Are there any new tissue biomarkers useful for diagnosis?

Luka Brcic
Diagnostic & Research Institute of Pathology
Medical University of Graz, Austria

31st European Congress of Pathology
07. – 11.09.2019, Nice, France
Problems in MPM diagnosis

Varying morphologies of MPM

+ Pleura is common site for metastasis

Wide differential diagnoses

Problems in MPM diagnosis

- Reactive versus neoplastic
- Mesothelioma versus metastatic carcinoma
- Mesothelioma versus sarcoma/other
OUTLINE

➢ OLD markers (used and not-used)

➢ “NEW” markers

➢ PROMISING markers
OUTLINE

- OLD markers (used and not-used)
- “NEW” markers
- PROMISING markers
Table 3. Immunohistochemical Markers Used in the Differential Diagnosis of Pleural Malignant Mesothelioma Versus Lung Adenocarcinoma Involving the Pleura

<table>
<thead>
<tr>
<th>Marker</th>
<th>Current Value/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelioid mesothelioma (positive mesothelioma markers)</td>
<td></td>
</tr>
<tr>
<td>Calretinin</td>
<td>Very useful. Is demonstrated in nearly all epithelioid mesotheliomas when antibodies to human recombinant calretinin are used. The staining is often strong and diffuse and is both nuclear and cytoplasmic; 5%–10% of lung adenocarcinomas are positive, but the staining is usually focal.</td>
</tr>
<tr>
<td>Cytokeratin 5 or 5/6</td>
<td>Very useful. Expressed in 75%–100% of mesotheliomas. About 2%–20% of lung adenocarcinomas can be focally positive.</td>
</tr>
<tr>
<td>WT1</td>
<td>Very useful. Approximately 70%–95% of mesotheliomas show nuclear positivity. Lung adenocarcinomas are negative.</td>
</tr>
<tr>
<td>Podoplanin (D2-40)</td>
<td>Very useful. About 90%–100% of mesotheliomas show positivity along the cell membranes; ≤15% of lung adenocarcinomas are focally positive.</td>
</tr>
<tr>
<td>Lung adenocarcinoma (positive carcinoma markers)</td>
<td></td>
</tr>
<tr>
<td>Claudin 4</td>
<td>Very useful. Essentially all lung adenocarcinomas are positive. Immunoreaction is often strong and diffuse and occurs along the cell membrane in a continuous or punctate pattern. Mesotheliomas are negative.</td>
</tr>
<tr>
<td>MOC31</td>
<td>Very useful. About 95%–100% of lung adenocarcinomas are positive; 2%–10% of mesotheliomas show focal staining.</td>
</tr>
<tr>
<td>CEA</td>
<td>Very useful. About 80%–100% of lung adenocarcinomas are positive; ≤5% of mesotheliomas are focally positive.</td>
</tr>
<tr>
<td>B72.3</td>
<td>Very useful. About 75%–85% of lung adenocarcinomas are positive. Very few mesotheliomas are positive.</td>
</tr>
<tr>
<td>BER-EP4</td>
<td>Very useful. About 95%–100% of lung adenocarcinomas are strongly positive; ≤20% of mesotheliomas are focally positive.</td>
</tr>
<tr>
<td>BG8 (Lewis')</td>
<td>Very useful. Approximately 90%–100% of lung adenocarcinomas are positive; 3%–7% of mesotheliomas show focal reactivity.</td>
</tr>
<tr>
<td>TTF-1</td>
<td>Very useful. About 75%–85% of lung adenocarcinomas show nuclear positivity (usually all nonmucinous lung adenocarcinomas are positive). It is not expressed in mesotheliomas.</td>
</tr>
<tr>
<td>Napsin A</td>
<td>Very useful. About 80%–90% of lung adenocarcinomas show cytoplasmic staining. It is not expressed in mesotheliomas.</td>
</tr>
</tbody>
</table>

Abbreviations: WT1, Wilms tumor-1; BG8, blood group 8; CEA, carcinoembryonic antigen; TTF-1, thyroid transcription factor-1.
<table>
<thead>
<tr>
<th>Marker</th>
<th>Current Value/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelioid mesothelioma (positive mesothelioma markers)</strong></td>
<td><strong>WT1</strong> Very useful. Up to 95% of mesotheliomas show nuclear positivity. Lung squamous carcinomas are negative.</td>
</tr>
<tr>
<td></td>
<td><strong>Calretinin</strong> Somewhat useful. Essentially all mesotheliomas are positive, often strongly and diffusely, with nuclear and cytoplasmic staining. About 40% of lung squamous carcinomas are positive, but the staining is often focal.</td>
</tr>
<tr>
<td></td>
<td><strong>Podoplanin (D2-40)</strong> Not useful. About 80%–100% of mesotheliomas are positive; 50% of lung squamous carcinomas also stain.</td>
</tr>
<tr>
<td></td>
<td><strong>Cytokeratin 5 or 5/6</strong> Not useful. Expressed in 75%–100% of mesotheliomas and 100% of lung squamous carcinomas.</td>
</tr>
<tr>
<td><strong>Lung squamous carcinoma (positive carcinoma markers)</strong></td>
<td><strong>p40 or p63</strong> Very useful. 100% of lung squamous carcinomas show strong and diffuse nuclear positivity for either marker. About 2.5% and 7% of mesotheliomas are focally positive for p40 and p63, respectively.</td>
</tr>
<tr>
<td></td>
<td><strong>Claudin 4</strong> Very useful. About 95% of squamous cell carcinomas are positive. Mesotheliomas are negative.</td>
</tr>
<tr>
<td></td>
<td><strong>MOC31</strong> Very useful. About 97%–100% of lung squamous carcinomas are positive; 2%–10% of mesotheliomas show focal staining.</td>
</tr>
<tr>
<td></td>
<td><strong>BG8 (Lewisδ)</strong> Very useful. About 80% of lung squamous carcinomas are positive; 3%–7% of mesotheliomas show focal staining.</td>
</tr>
<tr>
<td></td>
<td><strong>BER-EP4</strong> Useful. Approximately 85%–100% of lung squamous carcinomas are positive; ≤20% of mesotheliomas are focally positive.</td>
</tr>
<tr>
<td></td>
<td><strong>Cytokeratin 5 or 5/6</strong> Not useful. All lung squamous carcinomas (100%) and most mesotheliomas (75%–100%) are positive.</td>
</tr>
</tbody>
</table>

Abbreviations: WT1, Wilms tumor-1; BG8, blood group 8.
Other markers for differentiation of MPM and lung carcinoma

- **MUC4**, neg in EMM, pos in 83.3% lung AC and 89.3% lung SqCC
- **MUC21**, better than TTF-1, napsin A and MUC4. Pos in 3% MPM and in 96% lung AC
- **Glypican-1**, “highly sensitive marker for EMM”: 100% sensitivity, 97% specificity
  - However, Chiu et al. demonstrated it was positive in MPM, AC and MH
- **P63** pos in 23% and p40 in 4.6% of EMM!
- **Tenascin XB**, sensitivity 80%, specificity 69.5%. Positive in 19/24 MPM calretinin neg!

Other “used-to-be-promising” markers (MPM vs MH)

- **EMA**, sensitivity 41-79%, specificity 88-100%. Positive in benign lesions (30%)

- **GLUT-1**, increased in a variety of tumors, positive in erythrocytes.
  - Sensitivity 21-85%, specificity 90–100%. Positive in benign lesions

- **p53**, a tumor suppressor gene, infrequently mutated in MPM. Positive in MPM and MH

- **IMP3**, oncogene, a highly specific marker for malignant lesions. Sensitivity 37-94%.
  - Positive in benign lesions

- **CD146**, sensitivity 71%, specificity 98%

- **CD44**, involved in cancer progression, cell adhesion, cell migration. Pos in 57.7% MPM and 11.5% MH

For review see Bruno R et al, J Thorac Dis 2018
Other “used-to-be-promising” markers (MPM vs MH)

- **Desmin**, marker of benignity; sensitivity 48-84%, specificity up to 97%.
  - Positive in up to 50% MPM

- **β-catenin**, SMM vs spindle cell proliferations

- **Matrix metalloproteinase 14 (MMP14)**
- **Integrin alpha3 (ITGA3)**
- **Ki-67**, and other proliferation markers

➢ **Combinations:**
IMP3/EMA, GLUT-1/EMA, and IMP3/GLUT-1
Increased sensitivity and specificity.

For review see Bruno R et al, J Thorac Dis 2018
Best markers for differentiation of MPM and lung carcinoma?

- Best EMM marker WT-1 (85.1% diagnostic accuracy)
- Best carcinoma markers CEA (95.9%), p40 (94.6%) and claudin-4 (93.2%)
- Best combination
  - calretinin and WT-1 (86.5%) as pos MPM markers
  - p40 and CEA (97.3%) as neg MPM markers

Kushitani K et al, Histopathology 2017
However.....

...what is the best marker?
“Although ...... have been proposed as markers of mesothelial malignancy, in our experience they are not helpful for the individual case”

OUTLINE

➢ OLD markers (used and not-used)

➢ “NEW” markers

➢ PROMISING markers
CDKN2A (p16) homozygous deletion and BAP-1 loss
CDKN2A (p16) homozygous deletion and BAP-1 loss

- So far not reported in benign pleural lesions
- Not mesothelioma specific
9p21 deletion is very common in MPM (>70%)  
9p21 contains CDKN2A (p16), cDKN2B (p15^{INK4b}), p14^{ARF} and MTAP  

In EMM sensitivity 45-85%, in SMM up to 100%  

Homozygous or hemizygous deletion in sarcomatoid carcinomas  

None of the sarcomas showed homozygous deletion  

No deletion in reactive pleural mesothelial proliferations

Chiosea et al, Mod Pathol 2008; Tochigi N et al, Arch Pathol Lab Med 2013
CDKN2A (p16) homozygous deletion

- FISH for detection
- Cut-off 10-20% of cells with homozygous deletion
- Variable correlation to IHC
- P16 loss (IHC) associated with worse prognosis/aggressiveness

Chiosea et al, Mod Pathol 2008; Tochigi N et al, Arch Pathol Lab Med 2013; Chou A et al, Histology 2018

Retained p16 (upper) and deleted p16 (lower)
Methylthioadenosine Phosphorylase (MTAP)

- MTAP is co-deleted in 91-100% of MPM with homozygous deletion of CDKN2A
- High concordance between MTAP IHC and CDKN2A deletion
- MTAP loss predicts homozygous CDKN2A deletion in 84-100%
- Surrogate marker for CDKN2A deletion
- **Clone 2GA**, cytoplasmic staining (nuclear ignored)
- Could be used for distinction reactive vs malignant

BRCA-1 associated protein 1 (BAP 1)

- BAP 1- controls DNA repair and genes involved in cell proliferation and cell cycle, tumor-suppressor
- BAP-1 loss caused by mutations, deletions, epigenetic silencing
  - BAP-1 somatic mutation in MPM
  - BAP-1 germline mutation in BAP1 cancer syndrome (mesotheliomas, melanomas, renal cell tumors,...)

Testa JR et al, Nat Genetics 2011
BAP1 staining

- **Clone C-4**

- Biallelic mutations in BAP-1 result in loss if IHC expression

- Stromal cells always positive, even in germline mutated cases (normal cells have still one copy of BAP-1 gene)

Bononi et al, Nature 2017
BAP-1

Table 3: Frequency of BAP1 Loss by Immunohistochemistry in Benign Reactions

<table>
<thead>
<tr>
<th>Source, y</th>
<th>BAP1 Loss in Benign Reactions, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheffield et al, Churg (unpublished data, June 2015)</td>
<td>0/53 (0)</td>
</tr>
<tr>
<td>Galateau-Salle (unpublished data, June 2015)</td>
<td>0/23 (0)</td>
</tr>
<tr>
<td>Cigognetti et al, 2015</td>
<td>0/25 (0)</td>
</tr>
</tbody>
</table>

Series n=75

<table>
<thead>
<tr>
<th></th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/49</td>
<td>7/26</td>
</tr>
<tr>
<td>Sen (95% CI)</td>
<td>27% (17-31)</td>
<td></td>
</tr>
<tr>
<td>Spec (95% CI)%</td>
<td>100% (100-100)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: BAP1, BRCA1-associated protein 1.

Churg A et al, Arch Pathol Lab Med 2016

BAP1 facilitates diagnostic objectivity, classification, and prognostication in malignant pleural mesothelioma.

McGregor SM et al. Hum Pathol 2015

BAP1 is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations.

Cigognetti M et al, Mod Pathol 2015
BAP-1

<table>
<thead>
<tr>
<th>Source, y</th>
<th>BAP1 Loss in Benign Reactions, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheffield et al, 2015 and Churg (unpublished data, June 2015)</td>
<td>0/53 (0)</td>
</tr>
<tr>
<td>Galateau-Salle (unpublished data, June 2015)</td>
<td>0/23 (0)</td>
</tr>
<tr>
<td>Cigognetti et al, 2015</td>
<td></td>
</tr>
</tbody>
</table>

**Meta analysis- 1800 mesothelial biopsies**

**BAP-1 loss only in malignant cases**

BAP1 facilitates diagnostic objectivity, classification, and prognostication in malignant pleural mesothelioma.

McGregor SM et al. Hum Pathol 2015

BAP1 is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations.

Cigognetti M et al, Mod Pathol 2015

Benign

- Sen (95% CI): 95% (61-99)
- Spec (95% CI): 100% (100-100)

Malignant

- Sen (95% CI): 95% (17-31)
- Spec (95% CI): 100% (100-100)

Series n=75
### BAP-1

BAP1 facilitates diagnostic objectivity, classification, and prognostication in malignant pleural mesothelioma.  
**McGregor SM et al, Hum Pathol 2015**

---

### Table 3. Frequency of BAP1 Loss by Immunohistochemistry in Benign Reactions

<table>
<thead>
<tr>
<th>Source, y</th>
<th>BAP1 Loss in Benign Reactions, No.</th>
<th>Sen (95% CI)</th>
<th>Spec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheffield et al,39 2015 and Churg (unpublished data, June 2015)</td>
<td>0/53 (0)</td>
<td>27% (17 - 31)</td>
<td></td>
</tr>
<tr>
<td>Galateau-Salle (unpublished data, June 2015)</td>
<td>0/23 (0)</td>
<td>100% (100)</td>
<td></td>
</tr>
<tr>
<td>Cigognetti et al,38 2015</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviation:** BAP1, BRCAP1-like 1

---

BAP-1 loss is rare in adenocarcinoma. **Meta analysis- 1800 mesothelial data**  
**BAP-1 loss only in malignant cases**

---

BAP1 is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations.  
**Cigognetti M et al, Mod Pathol 2015**
CDKN2A (p16) homozygous deletion and BAP-1 loss

All sites and MM morphologies:
p16 deleted in 52%, BAP1 loss in 27%, one or the other in 58%

Pleural EMM:
p16 deleted in 58%, BAP1 loss in 56%, one or the other in 78%

Mesothelioma in situ:
All BAP1 loss/p16 not deleted- 70% of these changes progressed to MPM, BAP1 mutations are early event in mesothelioma development

Churg A et al, Arch Pathol Lab Med 2016; Churg A et al, Mod Pathol 2019
The main problem is sensitivity

- If BAP1 retained it still can be malignant lesion!
- If no p16 deletion it still can be malignant lesion!

CDKN2A (p16) homozygous deletion and BAP-1 loss

Churg A et al, Arch Pathol Lab Med 2016; Churg A et al, Mod Pathol 2019
CDKN2A (p16) homozygous deletion and BAP-1 loss in SMM

**SMM**
- BAP-1 loss in 0-15%
- p16 deletion 80 to 100%

**Sarcomatoid carcinoma**
- no BAP-1 loss, up to 53% p16 homozygous deletion

BAP-1 loss favors the diagnosis of SMM

Combination of MTAP and BAP-1 reliable and effective in distinguishing fibrous pleuritis from sarcomatoid mesothelioma (all FP samples had retained BAP1 and MTAP).

Sarcomatoid/desmoplastic MM vs sarcomatoid carcinoma

Morphology not helpful
SMM/DMM pos for keratin, neg for calretinin, CK 5/6, WT-1 and D2-40
Sarcomatoid carcinomas pos for keratin, neg for MOC-31, claudin-4 or TTF-1/napsin A

- GATA 3 expression identified in mesothelioma and reactive mesothelium and very low in lung AC and SqCC (0-8%)

19 sarcomatoid mesos vs 13 sarcomatoid carcinomas
19/19 SMM/DMM showed diffuse, strong staining
2/13 carcinomas showed patchy and weak staining

Morphology not helpful
SMM/DMM pos for keratin, neg for calretinin, CK 5/6, WT-1 and D2-40
Sarcomatoid carcinomas pos for keratin, neg for MOC-31, claudin-4 or TTF-1/napsin A

▪ GATA3, transcription factor, marker of breast and urothelial carcinoma
▪ GATA3 expression identified in mesothelioma and reactive mesothelium and very low in lung adenocarcinoma and SqCC (0-8%)

19 sarcomatoid mesos vs 13 sarcomatoid carcinomas
19/19 SMM/DMM showed diffuse, strong staining
2/13 carcinomas showed patchy and weak staining

when GATA3 positive- favor mesothelioma
when GATA 3 negative- favor diagnosis other than mesothelioma

OUTLINE

➢ OLD markers (used and not-used)

➢ “NEW” markers

➢ PROMISING markers
Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2)

➢ EZH2 marker of malignancy or increased cell proliferation
➢ Not organ specific
➢ Loss of BAP-1 enhance EZH2 expression, promoting proliferation of MPM cell lines

MPM vs MH
- BAP-1 cut off- 10%; EZH2 cut off- 50% (Both nuclear staining)
- MPM: 53% BAP-1 loss, 66% EZH2 high expression
- None of the benign lesions with BAP-1 loss or EZH2 high expression

Combination of BAP1 and EZH2 highly sensitive (90%) and specific (100%)

Shinozaki-Ushiku et al, Histopathology 2017; Yoshimura M et al, Lung Cancer 2019
NF2 loss

➢ Hemizygous loss of NF2 by FISH (18% cut-off) is useful for dg of mesothelioma, independent of p16 FISH

<table>
<thead>
<tr>
<th></th>
<th>MPM (n = 47)</th>
<th>RMH (n = 27)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loss or deleted (^b)</td>
<td>Retained</td>
<td>Loss or deleted (^b)</td>
<td>Retained</td>
</tr>
<tr>
<td><strong>NF2 FISH</strong></td>
<td>25</td>
<td>22</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>9p21 FISH</td>
<td>37</td>
<td>10</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>MTAP IHC(^a)</td>
<td>33</td>
<td>13</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>BAP1 IHC</td>
<td>27</td>
<td>20</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>BAP1 IHC/9p21 FISH</td>
<td>44</td>
<td>3</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>BAP1/MTAP IHC</td>
<td>42</td>
<td>5</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td><strong>NF2 FISH/ BAP1 IHC/9p21 FISH</strong></td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td><strong>NF2 FISH/ BAP1 IHC/MTAP IHC</strong></td>
<td>46</td>
<td>1</td>
<td>0</td>
<td>27</td>
</tr>
</tbody>
</table>

HEG homolog 1 (HEG1)

- *Heart of glass* gene regulate the concentric growth of the zebrafish heart
- A mesothelioma-related antigen, HEG1 expression supports the survival and proliferation of mesothelioma cells.

SKM9-2, a monoclonal anti-HEG1 antibody
  - Demonstrated specificity 99%, sensitivity 92% for MPM
  - No reaction with normal tissues

- Gene silencing of HEG1 suppressed the survival and proliferation of mesothelioma cells
- Potential target for function-inhibition drugs

Tsuji S et al, Sci Rep 2017
5-hydroxymethylcytosine (5-hmC)

- Involved in gene regulation through epigenetic methylation
- Loss of 5-hmC nuclear expression in different tumors
- Mean nuclear loss in MPM 84%, in reactive lesions 4%
- With cut-off of loss in >50% of tumor nuclei, sensitivity 92%, specificity 100%
- In combination with BAP-1 sensitivity 98%

CAM.2 (red)/5-hmC (brown) double stain, tumor cells show near-total loss of nuclear 5-hmC. Stromal and inflammatory cells show retained 5-hmC.
CONCLUSION
THANK YOU FOR YOUR ATTENTION