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• Dr. Günter Klöppel declares he has no conflict(s) of interest to disclose

Ki-67 in Pancreatic Neuroendocrine Neoplasms According to WHO 2017.

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Dept of Pathology, Technical University München, Germany

PRODUCTION OF A MOUSE MONOCLONAL ANTIBODY REACTIVE WITH A HUMAN NUCLEAR ANTIGEN ASSOCIATED WITH CELL PROLIFERATION
Johannes GERDES, Ulrich SCHWAB, Hilmar LEMKE and Harald STEIN
Institutes of Pathology and Biochemistry Christian Albrecht University, Kiel, Germany

The monoclonal antibody Ki-67 identifies a 359-kD non-histone nuclear protein (encoded by MKI67), which is not only expressed in the M-phase, but also in the G1, S and G2 phase.
Comparison between Ki67 and mitotic counts

26 counts

The function of the Ki-67 nuclear protein remains unclear

The original Ki-67 antibody worked only on frozen material, but was soon replaced by monoclonal antibodies which worked on formalin-fixed and paraffin-embedded tissues.

The original Ki-67 antibody worked only on frozen material, but was soon replaced by monoclonal antibodies which worked on formalin-fixed and paraffin-embedded tissues.

MIB-1 recognizes the Ki-67 antigen

Why and when was Ki-67 included into the PanNEN classification?
The WHO 2000 classification of PanNENs

Definition of grades

Well differentiated NENs
- Neuroendocrine tumour (NET) G1
  - Ki67 index: <3%
  - Mitotic index: <2/10 HPF
- Neuroendocrine tumour (NET) G2
  - Ki67 index: 3-20%
  - Mitotic index: 2-20/10 HPF
- Neuroendocrine tumour (NET) G3
  - Ki67 index: >20%
  - Mitotic index: >20/10 HPF
- Poorly differentiated NENs
  - Neuroendocrine carcinoma (NEC) G3
    - Ki67 index: >20%
    - Mitotic index: >20/10 HPF

Grade of pancreatic NENs and Ki67-index related to prognosis and therapy

- Well differentiated NEN - NET
  - Low proliferative activity
  - Hormone expression
  - Hormonal syndromes
  - Long survival
- Poorly differentiated NEN - NEC
  - High proliferative activity
  - No hormone expression
  - No hormonal syndromes
  - Short survival

Recommendations for counting Ki67 labeled cells

- Ki67 index is based on at least 500 cells in areas of higher nuclear labeling (‘hot spots’).
- For assessing Ki67, manual counting of printed images is suggested (Reid et al 2015); ‘eyeballing’ is not recommended.
New Features of the WHO 2017 classification of PanNENs

Definition of grades

<table>
<thead>
<tr>
<th>Grade</th>
<th>Ki67index%</th>
<th>Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated NENs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine tumour (NET) G1</td>
<td>&lt;3%</td>
<td>&lt;2/10 HPF</td>
</tr>
<tr>
<td>Neuroendocrine tumour (NET) G2</td>
<td>3-20%</td>
<td>2-20/10 HPF</td>
</tr>
<tr>
<td>Neuroendocrine tumour (NET) G3</td>
<td>&gt;20%</td>
<td>&gt;20/10 HPF</td>
</tr>
<tr>
<td>Poorly differentiated NENs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine carcinoma (NEC) G3</td>
<td>&gt;20%</td>
<td>&gt;20/10 HPF</td>
</tr>
<tr>
<td>Small cell type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large cell type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed neuroendocrine-nonneuroendocrine neoplasm (MiNEN)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pancreatic neuroendocrine neoplasms (PanNEN)

Ki67 > 55% predicts better the response to CIS-chemoTx
Ki67 > 55% longer survival and poor response to CIS-chemoTx

Pancreatic neuroendocrine neoplasms (PanNEN) with a Ki-67-index >20%

Liver Met of PanNET - primary tumor was G2, 16%

Ki-67: 37%

PanNEC, G3?

Are both G3 PanNECs?

Ki-67: 25%

Ki-67: 70%
Pancreatic neuroendocrine neoplasms (PanNEN) with a Ki-67-index >20%

- NETs or NECs of the pancreas?

Potential markers for PanNETs or PanNECs:
- ATRX / DAXX, MEN1, mTOR genes
- p53 / TP53
- Rb1 / rb1
- SSTR2A
- Islet 1
- Progesteron receptor

Jiao et al. Science 2011
Agaimy et al. Modern Pathol 2013
Mehmert et al. Gastroenterol 2014
Kuemmerer et al. Oncotarget 2015

Ki-67: 25%

Ki-67: 70%

NET G3 of the pancreas
Ki-67: 25%
p53

NET G3 of the pancreas
Ki-67: 70%
p53

NET G3

Small cell NEC
SSTR2A 2+

Large cell NEC
SSTR2A 0-1+

ATRX negative
MUT

ATRX positive
WT

Small cell NEC
NET G3

ATRX negative
MUT

ATRX positive
WT
Markers distinguishing between PanNETs G3 and PanNECs, large and small cell

<table>
<thead>
<tr>
<th>Organ</th>
<th>Diagnosis</th>
<th>n</th>
<th>Ki67-range</th>
<th>p53</th>
<th>p16</th>
<th>Rb1 loss</th>
<th>Dnmt3a-loss</th>
<th>Atrx loss</th>
<th>Daxx loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>NET G3</td>
<td>9</td>
<td>21%‐35%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>78%</td>
<td>11%</td>
<td>33%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>NEC</td>
<td>12</td>
<td>21%‐35%</td>
<td>75%</td>
<td>67%</td>
<td>43%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Konulszczak et al Modern Pathol 2017

PanNENs and Ki-67

<table>
<thead>
<tr>
<th>Well differentiated NENs - NETs</th>
<th>Poorly differentiated NENs - NECs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NET G1</td>
<td>NET G2</td>
</tr>
<tr>
<td>2%</td>
<td>20%</td>
</tr>
</tbody>
</table>

These tumors showed no p53/Rb1 abnormalities despite poor morphology and high proliferation. However,....

Take Home Message

- In PanNENs (as in many other tumors) the Ki-67 index strongly correlates with prognosis
- PanNENs are heterogeneous regarding morphology, proliferation and biology
- The new WHO classification therefore distinguishes among the PanNENs with Ki-67 >20% between well differentiated PanNENs (NETs) G3 and poorly differentiated PanNENs (NECs) G3

THANK YOU