Digital Image Analysis for Ki67 in breast cancer

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Disclosures: none
Ki67 protein

- Ki67 expressed in dividing cells (G1, S, G2 and M phase)
- Ki67 not expressed in resting cells (G0)
- Used to calculate ratio of positive cells = proliferation index (PI)
- Structural protein of condensed mitotic chromosomes
NordiQC Ki67 scoring challenge

198 participants
Influence of experience

198 participants
Digital Image Analysis

Criteria

• Identify nuclei
• Distinguish Ki67 positive and negative nuclei
• Exclude non-tumour cells from analysis
Virtuel Double Staining: concept

Cut serial sections (3µm):
• Slide stained for Ki67

• Neighboring slide stained for pancytokeratin
Image analysis for identification of tumor

Ki67

Pancytokeratin
Image analysis for identification of biomarker (Ki67)

Ki67

Pancytokeratin
Proliferation assessment in breast carcinomas using digital image analysis based on virtual Ki67/cytokeratin double staining

Rasmus Røge¹,² • Rikke Riber-Hansen³ • Søren Nielsen¹ • Mogens Vyberg¹,²

Digital Image Analysis – Ki67

VALIDATION OF VDS
Validation of Virtual Double Staining

• Validation of the Nuclear detection and segmentation (number of positive and negative nuclei)

• Validation of the alignment algorithm
  – Overlap/agreement between slides
  – Sensitivity to distance between slides
Experimental setup

• 3 TMAs containing more than 100 cores of breast carcinomas
• 2 slides were cut from each block, one stained for PCK, one for Ki67
• Ki67 PI indices were calculated using the VDS
• Areas were sampled from each core using SURS (systematic uniform randomized sampling) for manual counting
• Only a small percentage of total number of cells were counted (200-400)
Systematic Random Sampling

- Grid of frames randomly placed on core
- Positive and negative tumour cells counted manually in each frame
- Each frame extracted as an image for Virtual Double Staining
Stereological counting
Bland-Altman

ICCs > 0.9
NordiQC Ki67 scoring challenge

198 participants

PI score: ~20-80%
Median ~ DIA: ~40%

Boxplots: Participant Ki67 scores
Red dot: Digital Image Analysis
Digital Image Analysis – Ki67

ANTIBODY CLONE COMPARISON
# Ki67 stains assessed by NordiQC

## Performance in 5 NordiQC runs

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Participants</td>
<td>42</td>
<td>100</td>
<td>124</td>
<td>229</td>
<td>409</td>
</tr>
<tr>
<td>Sufficient</td>
<td>71%</td>
<td>73%</td>
<td>77%</td>
<td>89%</td>
<td>93%</td>
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## Performance marks in Run B22 (2016)

<table>
<thead>
<tr>
<th>Category</th>
<th>Optimal</th>
<th>Good</th>
<th>Borderline</th>
<th>Poor</th>
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<tbody>
<tr>
<td>Total</td>
<td>282</td>
<td>100</td>
<td>24</td>
<td>3</td>
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<tr>
<td>Proportion</td>
<td>69%</td>
<td>24%</td>
<td>6%</td>
<td>1%</td>
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</table>
Ki67 staining – the protocol trap

Serial sections stain for Ki67 in two labs
Antibody clone comparison

Original Article

Immunohistochemical assessment of Ki67 with antibodies SP6 and MIB1 in primary breast cancer: a comparison of prognostic value and reproducibility

Maria Ekholm, Sanda Beglerbegovic, Dorthe Grabau, Kristina Lövgren, Per Malmström, Linda Hartman, Mårten Fernö

Conclusions: SP6 was not superior to MIB1, but the two antibodies were comparable in the assessment of Ki67. Both MIB1 and SP6 could therefore be considered for prognostic use in primary breast cancer.
Antibody clone comparison

Comparative validation of the SP6 antibody to Ki67 in breast cancer

Lila Zabaglo¹,², Janine Salter¹,², Helen Anderson¹,², Emma Quinn¹, Margaret Hills¹, Simone Detre¹, Roger A’Hern³, Mitch Dowsett¹,²

Conclusions SP6 and MIB1 provide highly comparable measures of Ki67 that predict progression of advanced disease similarly. SP6 is substantially better suited than MIB1 to image analysis.
Ki67 antibody clone comparison

• The impact of different IHC methods (antibody clones, protocols and platforms) is rarely taken into account

• The aim of this study was to assess the impact of Ki67 antibody (Ab) clones, systems, protocols and stainer platforms on the Ki67 PI using VDS.
Experimental setup

- TMA with 40 breast cancers
- Commonly used mAbs: Mib1, SP6, 30.9, MM1
- Ready-To-Use and concentrated formats
- All major platforms
- Optimized were developed for all clone and platform combinations
- PI calculated using VDS
Concentrate

- 35.9
- 31.4
- 34.0
- 34.1
- 30.8

RTU

- 45.2
- 24.6
- 20.8

Absolute difference in PI (%)

Clone
- 30.9
- Mib1
- MM1
- SP6

Staining Platform
- Dako Autostainer
- Leica Bond
- Ventana Ultra
SP6 concentrate, Ventana platform

PI 38%

MM1 RTU, Leica platform

PI 12%
Ki67 PI in breast carcinoma

Conclusion

• Antibody (Ab) clones, systems, protocols and stainer platforms have great impact on the Ki67 PI
• VDS is an effective way of documenting the variation in PI related to immunoassays
• mAb MIB1 seems to give the most constant results across platforms when used with optimized protocols
Digital Image Analysis – Ki67

CONTROLS
Controls among NordiQC-participants
Figure 4.1: Tonsil control tissue material. A shows three different tonsil control tissues. B shows the variance within the same tonsil control tissue through the tissue block.
Figure 4.1: Ki67 staining of a tonsil control tissue and a cell line. A and C demonstrate stained tonsil control tissue and cell line. B and D demonstrate the same specimen with DIA performed. Red, orange and yellow colored elements indicate respectively strongly, moderately and weekly Ki67 stained nuclei. The blue elements are Ki67 negative cells.
Paraffin block from cell cultures

Stained for Ki67 (Mib1) in different antibody dilutions
Ki67 H-score across the block
Ki67 H-score, different Ab conc
Future studies:
NordiQC - B23 – Ki67

• 410 participants
• 93% sufficient
• But only 69% optimal!
• 3 breast cancers, pancreas, liver, tonsil AND 3 cell lines
Thank you for your attention!

Collaborators
Søren Nielsen
Rikke Riber-Hansen
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Marina Sørensen
Mogens Vyberg