Performance of chromogenic in situ hybridization (CISH) in HER-2 immunohistochemistry-equivocal cases

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Abstract
Background: Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the two assays currently used to determine HER-2 status of breast cancer, with FISH being the gold standard. CISH also allows the detection of HER-2 gene overexpression and amplification, a potential alternative to FISH. However, the effect of IHC-equivocal cases on CISH results has not been well studied.

Design: We studied three sets of breast carcinomas. Set 1 contained 60 tumors with equivocal HER-2 IHC score (antibody AB8, Neomarkers) during patient care at MD Anderson (site A). Set 2 contained 21 equivocal tumors determined by HercepTest (site A) kit at 60 tumors from set 1. Set 3 contained all the HercepTest-equivocal tumors from Set 2. Final IHC-equivocal cases showing 2+ IHC score previously with Neomarkers kit; set 2 contained the 60 tumors plus the 21 tumors showing 2+ HercepTest score (i.e., a total of 81 tumors); and set 3 contained all the tumors from set 2 that demonstrated 2+ HercepTest score (i.e., 36 for site A and 43 for site B). Both FISH and CISH assays were performed at site A and site B on duplicate paraffin sections of the three sets of samples using the same in situ hybridization (CISH) kit. The agreement rate for the 60 tumors at site A and 15% at site B, and by CISH, in 10% at site A and 15% at site B. Agreement between FISH and CISH in the three datasets at each site is shown below.

Results: Of the 60 IHC-equivocal tumors in set 1, HercepTest scores of 0, 1+, 2+, and 4+ were found in 4%, 12%, 23%, 25%, and 7%, at site A and in 2% at 2%, 10%, 15%, 3%, and 25%, at site B, respectively. Inter-site agreement on HercepTest results for set 1 was 85.7%, and Cohen's κ coefficient was 0.52. Gene amplification by FISH was found in 13% of the 60 tumors at site A and 15% at site B, and by CISH, in 10% at site A and 15% at site B. Table 1 shows the agreement between FISH and CISH in the three IHC-equivocal datasets at each test site.

Conclusions: Reproducibility of IHC-equivocal results was lower when different antibody was used. Inter-site agreement on HER-2 status was only fair using HercepTest to reproduce equivocal score determined with different peroxidase reaction between CISH and FISH copy. IHC-equivocal cases were good to strong at each test site, and a lower degree of agreement tended to be seen in data set containing a higher proportion of equivocal tumors determined by HercepTest.

Introduction
Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the two assays currently used to determine HER-2 status of breast cancer, with FISH considered the gold standard. Identification of HER-2 status is a prerequisite for eligibility for Herceptin treatment in patients with breast cancer. Specifically, only patients with HER-2 overexpression (as defined by IHC 3+ or HER2 gene amplification by FISH) are eligible for Herceptin treatment. Chromogenic in situ hybridization (CISH) is recently emerged as a potential alternative to FISH because CISH is technically simpler and more straightforward. CISH also allows detection of HER-2 amplification with conventional peroxidase reaction, enumeration of gene copy number with simultaneous histologic examination by regular bright-field microscopy. Our study showed an overall good agreement between CISH and FISH. We have evaluated the performance of CISH on tumors showing IHC-equivocal or 2+ score.

Materials and Methods
Resection specimens of invasive breast carcinomas with HER-2 equivocal status (i.e. IHC score of 2+) were obtained from several institutions. Final IHC-equivocal cases were confirmed to be 2+ score by routine patient care using an HER-2 monoclonal antibody, clone AB1 (1:100, Neomarkers, Fremont, CA). Repeat IHC staining using HercepTest (DakoCytomation, Carpinteria, CA) was performed at two test sites on duplicate paraffin sections of the 60 tumors at MD Anderson Cancer Center (site A) and of the 60 tumors at University of Tampere, Tampere, Finland (site B). A score of 2+ was interpreted as equivocal, and tumors were reclassified. Tissue blocks were cut for the two test sites on tumor samples consecutively obtained at the same 110 at site A and 116 at site B. Of the 220 cases, 21% (51%) demonstrated 2+ score with HercepTest.

Table 1. Agreement between FISH and CISH in the three IHC-equivocal datasets at each test site

<table>
<thead>
<tr>
<th>Test Site</th>
<th>Tumor Status</th>
<th>CISH/FISH</th>
<th>Agreement Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A</td>
<td>27/1</td>
<td>24/5</td>
<td>92.9%</td>
</tr>
<tr>
<td>Site B</td>
<td>21/2</td>
<td>18/4</td>
<td>85.7%</td>
</tr>
</tbody>
</table>

* Tumors that have invalid FISH or CISH results were excluded

Table 2. IHC-equivocal tumors showing discrepant HER-2 status between CISH and FISH

<table>
<thead>
<tr>
<th>Test Site</th>
<th>CISH/FISH</th>
<th>HER-2 Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A</td>
<td>27/1</td>
<td>24/5</td>
</tr>
<tr>
<td>Site B</td>
<td>21/2</td>
<td>18/4</td>
</tr>
</tbody>
</table>

* Tumors that have invalid FISH or CISH results were excluded

Conclusions
• Reproducibility of IHC-equivocal results was low when different antibody was used. Inter-site agreement on HER-2 status was only moderate using IHC/Herceptin to reproduce equivocal 2+ score determined with Neomarkers antibody (ABB).
• Agreement between CISH and FISH on HER-2 status was good to strong at each test site, and a lower degree of agreement tended to be seen in data sets containing a higher proportion of equivocal/2+ tumors determined with HercepTest.