Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.2023102131-137

Evaluation of HER2 Gene Amplification Using CISH in Patients with HER2 2+ (Equivocal) Breast Carcinoma Based on Immunohistochemistry

Behnaz Jahanbin[a], Vahid Soleimani*[a], Farid Azmoudeh-Ardalan[a], Samane Afshar[a], Masoumeh Safaei[a]

[a]*Department of Pathology, Imam Khomeini Hospital Complex, Tehran University of Medical Science, Tehran, Iran*

ARTICLE INFO

Received:
6 February 2023
Revised:
29 March 2023
Accepted:
3 April 2023

Keywords:
Breast Neoplasms; erbB-2; Estrogen Receptors; Progesterone; Chromogenic in Situ Hybridization; HER2

ABSTRACT

Background: Human epidermal growth factor 2 (HER2) is known to be an important prognostic factor in breast cancer. Numerous studies have shown HER2+ breast cancers have reduced overall survival and recovery time, as well as the efficacy of anti-HER2 therapies along with chemotherapy in improving disease outcomes. For this reason, it is recommended that all patients with breast cancer should be evaluated for HER2 status. This study aimed to assess the HER2 gene amplification by the CISH method in evaluating the HER2 status in patients with immunohistochemistry (IHC) 2+ (equivocal) results.

Methods: This retrospective cross-sectional study examined HER2 status based on the Chromogenic in situ hybridization (CISH) method in 280 breast carcinoma samples with an initial 2+ (equivocal) score in IHC. The relationship between HER2 amplification and hormone receptors (estrogen and progesterone) and Ki67 level was also investigated.

Results: In sixty samples (21.4%), the HER2 gene was amplified based on the CISH method. The majority (215, 76.8%) of the samples were negative and 5 (1.8%) samples were indeterminate. No significant relationship was observed between HER2 amplification, estrogen receptor (p=0.143), and Ki-67 protein level (p=0.977). There was a significant inverse relationship between HER2 amplification and progesterone receptor positivity (p=0.007).

Conclusion: These results demonstrate that CISH is a helpful method to assess HER2 status in equivocal breast cancer and is positive (amplified) in about 21.4% of them.

INTRODUCTION

Breast cancer is the most common cancer among women and is considered as the most important cause of death in women globally.[1] Breast cancer is a multifactorial disease and is under the influences of genes and environment.[1] One out of 10 cases of breast cancer is hereditary.[2] Nearly 2/3 of breast cancers are hormone-dependent.[3] Breast cancer treatment is based on a tumor's genetic and hormonal characteristics, grade, and stage.[4] One of the breast cancer's main genetic prognostic factors is human epidermal growth factor receptor 2 (HER2) gene status.[5] Other prognostic factors include the presence of estrogen and progesterone receptors and Ki-67 index.[6] The expression of estrogen and progesterone receptors are also important predictors of breast cancer prognosis. Estrogen receptors are present in 60 to 70% of breast cancer cases and progesterone receptors are present in 75% of breast cancer cases.

***Address for correspondence:**
Vahid Soleimani, M.D.,
Department of Pathology, Imam Hospital complex, Tehran University of Medical Sciences, Tehran, Iran
Tel: +989128158158
Email: vahidsmd2013@gmail.com

The presence of estrogen receptors is associated with a response to hormonal therapy with estrogen antagonists including tamoxifen.[5] The Ki-67 protein is considered as a cellular proliferation marker. It was previously shown that the Ki-67 concentration is associated with overall and disease-free survival.[7]

HER2 gene amplification is seen in about 30% of breast cancer cases and is related to reduced life expectancy and earlier recurrence.[8,9] On the other hand, HER2 gene amplification is associated with good response to chemotherapy and improved overall survival with medications including Trastuzumab.[10] Therefore, detection of HER2 amplification is important in predicting the survival and quality of life of breast cancer patients. Studies have showed that the expression of hormone receptors has been associated with reduced HER2 gene amplification.[11]

Based on the latest guideline by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP), HER2 status assessment should be performed in all newly diagnosed patients with breast cancer and patients with metastatic breast cancer.[12] The status of HER2 amplification is recommended to be assessed using immunohistochemistry (IHC) or in situ hybridization (ISH).[12] Based on the ASCO/CAP guideline, anti HER2 receptor antibody should be added to chemotherapy regimen in case of documented HER2 amplification (HER2 positive3+).[12] In case of ambiguous HER2 status, the ASCO/CAP recommends repeating HER2 status assessment on another tissue block or performing the assessment using another diagnostic method on the same tissue block.[12] There is a tendency towards performing IHC for detection of HER2 status in breast cancer due to its simplicity and lower cost. The ASCO/CAP has designed a scoring system for the assessment of HER2 status, where scores 0 and 1+ are considered HER2 negative, score 2+ as equivocal and 3+ as HER2 positive.[12] In equivocal cases, assessment using ISH is recommended.[12] It was previously shown that HER2 status was reported to be equivocal in 15 to 25% of breast cancer patients.[13,14] It was also shown that 20 to 30% of the patients with equivocal HER2 status based on IHC were HER2 positive in ISH investigation.[15,16]

Regarding the high cost of performing both tests in a large number of breast cancer cases, it is logical to improve the accuracy of IHC assessments in clinics. The primary objective of this study was to identify the prevalence of HER2+ breast cancer cases using chromogenic in situ hybridization (CISH) among tissue samples with equivocal HER2 status in IHC in a referral cancer center from all over the country. This study also aimed to assess the relationship between HER2 amplification and expression of estrogen and progesterone receptors and Ki-67 Ag among cases with equivocal HER status based on IHC.

METHODS

This cross-sectional study was conducted in the cancer institute of a tertiary hospital in Tehran, Iran. The hospital was chosen due to its high referral rate from all over the country; therefore, the cases represent various geographical regions within the country, and therefore the results might be generalizable to the whole country. The sampling method was non-random stratified probability sampling. The data for the study were collected confidentially from patient records. All breast cancer cases that referred to the hospital from 2015 to 2017 were assessed. The inclusion criteria were presence of breast cancer with equivocal HER2 status based on IHC assessment. Exclusion criteria were lack of data on the IHC assessment of HER2 status, being HER2 positive or HER2 negative based on IHC according to the ASOC/CAP guidelines (17).

Sample size was calculated based on the prevalence of HER2 positive cases based on ISH assessment (26.5%). Considering the minimum significant difference of 0.05, 5% precision and 80% power, the following equation was used.

$$n = \frac{Z^2_{1-\frac{\alpha}{2}}(p)(q)}{d^2}$$

Where n is the sample size, d is the minimum significant difference, p is the proportion of HER2 and q is calculated as 1-p. Z is a constant value (1.96 for 80% power). The sample size was calculated to be 280.

The extracted data included the results of HER2 CISH, estrogen (ER) and progesterone (PR) receptors as well as Ki-67 in tissue samples (Figure 1-3).

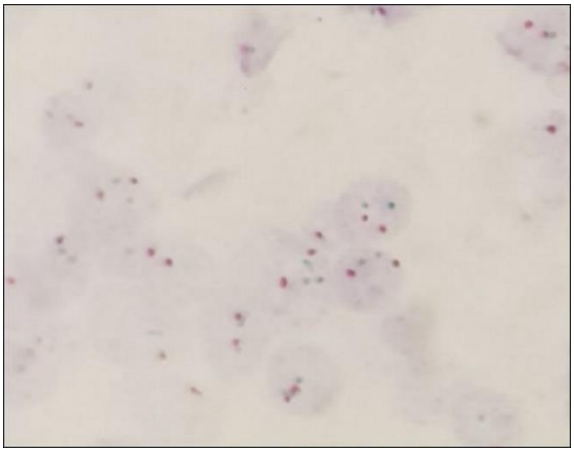

Figure 1. In situ hybridization (ISH) method using hematoxylin eosin (H&E) counterstaining showing tumoral cells without HER2 gene amplification (light green) and centromere 17 probe positive cells (red). Two light green and two red signals are seen in each cell.

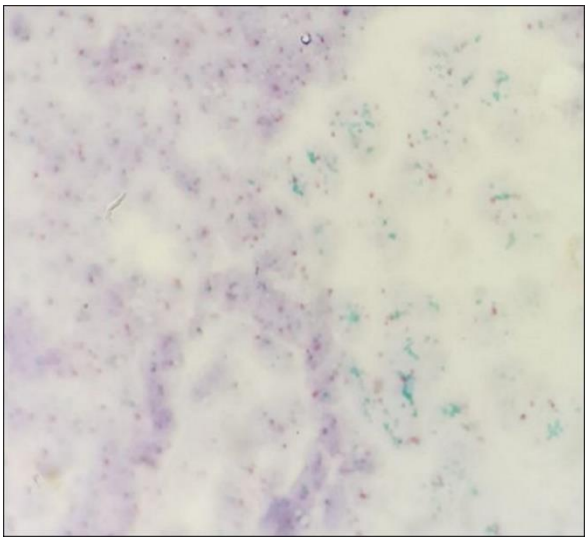

Figure 2. In situ hybridization (ISH) method using hematoxylin eosin (H&E)counterstaining showing non-tumoral cells without HER2 amplification (left) and tumoral cells with HER2 amplification (right). Light green signals indicate HER2 positivity and red signals indicate CEP17 positivity.

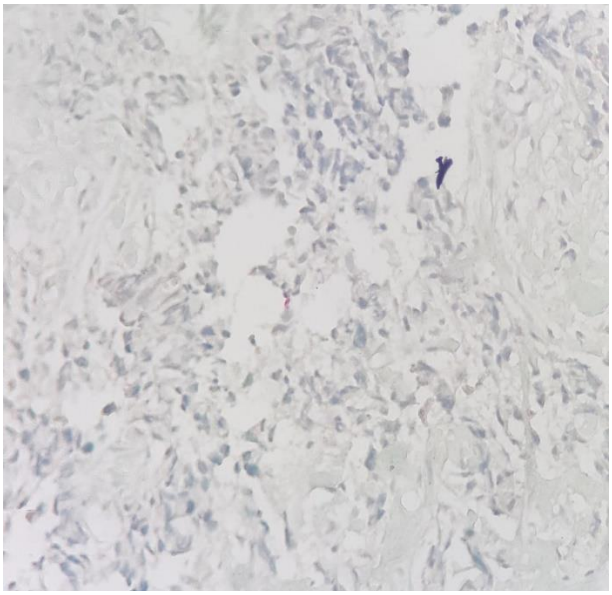

Figure 3. Hematoxyline staining of tumor cells stained by chromogenic in situ hybridization but no signal is identified that maybe due to poor primary fixation, increased cold ischemic time, bad processing or probably acid exposure, result is indeterminate or nondiagnostic for HER2gene amplification.

Statistical analysis

Data analysis was performed using the statistical package for social sciences (SPSS) software. HER2, ER and PR positivity were presented using frequency and percentage. The percentage of Ki-67 was normally distributed based on Kolmogorov-Smirnov test and was, therefore, presented using mean and standard deviation. The chi-square test was used to assess the relationship between HER2 status based on CISH and ER and PR, while the analysis of variance (ANOVA) was used to assess the relationship between HER2 status and Ki-67. The level of statistical significance was set at P<0.05.

RESULTS

A total of 280 breast cancer cases with equivocal HER2 status based on IHC were assessed in the study. HER2 amplification was evaluated in all samples using the CISH. From 280 cases, 215 (76.8%) were HER2 negative (not amplified) and 60 (21.4%) were HER2 positive (amplified), and 5 (1.8%) were indeterminate. The reason for the indeterminate findings included fixation problems, surgical electrocautery effect, increased cold ischemic time, and exposure of the tissue to acidic materials,high pepsin digestion as well as inappropriate primary tissue processing. The mean and standard deviation for Ki-67 percentage was 26.73 ± 18.56% ranging from 1% to 90% (Figure 4). The results of ER, and PR assessments are presented in Table 1.

Table 1. Characteristics of breast cancer in terms of HER2, ER, PR, and Ki.67 in the study samples

Variable		Frequency	Percentage
HER2	Positive	60	21.4
	Negative	215	76.8
	Indeterminate	5	1.8
ER+		243	86.8
PR+		210	75.0

HER2: Human Endothelial receptor 2, ER: Estrogen receptor, PR: progesterone receptor

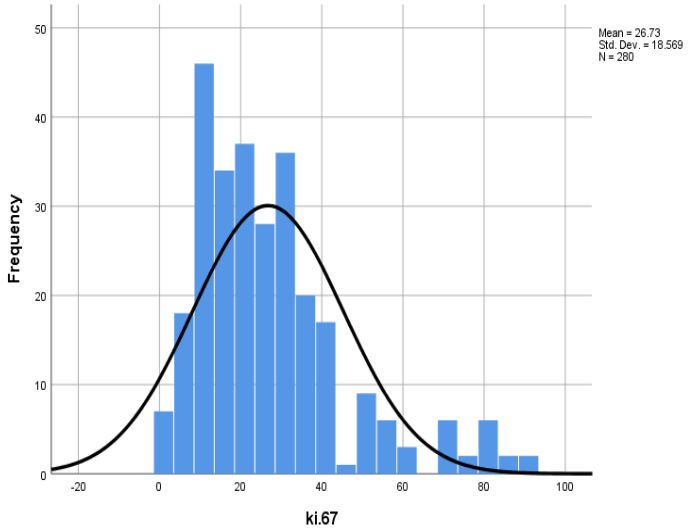

Figure 4. Frequency distribution of ki-67 status among study samples

Among the studied samples, 59 (21.0%) were classified as luminal A, while luminal B, HER2 enriched and triple negative classes were observed in 184 (65.7%), 10 (3.5%) and 27 (9.6%), respectively.

The chi-square test revealed no significant relationship between ER and HER2 status (p=0.373), while a significant relationship was observed between PR positivity and HER2 status (P=0.007) (Table 2).

Table 2. Relationship between HER2 status and ER and PR positivity among study samples

Variable		HER2+ Frequency (%)	HER2- Frequency (%)	P	OR	95% CI for OR	
ER	Positive	50 (18.1%)	189 (68.7%)	0.373	0.143	0.019	1.078
	Negative	10 (3.6%)	26 (9.4%)				
PR	Positive	37 (13.4%)	169 (61.4%)	0.007**	0.437	0.237	0.806
	Negative	23 (8.3%)	46 (16.7%)				

HER2: Human Endothelial receptor 2, ER: Estrogen Receptor, PR: progesterone Receptor, OR: Odds Ratio
Assessment of ER and PR was based on IHC, while HER2 assessment was performed using the chromogenic in situ hybridization (CISH) method
The chi square test was used for the analysis.
** Significant association at α=0.01

The chi-square test revealed no significant relationship between HER2 and hormonal receptor status defined as hormone receptor positive (i.e., ER+ and / or PR+) and hormone receptor negative (i.e., ER and PR negative) (P=0.752).

Table 3. Relationship between HER2 status and hormone receptor status among study samples

Variable		Hormone receptor + Frequency (%)	Hormone receptor - Frequency (%)	P	OR	95% CI for OR	
HER2	Positive	51 (86.4%)	8 (13.5%)	0.752	0.872	0.373	2.042
	Negative	190 (87.9%)	26 (12.0%)				

HER2: Human Endothelial receptor 2, ER: Estrogen Receptor, PR: progesterone Receptor, OR: Odds Ratio
Assessment of ER and PR was based on IHC, while HER2 assessment was performed using the chromogenic in situ hybridization (CISH) method
The chi square test was used for the analysis.

DISCUSSION

Currently, IHC is one of the most commonly used methods in the assessment of HER2 status in breast cancer cases.[18] The IHC is usually used as the primary method in breast cancer cases.[19] The fluorescent in situ hybridization (FISH) method is considered as the gold standard in detection of HER2 status in breast cancer cases.[20] The CISH procedure has lower cost compared to FISH method.[21] The CISH test has been considered valid and completely concordant in relation to FISH method in the detection of HER2 status in breast cancer in many studies.[22,23] Unlike IHC, CISH method is costly and requires specific knowledge and equipment.[21] The CISH method is based on chromogenic detection and has acceptable sensitivity and specificity for the detection of difficult cases.[21] The aim of this study was to count and assess HER2 amplification using CISH method among breast cancer cases with equivocal IHC findings.

The findings of our study revealed that among the 280 equivocal HER2 cases based on IHC method, 21.4% were positive (amplified) based on the CISH method, 76.8% were negative (not amplified) and 1.8% were indeterminate. This finding was in line with the findings of a study by Mohammed Ali *et al.* (2019), that reported 33.3% of HER2 indeterminate breast cancer cases based on IHC were HER2+ while only 0.32% were indeterminate.[24] In a study by Khanam *et al.* (2017), 60% of indeterminate HER2 cases based on IHC were HER2-, 30% were HER2+ and 10% were indeterminate in CISH assessment.[25] Meijer *et al.* (2011) reported 73.6% of indeterminate HER2 cases based on IHC were HER2- in CISH, while 21.3% were HER2+ and 4.9% were indeterminate.[16] Similar results were found in other studies.[26-28] These findings indicate that the use of CISH method is useful in indeterminate HER2 status breast cancer cases based on IHC.

The findings of our study revealed that 86.8% and 75.0% of the breast cancer cases were ER+ and PR+, respectively. Previous studies assessed the relationship between HER2 status and ER and PR expression and reported a negative relationship between HER2 positivity and ER and PR expression.[29-31] Although our study failed to report a significant relationship between HER2 status and ER positivity, a significant negative relationship was observed between HER2 status and PR positivity in our study. The negative relationship between HER2 status and steroid hormone receptors might justify the observation that HER2+ tumors do not respond to hormone receptor specific therapies.[32] On the other hand, the amplification of HER2 in patients with ER+ breast tumors was found to be associated with reduced PR expression.[33] It was previously shown that ER+/PR- breast cancers were mainly found among menopausal women.[34] This finding might be due to the fact that the endogenous estrogen is so low that it may not increase PR and suppress HER2 at menopause.[34]

The findings of our study revealed that the mean percentage for Ki-67 protein was 26.73 ± 18.56%,

which was lower than what was reported in previous studies.[35-38] Furthermore, our study failed to identify a significant relationship between HER2 status and Ki-67 among HER2 indeterminate breast cancer cases. In contrast a significant relationship was observed between HER2 status and Ki-67 in a previous study.[35] The difference between the findings of our study and the mentioned study might be due to different methodologiesas the later study was conducted on breast cancer cases while our study was conducted on breast cancer cases with indeterminate HER2 based on IHC.

On the other hand, the findings of our study revealed that luminal B (65.7%) was the most common tumor type followed by luminal A (21.0%), triple negative (9.5%) and HER2 enriched (3.5%). This finding was in line with the findings of the studies that followed more recent classification guidelines indicating that luminal B was the most common type with the prevalence of (57.1-68.5%).[39,40]

A strength of our study was the inclusion of breast cancer cases from different regions and cities of the country, which enables us to generalize the findings to the whole Iranian population. On the other hand, one of the limitations of this study was the exclusion of HER2+ and HER2- cases based on IHC in relationship analysis. As the relationships between HER2 status and ER, PR and Ki-67 were our secondary objective, sampling was based only on HER2 indeterminate cases based on IHC assessment. It is recommended for further researchers to assess the relationship between HER2 status and hormonal receptors and Ki-67 in a population with all types of HER2 status.

CONCLUSION

This study found that CISH can be of importance in determining the HER2 status of breast cancer tumors that are HER2 equivocal based on IHC assessment. This study also found a significant negative relationship between HER2 status based on CISH and PR among indeterminate HER2 status breast cancer cases based on IHC.

ETHICAL CONSIDERATIONS

This study was approved by the ethics committee at Cancer Institute, IKHC, TUMS, Tehran.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING

No funds, grants, or other support was received.

ACKNOWLEDGEMENTS

None.

REFERENCES

1. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast Cancer: Targets and Therapy*. 2019:151-64. doi: 10.2147/BCTT.S176070.
2. Beitsch PD, Whitworth PW, Hughes K, Patel R, Rosen B, Compagnoni G, et al. Underdiagnosis of hereditary breast cancer: are genetic testing guidelines a tool or an obstacle? *Journal of Clinical Oncology*. 2019;37(6):453. doi: 10.1200/JCO.18.01631.
3. Li N, Sun Y, Zhao S, Liu H, Zhao K. Study on the Efficacy Evaluation of Oral Sequential Tamoxifen in Postmenopausal Women with Hormone-dependent *Breast Cancer*. 2018.
4. Waks AG, Winer EP. Breast cancer treatment: a review. *JAMA*. 2019;321(3):288-300. doi: 10.1001/jama.2018.19323.
5. Allred DC. Issues and updates: evaluating estrogen receptor-α, progesterone receptor, and HER2 in breast cancer. *Modern Pathology*. 2010;23(2):S52-S9. doi: 10.1038/modpathol.2010.55.
6. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. *Cancer biology & medicine.* 2016;13(4):496. doi: 10.20892/j.issn.2095-3941.2016.0066.
7. Inwald E, Klinkhammer-Schalke M, Hofstädter F, Zeman F, Koller M, Gerstenhauer M, et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. *Breast cancer research and treatment.* 2013;139:539-52. doi: 10.1007/s10549-013-2560-8.
8. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *science*. 1987;235(4785):177-82. doi: 10.1126/science.3798106.
9. Smith K, Robbins P, Dawkins H, Papadimitriou J, Carrello S, Harvey J, et al. Detection of c-erbB-2 amplification in breast cancer by in situ hybridization. *The Breast.* 1993;2(4):234-8. doi: 10.1016/0046-8177(94)90152-x.
10. Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E, et al. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *The Lancet*. 2017;389(10075):1195-205. doi: 10.1016/S0140-6736(16)32616-2.
11. Sheikhpour R, Poorhosseini F. Relation between Estrogen and Progesterone receptor status with p53, Ki67 and Her-2 markers in patients with breast cancer. *Iranian Journal of Blood and Cancer*. 2016;8(4):93-7. Available from: http://ijbc.ir/article-1-666-en.html
12. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical

Oncology/College of American Pathologists clinical practice guideline update. *Archives of Pathology and Laboratory Medicine*. 2014;138(2):241-56. doi: 10.5858/arpa.2013-0953-SA.
13. Han X, Shi Y, Ma L, Lyu Z, Yang H, Yao J, et al. Comparison of immunohistochemistry with fluorescencein situhybridization in determining the human epidermal growth factor receptor 2 status of breast cancer specimens: a multicenter study of 3 149 Chinese patients. *Chinese medical journal.* 2014;127(2):246-53. doi: Not Available
14. Lee AH, Key HP, Bell JA, Hodi Z, Ellis IO. Breast carcinomas with borderline (2+) HER2 immunohistochemistry: percentage of cells with complete membrane staining for HER2 and the frequency of HER2 amplification. *Journal of clinical pathology.* 2011;64(6):490-2. doi: 10.1136/jcp.2011.089177.
15. Gheybi MK, Baradaran A, Mohajeri MR, Ostovar A, Hajalikhani P, Farrokhi S. Validity of immunohistochemistry method in predicting HER- 2 gene status and association of clinicopathological variables with it in invasive breast cancer patients. *Apmis*. 2016;124(5):365-71. doi: 10.1111/apm.12518.
16. Meijer SL, Wesseling J, Smit VT, Nederlof PM, Hooijer GK, Ruijter H, et al. HER2 gene amplification in patients with breast cancer with equivocal IHC results. *Journal of clinical pathology*. 2011;64(12):1069-72. doi: 10.1136/jclinpath-2011-200019.
17. Rakha EA, Starczynski J, Lee AH, Ellis IO. The updated ASCO/CAP guideline recommendations for HER 2 testing in the management of invasive breast cancer: a critical review of their implications for routine practice. *Histopathology*. 2014;64(5):609-15. doi: 10.1111/his.12357.
18. Bankfalvi A, Simon R, Brandt B, Bürger H, Vollmer I, Dockhorn- Dworniczak B, et al. Comparative methodological analysis of erbB- 2/HER- 2 gene dosage, chromosomal copy number and protein overexpression in breast carcinoma tissues for diagnostic use. *Histopathology*. 2000;37(5):411-9. doi: 10.1046/j.1365-2559.2000.00984.x.
19. Essmat MK, Abdelwanis MA, Mosad EZ, El-Maghraby TK, Othman AE. Assessment of human epidermal growth factor receptor 2/neu gene amplification and expression as a biomarker for radiotherapy and hormonal-treated breast cancer patients in upper Egypt. *Journal of Cancer Research and Therapeutics*. 2019;15(5):981-8. doi: 10.4103/jcrt.JCRT_42_17.
20. Dowsett M, Hanby AM, Laing R, Walker R. HER2 testing in the UK: consensus from a national consultation. *Journal of clinical pathology.* 2007;60(6):685-9. doi: 10.1136/jcp.2006.044321.
21. Ali AHM, Yahya AQ, Mohammed HL. Chromogenic in Situ Hybridization Technique versus Immunohistochemistry in Assessment of HER2/neu Status in 448 Iraqi Patients with Invasive Breast Carcinoma. Open Access *Macedonian Journal of Medical Sciences.* 2019;7(12):1917. doi: 10.3889/oamjms.2019.342.
22. Jacquemier J, Spyratos F, Esterni B, Mozziconacci M-J, Antoine M, Arnould L, et al. SISH/CISH or qPCR as alternative techniques to FISH for determination of HER2 amplification status on breast tumors core needle biopsies: a multicenter experience based on 840 cases. *BMC cancer*. 2013;13(1):1-11. doi: 10.1186/1471-2407-13-351.
23. Rosa F, Santos R, Rogatto SR, Domingues M. Chromogenic in situ hybridization compared with other approaches to evaluate HER2/neu status in breast carcinomas. *Brazilian Journal of Medical and Biological Research*. 2013;46(3):207-16. doi: 10.1590/1414-431x20132483.
24. Mohammed Ali A, Yahya A, Mohammed H. Chromogenic in situ hybridization technique versus immunohistochemistry in assessment of HER2/neu status in 448 Iraqi patients with invasive breast carcinoma. Open Access *Maced J Med Sci*. 2019. doi: 10.3889/oamjms.2019.342.
25. Khanam KF, Chowdhury T, Banu SG, Islam S. Determination of HER-2/neu gene Status by Chromogenic in Situ Hybridisation Assay on Borderline (2+) Immunohistochemistry Cases in Patients with Invasive Breast Carcinoma: An Experimental Study on Preserved Tissue. *Bangladesh Medical Research Council Bulletin.* 2017;43(1):08-15.
26. Bilous M, Morey A, Armes J, Cummings M, Francis G. Chromogenic in situ hybridisation testing for HER2 gene amplification in breast cancer produces highly reproducible results concordant with fluorescence in situ hybridisation and immunohistochemistry. *Pathology*. 2006;38(2):120-4. doi: 10.1080/00313020600561518.
27. Mayr D, Heim S, Weyrauch K, Zeindl- Eberhart E, Kunz A, Engel J, et al. Chromogenic in situ hybridization for Her- 2/neu- oncogene in breast cancer: comparison of a new dual- colour chromogenic in situ hybridization with immunohistochemistry and fluorescence in situ hybridization. *Histopathology*. 2009;55(6):716-23. doi: 10.1111/j.1365-2559.2009.03427.x.
28. van de Vijver M, Bilous M, Hanna W, Hofmann M, Kristel P, Penault-Llorca F, et al. Chromogenic in situ hybridisation for the assessment of HER2 status in breast cancer: an international validation ring study. *Breast Cancer Research*. 2007;9(5):R68. doi: 10.1186/bcr1776.
29. Huang H, Neven P, Drijkoningen M, Paridaens R, Wildiers H, Van Limbergen E, et al. Association between tumour characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. *Journal of clinical pathology*. 2005;58(6):611-6. doi: 10.1136/jcp.2004.022772.
30. Metib NJ, Kehiosh HJ, Hamzah SK. Correlation and frequency of HER-2/neu Status With Estrogen and Progesterone Receptors in Breast Carcinomas. *Karbala Journal of Medicine*. 2016;9(2):2588-99. doi: Not Available
31. Azizun-Nisa BY, Raza F, Kayani N. Comparison of ER, PR and HER-2/neu (C-erb B 2) reactivity pattern with histologic grade, tumor size and lymph node status

in breast cancer. *Asian Pac J Cancer Prev*. 2008;9(4):553-6. doi: Not Available
32. You SH, Chae BJ, Eom YH, Yoo T-K, Kim Y-s, Kim JS, et al. Clinical Differences in Triple-Positive Operable Breast Cancer Subtypes in Korean Patients: An Analysis of Korean Breast Cancer Registry Data. *Journal of breast cancer*. 2018;21(4):415-24. doi: 10.4048/jbc.2018.21.e53.
33. Rhodes A, Jasani B, Balaton A, Barnes D, Miller K. Frequency of oestrogen and progesterone receptor positivity by immunohistochemical analysis in 7016 breast carcinomas: correlation with patient age, assay sensitivity, threshold value, and mammographic screening. *Journal of clinical pathology*. 2000;53(9):688-96. doi: 10.1136/jcp.53.9.688.
34. Fernö M, Stål O, Baldetrop B, Hatschek T, Källström A-C, Malmström P, et al. Results of two or five years of adjuvant tamoxifen correlated to steroid receptor and S-phase levels. *Breast cancer research and treatment.* 2000;59(1):69-76. doi: 10.1023/a:1006332423620.
35. Elkablawy MA, Albasri AM, Mohammed RA, Hussainy AS, Nouh MM, Alhujaily AS. Ki67 expression in breast cancer. Correlation with prognostic markers and clinicopathological parameters in Saudi patients. *Saudi Med J*. 2016;37(2):137-41. doi: 10.15537/smj.2016.2.12285.
36. Ermiah E, Buhmeida A, Abdalla F, Khaled BR, Salem N, Pyrhönen S, et al. Prognostic value of proliferation markers: immunohistochemical ki-67 expression and cytometric s-phase fraction of women with breast cancer in Libya. *Journal of Cancer*. 2012;3:421. doi: 10.7150/jca.4944.
37. Agboola AO, Banjo AA, Anunobi CC, Salami B, Agboola MD, Musa AA, et al. Cell proliferation (KI-67) expression is associated with poorer prognosis in Nigerian compared to British breast cancer women. *International Scholarly Research Notices*. 2013;2013. doi: 10.1155/2013/675051.
38. Pathmanathan N, Balleine RL, Jayasinghe UW, Bilinski KL, Provan PJ, Byth K, et al. The prognostic value of Ki67 in systemically untreated patients with node-negative breast cancer. *Journal of clinical pathology*. 2014;67(3):222-8. doi: 10.1136/jclinpath-2013-201793.
39. Maisonneuve P, Disalvatore D, Rotmensz N, Curigliano G, Colleoni M, Dellapasqua S, et al. Proposed new clinicopathological surrogate definitions of luminal A and luminal B (HER2-negative) intrinsic breast cancer subtypes. *Breast Cancer Research.* 2014;16(3):R65. doi: 10.1186/bcr3679.
40. Li AQ, Zhou SL, Li M, Xu Y, Shui RH, Yu BH, et al. Clinicopathologic characteristics of oestrogen receptor-positive/progesterone receptor-negative/Her2-negative breast cancer according to a novel definition of negative progesterone receptor status: a large population-based study from China. *PLoS One*. 2015;10(5):e0125067. doi: 10.1371/journal.pone.0125067.

How to Cite This Article

Jahanbin B, Soleimani V, Azmoude-Ardalan F, Afshar S, Safaei Z. Evaluation of HER2 Gene Amplification Using CISH in Patients with HER2 2+ (Equivocal) Breast Carcinoma Based on Immunohistochemistry . Arch Breast Cancer. 2023; 10(2): 131-7.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/674