ORIGINAL ARTICLE

The Correlation between Immunohistochemistry and In Situ Hybridization for the Assessment of Human Epidermal Growth Factor Receptor 2 (HER-2) Status in Breast Cancer in Buddhasothorn Hospital

Buraphat Pengnoraphat, MD[1]

[1] Division of Medical Oncology, Department of Internal Medicine, Buddhasothorn Hospital, Chachoengsao, Thailand

Objective: To investigate the correlation of immunohistochemistry (IHC) and in situ hybridization (ISH) for the assessment of human epidermal growth factor receptor 2 (HER-2) in breast cancer in Buddhasothorn Hospital.

Materials and Methods: The present study design was a retrospective study. Breast cancer patients aged 18 years or older, diagnosed with stage I to IV according to the American Joint Committee on Cancer (AJCC) Eighth Edition between January 1, 2018 and September 30, 2023, in Buddhasothorn Hospital and had IHC and ISH for the assessment of HER-2 according to the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) 2018 were included. There were 131 breast cancer patients.

Results: The present study included 131 breast cancer patients with average age of 54 years, tumor size greater than 20 mm but not exceeding 50 mm (T2) at 45.4% (59/131), and 1 to 3 lymph nodes involvement (N1) at 34.6% (45/131). Early breast cancer was presented in 83.2% (109/131) of the cases, with estrogen receptor positivity at 42% (55/131). HER-2 testing by IHC showed equivocal (2+) in 52% (68/131) and positive (3+) in 48% (63/131). The Ki-67 proliferation index was 34±21.6%. The result of HER-2 testing by IHC positive (3+) highly correlates with ISH at 96.83% (61/63), which was statistically significant (Spearman's rho=0.54, p<0.001). The result of HER-2 testing by IHC positive (2+) correlated with ISH at 48.53% (33/68). These data demonstrated a correlation of HER-2 testing by IHC positive (3+) and ISH.

Conclusion: The HER-2 test using the IHC method at Buddhasothorn Hospital in the IHC positive (3+) group had a statistically significantly high correlation with the ISH test. Therefore, there is no need for ISH testing. In the IHC equivocal (2+) group, the correlation with ISH results did not differ from other studies. This needs to be verified using ISH according to the standard.

Keywords: Correlation; Human epidermal growth factor receptor 2 (HER-2); Immunohistochemistry (IHC); In situ hybridization (ISH)

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Breast cancer is the most common cancer in Thai women. The most common age at diagnosis is between 40 and 60 years[1]. Treatment of breast cancer includes surgery, radiation, and systemic treatment depending on staging and performance status. Systemic treatment comprises chemotherapy, hormonal therapy, targeted therapy[2-6], and immunotherapy.

In the current situation, targeted therapy[2-6] plays a role as the standard of care in human epidermal growth factor receptor 2 (HER-2) positive breast cancer. Locally advanced breast cancer is able to be prescribed an anti-HER-2 drug after positive results of HER-2 testing according to the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) 2018. It is unnecessary to investigate ISH testing for a confirmation test in HER-2 positive (3+). In the group of HER-2 equivocal (2+), there is a need to investigate ISH for confirmation test.

Anti-HER-2 drugs are highly effective and high-cost drugs, which promote longer overall survival[3,5,6] and disease-free survival[3,5,6], resulting in patients treated with these drugs having longer survival and good quality of life[6].

According to the protocol of The Comptroller General's Department and Social Security Office of Thailand, it allows the HER-2 positive breast cancer

Correspondence to:

Pengnoraphat B.
Division of Medical Oncology, Department of Internal Medicine, Buddhasothorn Hospital, Chachoengsao 24000, Thailand.
Phone: +66-38-814375
Email: Buraphat.p@gmail.com

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patients having positive results of HER-2 testing to be prescribed these medications. It is unnecessary to investigate ISH testing for confirmation test in HER-2 positive (3+), but in the group of HER-2 equivocal (2+), there is a need to investigate ISH for a confirmation test.

The author had plans to study the correlation of Immunohistochemistry (IHC) and in situ hybridization (ISH) for the assessment of HER-2 in breast cancer in Buddhasothorn Hospital. To ensure that medical oncologists make decisions on treatment as soon as the results showed HER-2 positive (3+), it is unnecessary to confirm ISH testing. Results in patients who were prescribed drugs early led to a high performance of treatment and reduced cost of investigation.

Objective

To investigate the correlation of IHC and ISH for the assessment of the HER-2 in breast cancer in Buddhasothorn Hospital:

1. Primary endpoint: The correlation of IHC positive (3+) and ISH for the assessment of HER-2
2. Secondary endpoint: The correlation of IHC equivocal (2+) and ISH for the assessment of HER-2

Materials and Methods

The study design was a retrospective study. Breast cancer patients who were 18 years old or older, diagnosed with stage I to IV according to the American Joint Committee on Cancer (AJCC) 8th Edition, between January 1, 2018 and September 30, 2023, in the Buddhasothorn Hospital and had IHC and ISH for the assessment of HER-2 according to ASCO/CAP 2018 were included. There were 131 breast cancer patients. Patients who had HER-2 results by IHC as negative (1+) or had no results of ISH for a confirmation test or had HER-2 results from other hospitals were excluded. The flow diagram of the patients included in the study is shown in Figure 1.

The age and Ki-67 proliferation index were calculated using mean average and standard deviation. Tumor size, lymph node status, stage of breast cancer, estrogen receptor status, and HER-2 status were calculated as percentages. The correlation of IHC and ISH for the assessment of HER-2 breast cancer was statistically analyzed by Spearman's rank-order correlation, with a p-value less than 0.05 indicating statistical significance.

Ethical approval

The present study protocol was approved by the Institutional Review Board of Buddhasothorn Hospital (number BSH-IRB 005/2567).

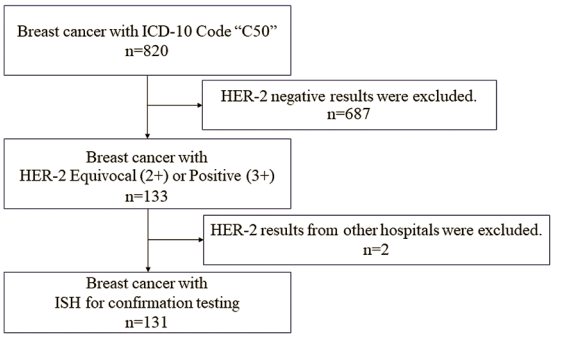

Figure 1. Patient selection flow diagram.

Results

The present study included 131 breast cancer patients with an average age of 54 years, with tumor size greater than 20 mm but not exceeding 50 mm (T2) at 45.4% (59/131), and 1 to 3 lymph nodes involvement (N1) at 34.6% (45/131). Early breast cancer was present in 83.2% (109/131) of the cases, with estrogen receptor positivity at 42% (55/131). HER-2 testing by IHC showed equivocal (2+) in 52% (68/131) and positive (3+) in 48% (63/131). The Ki-67 proliferation index was 34±21.6%, as shown in Table 1.

For IHC equivocal (2+) and positive (3+), there was a Classic HER-2 amplification (Group 1) in the ISH results, with percentages of 47.2% (32/68) and 92.1% (58/63), respectively, as shown in Table 2.

The result of HER-2 testing by IHC positive (3+) was highly correlated with ISH at 96.83% (61/63), which was statistically significant (Spearman's rho=0.54, $p<0.001$). Similarly, the result of HER-2 testing by IHC equivocal (2+) correlated with ISH at 48.53% (33/68). These data demonstrated the correlation of HER-2 testing by IHC positive (3+) and ISH, as shown in Table 3.

Discussion

The present study found that the correlation of HER-2 testing using IHC positive (3+) and ISH method is as high as 96.83% (61/63), while for IHC equivocal (2+), the correlation with ISH testing is only 48.53% (33/68). In comparison with the research by Ya-in[7], the correlation was close to 97.4% and 52.9% for IHC positive (3+) and IHC equivocal (2+), respectively. Similarly, when compared with a study conducted in China, which shares the same Asian descent as Thai populations, the research by

Table 1. Clinical characteristics of HER-2 breast cancer patients

Characteristic	HER-2 IHC equivocal (2+) (n=68)	HER-2 IHC positive (3+) (n=63)	Total (n=131)
Age (years); mean±SD	55±10.8	53±10.5	54±10.6
Tumor characteristics; n (%)			
T1 (≤ 20 mm)	12 (17.7)	11 (17.7)	23 (17.7)
T2 (>20 mm but ≤ 50 mm)	34 (50.0)	25 (40.3)	59 (45.4)
T3 (>50 mm)	8 (11.8)	15 (24.2)	23 (17.7)
T4 (local invasion)	14 (20.6)	11 (17.7)	25 (19.2)
Nodal characteristics; n (%)			
N0 (No regional lymph node)	17 (25.0)	5 (8.0)	22 (16.9)
N1 (1 to 3 nodal metastases)	20 (29.4)	25 (40.3)	45 (34.6)
N2 (4 to 9 nodal metastases or ipsilateral internal mammary node metastases)	18 (26.5)	15 (24.2)	33 (25.4)
N3 (>10 nodal metastases or ipsilateral supraclavicular node metastases)	13 (19.1)	17 (27.4)	30 (23.1)
Distant metastasis; n (%)			
M0	59 (86.8)	50 (79.4)	109 (83.2)
M1	9 (13.2)	13 (20.6)	22 (16.8)
Hormonal status; n (%)			
ER <10%	31 (45.6)	45 (71.4%)	76 (58.0)
ER ≥10%	37 (54.4)	18 (28.6%)	55 (42.0)
Ki-67 (%); mean±SD	35±23.1	32±19.9	34±21.6

HER-2=human epidermal growth factor receptor 2; IHC=immunohistochemistry; ER=estrogen receptor; Ki-67=Ki-67 proliferation index; SD=standard deviation

Table 2. Genomic HER-2 alteration clone characteristics by dual-probe ISH and IHC for HER-2 based on the 2018 ASCO/CAP guidelines

HER-2 clone groups	HER-2 IHC equivocal (2+) (n=68); n (%)	HER-2 IHC positive (3+) (n=63); n (%)
Classic HER-2 amplification	32 (47.2)	58 (92.1)
Monosomy 17	1 (1.5)	0 (0.0)
Co-amplification (previously polysomy 17)	1 (1.5)	3 (4.8)
Borderline HER-2 amplification	4 (5.9)	0 (0.0)
Classic HER-2 non-amplification	30 (44.1)	2 (3.2)

HER-2=human epidermal growth factor receptor 2; IHC=immunohistochemistry

Table 3. Distribution of HER-2 IHC results across reference test (ISH for HER-2)

HER-2 IHC	ISH for HER-2 negative	ISH for HER-2 positive	Total	Correlation (%)
Equivocal (2+)	35	33	68	48.53
Positive (3+)	2	61	63	96.83*
Total	37	94	131	

HER-2=human epidermal growth factor receptor 2; IHC=immunohistochemistry; ISH=In situ hybridization
* Spearman's rho=0.54, p<0.001

Zhao et al.[8] gave comparable results at 91.7% and 64.4% for IHC positive (3+) and IHC equivocal (2+), respectively.

Analyzing the correlation of HER-2 testing with IHC positive (3+) and ISH results in the Classic HER-2 amplification group showed a correlation of 92.1%, while for IHC equivocal (2+), the correlation with ISH results in the Classic HER-2 amplification group was 47.2%. Comparison with the research by Thambamroong[9] found a close correlation at 91.76% and 51.8% for IHC positive (3+) and IHC equivocal (2+), respectively.

The researcher believed that the data from Buddhasothorn Hospital was similar to the mentioned studies in both Thailand and China, supporting the reliability of the hospital's data. Factors contributing to the characteristics of the research results include accurate selection and sectioning of cancer tissue by

pathologists, leading to an efficient histopathological process, allowing knowledgeable pathologists to interpret the results accurately.

The limitations of the present study, being a retrospective study, prevent the collection of data for the HER-2 negative (0 or 1+) group for comparison, may have selection and information bias. The present study was conducted at a single hospital, which may impact the generalizability of the results to other settings or populations. Furthermore, the lack of sufficient follow-up on patients hindered a thorough investigation into clinical symptoms, disease-free survival periods, and overall survival rates for the sample group.

The results of the present study provide academic evidence that increases the confidence of oncologists to use anti-HER-2 drugs without the need for ISH confirmation, which is in line with the drug reimbursement supervision guidelines of the Comptroller General's Department and the Office of Universal Health Coverage. This has been approved for the use of targeted drugs in breast cancer patients who have a HER-2 positive (3+) IHC test without the need for ISH test results, resulting in patients having quickly access to treatment leads to the most effective treatment outcome and reduce public health costs as well.

Conclusion

The HER-2 test by IHC method at Buddhasothorn Hospital in the IHC positive (3+) group had a statistically significantly high correlation with the ISH test. Therefore, there is no need for ISH testing. However, in the IHC equivocal (2+) group, the correlation with ISH results was no different from the other studies, and it needs to be verified using ISH according to the standard.

What is already known on this topic?

IHC and ISH are both techniques used in molecular biology and pathology to study the expression levels of specific genes or proteins in tissue samples. In the case of evaluating the presence of HER-2 in breast cancer patients, both techniques can be used in conjunction to provide comprehensive information.

By combining the results of IHC and ISH, clinicians and pathologists can obtain a more comprehensive understanding of the HER-2 status of breast cancer patients. This information is crucial for determining the most appropriate treatment options, such as targeted therapies that specifically target HER-2 expression.

What does this study add?

The HER-2 test by IHC method at Buddhasothorn Hospital in the IHC positive (3+) group of patients had a statistically significant high correlation with the ISH test. Therefore, there is no need for ISH testing. This results in patients having fast access to treatment, which leads to the most effective treatment outcome and reduces public health costs.

Conflicts of interest

The authors declare no conflict of interest.

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