Sensitivity of HER-2/neu Antibodies in Archival Tissue Samples of Invasive Breast Carcinomas

Correlation With Oncogene Amplification in 160 Cases

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Key Words: Breast; Immunohistochemistry; Fluorescence in situ hybridization; HER-2; Gene amplification; FISH

Abstract

Overexpression and amplification of the HER-2 oncogene in patients with breast cancer has correlated with early onset of metastasis, resistance to hormonal therapy and some forms of chemotherapy, and shortened survival. Therefore, evaluation of this putative prognostic or predictive factor seems critical. Because different antibodies are used for the detection of the 185-kd HER-2 oncoprotein, we studied the sensitivity of 3 frequently used antibodies. Immunohistochemistry results were correlated with gene amplification level as assessed by fluorescence in situ hybridization. Protein overexpression was found in 17.2% and 12.5% of cases using antibodies against the external (TAB250) and internal (CB11) domains of the protein, respectively, and in 38.0% of cases using a rabbit polyclonal antibody. Fluorescence in situ hybridization was successful in all 160 tumors, and amplification was found in 37 tumors (23.1%). The monoclonal antibody TAB250 had the lowest misclassification rate, 9.6% (sensitivity, 67%; specificity, 97.5%).

Overexpression of the HER-2 oncogene occurs in 20% to 30% of invasive breast carcinomas and is correlated with a more aggressive phenotype and worse disease-free and overall survival.1-3 The HER-2 oncogene encodes a 185-kd epidermal growth factor receptor–like transmembrane glycoprotein with tyrosine kinase activity.4,5 Overexpression of the protein is found when there is gene amplification, which can be detected using fluorescence in situ hybridization (FISH).6,7 It is becoming an important method for routine assessment of oncogene amplification in breast and other cancers, and it seems to be more reproducible than immunohistochemistry for analysis of HER-2. However, it will not detect oncogene activation as a result of increased gene transcription or translation without a change in gene copy number. This procedure, which can be performed on fresh, frozen, or fixed paraffin-embedded tissue and provides analysis on a cell-by-cell basis, has proved as accurate as Southern blot analysis. In addition, it permits the measurement of the fraction of amplified cells and the intracellular heterogeneity within a given tumor cell population. In these instances, immunohistochemistry can be complementary by detecting overexpression of the gene product, the p185 HER-2 oncoprotein. Previous studies using a wide variety of different antibodies have reported various sensitivities and specificities using paraffin-embedded material,8 but none have compared results of immunohistochemistry with the data obtained by FISH.

As data on the putative predictive value of HER-2 accumulate, suggesting that HER-2 overexpression is associated with resistance to hormonal therapy and to cyclophosphamide, methotrexate, and fluorouracil but with sensitivity to adequately dosed anthracycline-based chemotherapy,9-15 it becomes important to develop a reliable and sensitive test to
identify patients with HER-2–overexpressing tumors to optimize their treatment. We studied HER-2 oncogene expression using 3 commonly used antibodies: monoclonal CB11 and TAB250, which recognize the internal and external domains of HER-2 oncoprotein, respectively, and a rabbit polyclonal antibody. The protein detection results were compared with gene amplification as assessed by FISH, which was considered the “gold standard.”

Materials and Methods

Breast Tumors

We performed a prospective study on 160 breast cancer cases. All cases were from the Institut Jules Bordet, Brussels, Belgium. The breast tumors were graded by the modified Bloom and Richardson score on H&E-stained slides.16,17

Cell Preparation and FISH

A sample of 0.5 cm² or a touch preparation was obtained from primary breast cancers immediately after surgery. Cells were disintegrated mechanically, and the suspension was centrifuged and treated with a 0.075-mol/L concentration of potassium chloride for 1 hour at 37 C. After washing in a methanol–acetic acid solution (3:1), the cell pellet was spread onto glass slides. FISH was performed on slides denatured in a 70% formamide–2· standard saline citrate (SSC) solution, pH 7, at 73 C for 10 minutes. After dehydration in an ethanol series, 10 µL of hybridization mixture containing HER-2 labeled with Spectrum Orange and chromosome enumeration probe (CEP) 17 labeled with Spectrum Green (SO LSI HER-2/SG CEP17, Vysis, Downers Grove, IL) was applied. Slides were incubated overnight in a moist chamber at 37 C and then washed in 0.4·SSC, pH 7, at 73 C and a 0.4·SSC–0.1% Nonidet P-40 solution (Sigma-Aldrich, Bornem, Belgium) at room temperature for 2 minutes each. Nuclei were counterstained with 1 µg/mL of 4¢,6-diamidino-2 phenylindole dihydrochloride (DAPI) in the antifade p-phenylendiamine dihydrochloride.

Analysis of Interphase FISH

Hybridization signals were enumerated in at least 150 (touch preparations) or 250 (pulverized tumors) nuclei per specimen. Overlapping nuclei and nuclei lacking hybridization signals were excluded from analysis. Individual signals (eg, 1, 2, 3, 4) for HER-2 and CEP17 were evaluated for each nucleus. HER-2 gene amplification was defined as a HER-2/chromosome 17 ratio greater than 2.0 using the Vysis probe (catalog no. 32-190007). Analysis and photography were performed using a fluorescence microscope (Leitz DMRB, Leica, Wetzlar, Germany) equipped with a triple bandpass filter for simultaneous detection of Spectrum Green, Spectrum Orange, and DAPI (DAPI, FITC, TRITC, Chroma, San Rafael, CA). Photographs were obtained after digitization of the images with a charge-coupled device camera, under oil immersion at -1,000 magnification.

Immunohistochemical Analysis

Sections (4 µm) from embedded blocks of breast carcinomas that had been fixed routinely (overnight) in neutral buffered formalin were cut on poly-L-lysine–coated slides. HER-2 protein expression was evaluated immunohistochemically using 2 monoclonal antibodies for the external (TAB250, dilution, 1:40; Zymed Laboratories, San Francisco, CA) and the internal (NCL-CB11, dilution, 1:50; Novocastra Laboratories, Newcastle upon Tyne, England) domains of the protein and 1 rabbit polyclonal antibody (catalog no. 28-0004, dilution, 1:30; Zymed). The slides were dewaxed and rehydrated, and immunoreactivity was enhanced by antigen retrieval treatment for the rabbit polyclonal antibody. This consisted of heating the slides in a microwave oven (Panasonic NN-5252B, Matsushita Electric Ltd, England) in a 10-mmol/L concentration of sodium citrate buffer, pH 7, 4 times for 5 minutes each time at 600 W and followed by cooling for 20 minutes at room temperature.

The sections were stained using the Ventana NexES Staining System (Ventana Medical Systems, Tucson, AZ). Briefly, after a 30-minute incubation at 37 C with the primary antibody, sections were incubated for another 10 minutes at 37 C with a secondary biotinylated antibody and then with avidin-peroxidase for another 10 minutes; 3¢,3¢-diaminobenzidine was used as the chromogen. All products needed for these steps are included in the DAB detection kit provided by the manufacturer (Ventana Medical Systems). Slides were counterstained in Mayer hematoxylin, dehydrated, and mounted. The stained slides were analyzed microscopically by 2 independent observers (D.G. and D.L.), and results were expressed on a plus-minus scale on the basis of the proportion of cells stained: 0%, –; more than 0% to 10%, +; more than 10% to 50%, ++; and more than 50% to 100%, ++++. Tumors in which there was cytoplasmic staining without distinct cell membrane staining were scored as negative. The SK-BR-3 cell line, which is known to show a 6- to 8-fold amplification of HER-2,18 served as the positive control for FISH and immunohistochemistry.

Statistical Methods

For the statistical analysis of HER-2 amplification and HER-2 overexpression, we used binary variables reflecting the positivity status of the measures (yes or no). For each antibody tested, low positivity results (10% or fewer of stained cells) were considered alternatively as positive (A) or as negative (B). The association between HER-2 amplification
and HER-2 overexpression as assessed by the different techniques was tested using a chi-square test for homogeneity. Association with the other parameters measured, all categorical, also was assessed using a chi-square test for homogeneity or for a linear trend for ordinal variables. The level required to reach significance was set at 5%. To compare the performance of the different techniques, HER-2 amplification, as assessed by FISH, was used as the gold standard (positive cases by this method were considered as true positive and negative cases as true negative). Specificity and sensitivity were calculated for each antibody. The antibody with the lowest misclassification rate was selected as the best choice to detect HER-2–positive tumors.

**Results**

**Fluorescence In Situ Hybridization**

The level of oncogene amplification in pulverized specimens or touch preparations from 160 breast tumors was assessed quantitatively by enumerating HER-2 signals using FISH. To control for aneuploidy of the chromosome 17, on which HER-2 is located, an alpha-satellite centromere probe for chromosome 17 was cohybridized with the HER-2 probe. Although Press et al. suggested the use of a single HER-2 probe to detect amplification, we found this approach was justified by the fact that, in our hands, chromosome 17 aneuploidy was frequent (>40% of cases). Aneuploidy was associated commonly with an average of 3 to 5 HER-2 copies. These tumors typically had scattered HER-2 signals and an equal number of chromosome 17 centromere signals. We did not consider such cases to be amplified. Using a ratio of more than 2 oncogene signals/centromere control signals to define amplification, we found that 23.1% of cases had amplification of HER-2. We found 3 patterns of HER-2 gene distribution or amplification: normal diploidy, aneuploidy, and amplification (weak or strong). In 23 tumors (14.4%), a majority of cancer cells had a high amplification of the HER-2 gene with the signals forming 1 or 2 clusters, suggesting that the amplified gene was present in homogeneously staining regions. In 14 other tumors, cancer cells had multiple scattered HER-2 signals, suggesting that the amplicon was within double-minute chromosomes. We found a correlation between tumor grade and HER-2 amplification ($P < .006$); the higher the grade, the greater the number of cases with oncogene amplification. Of note, HER-2 amplification was never detected in lobular carcinomas ($n = 20$, 12.5%).

In addition to determining a relative level of oncogene amplification vs control signal, the pattern of distribution of signals in each tumor also was analyzed. One pattern consisted of 2 red and 2 green signals, reflecting a normal disomic complement for HER-2 and centromere 17 (Image 1A). In the strongly amplified cases, more than 20 HER-2 signals were seen along with 2 to 10 centromere 17 signals (Image 1B). Finally, more than 40% of cases exhibited chromosome 17 aneuploidy and a corresponding decrease or increase in HER-2 signals, resulting in an approximate 1:1 ratio of HER-2/centromere signals (Image 1C).

As the probe we used for determination of HER-2 amplification is a mix of alpha-satellite and unique sequence probes, one might postulate that chromosome 17 signals include artifacts due to nonoptimal hybridization or washing conditions. To address this issue, we performed a hybridization with the alpha-satellite probe alone in the appropriate conditions (in one fourth of aneuploid samples). This demonstrated a perfect correspondence with the previous results obtained using a dual-color probe (data not shown).

**Table II**

**HER-2 Amplification Correlated With Grade** and Tumor Type

<table>
<thead>
<tr>
<th>HER-2 Amplification</th>
<th>Low</th>
<th>High</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive ductal carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 (n = 23)</td>
<td>3</td>
<td>1</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Grade 2 (n = 57)</td>
<td>5</td>
<td>7</td>
<td>12 (21)</td>
</tr>
<tr>
<td>Grade 3 (n = 60)</td>
<td>6</td>
<td>15</td>
<td>21 (35)</td>
</tr>
<tr>
<td>Total (n = 140)</td>
<td>14</td>
<td>23</td>
<td>37 (26.4)</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 (n = 3)</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Grade 2 (n = 16)</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Grade 3 (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total (n = 20)</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total (n = 160)</td>
<td>14</td>
<td>23</td>
<td>37 (23.1)</td>
</tr>
</tbody>
</table>

* $P = .02$.  
† $P = .009$.  

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Immunohistochemistry

The relationship between gene amplification and protein overexpression of HER-2 is illustrated in Table 2. In the present study, we considered only membrane staining as associated with HER-2 overexpression.20 With few exceptions for CB11 (n = 1), TAB250 (n = 3), and rabbit polyclonal (n = 1) antibodies, all the cases with high gene amplification also overexpressed HER-2 protein (data not shown). Cases of weak protein expression (+, 10% or less) were considered as positive (A) or negative (B) to determine the most sensitive and specific antibody and to compare the results with those of previous studies. This approach was justified by the fact that weak protein expression might reflect a low amplification of the oncogene (5 or less).

When staining of 10% or less was considered positive (Table 2), detection with monoclonal antibodies CB11 and TAB250 was positive in 19 (51%) of 37 cases and 24 (67%) of 36 cases, respectively, amplified by FISH. This percentage reached 83% (29/35) when the polyclonal antibody was used, suggesting that antigen retrieval and polyclonal antibody is the most sensitive combination to detect HER-2 amplification. However, with this antibody, 28 (24.3%) of 115 cases had overexpression of the protein in the absence of gene amplification. Although this situation has been reported,21 the percentage of such cases was much lower than in our experience.

Immunohistochemistry using all 3 antibodies (with a threshold >10% staining) failed to show protein overexpression in 14% (5/37) of samples that showed amplification by FISH, an observation that can be explained by suboptimal sensitivity of the antibody, overfixation of the tissue, or inadequate antigen retrieval.

When weak protein expression was considered negative (Table 2), no protein detection was found in a greater...
proportion of the cases that were amplified by FISH, as expected. However for the polyclonal antibody, the specificity increased with an important decrease (from 18.7% [28/150] to 7.3% [11/150]) in the number of cases exhibiting protein expression without gene amplification.

The aforementioned results were used to determine antibody specificity, sensitivity, and the percentage of false or discordant results. These are summarized in Table 3. Analysis of these variables shows that the monoclonal antibody TAB250 is the most reliable antibody to assess HER-2 protein expression when weak expression (+, 10% or less) is considered as positive (TAB250A), with a sensitivity of 67%, specificity of 97.5%, and 9.6% false or discordant results. Our data also indicate that for optimal sensitivity using the monoclonal antibody CB11, even weak positivity should be considered positive for HER-2 protein overexpression. The use of the polyclonal antibody increases the sensitivity but significantly decreases the specificity. No antibody detected HER-2 overexpression at low levels (2:5 ratio) of gene amplification (as detected by FISH).

An additional analysis was performed to assess whether the use of 2 antibodies on adjacent sections could enhance the sensitivity, specificity, or both of immunohistochemistry. The results given in Table 4 show that the combination of the monoclonal antibodies TAB250 and CB11, considering any stained cell as a positive result, does not improve the sensitivity or specificity obtained by using the TAB250 antibody alone (Table 3). However, results obtained by using the polyclonal antibody at the more than 10% cutoff (polyclonal B) in combination with the TAB250 antibody enhanced the sensitivity in comparison with both antibodies used separately. The specificity and false results rate were similar to those of the polyclonal antibody used alone.

Discussion

Detection of HER-2 overexpression has prognostic and therapeutic implications. In the present study, a cohort of 160

**Table 2**

HER-2 Amplification Detected by Fluorescence In Situ Hybridization Compared With Overexpression Measured by Immunohistochemistry*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Amplification</th>
<th>No</th>
<th>Yes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB11</td>
<td>No</td>
<td>122</td>
<td>18</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>123</td>
<td>37</td>
<td>160</td>
</tr>
<tr>
<td>TAB250</td>
<td>No</td>
<td>118</td>
<td>12</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>121</td>
<td>36</td>
<td>157</td>
</tr>
</tbody>
</table>

* For each antibody tested, low positivity results (10% or less of stained cells) were considered alternatively as positive (A) or as negative (B).

**Table 3**

Comparison of Sensitivity, Specificity, and False or Discordant Results of Anti–HER-2 Antibodies*

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>False or Discordant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB11</td>
<td>B 16/37 (43)</td>
<td>123/123 (100)</td>
<td>21/160 (13.1)</td>
</tr>
<tr>
<td></td>
<td>A 19/37 (51)</td>
<td>122/123 (99.2)</td>
<td>19/160 (11.9)</td>
</tr>
<tr>
<td>TAB250</td>
<td>B 19/36 (53)</td>
<td>119/121 (98.3)</td>
<td>19/157 (12.1)</td>
</tr>
<tr>
<td></td>
<td>A 24/36 (67)</td>
<td>118/121 (97.5)</td>
<td>15/157 (9.6)</td>
</tr>
<tr>
<td>Polyclonal</td>
<td>B 23/35 (66)</td>
<td>104/115 (90.4)</td>
<td>23/150 (15.3)</td>
</tr>
<tr>
<td></td>
<td>A 29/35 (83)</td>
<td>87/115 (75.6)</td>
<td>34/150 (22.7)</td>
</tr>
</tbody>
</table>

* Data are given as number/total number (percentage).
* For each antibody tested, low positivity results (10% or less of stained cells) were considered alternatively as positive (A) or as negative (B).
breast tumors with levels of HER-2 gene amplification detected by FISH was used to demonstrate variable detection rates of immunostaining in archival tissues for 3 HER-2–specific antibodies. By using a 2-color FISH method, simultaneous detection of oncogene and chromosome copy numbers was ascertained. We defined amplification as a ratio of HER-2 signals/alpha-satellite signals greater than 2. Aneuploidy was frequent (>40%), suggesting that duplication of the chromosome 17 centromeric region (and probably chromosome 17 number) might be involved in cancer progression or development. Aneuploidy of chromosome 17 or the 17q region has been reported using FISH or comparative genomic hybridization, but with lower frequency (around 20%). Higher rates of amplification were associated with higher grade tumors, and amplification was never found in lobular carcinoma, supporting the view that such tumors represent a separate subtype of breast carcinoma.

The detection of protein overexpression using the polyclonal antibody gave 22.7% false (negative or positive) results compared with gene amplification by FISH (using any staining as positive). This might be due to antigen retrieval problems or the selection of too low a threshold (10% or less) for protein detection. A previous report concluded that when using a polyclonal antibody, less than 25% positively staining cells should be considered negative for protein overexpression. Our results concur with this recommendation since, in our hands, weak staining with the polyclonal antibody also occurred focally in in situ carcinomas and hyperplastic epithelium (data not shown). Taking into account these considerations, we found only 15.3% (23/150) of the results were false or discordant when we used 10% or less as a threshold for positive protein detection.

An alternative explanation for these findings is that aneuploidy, which was not considered as amplification in this study, might lead to increased protein expression detected by immunohistochemistry. To resolve this problem, we counted the number of aneuploid cases among the cases positive by immunohistochemistry for each antibody. For the polyclonal, monoclonal TAB250, and monoclonal CB11 antibodies, 18 of 28 cases, 2 of 3 cases, and 1 of 1 case, respectively, exhibited aneuploidy. This observation suggests that chromosome 17 aneuploidy might be associated with immunoreactivity, resulting in detection of true protein expression. The polyclonal antibody seems particularly promising for detecting such abnormalities.

In comparing the 3 antibodies, we found the monoclonal antibody TAB250 to be more sensitive and specific than the monoclonal antibody CB11 and the polyclonal rabbit antibody, giving only 9.6% false results. Although the polyclonal antibody was the most sensitive (83%), its specificity was not optimal (75.6%). Monoclonal antibody CB11, often used in HER-2 studies, is highly specific but less sensitive than TAB250. Our results are in concordance with those of Penault-Llorca et al and are complementary to those of Press et al, who reported a 97% specificity and 36% sensitivity for antibody TAB250 and 100% specificity and 51% sensitivity for CB11, as determined by Southern, Northern, and Western blot hybridization. In the work by Press et al, the sensitivity of antibody TAB250 could be improved (to reach 70%) by using protease pretreatment; however we elected not to pretreat, as this might have resulted in false-positive staining.

We found that the results obtained by combining the 2 monoclonal antibodies did not improve sensitivity or specificity. However, when the polyclonal antibody was considered positive at a level of more than 10% staining, its combination with monoclonal antibody TAB250 led to 74% sensitivity and 89.5% specificity. We therefore suggest the use of the monoclonal antibody TAB250 with weak staining considered positive, alone or in conjunction with the polyclonal antibody, with a positive test being more than 10% cell staining, to reach a good balance between specificity and sensitivity.

Optimal results using immunohistochemistry depend on the quality of the examined tissue. For prospective studies that use fresh or recently archived tissues, the use of less sensitive and more specific antibodies may be preferable; while for retrospective studies, implying the use of archival samples, a more sensitive antibody might be better.

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**Table 4**

Comparison of Sensitivity, Specificity, and False or Discordant Results of Anti–HER-2 Antibody Combinations

<table>
<thead>
<tr>
<th>Antibody Combination</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>False or Discordant Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB11 A–TAB250 A</td>
<td>24/36 (67)</td>
<td>117/121 (96.7)</td>
<td>16/157 (10.2)</td>
</tr>
<tr>
<td>CB11 A–Polyclonal B</td>
<td>25/35 (71)</td>
<td>103/115 (89.6)</td>
<td>22/150 (14.7)</td>
</tr>
<tr>
<td>TAB250 A–Polyclonal B</td>
<td>26/35 (74)</td>
<td>101/114 (88.6)</td>
<td>22/149 (14.8)</td>
</tr>
<tr>
<td>CB11 B–Polyclonal B</td>
<td>25/35 (71)</td>
<td>104/115 (90.4)</td>
<td>21/150 (14.0)</td>
</tr>
<tr>
<td>TAB250 B–Polyclonal B</td>
<td>26/35 (74)</td>
<td>102/114 (89.5)</td>
<td>21/149 (14.1)</td>
</tr>
</tbody>
</table>

* Data are given as number/total number (percentage).

† For each antibody tested, low positivity results (10% or less of stained cells) were considered alternatively as positive (A) or as negative (B).
Antibody specificity might be further optimized by using a scoring system that combines intensity of staining and percentage of positive cells. A report by Jacobs et al. suggests an improved scoring system that considers the membrane staining of benign breast epithelium as the zero-baseline, which makes sense. Either a final subtracted score more than 2+ or tumor cell staining of 3+ or greater was required for the case to be considered positive. With this method, the investigators succeeded in increasing the specificity for immunohistochemistry from 41.6% to 93.2%. However, our scoring technique for immunohistochemistry, also used in many other studies, is more adapted for comparison with FISH on pulverized tissue. Indeed, amplification analyses consist of an average count in the tumor population and not of a few cells with a high level of amplification. Moreover, we found no staining of benign breast epithelium when using the monoclonal antibodies.

The use of pulverized tumor tissue might be a major cause of false-positive amplification, as ductal carcinomas in situ more commonly exhibit HER-2 amplification than invasive carcinomas. On review of our series, it seems that only 5 of 160 cases presented weak staining in the in situ but not the invasive part of the tumor. As previous major studies do not distinguish invasive and in situ components when studying HER-2 amplification, touch preparations or pulverized tumor tissue may be as good as paraffin-embedded sections for the evaluation of HER-2 amplification.

A previous report suggests that overexpression of HER-2 might be an early step in the development of a distinct histologic type of breast carcinoma. In vitro and in vivo studies suggest that HER-2 amplified oncogene in tumor cells confers motility and invasion properties. HER-2 gene amplification in the absence of adjuvant therapy is an independent predictor of poor clinical outcome and is, in some studies, a stronger discriminant than tumor size. Circulating levels of the extracellular domain of the HER-2–related protein are elevated in 20% to 40% of patients with advanced-stage or metastatic breast cancer, and HER-2 overexpression, identified by immunohistochemistry, has been shown to correlate with early onset of metastasis. However, on a positive note, it also seems to correlate with response to combined cyclophosphamide, doxorubicin, and fluorouracil chemotherapy in women with node-positive breast cancer.

Therefore, HER-2 expression may have prognostic and predictive usefulness for breast cancer management. The latter role of HER-2 will require more studies before implementation in daily practice. Phase 2 clinical trials of immunotherapy directed at HER-2–overexpressing carcinomas have been completed and show that HER-2 is a suitable target for this kind of therapy. Thus, a reliable method of detecting HER-2 overexpression is necessary. In our study, FISH was the most reliable. The recent computerized automation improves the counting of gene amplification over the more time-consuming manual method and, thus, may make it more widely applicable.

Standardized immunohistochemical methods using a single antibody can result in good reproducibility in paraffin-embedded tissue sections. The study of Jacobs et al., which compared the immunohistochemical and FISH methods for evaluation of HER-2 in breast cancer, demonstrates equivalence of both techniques and, therefore, recommends immunohistochemistry as a less time-consuming and less expensive method for routine use in laboratories.

In our study, significant variability in sensitivity was found among 3 commonly used antibodies, and, consequently, these techniques are less reproducible than FISH. The combination of polyclonal and monoclonal antibodies might improve results obtained with a polyclonal antibody, leading to increased sensitivity. In fact, in addition to the choice of the antibody, the great variability in antigen retrieval, tissue fixation, and detection methods makes it difficult to compare results from multicenter studies. Therefore, we suggest that FISH performed with a probe approved by the US Food and Drug Administration for HER-2 oncogene (from Vysis or Ventana) may be the best way to allow comparison of results between different clinical studies because this method may be less technique-dependent than immunohistochemistry. Additional comparative studies of immunohistochemistry and FISH for HER-2 oncogene detection in breast cancers are needed to assess the respective prognostic or predictive reliability of both methods, keeping in mind that one technique detects gene duplication or amplification and the other, protein overexpression.

Our data suggest that staining with monoclonal antibody TAB250 (even if weak) is the most appropriate way to detect HER-2 expression. While allowing a small percentage of false or discordant results, it is more sensitive than the monoclonal antibody CB11 (at similar specificity) and more specific (at similar sensitivity) than the polyclonal antibody. Nevertheless, the polyclonal antibody was the only antibody able to detect cases of aneuploidy, which may represent an additional mechanism of HER-2 overexpression.

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