Digital image analysis in HER2 immunostained breast carcinomas

Anja Brügmann MD, PhD

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Fab fragment of trastuzumab, a monoclonal antibody, bound to the extracellular domain of HER2

In patients with primary breast cancer the HER2 is assessed in order to select the patients who can benefit from HER2 targeted treatment.
Choice of assay

IHC and FISH are reference standards in HER2 assessment due to the initial clinical trials using trastuzumab in first line treatment of HER2-overexpressing metastatic breast cancer


Today, trastuzumab is a recommended adjuvant therapy in early breast cancer and evaluation of HER2 status is routine in all primary breast carcinomas and guidelines for HER2 testing have emerged.

The HER2 status is performed on tumor tissue specimens at pathology laboratories and different methods are available.

In Situ Hybridization

IHC  BRISH  FISH

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**HER2 IHC and ISH classifications modified from the American Society of Clinical Oncology/ College of American Pathologists guideline recommendations**


<table>
<thead>
<tr>
<th>Classification</th>
<th>IHC score</th>
<th>IHC staining pattern</th>
<th>ISH HER2/CEN17 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0/1+</td>
<td>No, or weak incomplete membrane staining in tumor cells</td>
<td>&lt;1.8 Non-amplified</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2+</td>
<td>Complete membrane staining with non-uniform or weak intensity in at least 10% of tumor cells</td>
<td>1.8.-2.2 Equivocal</td>
</tr>
<tr>
<td>Positive</td>
<td>3+</td>
<td>Uniform intense membrane staining of &gt;30% of tumor cells</td>
<td>&gt;2.2 Amplified</td>
</tr>
</tbody>
</table>
The challenges in assessment of HER2 status

1) GET AN OPTIMAL STAINING RESULT

2) MAKE A CORRECT EVALUATION
   - In the routine testing of HER2 in pathology laboratories both technical aspects and evaluation cause problems. One way it has been documented is via the reports of the quality assessment schemes [www.NordiQC.org](http://www.NordiQC.org) and [www.ukneqas.org.uk](http://www.ukneqas.org.uk) and [www.ciqc.ca](http://www.ciqc.ca)

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Sources of HER2 testing variation – as described by ASCO/CAP Wolff, J Clin Oncol, 2007

- **Preanalytic**
  - Time to fixation
  - Method of tissue processing
  - Time of fixation
  - Type of fixation

- **Analytic**
  - Assay validation
  - Standardized laboratory procedures
  - Type of antigen retrieval
  - Test reagents
  - Use of standardized control materials
  - Use of automated laboratory methods

- **Postanalytic**
  - Interpretation criteria
  - Use of image analysis
  - Reporting
  - Quality assurance
  - Laboratory accreditation
  - Proficiency testing
  - Pathologist competency assessment
Results of the latest 3 runs of HER2 assessment in NordiQC [www.NordiQC.org](http://www.NordiQC.org)

<table>
<thead>
<tr>
<th>IHC RUN</th>
<th>Run B13 2012</th>
<th>Run B14 2012</th>
<th>Run B15 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n=</td>
<td>253</td>
<td>263</td>
<td>272</td>
</tr>
<tr>
<td>Sufficient staining results</td>
<td>83 %</td>
<td>79 %</td>
<td>90 %</td>
</tr>
<tr>
<td>Score concordant with NordiQC</td>
<td>79%</td>
<td>84%</td>
<td>87%</td>
</tr>
</tbody>
</table>
Digital Image Analysis Improves the Quality of Subjective HER-2 Expression Scoring in Breast Cancer

Ivar Skaland, MSc,* Irene Øvestad, MSc,* Emiel A. M. Janssen, MSc,* Jan Klos, MD,* Kjell H. Kjellevold, MD,* Tove Helliesen, MD,* and Jan P. A. Baak, MD, PhD, FRCPath, FIAC(Hon)*†‡
App I Immunohistochem Mol Morphol

Image analysis as an adjunct to manual HER-2 immunohistochemical review: a diagnostic tool to standardize interpretation
Histopathology 2010

Lynne Dobson, Catherine Conway, Alan Hanley, Alex Johnson, Sean Costello, Anthony O’Grady,¹ Yvonne Connolly,² Hilary Magee,² Daniel O’Shea, Michael Jeffers² & Elaine Kay¹

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Table 5. Comparison of SlidePath’s Tissue IA system with other commercially available systems for HER-2 analysis

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>System</th>
<th>Aperio</th>
<th>Biolimagene</th>
<th>Dako (Chromavision)</th>
<th>Genetix (Applied Imaging)</th>
<th>Ventana (TriPath Imaging)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SlidePath</td>
<td>Tissue IA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aperio</td>
<td>Scanscope XT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biolimagene</td>
<td>Pathiam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dako</td>
<td>ACIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Chromavision)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Genetix</td>
<td>Ariel</td>
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</tr>
<tr>
<td>(Applied Imaging)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ventana</td>
<td>VIAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(TriPath Imaging)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Assay**
- Dako HercepTest®
- Leica Oracle Bond™
- Ventana Pathway® (4b5)
- Dako HercepTest®
- Dako HercepTest®
- Dako HercepTest®
- Dako HercepTest®
- Ventana Pathway® (4b5)
- Ventana Pathway® (cb11)

Concordance with manual review (sample size)
- 91% (n = 275)
- 86%* (n = 180)
- 81%* (n = 176)
- 75%* (n = 90)
- a* (n = 124)
- 86%* (n = 206)
- 77%* (n = 201)

Image format support
- ●
- ○
- ○
- ○
- ○
- ○
- ○

Dependence on manual selection
- ●
- ●
- ●
- ●
- ●
- ●
- ●

Quantitation base
- Intensity, continuity
- Intensity
- Morphology, intensity
- Intensity
- Intensity
- Intensity
- Intensity

High ●, Intermediate ○, Low ○.
*Data from Food and Drug Administration 510 k substantial equivalence reports (http://www.fda.gov).
a: The likelihood of the image analysis system producing a consistent score on a given slide is as likely as the pathologists are to agree with each other.

Dobson et al, *Histopathol.* 2010

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Digital image analyses in HER2 assessment. The HER2-CONNECT algorithm was aligned to match manual digital image readings of expert assessors

Brügmann et al., Breast Cancer Res Treat, 2011
How does it work?
The HER2-CONNECT algorithm was aligned to match manual digital image readings of expert assessors

Brügmann et al., *Breast Cancer Res Treat*, 2011

**Pre-processing:**
Identifies brown pixels in linear structures only.

**Segmentation:**
Statistical rules define intensity of brown and dimensions of linearity to classify the relevant pixels.

**Post-processing:**
Skeletonizes the membrane, merges membrane segments, and eliminates small segments by a user-specified cut-off.
How does it work? Video from HER2-CONNECT application showing how to:

- Mark a region of interest (ROI)
- Let the computer software determine the IHC score
How connectivity is defined

\[
\int_0^1 \left( \sum \frac{A_x}{\sum A} \, dx \right)
\]

Area under the curve: Connectivity = 0.680

\[
X = \frac{(A_{\text{dyn}} - A_{\text{min}})}{(A_{\text{max}} - A_{\text{min}})}
\]

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#### Data from study

<table>
<thead>
<tr>
<th>Connectivity</th>
<th>Automated image analysis</th>
<th>Digital reading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/1+</td>
<td>2+</td>
</tr>
<tr>
<td>ROI*</td>
<td>236</td>
<td>37</td>
</tr>
<tr>
<td>Mean</td>
<td>0.008</td>
<td>0.28</td>
</tr>
<tr>
<td>Std. dev.</td>
<td>0.0026</td>
<td>0.14</td>
</tr>
<tr>
<td>Range</td>
<td>0–0.12</td>
<td>0.13–0.55</td>
</tr>
</tbody>
</table>

* Number of ROI in the validation set (total $n = 430$)

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Key results from our study testing and validating HER2-CONNECT

Validation set:

- Percentage agreement, IA vs. DR: 92.3%
- Cohen’s kappa, IA vs. DR: 0.86
- Specificity*, IA vs. FISH: 99.2%
- Sensitivity*, IA vs. FISH: 100%

*excluding IHC score 2+
Data in summary
HER2 connectivity versus FISH ratios.

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Membrane connectivity estimated by digital image analysis of HER2 immunohistochemistry is concordant with visual scoring and fluorescence in situ hybridization results: algorithm evaluation on breast cancer tissue microarrays

Aida Laurinaviciene¹,²*, Darius Dasevicius²,³, Valerijus Ostapenko¹, Sonata Jarmalaite⁴, Juozas Lazutka⁴ and Arvydas Laurinavicius²,³

DIAGNOSTIC PATHOLOGY, 2011

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ImmunoMembrane: a publicly available web application for digital image analysis of HER2 immunohistochemistry

Vilppu J Tuominen,¹ Teemu T Tolonen¹,² & Jorma Isola¹
¹Institute of Biomedical Technology, University of Tampere, Tampere, Finland, and ²Department of Pathology, Centre for Laboratory Medicine, Tampere University Hospital, Tampere, Finland

go to
http://153.1.200.58:8080/immunomembrane/
In Conclusion
Digital image analysis as a supplementary diagnostic tool

- Standardized quantification of semi-quantitative assays
- Motivates use of standardized staining protocols
- Lowers interobserver variability
- Reduces of the number of cases in the IHC score 2+ category and thereby workload
- Can be performed on full slide tissue sections

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In Conclusion

Limitations to the use of digital image analysis

• Has to be reviewed to check that the result is concordant with both the full slide and focal "hotspots"
• Potentially adds to the list of parameters which can cause inaccuracy
• Caution in DCIS which often has a high HER2 expression
• Caution in invasive lobular carcinomas
Discussion and future perspectives

- Potential general applicability in scoring IHC quantitatively

- HER2, and other biomarkers, is requested in an increasing number of epithelial cancers (gastric cancer, lung cancer)