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Dr. Andrew Churg declares he/she has no conflict(s) of interest under ACCME rules, but he does consult with law firms in asbestos litigation.

Case 4 History

• A 57 yo woman presented with abdominal pain and increasing girth. CT scan showed ascites and small areas interpreted as tumor surrounding various abdominal organs. A paracentesis was performed and a cytology specimen obtained.
• The clinician asked whether a definitive diagnosis was possible on this material or whether a biopsy was needed.
Diagnosis Case 4

- Epithelial mesothelioma of the peritoneum, diagnosed purely on effusion cytology
**Issues in the Cytologic Diagnosis of Mesothelioma in Effusions**

- Can you ever make a definitive diagnosis of mesothelioma on an effusion cytology specimen?
- Useful clinical features
- Published criteria for MM in effusion cytology
- How to separate mesothelial cells from carcinoma cells
- Markers that generally don’t work
- New and useful markers: BAP1 and p16 FISH

---

**Do Clinical Features Help?**

- Nodular pleural thickening strongly suggestive of malignancy
- Pleural thickening involving the mediastinal pleura strongly suggestive of malignancy
- Be very cautious in diagnosing mesothelioma in the absence of pleural thickening on CT scan or direct visualization of tumor by operator
- An effusion by itself could be anything

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**Issues in the Diagnosis of Mesothelioma in Effusion Cytology Specimens**

- Is the process mesothelial?
  - Should not be a problem if you have a cell block to run IHC
  - Be wary of long CytoLyt fixation: leaches calretinin and WT-1 from cells (and BAP1 to a lesser extent as well)
- If mesothelial, is the process malignant?
  - Morphology
  - Immunohistochemistry
  - FISH

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**The Diagnosis of Malignant Mesothelioma in Effusion Cytology**

A Reappraisal and Results of a Multi-Institutional Survey

Ajit Paatni, MD1; Kirkee Rasara, MD2; Maureen F. Zukowski, MD2; and Ritu Nayar, MD2

Cancer Cytopathol 2013

BACKGROUND: The diagnosis of malignant mesothelioma (MM) in effusion specimens is controversial. A multi-institutional (Northwestern University), a primary diagnosis of MM was made on fluid cytology specimens. In an effort to estimate the practice at other institutions, a survey was disseminated regarding the diagnosis of MM. The authors also reviewed their own institutions' experience with primary cytologic diagnosis of MM. METHODS: Patients with MM at the study institution were identified from 1992 through 2001. Fluid cytology specimens providing histologic diagnoses were reviewed. A survey was sent to a number of authors to assess practice patterns regarding the diagnosis of MM. RESULTS: At this study institution, 19 cases of MM had effusion specimens providing the diagnosis: 11 were malignant; 7 were malignant. Almost all laboratories (93) agreed to diagnosis MM in effusion specimens in situ hybridization for p16 and/or bcl-2 in situ hybridization for p53 to help exclude benign from malignant mesothelioma were assessed. In 15 (78%) of the laboratories, one or more additional immunostains were used, with a median of 3. The overall median diagnostic evaluation was 1 week. Most laboratories (77%) felt that 65% of labs will make a definitive dx on cytology alone; 35% will not.
**Recommended Antibodies for Separating Mesothelioma from Adenocarcinoma**

- **Mesothelioma Markers**
  - Calretinin
  - Cytokeratin 5/6\(^1\)
  - WT-1\(^2\)
  - D2-40
  - Mesothelin

- **Carcinoma Markers**
  - Claudin-4
  - MOC-31\(^3\)
  - TTF-1
  - p40 (for squamous ca)
  - PAX-8 (gyne tumors)

\(^1\)Positive in many squamous carcinomas
\(^2\)Not in the peritoneum in women
\(^3\)May cross reaction with mesotheliomas

**Morphologic Criteria for an Effusion Cytologic Dx of Mesothelioma -1**

- High cellularity
- Balls/papillary clusters
- Numerous cytoplasmic vacuoles
- Balls > 50 cells
- Very large cells
- Single population ranging from normal to atypical
- Cell within cell arrangement
- Macronucleoli

Hjerpe Welker Henderson Nguyen

**Morphologic Criteria for an Effusion Cytologic Dx of Mesothelioma -2**

- Extracellular matrix cores within tissue
- Blebbing of cell membrane
- Windows between cells
- Microrvilli on cell surface
- Gradation of staining
- Cytoplasm
- Low N:C ratio
- Single or double nuclei

Hjerpe Welker Henderson Nguyen

**IHC Markers Proposed to Be Useful for Separating Benign from Malignant Mesothelial Processes**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Proposed Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pankeratin</td>
<td>Seen in both benign and malignant mesothelial processes</td>
</tr>
<tr>
<td>EMA</td>
<td>Claimed to be marker of malignancy</td>
</tr>
<tr>
<td>p53</td>
<td>Claimed to be marker of malignancy</td>
</tr>
<tr>
<td>Desmin</td>
<td>Claimed to be marker of benign mesothelial cells</td>
</tr>
<tr>
<td>GLUT-1</td>
<td>Claimed to be marker of malignancy</td>
</tr>
<tr>
<td>X-linked inhibitor of apoptosis</td>
<td>Claimed to be marker of malignancy</td>
</tr>
<tr>
<td>IMP-3</td>
<td>Claimed to be marker of malignancy</td>
</tr>
</tbody>
</table>

Abbreviations: EMA, epithelial membrane antigen; GLUT-1, glucose transporter-1.

Churg and Galateau-Salle Arch Path 2012
Survival in Atypical Mesothelial Proliferations at 5 Years by IHC Stain Result (Cutoff: 10% Staining) (Churg and Galateau-Salle, Arch Path 2012)

- **Desmin**
  - <10%
  - >10%
- **EMA**
  - <10%
  - >10%
- **p53**
  - <10%
  - >10%

Survival at 5 Years Using a 10% Staining Cutoff

- **Surviving**

Collaborators

- Harry Hwang, PhenoPath Labs
- Allen Gown, PhenoPath Labs
- Brandon Sheffield, Vancouver General Hospital

Function & Detection of Genes Mutated or Lost in Mesotheliomas

- **BAP1**: controls DNA repair and genes related to cell proliferation, cell cycle, cell death. Believed to function as a tumor suppressor.
  - Wild type BAP1 protein is detectable by IHC
  - Deletions or mutations in BAP1 lead to loss of IHC detectable nuclear protein (protein either lost or sequestered in cytoplasm)
- **p16^{INK4a} (CDKN2A)**: Prevents cell cycle progression. Acts as a tumor suppressor.
  - Loss of p16 (9p21 locus) can be detected by FISH
  - p16 IHC does not give the same information as p16 FISH!
- **NF2**: Regulates cell proliferation in response to adhesion. Functions as a tumor suppressor
  - Detection of abnormal NF2 in tissue sections problematic

Frequency of BAP1 Loss by IHC in Mesotheliomas (Tissue)

- **Report**
  - **N**
  - **Epi**
  - **Mixed**
  - **Sarc**
- **Nasu 2015**
  - 70
  - 74%
  - 59%
  - 50%
- **Farzin 2015**
  - 229
  - 63%
  - 42%
  - 18%
- **Yoshikawa 2015**
  - 23
  - 81%
  - -----14%-----
- **Sheffield 2015**
  - 22
  - 56%
  - 10%
- **Cigognetti 2015**
  - 212
  - 70%
  - 60%
  - 15%
- **Hwang 2016**
  - 15%
- **Singhi 2015**
  - 75
  - 64%
  - -----10%-----
- **pleural** **peritoneal**

(Churg et al: Arch Path 2016)
**Frequency of BAP1 Loss by IHC in Benign Reactions (Tissue)**

- Sheffield AJSP 2015
- & Churg unpublished 0/53
- Galateau-Salle unpublished 0/23
- Cigognetti Mod Path 2015 0/25
- McGregor Human Path 2016 0/20

*Churg et al: Arch Path 2016*

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**Epithelial Mesotheliomas (Tissue) Showing Homozygous Deletion of p16 by FISH**

<table>
<thead>
<tr>
<th>Report</th>
<th>Pleural</th>
<th>Peritoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiosea 2008</td>
<td>67%</td>
<td>25%</td>
</tr>
<tr>
<td>Monaco 2011</td>
<td>70%</td>
<td>51%</td>
</tr>
<tr>
<td>Kasinskas 2010</td>
<td>45%</td>
<td>36%</td>
</tr>
<tr>
<td>Chung 2010</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>Takeda 2010</td>
<td>86%</td>
<td></td>
</tr>
<tr>
<td>Wu 2013</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>Hwang 2014</td>
<td>45%</td>
<td>14%</td>
</tr>
<tr>
<td>Sheffield 2015</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>Ito 2015</td>
<td></td>
<td>48%</td>
</tr>
<tr>
<td>Singhi 2015</td>
<td>58%</td>
<td>29%</td>
</tr>
</tbody>
</table>

*Churg et al: Arch Path 2016*

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**Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas**

Simion Chiosea1, Alyssa Kasinskas2, Philip J Gaglio3, Kish A Mitchell4, Dani S Zander5 and Sanja Bacic6

1Department of Anatomic Pathology, Department of Pathology, University of Pittsburgh Medical Center, Presbyterian University Hospital, Pittsburgh, PA, USA; 2Department of Pathology, The Methodist Hospital, Houston, TX, USA and 3Department of Pathology, Penn State Milton S. Hershey Medical Center, Hershey, PA, USA.

Definitive diagnosis of malignant mesothelioma in small specimens can be extremely difficult based on morphology alone. Homozygous deletion of 9p21, the locus harboring the p16 gene, has been reported as the most common genetic alteration in malignant mesothelioma. Recent studies demonstrated that this deletion may be useful for differentiating benign from malignant mesothelial proliferations in cytology specimens. The aim of this study was to evaluate the diagnostic utility of homozygous deletion of 9p21 assessed by fluorescence in situ hybridization (FISH) in mesothelial proliferations involving normal surfaces in pleural-embodied tissue. p16 protein immunexpression was also explored as a potential diagnostic aid. FISH analysis demonstrated homozygous deletion of the 9p21 locus in 35 of 52 cases (67%) of pleural mesotheliomas and in 3 of 20 cases of peritoneal mesotheliomas (15%) (P=0.006). Loss of immunexpression of p16 was observed in 71% of the peritoneal mesotheliomas, 60% of the pleural malignant mesotheliomas and 19% of the reactive mesothelial cells. Homozygous deletion did not correlate with p16 protein expression in any of the studied groups. Our study suggests that 9p21 homozygous deletion assessed by FISH in pleural-embodied tissue may be helpful for differentiating between malignant mesotheliomas and reactive mesothelial proliferations. A discrepancy between p16 protein expression and homozygous deletion suggests that other molecular mechanisms may play a role in p16 protein expression in mesothelial proliferations.

*Arch Path (2008) 71: 740-747, 10.1093/iodpath/iod045 published online 7 March 2008*

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**Fraction of Benign Mesothelial Reactions (Tissue) Showing Homozygous Deletion of p16 by FISH**

<table>
<thead>
<tr>
<th>Report</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illei 2003</td>
<td>0/19</td>
<td>0%</td>
</tr>
<tr>
<td>Chiosea 2008</td>
<td>0/40</td>
<td>0%</td>
</tr>
<tr>
<td>Monaco 2011</td>
<td>0/70</td>
<td>0%</td>
</tr>
<tr>
<td>Chung 2010</td>
<td>0/11</td>
<td>0%</td>
</tr>
<tr>
<td>Wu 2013</td>
<td>0/10</td>
<td>0%</td>
</tr>
<tr>
<td>Sheffield 2015</td>
<td>0/40</td>
<td>0%</td>
</tr>
<tr>
<td>Ito 2015</td>
<td>0/30</td>
<td>0%</td>
</tr>
</tbody>
</table>

Total 0/220 0%

*Churg et al: Arch Path 2016*
BAP1 lost mesothelioma. Arrows indicate stromal cells and lymphocytes.

BAP1 wild type-mesothelioma. Arrows indicate stromal cells and lymphocytes.

Utility of BAP1 Immunohistochemistry and p16 (CDKN2A) FISH in the Diagnosis of Malignant Mesothelioma in Effusion Cytology Specimens

Harry C. Hoang, MD,* Brandon S. Sheffield, MD,†‡ Stephanie Rodriguez, HTL, MB, ASCP,* Kim Thornton, ASCP, QIMHC,* Christopher H. Yee, MBBX,* Allen M. Goss, MD,* and Andrew Chay, MD†


Abstract: The diagnosis of malignant mesothelioma in effusion cytopathology specimen is controversial. Intraepithelial BAP1 immunohistochemistry and p16 fluorescence in situ hybridization (FISH) have recently been reported as reliable markers of malignancy in biopsies of mesotheliomas. In addition, recent studies in biopsies and in situ hybridization, we examined 40 biopsies of epithelial mesotheliomas and 3 benign mesothelial tumors, and found that all samples showed retention of BAP1 or p16 in the benign mesothelial cells. Four cytology specimens were tested for the same BAP1 and p16 retention or loss as in the biopsy specimens.
Frequency of BAP1 Loss by IHC in Effusion Cytology Specimens of Mesotheliomas

- Hwang AJSP 2016
  - 10/15 (67%) CytoLyt fixed cell blocks
- Andrici Mod Path 2015
  - 43/75 (57%) of effusions associated from definite mesotheliomas
  - 4/100 (4%) effusions thought to be benign—but technically poor preps with negative internal positive control—authors discount these results
- Gigognetti Mod Path 15
  - 27/45 (64%)

Frequency of p16 Homozygous Deletion by FISH in Effusion Cytology Specimens of Mesothelioma

- Illei 2003 6/7 (86%) CytoLyt fixed cytospins
- Onofre 2008 16/33 (49%) Air dried smears
- Monaco 2011 11/16 (69%) Formalin fixed cell blocks
- Hida 2015 14/20 (70%) Alcohol fixed smears
- Hwang 2016 8/11 (73%) CytoLyt fixed cell blocks
Cells mark as mesothelial
Cellular: Yes
Balls of cells: Yes
Cytologic atypia: No
Macronucleoli: No

BAP1 lost, p16 not deleted

Corresponding biopsy

70M with pleural effusion
CT appearance after thoracentesis = tumor in pleura
Cells mark as mesothelial
Cellular: No
Balls of cells: a few
Cytologically atypical: Yes

BAP1 not lost, p16 deleted
p16
BAP1
Corresponding biopsy

72M pleural effusion. This is the CT available with the cytology specimen. No biopsy

Cells mark as mesothelial
Cellular: yes
Balls of cells: yes
Cytologic atypia: yes
Cell within cell: yes
BAP1 lost, p16 FISH not done. Dx = mesothelioma despite no evidence of tumor on CT

Important Caveats
- p16 deletion is seen in many types of tumors
- BAP1 loss is seen in other types of tumors
- You must confirm process is mesothelial first!
- The sensitivity of both BAP1 loss and p16 FISH deletion in pleural epithelial mesotheliomas is only around 60 to 70%
- p16 FISH deletion is less common in peritoneal epithelial mesotheliomas
- p16 FISH on tissue sections requires correction for truncation artifacts and counting of 50 to 100 cells
- BAP1 staining requires a positive internal control to be interpretable
- Loss of BAP1 by IHC or p16 by FISH makes a mesothelial process malignant; however, failure to find loss/deletion does not make a mesothelial process benign!

Take Home Points
- CT information is very helpful in evaluating effusion cytology specimens of potential mesotheliomas
  - Be cautious if there is no clinical or radiologic evidence of tumor
- Provided a cell block is available, separation of mesothelial cells from carcinoma cells is no longer a problem
- Morphology can be helpful but many mesotheliomas have deceptively bland cytology
- BAP1 IHC and p16 FISH confirm a diagnosis of mesothelioma if they are lost/deleted, but many mesotheliomas do not show loss of these markers

Establish mesothelial nature of atypical cells by immunohistochemistry
- Cells are mesothelial
  - Run BAP1 IHC
  - Stop: Do not run BAP1 or p16 FISH
- Cells are not mesothelial
  - BAP1 not lost
    - Diagnosis of mesothelioma established
    - Run p16 FISH
    - Tests not useful (process may still be a mesothelioma)
  - BAP1 lost
    - p16 deleted
      - Diagnosis of mesothelioma established
    - p16 not deleted
      - Diagnosis of mesothelioma established

Helpful Caveats
- p16 deletion is seen in many types of tumors
- BAP1 loss is seen in other types of tumors
- You must confirm process is mesothelial first!
- The sensitivity of both BAP1 loss and p16 FISH deletion in pleural epithelial mesotheliomas is only around 60 to 70%
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