Original Article

# Clinicopathological variables predicting *HER-2* gene status in immunohistochemistry-equivocal (2+) invasive breast cancer

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**Background and objective:** Human epidermal growth factor receptor-2 (*HER-2*) gene status is crucial to guide treatment decisions regarding the use of HER-2-targeted therapies in breast cancer. An invasive breast cancer with *HER-2* 2+ score is regarded as *HER-2* status equivocal and should further determine by fluorescent in situ hybridization (FISH), which is considered the standard test for HER-2 status. Here, we aimed to establish a risk score to allow for prediction of the presence of *HER-2* gene status.

**Methods:** A total of 182 HER-2 2+ by immunohistochemistry (IHC) invasive breast cancer cases were enrolled in this study. The association between clinicopathological variables like age, sex, tumor grade, hormone receptor (HR) status, P53 and proliferation index (Ki67), and FISH result using US Food and Drug Administration (FDA) criteria was evaluated. Also, we compared the HER-2 FISH results using FDA criteria and 2013 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline.

**Results:** The study population had a median age of 48 years (range, 29-78 years). Estrogen receptor (ER) was expressed in 131 (72.0%) patients. 73.1% of patients (133/182) were progesterone receptor (PR) positive. The median Ki67 value was 20% (range, 3-90%). There was good agreement between the FDA and 2013 ASCO/CAP guideline. Sixty-three of all patients were *HER-2* FISH amplified (positive) based on FDA criteria. Tumors with *HER-2* amplified were more likely to harbor ER negative (58.8% *vs.* 25.2%, P<0.001) or PR negative (57.1% *vs.* 26.3%, P<0.001) or P53 negative (44.8% *vs.* 29.8%, P=0.048). A significant high level of Ki67 was detected in *HER-2* amplified groups (P=0.006). We created a risk score that comprised HR, P53 and Ki67. A significant association between risk score and *HER-2* FISH amplification was observed ($\chi^2$=30.41, P<0.001).

**Conclusions:** This novel immunohistochemical risk score could be highly useful to predict the presence of *HER-2* gene status in invasive breast cancer.

**Keywords:** Invasive breast cancer; human epidermal growth factor receptor-2 (HER-2); immunohistochemistry (IHC); fluorescent in situ hybridization (FISH); prediction

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## Background

Breast cancer is regarded as a heterogeneous group of tumors that are diverse in terms of underlying biology, pathological characteristics, response to therapy, and clinical outcome (1). Breast cancer is divided into at least five distinct molecular subtypes [luminal A, luminal B, human epidermal growth factor receptor-2 (*HER-2*), normal-like, and basal] by gene expression analysis (2). Breast cancer with *HER-2* overexpression currently comprises 15% to 20% of all cases in the world (3). *HER-2*/neu, located on chromosome 17q21, encodes for the 185 kD transmembrane glycoprotein *HER-2*, which is one of the most targeted proteins. Studies indicate that *HER-2* is involved in the activation of

intracellular signal transduction pathways that regulate cell growth, proliferation, adhesion, and motility (4). *HER-2* overexpression or amplification in breast cancer has been extensively studied worldwide (5-7). Overexpression or amplification of *HER-2* has been demonstrated to be an independent parameter for bad prognosis, and is shown to be associated with resistance to certain chemotherapeutic agents (8-11). *HER-2*-targeted therapies have significantly improved disease-free survival in women with *HER-2*-positive cancers both in early and metastatic breast cancer (12,13). Three *HER-2*-targeted agents, trastuzumab (Herceptin), lapatinib (Tykerb), and pertuzumab (Perjeta), have been made available in the past decade for the treatment of *HER-2*-positive metastatic breast cancer (14). Combinations of *HER-2*-directed agents may yield additive or synergistic effects that lead to better prognosis (15).

Overexpression of the *HER-2* protein has become a marker for eligibility for *HER-2*-directed treatments. False positive or false negative results in *HER-2* patients may lead to inappropriate treatment administration (16). Therefore, *HER-2* status is crucial in the guidance of treatment decisions for the use of trastuzumab and is becoming a standard recommendation in the pretreatment work-up of patients with invasive breast cancer. Two conventional methods are used for determining *HER-2* status, namely, immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH). IHC is most frequently used in initial pathological tests for *HER-2* protein expression, and is convenient and inexpensive. *HER-2* IHC results are generally divided into four scale scores (range, 0-3+) on the basis of percentage of positive tumor cells and staining intensity. The US Food and Drug Administration (FDA) and American Society of Clinical Oncology/College of American Pathologists (ASCO/CAPs) recommends that *HER-2* IHC scores of 0 and 1+ should be regarded as *HER-2* negative and those with *HER-2 3*+ scores should be considered *HER-2* positive. An invasive breast cancer with *HER-2 2*+ score is regarded as *HER-2* equivocal and should be further assessed by FISH, which is considered the standard test for *HER-2* status. FISH is more accurate and reliable than IHC; however, its use for routine testing is hindered by drawbacks such as high cost, need for a skilled operator, long procedure, need for special equipment, and difficult preservation of slides for later review.

Invasive breast cancer with *HER-2 2*+ IHC status can be divided into two groups: those that have been possibly *HER-2* amplified and those that have not been *HER-2* amplified. Going *et al.* (17) interpreted 4,343 assessable HercepTests on successive breast cancer tissues and found that 35.7% (315/883) of patients with *HER-2 2*+ were *HER-2* amplified. A few studies have reported the possibility of predicting *HER-2* positivity from *HER-2 2*+ IHC samples (18,19). In our present study, we designed a retrospective clinical analysis to develop a multivariate logistic regression analysis that predicts the presence of *HER-2* amplification in *HER-2 2*+ invasive breast cancer patients.

## Materials and methods

### *Patients*

The present study enlisted 277 operable patients diagnosed with invasive breast cancer between October 2006 and December 2012 at Zhejiang Cancer Hospital, China. All patients were newly confirmed for invasive breast cancer status and have not received treatment. A total of 182 patients with *HER-2 2*+ IHC evaluation were included in this study. The extent of disease was determined by TNM staging according to the new staging system of the American Joint Committee on Cancer/International Union against Cancer (AJCC/UICC) (20). Patient clinical history and tumor characteristics were obtained from histopathology reports and medical records. Gathered data included patient age, tumor location, histological grade, tumor size, regional lymph node status, lympho-vascular invasion (LVI), estrogen receptor (ER), progesterone receptor (PR), HER-2 status, and Ki-67 index. This study was approved by the Institutional Review Board of the hospital. All patients provided informed consent prior to surgery.

### *Immunohistochemistry (IHC)*

All surgical specimens were routinely fixed in 10% buffered formalin solution and embedded in paraffin. Each specimen was verified by two pathologists before inclusion in this study. *HER-2* IHC was performed on unstained sections from representative paraffin blocks using HercepTest. After deparaffinization and dehydration, tissue sections were placed in 0.1 M sodium citrate buffer (PH 6) for 40 min at 99 °C, after which the antigen was retrieved. The slides were cooled at room temperature, rinsed with distilled water, incubated with rabbit monoclonal anti-human *HER-2*/neu antibody for 1 h, then applied with biotinylated secondary antibody for 10 min. The signal was visualized using avidin-peroxidase. The slides were counterstained with Mayer's hematoxylin solution, dehydrated, and

 

mounted. *HER-2* positivity was defined by membranous staining.

*HER-2* immunoreactivity was localized in the cell membrane. *HER-2* expression was scored using HercepTest according to manufacturer's recommendations. Guidelines for scoring were as follows: 0, no immunostaining; 1+, faint perceptible staining of the tumor cell membranes; 2+, weak to moderate complete membrane staining in more than 10% of the tumor cells; and 3+, strong circumferential staining of the entire tumor cell membrane.

All cases also underwent ER, PR, and proliferation index (Ki67) IHC testing. A cut-off level of 10% or greater was defined as positive for ER and PR expression. Positivity for Ki67 was defined by a cut-off level of 15% or greater.

### *Fluorescence in situ hybridization (FISH)*

*HER-2*/neu FISH were assessed on all specimens with *HER-2* IHC 2+. The selected paraffin-embedded tissues sections (4 μm) containing representative invasive breast cancer cells were analyzed by dual-color FISH (a mixture of a spectrum orange DNA probe, covering a 218 kb region that includes the *HER-2* gene, and a spectrum green probe for the chromosome 17 centromere) using the PathVysion *HER-2* DNA Probe kit (Vysis, Inc., USA) according to the manufacturer's instructions. After 5 min denaturation at 82 ℃, the slides and probe mix were incubated overnight at 45 ℃ in a humidified hybridization chamber. The following morning, a fluorescence-mounting medium containing DAPI was applied after a series of stringent washes. The FISH specimens were analyzed on a Nikon Eclipse 80i fluorescence microscope with special filters.

The screening protocol included two independent observers. For each specimen, orange and green signals were counted from a minimum of 80 tumor cell nuclei in at least two distinct areas. *HER-2* gene status was evaluated based on the ratio of *HER-2* signals and chromosome 17 centromic signals. In our study, a case was regarded *HER-2* gene amplified if the ratio of *HER-2/CEP17* was equal to or more than 2.0 as FDA recommendation. Also, the result were classified following 2013 ASCO/CAP guideline: positive (*HER-2/CEP*7 ratio ≥2.0 with an average *HER-2* copy number ≥4.0 signals per cell; *HER-2/CEP*7 ratio ≥2.0 with an average *HER-2* copy number <4.0 signals per cell; *HER-2/CEP*7 ratio <2.0 with an average *HER-2* copy number ≥6.0 signals per cell.), equivocal (*HER-2/CEP*7 ratio <2.0 with an average *HER-2* copy number ≥4.0 and <6.0 signals per cell.) and negative (*HER-2/CEP*7 ratio <2.0 with an average *HER-2* copy number <4.0 signals per cell).

### *Statistical analysis*

Pearson's chi-square test was performed to evaluate the association between clinicopathological variables and *HER-2* FISH positivity. Student's *t*-test was used to compare the Ki67 between the *HER-2* negative and positive group. Risk factors influencing *HER-2* FISH positivity were evaluated by unconditional logistic regression analysis. All statistical calculations were performed with SPSS 13.0 for Windows (Chicago, IL, USA). A P value <0.05 was considered statistically significant.

## Results

This study included 182 invasive breast cancer patients with *HER-2* IHC score of 2+. The characteristics of these patients are summarized in *Table 1*. The study population had a median age of 48 years (range, 29-78 years). Tumor cell grade was available in 153 patients (84.1%), 105 being grade 1 or 2 (57.7%) and 48 being grade 3 (26.4%). Hormone receptor (HR) status was available in all patients. ER was expressed in 131 (72.0%) patients. PR positivity was shown in 73.1% of the patients (133/182). Median Ki67 value was 20% (range, 3-90%). A total of 121 patients had high and 61 had low Ki67 value, according to the Ki67 cut-off value of 15%. According to the new TNM staging system, 19 of all the cases (10.4%) were stage I, 132 (72.5%) were stage II, and 31 (17.0%) were stage III.

The distribution of *HER-2* FISH results according to both FDA and 2013 ASCO/CAP recommendation are shown in *Table 2*. *HER-2* FISH amplified (positive) was found in 34.6% (63/182) according to FDA criteria and 32.9% (60/182) with 2013 ASCO/CAP guideline. There was good agreement between the FDA and 2013 ASCO/CAP guideline. Some changes have been also observed. There were only three patients who had positive according to FDA criteria that changed to negative according to ASCO/CAP guideline, and five patients with positive based on ASCO/CAP cut-off changed to negative with FDA recommendation. The majority of *HER-2* equivocal (ASCO/CAP guideline) patients had *HER-2* negative (90.9%, 10/11).

Then, we take the *HER-2* test guideline of FDA as the major guideline. Sixty-three of all patients were *HER-2* FISH amplified (positive). Patients with *HER-2* FISH amplified tumors were more likely to have higher histological grades ($\chi^2$=8.73, P=0.033) compared with

 

**Table 1** Correlation of HER2 FISH results with clinicopathological features in 182 IHC score 2+ breast cancer

| Factors | FDA | | | 2013 ASCO/CAP | | | |
|---|---|---|---|---|---|---|---|
| | HER2 positive, n (%) | HER2 negative, n (%) | P | HER2 positive (%) | HER2 equivocal (%) | HER2 negative (%) | P |
| Patients, N | 63 | 119 | | 60 | 11 | 111 | |
| Age (years) | | | 0.182 | | | | 0.130 |
| <50 | 69 (69.7) | 30 (30.3) | | 27 (27.3) | 5 (5.1) | 67 (67.7) | |
| ≥50 | 50 (60.2) | 33 (39.8) | | 33 (39.8) | 6 (7.2) | 44 (53.0) | |
| Location | | | 0.280 | | | | 0.573 |
| Left | 61 (69.3) | 27 (30.7) | | 28 (31.8) | 7 (8.0) | 53 (60.2) | |
| Right | 58 (61.7) | 36 (38.3) | | 32 (34.0) | 4 (4.3) | 58 (61.7) | |
| Histological grade | | | 0.033 | | | | 0.405 |
| Grade 1-2 | 73 (69.5) | 32 (30.5) | | 30 (28.6) | 5 (4.8) | 70 (66.7) | |
| Grade 3 | 25 (52.1) | 23 (47.9) | | 20 (41.7) | 4 (8.3) | 24 (50.0) | |
| Not evaluable | 21 (72.4) | 8 (27.6) | | 10 (34.5) | 2 (6.9) | 17 (58.6) | |
| LVI | | | 0.299 | | | | 0.115 |
| Negative | 70 (68.6) | 32 (31.4) | | 28 (27.5) | 5 (4.9) | 69 (67.6) | |
| Positive | 49 (61.3) | 31 (38.7) | | 32 (40.0) | 6 (7.5) | 42 (52.5) | |
| T stage | | | 0.541 | | | | 0.001 |
| T1 | 19 (73.1) | 7 (26.9) | | 7 (26.9) | 1 (3.8) | 18 (69.2) | |
| T2 | 84 (64.6) | 46 (35.4) | | 43 (33.1) | 4 (3.1) | 83 (63.8) | |
| T3 | 14 (66.7) | 7 (33.3) | | 7 (33.3) | 6 (28.6) | 8 (38.1) | |
| T4 | 2 (40.0) | 3 (60.0) | | 3 (60.0) | 0 (0) | 2 (40.0) | |
| N stage | | | 0.498 | | | | 0.698 |
| N0 | 55 (71.4) | 22 (28.6) | | 22 (28.2) | 52 (66.7) | 4 (5.1) | |
| N1 | 33 (61.1) | 21 (38.9) | | 35 (37.2) | 53 (56.4) | 6 (6.4) | |
| N2 | 17 (63.0) | 10 (37.0) | | 3 (30.0) | 6 (60.0) | 1 (10.0) | |
| N3 | 12 (57.1) | 9 (42.9) | | | | | |
| Clinical stage | | | 0.370 | | | | 0.082 |
| I | 15 (78.9) | 4 (21.1) | | 4 (21.0) | 1 (5.3) | 14 (73.7) | |
| II | 83 (62.9) | 49 (37.1) | | 46 (34.8) | 5 (3.8) | 81 (61.4) | |
| III | 21 (67.7) | 10 (32.3) | | 10 (32.2) | 5 (16.1) | 16 (51.6) | |
| ER status | | | <0.001 | | | | <0.001 |
| Negative | 31 (41.2) | 30 (58.8) | | 30 (58.8) | 1 (2.0) | 20 (39.2) | |
| Positive | 98 (74.8) | 33 (25.2) | | 30 (22.9) | 10 (7.6) | 91 (69.5) | |
| PR status | | | <0.001 | | | | <0.001 |
| Negative | 21 (42.9) | 28 (57.1) | | 27 (55.1) | 3 (5.1) | 19 (38.8) | |
| Positive | 98 (73.7) | 35 (26.3) | | 33 (24.8) | 8 (6.0) | 92 (69.2) | |
| P53 status | | | 0.048 | | | | 0.138 |
| Negative | 32 (55.2) | 26 (44.8) | | 25 (43.1) | 3 (5.2) | 30 (51.7) | |
| Positive | 87 (70.2) | 37 (29.8) | | 35 (28.2) | 8 (6.5) | 81 (6.5) | |
| Ki-67 | | | <0.001 | | | | 0.003 |
| 0-15% | 51 (83.6) | 10 (16.4) | | 47 (77.0) | 4 (6.6) | 10 (16.4) | |
| ≥15% | 68 (56.2) | 53 (43.8) | | 64 (52.9) | 7 (5.8) | 50 (41.3) | |

IHC, immunohistochemistry; LVI, lympho-vascular invasion; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor-2; FDA, Food and Drug Administration; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists.

**Table 2** Distribution of HER2 FISH results based on FDA guideline and 2013 ASCO/CAP guideline

| 2013 ASCO/CAP | FDA | |
|---|---|---|
| | Negative | Positive |
| Negative | 106 | 5 |
| Equivocal | 10 | 1 |
| Positive | 3 | 57 |

HER2, human epidermal growth factor receptor-2; FISH, fluorescent in situ hybridization; FDA, Food and Drug Administration; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists.

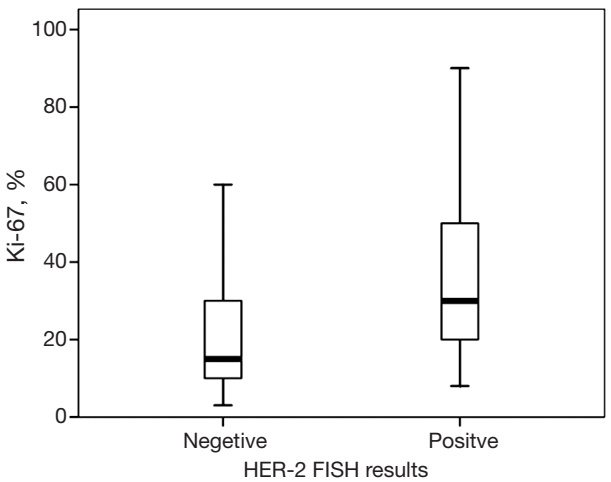


**Figure 1** Box plots showing that higher Ki-67 in cancer with HER-2 FISH positive than in cancer with HER-2 FISH negative. HER-2, human epidermal growth factor receptor-2; FISH, fluorescent in situ hybridization.

patients with unamplified tumors. No significant difference between the groups were found with respect to age (<50 *vs.* ≥50 years, P=0.182), LVI (P=0.299), cancer location (P=0.280), or clinical stage (P=0.370). Tumors with *HER-2* amplification were more likely to be ER-negative (58.8% *vs.* 25.2%, P<0.001), PR-negative (57.1% *vs.* 26.3%, P<0.001), or P53-negative (44.8% *vs.* 29.8%, P=0.048). The median percentage of Ki67 was 15% in the non-*HER-2*-amplified group and 30% in the *HER-2*-amplified group. A significantly high level of Ki67 was detected in the *HER-2*-amplified groups (P=0.006, *Figure 1*). Based on the Ki67 cut-off value of 15%, patients were classified into either of two groups: relatively high Ki67 or low Ki67. A positive correlation was found between Ki67 and *HER-2* status ($\chi^2$=13.46, P<0.001).

A logistic regression model was used to reveal risk factors for *HER-2* amplification. The association between clinicopathological variables and *HER-2* amplification is shown in *Table 3*. Cases with high Ki67 had significantly higher risk of *HER-2* amplification than those with low Ki67 (OR =3.975; 95% CI, 1.846-8.560; P<0.001). Subjects with ER positive expressions were less likely to exhibit *HER-2* amplification compared with those with ER negative expression (OR =0.236; 95% CI, 0.119-0.467; P<0.001). The risk was also much reduced in cases with PR positive expressions than those with PR negative expressions (OR =0.268; 95% CI, 0.135-0.531; P<0.001). Subjects with P53 positive expressions were less likely to develop *HER-2* amplification (OR =0.523; 95% CI, 0.275-0.997; P=0.049).

We created a risk score that comprised the following factors: ER (score 1 when IHC negative; 0 when positive), PR (score 1 when IHC negative; 0 when positive), P53 (score 1 when IHC negative; 0 when positive), and Ki67 (score 0 when IHC negative; 1 when positive). The sum of the above parameters allowed the establishment of a risk score for *HER-2* FISH amplification (*Table 4*). A significant association between risk score and *HER-2* FISH amplification was observed ($\chi^2$=30.41, P<0.001, *Figure 2*). Receiver operator characteristic curves were constructed to compare the ability of the four tumor markers to differentiate between patients with or without *HER-2* FISH amplification. AUC was 0.64±0.04, 0.35±0.05, 0.37±0.05, and 0.43±0.05 for Ki67, ER, PR, and P53. AUC was 0.74±0.04 (95% CI, 0.66-0.81) for the sum of all four markers (*Figure 3*).

## Discussion

Using trastuzumab supplement for neoadjuvant or adjuvant chemotherapy provides significant survival benefit in invasive breast cancer with *HER-2*-overexpressing tumor cells. However, for *HER-2*-negative cases, trastuzumab offers no benefit and only contributes cardiotoxicity and waste of money. Therefore, accurate determination of *HER-2* status in breast cancer patients is an important part of routine practice in pathological reporting. Cases with weak positive staining (2+) by *HER-2* IHC represent a subgroup of patients that requires additional assessment with FISH.

A variety of IHC antibodies and other methods have been developed to determine *HER-2* status in breast cancer patients. Ciftlik *et al.* (21) designed a glass/silicon micro-machined structure for applying microfluidic tissue

**Table 3** Logistic regression analysis of risk factors for HER2 FISH positive (based on FDA guideline) in HER-2 IHC scores 2+ breast cancer patients

| Factors | HR | 95% CI | P |
|---|---|---|---|
| Age (years) | | | |
| <50 | Ref | | |
| ≥50 | 1.518 | 0.822-2.805 | 0.183 |
| Location | | | |
| Left | Ref | | |
| Right | 1.402 | 0.758-2.594 | 0.281 |
| Histological grade | | | |
| Grade 1-2 | Ref | | |
| Grade 3 | 2.099 | 1.040-4.236 | 0.039 |
| Not evaluable | 0.869 | 0.348-2.168 | 0.763 |
| LVI | | | |
| Negative | Ref | | |
| Positive | 1.384 | 0.749-2.558 | 0.300 |
| T stage | | | |
| T1 | Ref | | |
| T2 | 1.486 | 0.582-3.798 | 0.408 |
| T3 | 1.357 | 0.387-4.759 | 0.633 |
| T4 | 4.071 | 0.558-29.725 | 0.166 |
| N stage | | | |
| N0 | Ref | | |
| N1 | 1.591 | 0.761-3.326 | 0.217 |
| N2 | 1.471 | 0.583-3.707 | 0.414 |
| N3 | 1.875 | 0.693-5.075 | 0.216 |
| Clinical stage | | | |
| I | Ref | | |
| II | 2.214 | 0.695-7.048 | 0.179 |
| III | 1.786 | 0.470-6.789 | 0.395 |
| ER status | | | |
| Negative | Ref | | |
| Positive | 0.236 | 0.119-0.467 | <0.001 |
| PR status | | | |
| Negative | Ref | | |
| Positive | 0.268 | 0.135-0.531 | <0.001 |
| P53 status | | | |
| Negative | Ref | | |
| Positive | 0.523 | 0.275-0.997 | 0.049 |
| Ki-67 | | | |
| 0-15% | Ref | | |
| ≥15% | 3.975 | 1.846-8.560 | <0.001 |

HER-2, human epidermal growth factor receptor-2; FISH, fluorescent in situ hybridization; FDA, Food and Drug Administration; IHC, immunohistochemistry; LVI, lympho-vascular invasion; ER, estrogen receptor; PR, progesterone receptor; HR, hormone receptor; Ref, Reference.

**Table 4** Distribution of *HER2* gene amplification according to different risk score based on FDA guideline

| Risk index | Patients, n (%) | Cases with HER-2 amplification |
|---|---|---|
| 0 | 35 (19.2) | 3 |
| 1 | 72 (40.0) | 18 |
| 2 | 28 (15.4) | 13 |
| 3 | 37 (20.3) | 23 |
| 4 | 10 (5.5) | 6 |

HER-2, human epidermal growth factor receptor-2; FDA, Food and Drug Administration.

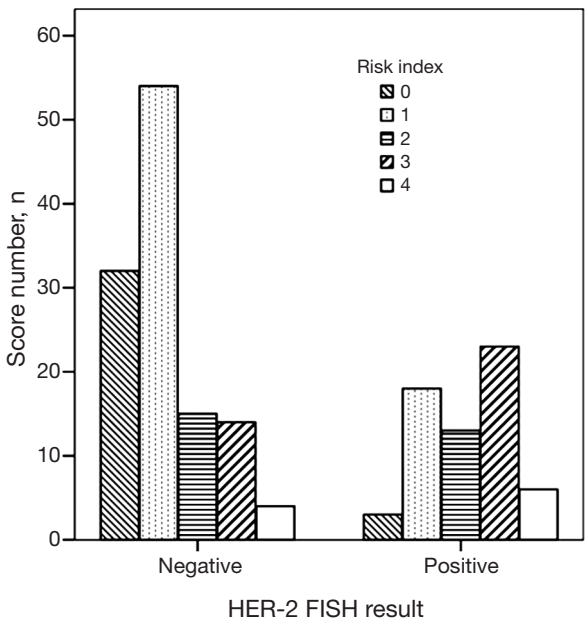


**Figure 2** Distribution of HER2 gene status according to risk index. HER2, human epidermal growth factor receptor 2.

processing protocols, thus allowing rapid IHC processing of breast carcinomas and correct determination of *HER-2* status. The concordance rate between microfluidic processor results and subsequent in situ hybridization (ISH) of the same samples was 100%, although the number of cases included in this study was relatively small (score IHC 2+, n=27). SP3, a rabbit monoclonal antibody, was proven to have a high level of agreement with ISH methods (22). The concordance rates reported by D'Alfonso (23) from 100 breast cancer patients between SP3 and FISH in needle core biopsy and excisional biopsy specimens were 96% (95% CI, 91.9-99.7%) and 97% (95% CI, 90.3-99.3%), respectively. Despite the steps that have been made to

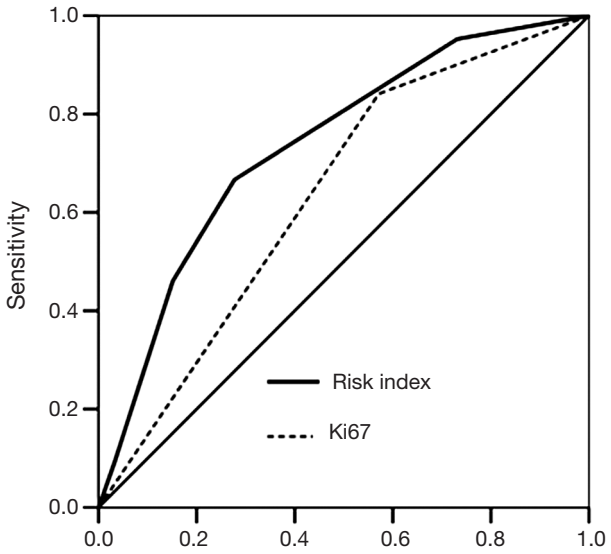


**Figure 3** Receiver operator characteristic curves were constructed to compare the ability of risk index to differentiate between patients with or without HER2 FISH amplification. HER2, human epidermal growth factor receptor 2; FISH, fluorescent in situ hybridization.

standardize the process of IHC assessment, intra- and inter-observer variability in scoring is not uncommon (24). Computer-assisted analysis on *HER-2* IHC slides may be an effective supplement to conventional IHC analysis (25); however, this method requires special materials and could not be widely implemented for use within a short time.

Although numerous previous studies have reported that *HER-2* overexpression (IHC 3+) or *HER-2* amplification is associated with high tumor cell grade, absence of ER or PR expression, DNA aneuploidy, and high Ki67 (26-29), published evidence on the correlation between relevant prognostic factors and FISH-determined *HER-2* status in *HER-2* IHC 2+ cases is still lacking. A method with high discriminatory power will help clinical physicians obtain results faster without the performance of FISH. To date, only three publications have studied this relationship. Lee (30) recently characterized a relatively large series of 1735 invasive breast cancer tissues, among which 419 (24%) were scored *HER-2* 2+ by IHC. Additionally, 14% (57/413) were *HER-2* amplified according to FDA criteria (ratio of *HER-2* to chromosome 17≥2.0). *HER-2* amplification was related to the percentage of complete membrane staining. Chibon (31) selected 108 breast cancers with *HER-2* IHC score of 2+ to predict *HER-2* gene status. FISH amplification rate was determined to be 33%. Tumor grade and percentage of membrane staining were indicators of *HER-2* status. A study by Dieci *et al.* (32) analyzed 480 HER-2 2+ breast cancer samples, resulting in high tumor grade and high Ki67 being significantly associated with HER-2 FISH amplification. However, the ER and PR statuses were not determined in all cases. HR positivity is related with better prognosis in breast cancer patients. Furthermore, although the association between pathological variables (tumor grade and Ki67) and *HER-2* status has been well established, the power of these studies has been relatively low. To ensure that all women with *HER-2* amplified cancers receive adequate treatment, a powerful method for assessing *HER-2* amplification is imperative. In our study, we integrated clinical and pathological factors from 182 invasive breast cancer cases with IHC score of 2+ to develop a risk score that better predicts the occurrence of *HER-2* amplification. All samples were routinely submitted for FISH analysis to determine the *HER-2* gene status. We found that 34.6% (63/182) of all cases were *HER-2* amplified. A positive correlation was found between the HR, P53, and Ki67 and *HER-2* status. The risk score, derived by the sum of HR, P53, and Ki67, was a highly significant predictor of *HER-2* status ($\chi^2$=30.41, P<0.001). Overall, compared with previous studies, this study examined cases that were all from surgical specimens, and incorporated multiple clinicopathological parameters for the development of a powerful predictive model for *HER-2* status. The additional variables allow for higher accuracy for validation of *HER-2* status.

Some limitations were observed in this study. First, our analysis focused on invasive breast cancer, thereby limiting our analysis from other histological classifications; second, this study was based on patients from one center, and results may not apply to other medical settings. Before clinical use, the evaluation of ER, PR, Ki67 should be standardized; third, any predictive model incorporates a certain degree of uncertainty, so predicting the status of an individual patient remains imperfect. More studies that address these issues are needed for confirmation. Despite the statistical accuracy for the prediction of *HER-2* amplification in invasive breast cancer, FISH analysis remains the gold standard for determining *HER-2* status.

Accurately evaluating the breast cancer *HER-2*/neu genotype has become an important task as emerging date showing that the benefit of using Herceptin in the treatment for *HER-2* positive patients. Subgroup of breast cancer patients achieves a pCR after the neoadjuvant chemotherapy. There is no residual tumor cell in the

 

surgical biopsy for examination. Tissue accessibility prohibits patients from obtaining *HER-2* status. Preoperation needle core biopsy tissue becomes the only available material in this group of patients. In such cases, our risk score can be used to prioritise the treatment of Herceptin. In a recently meta-analysis (33), *HER-2* IHC 0/1+ and 3+ cannot be absolutely considered as negative and positive. The discordance rates are 4% and 9% in 0/1+ and 3+ *HER-2* IHC score, respectively. In such instances, this IHC risk score would help physician to select those patients who will benefit from the target therapy.

Based on the results of our study, we present a novel IHC risk score that will help determine *HER-2* status accurately. In the future, we hope to validate this model by analyzing a larger series of invasive breast cancer tissues.

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