Quantitative Image Analysis of HER2 Immunohistochemistry for Breast Cancer

CAP Guideline Update and Review of Draft Recommendations

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Disclosures

• There is no financial disclosure or conflict of interest.
• The presentation represents my personal and professional opinion only.
• Member of Digital Pathology Association Board of Directors & Executive Committee, Editorial Board member of Journal of Pathology Informatics, Member of Association of Pathology Informatics, CAP Digital Pathology Committee and contributing editor of CAP Digital Pathology Resource Guide 2014-2017, Chair of the CAP Pathology and Laboratory Quality Center Expert Panel of the HER2 IHC Quantitative Image Analysis guideline.
Outline

• Introduce quantitative image analysis (QIA)
• Discuss some of the challenges of QIA and HER2 IHC for breast cancer interpretation and reporting
• Review draft recommendations from CAP guideline on HER2 IHC QIA in progress
Introduction

Quantitative image analysis (QIA) = Quantitative extraction of meaningful information from images

QIA is a powerful advantage of digital pathology

- When the slides are digitalized, they can be numerically analyzed using computer algorithms.
- Algorithms can be used to automate the manual counting of structures, or for classifying the condition of tissue, like algorithms used in grading tumors.
- This could reduce human error and improve accuracy of diagnoses.
The power of image analysis

Four exact copies of the same rectangle cut from square A are laid out in an overlapping fashion to form a continuous color bridge to B.

Reliable
Measurable
Repeatable
Quantifiable
Benefits of image analysis

- Better accuracy (more precise quantitative measurements)
- Standardization (more reproducible results, especially for intermediate categories & complex scoring systems)
- Automation (reduce time consumption for pathologists, especially for performing mundane tasks like counting)
- Enhanced efficiency (triage cases – eg, weed out negative cases)
- CAD (eg, help pathologists find, diagnose & grade disease like cancer)
- Enable big data projects (eg, image analysis for biomarker discovery)
Current state of QIA

• Advancements in genomics, computing and imaging technology have spurred new opportunities to use QIA in diagnostic medicine
• Current shift from research to clinical applications, especially in diagnostic testing
• Diagnostic pathology transition from qualitative (descriptive, analog) to quantitative (automated) science
• Precision medicine currently demands precision diagnostics
• Most widely employed clinical diagnostic algorithms are for breast cancer biomarkers (ER, PR, HER2, Ki-67 and p53)
# Image analysis tools

<table>
<thead>
<tr>
<th>Software Type</th>
<th>Image Format Compatibility</th>
<th>Technical Knowledge Level</th>
<th>Customization Level</th>
<th>Features</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic Science Image Analysis</strong></td>
<td>Most Image Formats</td>
<td>Moderate</td>
<td>High</td>
<td>Variety of measurement tools, Access to image processing tools, Some automation</td>
<td>Image Pro Premier, Metamorph, ImageJ/FIJI, Cell Profiler</td>
</tr>
<tr>
<td><strong>Slide Scanner Based</strong></td>
<td>Limited Image Formats</td>
<td>Low</td>
<td>Low-Moderate</td>
<td>Direct access to images, Access to common algorithms, US IVD for HER2/ER Pattern recognition, Batch processing, Designed for Digital Pathology</td>
<td>Roche/Ventana, Leica/Aperio, 3D Histech, HALO, PathXL, TissueMark</td>
</tr>
<tr>
<td><strong>Digital Pathology Inspired</strong></td>
<td>Most Image Formats</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Workflow based, Easily adjustable parameters, Batch processing, Pattern recognition, Access more feature data, Designed for Digital Pathology</td>
<td>InForm, Visiopharm, Definiens Tissue Studio</td>
</tr>
<tr>
<td><strong>Algorithm Based</strong></td>
<td>Most Image Formats</td>
<td>High</td>
<td>High</td>
<td>Fully customizable, Unique algorithms, Even more feature data, Batch processing</td>
<td>MatLab, Visiopharm, Definiens Developer</td>
</tr>
</tbody>
</table>
Image analysis tools

- Examples of whole slide image analysis:
  - Positive pixel count
Image analysis tools

- Examples of whole slide image analysis:
  - Nucleus analysis
Image analysis tools

- Examples of whole slide image analysis:
  - Cytoplasm analysis
Image analysis tools

- Examples of whole slide image analysis:
  - Membrane
## Algorithms for QIA

<table>
<thead>
<tr>
<th>RESEARCH</th>
<th>CLINICAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many apps</td>
<td>Limited algorithms</td>
</tr>
<tr>
<td>Modifiable parameters</td>
<td>Locked down apps</td>
</tr>
<tr>
<td>Lab developed tests</td>
<td>Approved (FDA)</td>
</tr>
<tr>
<td>Research environment</td>
<td>Regulated lab (CLIA)</td>
</tr>
<tr>
<td>Continuous data</td>
<td>Discrete results</td>
</tr>
<tr>
<td>Variable output</td>
<td>Match manual scores</td>
</tr>
<tr>
<td>Researchers</td>
<td>Pathologist oversight</td>
</tr>
<tr>
<td>Financial benefit</td>
<td>Questionable ROI (CPT code)</td>
</tr>
<tr>
<td>Stand-alone system</td>
<td>Integrated workflow</td>
</tr>
<tr>
<td>Widespread use</td>
<td>Slow adoption</td>
</tr>
</tbody>
</table>
Challenges
A patient’s medical journey begins with their diagnosis...

...Pathologists provide forecast of

*Diagnosis*
*Prognosis*
*Therapeutic selection & Prediction of response*

Courtesy of Dr. Mark Lloyd
ASCO/CAP HER2 guideline

- HER2 status must be determined in all patients with invasive breast cancer.
- In the US, recommend using an assay that has received FDA approval, although a CLIA-certified laboratory may choose instead to use a LDT.
- If results are equivocal (revised criteria), reflex testing should be performed using an alternative assay (IHC or ISH).
- Must ensure that interpretation and reporting guidelines for HER2 testing are followed.
HER2 status must be determined in all patients with invasive breast cancer.

In the US, recommend using an assay that has received FDA approval, although a CLIA-certified laboratory may choose instead to use a LDT.

If results are equivocal (revised criteria), reflex testing should be performed using an alternative assay (IHC or ISH).

Must ensure that interpretation and reporting guidelines for HER2 testing are followed.

Image analysis can be used to achieve consistent interpretation.

However, a pathologist must confirm the image analysis result.

Image analysis procedures must be validated before implementation.

Image analysis equipment, just as other laboratory equipment, must be calibrated and subjected to regular maintenance and internal quality control evaluation.
Digital image analysis outperforms manual biomarker assessment in breast cancer

Gustav Stålhammar1,2, Nelson Fuentes Martinez1,3, Michael Lippert4, Nicholas P Tobin5, Ida Mölholm6,7, Lorand Kis7, Gustaf Rosin1, Mattias Rantalainen8, Lars Pedersen4, Jonas Bergh1,5,9, Michael Grunkin4 and Johan Hartman1,5,7

1Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden; 2St Erik Eye Hospital, Stockholm, Sweden; 3Södersjukhuset, Stockholm, Sweden; 4Visiopharm A/S, Hoersholm, Denmark; 5Cancer Center Karolinska, Stockholm, Sweden; 6Department of Applied Mathematics and Computer Science, Technical University of Denmark, Kongens Lyngby, Denmark; 7Department of Clinical Pathology, Karolinska University Hospital, Stockholm, Sweden; 8Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden and 9Department of Oncology, Karolinska University Hospital, Stockholm, Sweden

• In conclusion, the system for DIA evaluated here was in most aspects a superior alternative to manual biomarker scoring.
• It also has the potential to reduce time consumption for pathologists, as many of the steps in the workflow are either automatic or feasible to manage without pathological expertise.

Table 2 Molecular ‘intrinsic’ breast cancer subtypes and surrogate definitions by immunohistochemical profile

<table>
<thead>
<tr>
<th>Intrinsic subtype</th>
<th>Surrogate IHC classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal subtype</td>
<td>ER ≥ 1% and/or PR ≥ 20% and HER2 ‘negative’ and Ki67 ‘low’</td>
</tr>
<tr>
<td>Luminal A</td>
<td>1. ER ≥ 1% and/or PR ≥ 20% and HER2 ‘negative’ and Ki67 ‘high’ or</td>
</tr>
<tr>
<td>Luminal B</td>
<td>2. ER ≥ 1% and PR &lt; 20% and HER2 ‘negative.’ Any Ki67 or</td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>3. ER ≥ 1% and PR ≥ 1% and HER2 ‘positive.’ Any Ki67</td>
</tr>
<tr>
<td>Basal-like</td>
<td>ER &lt; 1% and PR &lt; 1%. HER2 ‘positive.’ Any Ki67</td>
</tr>
<tr>
<td></td>
<td>ER &lt; 1% and PR &lt; 1%. HER2 ‘negative.’ Any Ki67</td>
</tr>
</tbody>
</table>

% = Proportion of tumor cells stained with the respective biomarker. ‘Positive’ and ‘negative’ = as defined by the American Society of Clinical Oncology and College of American Pathologists recommendations for human epidermal growth factor receptor 2-testing in breast cancer.30 ‘High’ and ‘low’ = as defined by each laboratory’s own reference data,3,6,17 with threshold generally in the range of 14–29%.15,19–21
HER2 image algorithms

Quantitation of Results (Membrane)

<table>
<thead>
<tr>
<th>Date</th>
<th>K-Number</th>
<th>Tissue - Stain</th>
<th>Reagent</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010/10</td>
<td>KD92333</td>
<td>Breast - P53K-67</td>
<td>Dako</td>
<td>FDA-approved</td>
</tr>
<tr>
<td>2009/02</td>
<td>KD061010</td>
<td>Breast - HER2/neu</td>
<td>Dako</td>
<td>Image Analysis</td>
</tr>
<tr>
<td>2007/02</td>
<td>KD02756</td>
<td>Breast - HER2/neu</td>
<td>Dako</td>
<td>Image Analysis (SW only)</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>ScanScope XT System (Aperio Technologies, Vista, CA)</td>
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<tr>
<td>2009/06</td>
<td>KD06594</td>
<td>Breast - HER2/neu</td>
<td>Dako</td>
<td>Turntable Image Analysis</td>
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<tr>
<td>2009/04</td>
<td>KD06054</td>
<td>Breast - ER/PR</td>
<td>Dako</td>
<td>Reading on Monitor</td>
</tr>
<tr>
<td>2008/08</td>
<td>KD73987</td>
<td>Breast - ER/PR</td>
<td>Dako</td>
<td>Image Analysis</td>
</tr>
<tr>
<td>2007/12</td>
<td>KD71571</td>
<td>Breast - HER2/neu</td>
<td>Dako</td>
<td>Reading on Monitor</td>
</tr>
<tr>
<td>2007/10</td>
<td>KD71128</td>
<td>Breast - HER2/neu</td>
<td>Dako</td>
<td>Image Analysis</td>
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<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td>VIAS (Tripath Imaging, Burlington, NC)</td>
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<td></td>
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<tr>
<td>2006/09</td>
<td>KD04248</td>
<td>Breast - P53</td>
<td>Ventana</td>
<td>Image Analysis</td>
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<tr>
<td>2005/04</td>
<td>KD53520</td>
<td>Breast - Ki-67</td>
<td>Ventana</td>
<td>Image Analysis</td>
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<tr>
<td>2005/04</td>
<td>KD51282</td>
<td>Breast - HER2/neu</td>
<td>Ventana</td>
<td>Image Analysis</td>
</tr>
<tr>
<td>2005/05</td>
<td>KD50126</td>
<td>Breast - ER/PR</td>
<td>Ventana</td>
<td>Image Analysis</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ARIOL (Applied Imaging, Santa Clara, CA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004/03</td>
<td>KD32200</td>
<td>Breast - ER/PR</td>
<td>Dako</td>
<td>Image Analysis</td>
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<tr>
<td>2004/01</td>
<td>KD31715</td>
<td>Breast - HER2/neu</td>
<td>Dako</td>
<td>Image Analysis</td>
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<tr>
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<tr>
<td>ACIS (Clarient, Aliso Viejo, CA/Chroma Vision, San Juan Capistrano, CA)</td>
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<td></td>
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<tr>
<td>2004/02</td>
<td>KD12138</td>
<td>Breast - ER/PR</td>
<td>Dako</td>
<td>Image Analysis</td>
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<tr>
<td>2003/12</td>
<td>KD31113</td>
<td>Breast - HER2/neu</td>
<td>Dako</td>
<td>Image Analysis (system)</td>
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<tr>
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<tr>
<td>QCA (Cell Analysis, Highland Park, IL.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003/12</td>
<td>KD31363</td>
<td>Breast - ER</td>
<td>Dako</td>
<td>Image Analysis (SW only)</td>
</tr>
</tbody>
</table>
Virtuoso Image Analysis Algorithm

Courtesy of Roche Diagnostic Corporation
Validation

- Parameters to consider validating:
  - **System** (software, etc.)
  - **Test** (IHC platform)
  - **Pathologist** (reader)
  - **Result** (comparison)

- **Gold standard** = alternative, validated method
  - eg, FISH, another algorithm?
  - **Accuracy** (concordance/correct)
Accuracy: the closeness of a measured value to a standard or known value.

Precision: the closeness of two or more measurements to each other; precision is independent of accuracy.
Precision

• Repeatability or reproducibility
  o Assay variations (batches/runs)
  o Technical variations (image acquisition)
  o Operator variability (ROI selection)
Same scanner variability
WSI scanner reproducibility

- HER2/neu algorithms
  - Commercial algorithm
  - Preset parameters
- WSI from 3 scanners
- Inter-scanner variability
  - Different image properties
- Reducing discrepancies
  - Re-training (calibration)

<table>
<thead>
<tr>
<th>Classifier</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathologist panel</td>
<td>21</td>
<td>137</td>
<td>83</td>
</tr>
<tr>
<td>Algorithm 1 on Aperio-CS</td>
<td>46</td>
<td>120</td>
<td>75</td>
</tr>
<tr>
<td>Algorithm 1 on Aperio-T2</td>
<td>65</td>
<td>101</td>
<td>75</td>
</tr>
<tr>
<td>Algorithm 1 on Hamamatsu</td>
<td>24</td>
<td>119</td>
<td>98</td>
</tr>
<tr>
<td>Algorithm 2 on Aperio-CS</td>
<td>13</td>
<td>145</td>
<td>83</td>
</tr>
<tr>
<td>Algorithm 2 on Aperio-T2</td>
<td>13</td>
<td>146</td>
<td>82</td>
</tr>
<tr>
<td>Algorithm 2 on Hamamatsu</td>
<td>14</td>
<td>149</td>
<td>78</td>
</tr>
</tbody>
</table>

HER2/neu: (Human epidermal growth factor receptor 2)

Keay et al. J Pathol Inform 2013, 4:19
The gap in practice

- QIA has been shown to improve consistency and accuracy of interpretation than manual scoring by pathologists, but has not gained widespread acceptance
  - In 2016, of the 826 laboratories enrolled in the CAP HQIP-A mailing, 183 (22.1%) reported using QIA
- While the ASCO/CAP HER2 testing guidelines addressing the key pre-analytical and IHC related issues, there is a need of guideline for HER2 IHC QIA
CAP QIA guideline

Scope:

• to provide recommendations for improving reproducibility, precision, and accuracy in the interpretation of HER2 IHC where QIA is employed

Methods:

• process follows the National Academy of Science (formerly IOM) standards for developing clinical practice guidelines
  • Built on systematic literature review
  • Draft recommendations by an expert panel with the input of an advisory panel
  • Public comment period
  • Grades provided for strength of evidence and strength of recommendation
CAP Center guideline life cycle

1. Submit and Select Ideas
2. Determine Scope and Form Workgroup
3. Research and Review Evidence/Draft Recommendations
4. Solicit Comment
5. Complete Recommendations
6. Review and Approve
7. Publish and Implement
8. Maintain
Key Questions

1. What equipment validation and daily performance monitoring is needed?

2. What training of staff and pathologists is required? What are the competency assessments needs over time?

3. How does one select or develop an appropriate algorithm for interpretation?

4. How does one determine the performance of the image analysis?

5. How should image analysis be reported?
# Guideline Panel Members

## Advisory panel
- Kenneth J. Bloom, MD
- M. Elizabeth Hammond, MD
- Stephen Hewitt, MD, PhD
- Richard Levenson, MD
- David Rimm, MD, PhD
- Mogens Vyberg, MD

## Expert panel
- Marilyn Bui, MD, PhD, Chair
- Kimberly H. Allison, MD
- Elizabeth Chlipala, BS, HTL(ASCP) QIHC
- M. Elizabeth Hammond, MD
- Andrea Kahn, MD
- Anant Madabhushi, PhD
- Liron Pantanowitz, MD

## Staff
- Carol Colasacco, MLIS, SCT(ASCP), Medical Librarian
- Nicole Thomas, MPH, CT(ASCP), Sr. Guideline Development Manager

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CAP
Results of the systematic review

- 248 references for title/abstract screening
- 52% (130) included in full text screening
- 64% (65) included in data extraction

Total number of included studies to be determined after the literature refresh
Draft recommendations

- 11 draft recommendations
  - 7 recommendations (based on laboratory accreditation requirements)
  - 4 expert consensus opinions

- Data was difficult to synthesize
  - Various imaging systems reported in the literature
  - Data not reported for many of the outcomes of interest
Results of comment period

• CAP hosted a three week comment period in March 2017 for any stakeholder to provide feedback to the draft recommendations

• More than 150 participants and more than 180 comments received
Draft guideline statements

1. Laboratories should select a quantitative image analysis system for HER2 immunohistochemistry that is capable of meeting the standards for reporting as set forth by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) in the guideline “Recommendations for Human Epidermal Growth Factor 2 Testing in Breast Cancer.” – Expert Consensus Opinion

85. 27% agree

14.73% disagree

Final statement will be revised
2. Laboratories should validate their quantitative image analysis results for clinical use by comparing them to an alternative, validated method. – Recommendation

93.64% agree
6.36% disagree

Final statement will be revised
3. Laboratories should ensure that the results produced by a quantitative image analysis system are reproducible within and between different batch analyses. – Recommendation

95.10% agree
4.90% disagree
No revision
Draft guideline statements, continued

4. Laboratories should ensure that the results produced by a quantitative image analysis system are reproducible between operators when they select regions of interest for analysis and/or perform annotation. – Expert Consensus Opinion

89.90% agree
10.10% disagree
No revision
Draft guideline statements, continued

5. Laboratories should continuously monitor and document the performance of their quantitative image analysis system. – Recommendation

89.8% agree
10.2% disagree

Final statement will be revised
Draft guideline statements, continued

6. Laboratories should have procedures in place to address changes to the quantitative image analysis system that could impact clinical results. – Recommendation

93.88 % agree
6.12% disagree
No revision
7. Laboratories should report that quantification was obtained by image analysis, the image analysis methods used, and at minimum, utilize the scoring schema recommended by the ASCO/CAP “Recommendations for Human Epidermal Growth Factor 2 Testing in Breast Cancer” guideline. – Expert Consensus Opinion

95.88% agree
4.12% disagree

Final statement will be revised
8. Personnel involved in the quantitative image analysis process should be trained specifically in the use of the technology. – Recommendation

92.78% agree
7.22% disagree
No revision
9. Laboratories should retain at minimum, the regions of an image that were analyzed and the metadata generated in adherence to local requirements and applicable regulations. –Expert Consensus Opinion

83.16% agree
16.84% disagree

No revision
Draft guideline statements, continued

10. A pathologist trained in QIA should oversee the entire process of quantitative image analysis used for clinical practice. – Recommendation

74.47% agree
25.53% disagree

Final statement will be revised
11. A pathologist trained in QIA must visually verify the image, the annotated image analysis output, and the algorithm results prior to finalizing the report. — Recommendation

78.72% agree

21.28% disagree

Final statement will be revised
Next steps

• Manuscript and Methods Supplement
  – Expected submission in August, 2017
  – Publication (early online release) estimated in October, 2017
Summary

- QIA has been shown to improve consistency and accuracy of interpretation than manual scoring by pathologists, but has not gained widespread acceptance.
- Lack of a guideline is a practical gap.
- This guideline is to provide recommendations for improving reproducibility, precision, and accuracy in the interpretation of HER2 IHC where QIA is employed.
- This is an evidence-based guideline with public input to ensure the recommendations are clinically sound, practical and implementable.
Acknowledgement

• CAP Quality Center and member of the Expert Panel and Advisory Panel of the Quantitative Image Analysis Guideline
• Digital Pathology Association
• Moffitt Cancer Center Analytic Microscopy Core (Joseph Johnson, slides sharing and Jonathan Nguyen, file transferring)
• Dr. Liron Pantanowitz, slides sharing
• Ms. Nicole Thomas, slides preparation
COLLABORATION

- support digital pathology education initiatives
- define best practices
- influence standards and interfaces
- organize an annual conference that addresses diverse needs within the industry

PATHOLOGY VISIONS

DIGITAL PATHOLOGY: THE POWER OF PIXELS
OCTOBER 1 - 3 | SAN DIEGO, CA
Questions?