**Background**

- HER2 testing by immunohistochemistry (IHC) is prone to inter-observer variability and subjective interpretation.
- Pathologist-assisted digital image analysis is recommended in the CAP/ASCO guidelines to improve interpretive consistency.
- Benchmark concordance for HER2 IHC - HER2 FISH is >95% for positive (3+) and negative (0,1+) cases.
- HER2-CONNECT (Visiopharm, Denmark) is an algorithm that scores stained membrane interconnectivity rather than individual cell staining patterns.
- As such, it is a surrogate for the so-called “chickenwire” pattern that is characteristic of true HER2 positive tumors.
- We applied HER2-CONNECT analysis with pathologist quantitative interpretation (HC+PQI) to a set of breast tumors with known HER2 FISH status to assess concordance.

**Design**

- HER2 IHC (HercepTest) slides previously interpreted manually were re-submitted for HC+PQI.
- These included core and excisional biopsies from primary and metastatic sites.
- HER2 FISH (PathVysion) was performed on all cases.
- Using connectivity scores on a scale of 0-1.0 from the analyzed regions, a pathologist determined the overall % of HER2 positivity (since HER2-CONNECT does not perform cell quantification) and reported a final score per CAP/ASCO guidelines (<10% = 1+, 10-30% = 2+, >30% = 3+).

**HER2 IHC-FISH Correlation by “Chicken-wire” Pattern**

<table>
<thead>
<tr>
<th>Sensitivity Predicting HER2 Amplification</th>
<th># of cases %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Wire</td>
<td>78/115 67.8</td>
</tr>
<tr>
<td>Uniformity</td>
<td>86/88 97.7</td>
</tr>
<tr>
<td>Both</td>
<td>78/78 100</td>
</tr>
</tbody>
</table>

*K Hansen, ASCO 2020

**HER2 IHC-FISH Correlation Using HER2-CONNECT™**

<table>
<thead>
<tr>
<th>Manual Read</th>
<th>HER2-CONNECT alone</th>
<th>HC+PQI</th>
<th>HC score</th>
<th>HC+PQI Decision Tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+</td>
<td>64/77</td>
<td>83%</td>
<td>66/92</td>
<td>72%</td>
</tr>
<tr>
<td>2+</td>
<td>2/73</td>
<td>2.7%</td>
<td>0/39</td>
<td>0%</td>
</tr>
<tr>
<td>0/1+</td>
<td>0/40</td>
<td>0%</td>
<td>0/59</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Results**

- 190 cases comprised the analysis set.
- 66 cases were HER2 FISH amplified.
- 6 cases FISH equivocal:
  - 1 “1+”, 5 “2+”
- Overall concordance:
  - HER2-CONNECT™ alone = 83%.
  - Manual read = 88.9%.
  - HC+PQI = 97.5%.
- Using FISH as the gold standard, HC+PQI reduced “false positives” (compared to manual) from 13% (15/108) to 3% (4/108).
- 17 of 63 HER2 nonamplified samples manually scored 2+ were 1+ by HC+PQI (i.e. FISH testing reduced by 27%).

**Conclusion**

- Relative to manual interpretation, HC+PQI improved correlation with HER2 FISH and reduced the overall number of 2+ results, underscoring the specificity of the uniform “chicken wire” pattern of HER2 IHC predicting HER2 amplification by FISH.