Computational Pathology: Bringing Algorithms to the Forefront of Improving Pathology Resulting

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Human Epidermal Growth Factor Receptor Type 2 (HER2/ERBB2)

- Receptor tyrosine kinase
- Amplified/overexpressed in 15-20% of primary breast cancers
- Testing methods: IHC and ISH
- HER2 targeted therapies
  - Trastuzumab (Herceptin), pertuzumab (Perjeta), lapatinib (Tykerb)
2013 ASCO/CAP Guidelines: Immunohistochemistry Criteria*

HER2 testing (invasive component) by validated IHC assay

Batch controls and on-slide controls show appropriate staining

Circumferential membrane staining that is complete, intense, and within > 10% of tumor cells*

- IHC 3+ positive

Circumferential membrane staining that is incomplete and/or weak/moderate and within > 10% of tumor cells*

- IHC 2+ equivocal

Incomplete membrane staining that is faint/barely perceptible and within > 10% of tumor cells*

- IHC 1+ negative

No staining is observed* or membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of tumor cells

- IHC 0 negative

Must order reflex test (same specimen using ISH) or order a new test (new specimen if available, using IHC or ISH)

These were updated in 2018 after this work was completed

* These were updated in 2018 after this work was completed.
2013 ASCO/CAP Guidelines: ISH Criteria

HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization

**HER2/CEP17 ratio \( \geq 2.0 \)**

- **Average HER2 copy number \( \geq 4.0 \) signals/cell**
  - ISH positive

- **Average HER2 copy number < 4.0 signals/cell**
  - ISH positive

**HER2/CEP17 ratio < 2.0**

- **Average HER2 copy number \( \geq 6.0 \) signals/cell**
  - ISH positive

- **Average HER2 copy number \( \geq 4.0 \) and < 6.0 signals/cell**
  - ISH equivocal

- **Average HER2 copy number < 4.0 signals/cell**
  - ISH negative

Must order a reflex test (same specimen using IHC), test with alternative ISH chromosome 17 probe, or order a new test (new specimen if available, ISH or IHC)
Test Validation and Concordance

• ASCO/CAP 2007 guidelines
  – “Proof of initial testing validation in which positive and negative HER2 categories are 95% concordant with alternative validated method or same validated method for HER2.”

• ASCO/CAP 2013 guidelines
  – “Laboratories are responsible for ensuring the reliability and accuracy of their testing results, by compliance with accreditation and proficiency testing requirements for HER2 testing assays. Specific concordance requirements are not required.”
### Table 2. Histopathologic Features Suggestive of Possible HER2 Test Discordance

<table>
<thead>
<tr>
<th>Criteria to Consider*</th>
</tr>
</thead>
<tbody>
<tr>
<td>New HER2 test should not be ordered if the following histopathologic findings occur and the initial HER2 test was negative:</td>
</tr>
<tr>
<td>Histologic grade 1 carcinoma of the following types:</td>
</tr>
<tr>
<td>Infiltrating ductal or lobular carcinoma, ER and PgR positive</td>
</tr>
<tr>
<td>Tubular (at least 90% pure)</td>
</tr>
<tr>
<td>Mucinous (at least 90% pure)</td>
</tr>
<tr>
<td>Cribriform (at least 90% pure)</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma (90% pure) and often triple negative</td>
</tr>
</tbody>
</table>

Similarly, a new HER2 test should be ordered if the following histopathologic findings occur and the initial HER2 test was positive:

| Histologic grade 1 carcinoma of the following types: |
| Infiltrating ductal or lobular carcinoma, ER and PgR positive |
| Tubular (at least 90% pure) |
| Mucinous (at least 90% pure) |
| Cribriform (at least 90% pure) |
| Adenoid cystic carcinoma (90% pure) and often triple negative |

If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test must be ordered on the excision specimen if one of the following is observed:

| Tumor is grade 3 |
| Amount of invasive tumor in the core biopsy is small |
| Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core |
| Core biopsy result is equivocal for HER2 after testing by both ISH and IHC |
| There is doubt about the specimen handling of the core biopsy (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error |

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; PgR, progesterone receptor.

*Criteria to consider if there are concerns regarding discordance with apparent histopathologic findings and possible false-negative or false-positive HER2 test result.
Published Discordance Rates between IHC and Her2neu

• 7% of 0,1+ tumors (Petroni et al, *Oncology Letters*, 2016)

• 1.6% of 0,1+ tumors (Solomon et al., *AJCP*, 2017)

• 4% of 0,1+ (Kaufman et al., *Cancer*, 2014)
  – recommended reflex to FISH for all negative or equivocal cases (0, 1+, 2+)
MDACC Algorithm

- Reflex FISH testing on all cases that do not have IHC score of 0
  - Based on published discordance rates for IHC 1+ and 3+
  - Reflex testing on IHC 2+ is required
Objectives

• To determine the positive and negative concordance rates for HER2 IHC/FISH tests at MDACC.

• To derive an algorithm that will help select HER2 IHC 1+ cases for reflex FISH testing, to reduce health care costs while minimizing patient impact.
Methods

  – Regular expression-based ETL (extract, transform, load) tool
  – Query structured data elements in breast biomarker worksheets, e.g. tumor block, HER2, ER, PR, Ki67 index

• FISH data from the Cytogenetics Lab (Apr. 2013-Aug. 2017)
  – HER2/CEP17 ratio, average HER2 copy number/cell, average CEP17 copy number/cell, etc.

• Cases with more than one biomarker worksheet/FISH test excluded
  – Bilateral tumors and those with repeat IHC

• Visual Basic for Applications to merge data
Methods

  – Regular expression-based ETL (extract, transform, load) tool
  – Query structured data elements in breast biomarker worksheets, e.g. tumor block, HER2, ER, PR, Ki67 index
    • SQL query of table with data modified to conform to varying structures for different breast versions
    • Cases with more than one worksheet excluded
      – Bilateral tumors, repeat IHC, repeat FISH tests
• FISH data from the Cytogenetics Lab (Apr. 2013-Aug. 2017)
  – HER2/CEP17 ratio, average HER2 copy number/cell, average CEP17 copy number/cell, etc.
• Visual Basic for Applications to merge data based on matching Surgical Accession numbers
Methods

• Cases with incomplete IHC biomarker data excluded

• IBM SPSS v23.0 used to generate a decision tree using the CHAID (Chi-square automatic interaction detection) growing method
  – Significance level of $p = 0.05$ for splitting nodes
  – Split-sample validation: 2/3 data as the training set, and 1/3 data as test set
## HER2 FISH vs IHC Results

<table>
<thead>
<tr>
<th>FISH</th>
<th>IHC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Negative</td>
<td>79</td>
<td>467</td>
</tr>
<tr>
<td>Equivocal</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>87 (8.2%)</td>
<td>516 (48.7%)</td>
</tr>
</tbody>
</table>
Cases with Complete Biomarker Data

• False negative rate: 10.0% (29/289)
• False positive rate: 0.1% (1/728)
• Excluding IHC/FISH equivocal cases
  – Positive concordance rate: 99.4% (1/158)
  – Negative concordance rate: 95.0% (546/575)
  – Overall concordance rate: 95.9% (703/733)
Discussion

• Discontinuing reflex testing on HER2 IHC 3+ cases:
  – Miss 0.1% (1/728) true FISH-negative cases
  – Spare 159 FISH tests
  – Potential health care savings of $242,634 ($1526/test) over 4 years
Decision Trees for HER2 IHC 1+ Cases

Figure 1: Decision tree analysis for the training sample.
Reflexed 94.4% (17/18) of IHC 1+ false negative to FISH

Figure 2: Decision tree analysis for the test sample.
Reflexed 80% (8/10) IHC 1+ false negative to FISH in Test
Discussion

• Using cut-off of ER 95% for reflex testing on HER2 IHC 1+ cases:
  – Miss 1.0% (3/289) true FISH-positive cases
  – Spare 147 FISH tests
  – Potential health care savings of $224,322 ($1526/test) over 4 years
Discussion

• Overall, potential health care savings of $116,739/per year
• Reduce FISH tests by 29% (306/1059)
However...

• HER2 IHC antibody change
  – Prior to Aug. 2016: HER2/neu Ab-8 (Neomarkers, clone e2-4001)
  – After Aug. 2016: PATHWAY anti-HER2/neu (Ventana, clone 4B5)
Objectives

• Compare the positive and negative concordance rates for HER2 IHC/FISH tests at MDACC using the two primary HER2 antibodies.
Methods

• Same as previous, now including (manual review):
  – All breast cancer cases with in-house HER2 IHC
  – Cases with more than one biomarker worksheet
  – Repeat FISH tests

• Extensive database clean-up (manual review):
  – Non-breast cases
  – Cases with bilateral tumors/repeat biomarkers
  – Cases with duplicate FISH tests
  – Typographical errors in biomarker worksheets and cytogenetics database
    • Lack of controlled vocabulary and internal error checking
  – Cytogenetics cases with inconsistent accession formatting
    • Lack of controlled vocabulary
## Results

<table>
<thead>
<tr>
<th></th>
<th>FISH</th>
<th>IHC</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ab-8</td>
<td>239</td>
<td>640</td>
<td>361</td>
<td>179</td>
<td></td>
<td></td>
<td>1419</td>
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<tr>
<td></td>
<td>Negative</td>
<td>224</td>
<td>567</td>
<td>201</td>
<td>3</td>
<td></td>
<td>995</td>
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<tr>
<td></td>
<td>Equivocal</td>
<td>8</td>
<td>32</td>
<td>23</td>
<td>2</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>7</td>
<td>41</td>
<td>137</td>
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<tr>
<td>4B5</td>
<td>62</td>
<td>212</td>
<td>214</td>
<td>86</td>
<td></td>
<td></td>
<td>574</td>
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<tr>
<td></td>
<td>Negative</td>
<td>58</td>
<td>193</td>
<td>157</td>
<td>1</td>
<td></td>
<td>409</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>3</td>
<td>14</td>
<td>18</td>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1</td>
<td>5</td>
<td>39</td>
<td>85</td>
<td></td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>Grand Total</td>
<td>301</td>
<td>852</td>
<td>575</td>
<td>265</td>
<td></td>
<td>1993</td>
</tr>
</tbody>
</table>
Results

• Neomarkers Ab-8 (Aug. 2013-2016)
  – False negative rate: 13.4% (48/359)
  – False positive rate: 0.3% (3/995)
  – Excluding IHC/FISH equivocal cases
    • Positive concordance: 98.3% (174/177)
    • Negative concordance: 94.3% (791/839)
    • Overall concordance: 95.0% (965/1016)

  – False negative rate: 4.6% (6/130)
  – False positive rate: 0.2% (1/409)
  – Excluding IHC/FISH equivocal cases
    • Positive concordance: 98.8% (85/86)
    • Negative concordance: 97.7% (251/257)
    • Overall concordance: 98.0% (336/343)
Discussion

• Insufficient data to train and test a model
  – Cannot use 4B5 data as test data set as it was used in previous training set
  – Training on old antibody and testing on new antibody not appropriate
Learning Points

• Cytogenetics database
  – Surgical accession number
  – Block ID
  – Controlled fields for primary site
• Biomarker worksheets
  – Block ID (in-house and outside cases)
  – Tumor grade
• Potential use of error checking logic in biomarker worksheets in QI
  – HER2 IHC/FISH discordance
  – Typographical/clicking errors
  – IHC interpretation by pathologist
Future Directions for Her2neu Testing

• How to work around multiple worksheets without manual database clean-up
• Accumulate data to develop an algorithm for new Ventana antibody
  • Collect more false negative data and develop algorithm
  • Determine if there is systematic variation by pathologists and work for a higher level of concordant criteria in readout
• Consider not reflexing to FISH for 3+ cases (savings...)
• Try See5, a Decision Tree algorithm
Computational Pathology in Pathology

• Computational algorithms can improve
  – QI
  – Development of reflexive algorithms
  – Predictors of disease
    • Sepsis prediction algorithms
  – Anatomic Pathology Diagnosis
**AI in Anatomic Pathology Diagnosis**

- **Automated histological diagnosis**
  - Cost benefit
    - What is the rate limiting step in completing a report
      - Diagnosis instantaneous
        » And when it isn’t, will you bypass the special studies?
      - Qualifiers such as grade, size, margins etc. are not
        » Focus on biopsies
      - Preparation of report is not
        » Room for improved resulting - prepare the report
        » AP LIMS and HER systems have a long way to go
AI

• Recent ethical considerations
  – PAIGE (NY Times 9/20/2018)
  – FDA approval rules vary
    • Imbedded in HER vs standalone
  – Exclusivity of use of data
    • Data belongs to the institution that created it
Data Sources

• Need for discretized data
  – Electronic data capture

• Need for interoperable data
  – Amass data across multiple institutions
  – ONC, SDC (structured data capture transmitted with form metadata for collection and output)

• Need for testing multiple decision algorithms
  – False positives can vary by types of studies
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