accurate tumor typing is critical!

References

- DeMay RM. The Art of Science of Cytopathology Chicago, IL: ASCP Press 1996
- Felix G. Fernandez, MD and Richard J. Bartolozzi, MD, PhD. Large-Cell Neuroendocrine Carcinoma of the Lung October 2006, Vol. 13, No. 4, 270-275
- Gabriel Sica, MD, PhD, Madeline F. Vazquez, MD, Nassar Altorki, MD, Jeffrey Port, MD, Paul C. Lee, MD, Wing Y. Ng, MD; Elizabeth Heimpel, MD and Angela Sepp, MD. PAX-5 Expression in Pulmonary Neuroendocrine Neoplasms, Its Usefulness in Surgical and Fine-Needle Aspiration Specimens Am J Clin Pathol 2008; 129, 556-562
Problematic Cases in Pulmonary Cytopathology

As Identified from the ASCP NonGYN Assessment Program

Adebowale J. Adeniran, MD
ASCP NonGYN Assessment Committee
Medical Member
Assistant Professor of Pathology
Yale University School of Medicine
New Haven, CT

ASCP CME Disclosure of Relevant Financial Relationships

In accordance with ACCME Standards, I verify that I have no relevant financial relationships to disclose, with proprietary entities producing health care goods or services, that are discussed in this CME activity.

Guidelines for interpreting body cavity fluids

We need to know:
1. The significance of a positive diagnosis
2. The most reliable criteria for a diagnosis of malignancy
3. The common pitfalls
4. When to be cautious
5. When to look really hard for the foreign cells
6. How to deal with the problematic case

Pleural fluid cytology

The problem

Metastatic adenocarcinoma versus benign/reactive mesothelial cells
Metastatic adenocarcinoma versus mesothelioma
Mesothelioma versus reactive mesothelial cells

Guidelines for body cavity fluids

- Cells in 3-D
- Large groups of cells with complex arrangement
- A discrete population of cells distinct from mesothelial cells
Case #1
An 86-year-old female with a history of pneumonia and empyema has persistent bilateral pleural effusions and pleural thickening. Thoracocentesis was performed and the pleural fluid was submitted for evaluation.

Key diagnostic features
• Mostly single cell presentation with occasional clusters
• Predominantly mononuclear or binuclear cells
• Round cells with low nuclear/cytoplasmic ratios
• Round, bland nuclei with small inconspicuous nucleoli
• Dense cytoplasm with clear outer rim (“lacy skirt”)
• Evidence of window-like slits between cells

Statistical Performance of Problematic Cases
Cases from the ASCP NonGYN Assessment Program
Pleural Fluid, NonGYN, Benign/Reactive Mesothelial Cells

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Total</th>
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<td>945</td>
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<td>Infectious/Inflammatory Process</td>
<td>46</td>
<td>4.1%</td>
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<td>Benign Neoplasm</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Lesion of Uncertain Biologic Potential</td>
<td>23</td>
<td>2.2%</td>
</tr>
<tr>
<td>Positive for Malignancy</td>
<td>45</td>
<td>4.2%</td>
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<tr>
<td>Total</td>
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Reference Interpretation Choices

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<th>Cause</th>
<th>Total</th>
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</tr>
</thead>
<tbody>
<tr>
<td>ADENOCARCINOMA, NOS</td>
<td>35</td>
<td>4.8%</td>
</tr>
<tr>
<td>ATYPICAL LYMPHOID CELLS, R/O MALIGNANCY</td>
<td>16</td>
<td>2.2%</td>
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<tr>
<td>BENIGN/REACTIVE MESOTHELIAL CELLS</td>
<td>669</td>
<td>86.2%</td>
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<tr>
<td>GRANULOMATOUS INFLAMATION</td>
<td>23</td>
<td>3.2%</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2</td>
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<tr>
<td>MALIGNANCY</td>
<td>6</td>
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<td>1</td>
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</tr>
<tr>
<td>Total</td>
<td>723</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Causes and key cytologic features of Non-neoplastic effusions
• Congestive heart failure
• Occasional hemosiderophages
• Pulmonary infarction
• Non-specific mixed inflammation
• Pneumonia
  • Inflammatory cells of various types depending on the nature and duration of the pneumonia
  • Chemotherapy and Radiation pneumonitis
  • No consistent and distinctive changes
• Autoimmune serositis (e.g. SLE)
  • Characteristic LE cells and moderate amount of neutrophils
• Rheumatoid pleuritis
  • Abundant clumps of granular debris and macrophages
• Tuberculosis
  • High proportion of lymphocytes and few mesothelial cells
• Others: Hypothyroidism, Nephrotic syndrome, Cirrhosis
Typical immunostaining patterns of Reactive mesothelial cells and Adenocarcinoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Reactive mesothelial cells</th>
<th>Adenocarcinoma</th>
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</thead>
<tbody>
<tr>
<td>AE1/AE3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CK5/6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calretinin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HBME-1</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>WT-1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D2-40</td>
<td>+</td>
<td>-</td>
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<tr>
<td>TTF-1</td>
<td>-</td>
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</table>

Case #2

An 85-year-old man presented with increasing shortness of breath. Chest film revealed a large right pleural effusion with compressive atelectasis of the right lung. Thoracocentesis yielded 800cc of serosanguinous fluid which was centrifuged and prepared for cytologic evaluation.

Key diagnostic features

- Cellular sample with 2-cell population
- 3-D cell groups with common cell border
- Numerous large clusters
- Eccentric nuclei with marked hyperchromasia and prominent irregular nucleoli
- Homogeneous delicate cytoplasm with large randomly distributed secretory vacuoles
- Lacunae (cell block sections)
Most common tumors that cause malignant pleural effusions by sex

<table>
<thead>
<tr>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Breast</td>
</tr>
<tr>
<td>Lymphoma/leukemia</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Lymphoma/leukemia</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Ovary</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>Genitourinary (kidney, bladder, prostate)</td>
<td>Endometrium</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Mesothelioma</td>
</tr>
</tbody>
</table>

Cytologic differences between Malignant Mesothelioma and Adenocarcinoma

<table>
<thead>
<tr>
<th>Feature</th>
<th>Malignant Mesothelioma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell population</td>
<td>Monotonic mesothelial cells</td>
<td>2-cell population</td>
</tr>
<tr>
<td>Cellular groups</td>
<td>3-D, tight spheres and loose clusters with knobby borders</td>
<td>Windows commonly seen</td>
</tr>
<tr>
<td>Psammoma bodies</td>
<td>Few in number when present</td>
<td>Numerous when present</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Usually central or paracentral</td>
<td>Usually eccentric</td>
</tr>
<tr>
<td></td>
<td>Mild hyperchromasia</td>
<td>Marked hyperchromasia</td>
</tr>
<tr>
<td></td>
<td>Small or very prominent nucleoli</td>
<td>Frequently prominent irregular nucleoli</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Dense center with fuzzy edges</td>
<td>Delicate, homogenous</td>
</tr>
<tr>
<td></td>
<td>2-tone staining</td>
<td>Uniform stain</td>
</tr>
<tr>
<td>Vacuoles</td>
<td>Perinuclear and submembranous</td>
<td>Secretory, large</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Randomly distributed</td>
</tr>
<tr>
<td>Multinucleated giant cells</td>
<td>Common</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Typical immunostaining patterns of Malignant Mesothelioma and Adenocarcinoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Malignant mesothelioma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE1/AE3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CK5/6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Calretinin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HMBE-1</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>WT-1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D2-40</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CEA</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ber-EP4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B72-3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD15</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MOC-31</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TTF-1</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Case #3

A 79-year-old man was seen at the emergency room for possible myocardial infarction. On exam a large right pleural fluid was discovered as was thickening of the chest wall. Thoracentesis yielded 1000cc of bloody fluid and was submitted for processing and cytologic evaluation.
Key diagnostic features

- Large clusters with scalloped (“knobby”) edges
- Giant mesothelial cells, including binucleated multinucleated forms
- Cellular clasping and “cell within cell” appearance
- Round, centrally placed nucleus and prominent nucleolus
- Dense cytoplasm with peripheral “halo”
- Normal nuclear-to-cytoplasmic ratio
- Windows

Statistical Performance of Problematic Cases

Cases from the ASCP NonGYN Assessment Program

Pleural Fluid, NonGYN, Mesothelioma

Cumulative Statistics from Reference Cases

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative/Reactive/Hyperplasia/Developmental</td>
<td>264</td>
<td>0.0%</td>
</tr>
<tr>
<td>Infectious/Inflammatory Process</td>
<td>79</td>
<td>2.4%</td>
</tr>
<tr>
<td>Benign Neoplasm</td>
<td>10</td>
<td>0.3%</td>
</tr>
<tr>
<td>Lesion of Uncertain Biologic Potential</td>
<td>36</td>
<td>1.1%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2925</strong></td>
<td><strong>88.3%</strong></td>
</tr>
</tbody>
</table>

Reference Interpretation Choices

Common Differential Diagnoses of Mesothelioma

- Reactive mesothelial cells
- Metastatic tumor
  - Adenocarcinoma
  - Squamous cell carcinoma
  - Epithelioid hemangioendothelioma
**Mesothelioma versus Reactive mesothelial cells**

- Mesothelioma cells are markedly larger in size
- Chromatin in mesothelioma stains variably darker and may be irregular in distribution
- Nucleoli are usually present and may be enlarged and multiple
- Macronucleoli are associated with malignancy and may be the sole criterion of malignancy
- Reactive mesothelial proliferations may show high cellularity, cytologic atypia, papillary excrescences, and entrapment
- Malignant mesotheliomas may appear bland

**Ancillary studies** are of little value in this scenario

- Immunohistochemistry
  - Desmin
  -EMA
  - GLUT-1
  - P53
  - Ki67
  - Oncofetal Protein IMP3
- Molecular studies
  - 9p21 homozygous deletion
- Distinction remains a clinicopathologic one

**Pathologic parameter** is morphologic assessment by standard H&E light microscopy

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**Effusion cytology caution!!!**

*Positive effusion = stage 4 metastatic disease*

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**Pitfalls in body cavity fluids**

- Single malignant cells
- Uniform population of tumor cells with virtually no mesothelial cells
- Reactive atypia

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**Clues to identifying single malignant cells**

- Intracytoplasmic mucin may be a distinct, well-defined vacuole i.e. target-like, or may be multiple small, fine vacuoles
- High nuclear/cytoplasmic ratio (N/C) cells
- History-breast, gastric or other GI primaries are especially notorious for producing dispersed malignant cells
- Always look for the lurking, hiding malignant cells

---

**Key Take Away Points**

- **Approach to a difficult effusion**
  - History
  - Cell arrangement
  - Compare with obvious mesothelial cells
  - Cytomorphology
  - Prepare additional smears
  - Prepare cell block
  - Ancillary studies
  - Consultation
  - Communicate limitations
- **Therapeutic Implications**
  - Positive effusion = stage 4 metastatic disease
  - Mesothelioma = radical surgery + radiation & chemotherapy
References


