Iranian Journal of Pathology | ISSN: 2345-3656

Optimizing HER2/neu Detection in Equivocal (Score +2) Breast Cancer by Immunohistochemistry (IHC) via Fluorescence in Situ Hybridization (FISH): A Single-Center Experience

Azad M. Ahmed

Department of Dental Basic Sciences, College of Dentistry, University of Duhok, Kurdistan Region, Iraq

KEYWORDS

breast cancer, HER2/neu, immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), equivocal HER2 score, molecular profiling

Scan to discover online

Main Subjects:
GI, Liver & Pancreas Pathology

Received 25 Oct 2025;
Accepted 03 Feb 2026;
Published Online 20 Feb 2026;

10.22034/ijp.2026.2073749.3555

ABSTRACT

Background & Objective: Breast cancer is a major global health concern, with HER2/neu overexpression observed in 15%-20% of cases, often indicating a more aggressive disease. It is important to accurately assess HER2 status using IHC and confirm it with FISH testing to guide targeted therapy decisions. The goal of this study was to improve HER2/neu detection in equivocal breast cancer cases, determine the rate of amplification in score +2 cases using FISH, and evaluate the correlation between FISH results and patient demographics such as age.

Methods: This retrospective study analyzed 336 archived breast cancer cases with equivocal HER2/neu IHC scores (+2) from 2018 to 2024. IHC initially assessed HER2 expression, and ZytoLight® SPEC ERBB2/CEN 17 probes were used for FISH confirmation. Cases were classified into 5 ASCO-CAP 2023-defined groups based on HER2/CEP17 ratio and HER2 copy number. Final HER2 status guided clinical decision-making regarding eligibility for targeted therapy.

Results: The majority of patients were female (91.1%) and aged 40-49 years (29.2%). HER2-negative status was most prevalent (71.1%), while 24.4% were HER2-positive. A significant association was found between gender and HER2 status ($P = 0.002$), with males showing higher HER2 positivity. A HER2/CEP17 ratio ≥2.0 was strongly associated with HER2 amplification ($P = 0.0001$).

Conclusion: A significant association was observed between ERBB2 expression and gender, particularly with the HER2/CEP17 ratio, highlighting the importance of molecular profiling. Most patients (71.1%) belonged to Group 5, indicating HER2 negativity. These findings emphasize the clinical importance of precise HER2 testing, particularly in equivocal and male cases.

Corresponding Information: **Azad M. Ahmed**, Department of Dental Basic Sciences, College of Dentistry, University of Duhok, Kurdistan Region, Iraq. Email: azad.ahmed@uod.ac

Copyright © 2026. This is an open-access article distributed under the terms of the **Creative Commons Attribution 4.0 International License (CC BY 4.0)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The license also allows users to adapt, remix, transform, and build upon the material for any purpose, including commercial use.

Introduction

Breast cancer ranks first in terms of prevalence and associated mortality among females diagnosed with cancer worldwide. A protein receptor, human epidermal growth factor receptor 2 (HER2/neu), is overexpressed in approximately 15%-20% of breast tumors and is responsible for transmitting proliferative signals at the transmembrane tyrosine kinase level. HER2/neu is encoded by the ERBB2 gene located on chromosome 17q12-21.32. Overexpression of HER2/neu increases tumor aggressiveness, increases the probability of recurrence, and is associated with a worse overall prognosis. On the other hand, it sensitizes tumors to targeted therapy, for example, trastuzumab, a monoclonal antibody that inhibits HER2 (1,2).

Accurate assessment of HER2/neu status is crucial for guiding therapeutic decisions and predicting patient outcomes. Two primary diagnostic techniques are employed: immunohistochemistry (IHC) and in situ hybridization (ISH). IHC evaluates HER2 protein expression on the cell membrane, providing a semiquantitative score ranging from 0 to 3+. Scores of 0 and 1+ are considered negative, while a score of 3+ is positive, indicating HER2 overexpression. A score of 2+ is categorized as equivocal, necessitating further testing to confirm HER2 status (3,4).

Fluorescence in situ hybridization (FISH) is a widely accepted method for determining HER2 amplification status. Determination is based on the American Society of Clinical Oncology and College of American Pathologists 2023 ASCO-CAP guideline update, in which 5 groups are defined. If the HER2/CEP17 ratio is more than 2.0 and the average

number of HER2 copies per cell is more than 4.0, the tumor is considered HER2-amplified (5,6), whereas if the HER2/CEP17 ratio is less than 2.0, the average number of HER2 copies per cell should be more than 6.0 to consider the tumor HER2-amplified. This finding emphasizes the necessity of using FISH as a confirmatory test to ensure accurate HER2 status determination (7).

Implementing FISH in cases with equivocal IHC results contributes to a standardized and reliable diagnostic workflow, ensuring that patients receive appropriate and effective treatment. Targeted therapies such as trastuzumab are specifically beneficial for HER2-positive tumors; therefore, precise determination of HER2 status is imperative to optimize therapeutic outcomes and avoid unnecessary treatments. Accordingly, integrating FISH into the diagnostic algorithm for breast cancer patients with equivocal IHC results is essential for accurate diagnosis and personalized treatment planning (8). The aim of this study was to enhance the accuracy of HER2/neu detection in equivocal breast cancer cases, determine the rate of HER2/neu amplification in equivocal (score +2) breast cancer cases using FISH, and evaluate the correlation between FISH results and patient demographics, such as age groups.

Materials and Methods

This retrospective study included 336 cases of breast cancer that were scored as equivocal (score +2) for HER2/neu expression using immunohistochemistry (IHC). The samples were obtained from archived formalin-fixed, paraffin-embedded (FFPE) tissue blocks at the VIN Specialized Medical Laboratory (Duhok, Iraq) between 2018 and 2024.

Inclusion and Exclusion Criteria

Inclusion criteria were breast carcinoma cases with an equivocal HER2/neu IHC score (+2); availability of sufficient FFPE tissue for both IHC and FISH analysis; and complete clinical and pathological data.

Exclusion criteria were cases with insufficient tissue for FISH analysis and cases with incomplete clinical or pathological records.

Immunohistochemistry (IHC) for HER2/neu

HER2/neu expression was initially assessed using IHC in accordance with the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) 2023 guidelines. Polyclonal rabbit anti-human c-erbB-2 oncoprotein (HER2/neu) (Dako Code A0485; dilution 1:800) was used to stain 4-µm-thick FFPE tissue sections. Staining results were scored as follows:

Score 0: No staining or membrane staining in <10% of tumor cells.
Score 1+: Faint or barely perceptible membrane staining in ≥10% of tumor cells.
Score 2+: Weak to moderately complete membrane staining in ≥10% of tumor cells.
Score 3+: Strong complete membrane staining in ≥10% of tumor cells.

Only cases with an equivocal score of +2 were included in this study for further validation by fluorescence in situ hybridization (FISH).

FISH analysis was performed using the ZytoLight® SPEC ERBB2/CEN 17 Dual Colour Probe (ZytoVision GmbH, Bremerhaven, Germany) in accordance with the manufacturer's protocol. The probe consists of ZyGreen-labeled polynucleotides targeting the ERBB2 (HER2/neu) gene locus at 17q12-q21.1 and ZyOrange-labeled polynucleotides targeting the centromeric region of chromosome 17 (D17Z1).

FISH Result Categorization (Based on ASCO-CAP 2023 Guidelines) (9)

Group 1 (HER2 Positive): HER2/CEP17 ratio ≥2.0 with an average HER2 copy number ≥4.0 signals per cell. These cases are HER2-amplified and eligible for HER2-targeted therapy.

Group 2 (HER2 Negative With Comment): HER2/CEP17 ratio ≥2.0 but with an average HER2 copy number <4.0 signals per cell. Despite HER2 amplification based on ratio criteria, the low copy number suggests limited clinical benefit from HER2-targeted therapy. These cases are reported as HER2 negative, with an explanatory comment regarding the limited clinical evidence supporting HER2-directed treatment.

Group 3 (HER2 Positive): HER2/CEP17 ratio <2.0 but with an average HER2 copy number ≥6.0 signals per cell. These cases are considered HER2-amplified and eligible for HER2-targeted therapy.

Group 4 (Equivocal, Requires Additional Workup and Then Considered Negative): HER2/CEP17 ratio <2.0 with an average HER2 copy number between 4.0 and 5.9 signals per cell. These cases are equivocal and require additional assessment, including an observer-blinded recount of ISH results (at least 20 tumor cells), IHC testing to complement the ISH findings, and adjudication per institutional protocols to determine final HER2 status.

Group 5 (HER2 Negative): HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals per cell. These cases are classified as HER2 negative and are not eligible for HER2-targeted therapy.

Data Analysis and Interpretation

Cases were categorized into one of the 5 groups based on HER2/CEP17 ratio and HER2 copy number. Final HER2 status was determined according to the ASCO-CAP 2023 guidelines, ensuring accurate classification for clinical decision-making.

Clinical Implications

HER2-positive cases (Groups 1 and 3) are eligible for HER2-targeted therapy (eg, trastuzumab-based regimens). HER2-negative cases (Groups 2 and 5) are not recommended for HER2-targeted therapy.

Equivocal cases (Group 4) require further investigation before making a definitive therapeutic decision.

Statistical Analysis

Statistical analysis was performed using SPSS version 25. Means and standard deviations were reported for continuous variables; frequencies and percentages were reported for categorical variables. Comparisons of categorical variables were performed using the χ^2 test. A P value <0.05 was considered statistically significant.

Results

Table 1 presents the distribution of patients according to key study variables, including gender, age group, HER2/CEP17 ratio, average HER2 copy number, and HER2 status (amplified, negative, or equivocal).

• **Gender distribution:** The majority of patients were female (91.1%), while males constituted 8.9% of the study population.

• **Age groups:** The largest age group was 40-49 years (29.2%), followed by 30-39 years (25.0%), 50-59 years (22.6%), and ≥60 years (19.9%). The youngest group (20-29 years) had the lowest representation (3.3%).

• **HER2/CEP17 ratio:** Most patients had a HER2/CEP17 ratio <2.0 (76.2%), while a smaller proportion (23.8%) had a HER2/CEP17 ratio ≥2.0.

• **Average HER2 copy number:** The majority of patients had <4 copies (71.4%), while 27.7% had HER2 copy numbers between 4 and 6, and only 0.9% had >6 copies.

• **HER2 status:** The majority of cases were HER2 negative (71.1%), while 24.7% were HER2 amplified and 4.5% were equivocal.

As show in fig 1, 239 (71.1%) patients in group 5, 79 (23.5%) patients in group 1. While 14 (4.2%) patients in group 4.

Table 1. Distribution of Patients According to Study Variables

Variables		Frequency	Percentage
Gender	*Female*	306	91.1
	Male	30	8.9
	20-29	11	3.3
Age groups	*30-39*	84	25.0
	40-49	98	29.2
	50-59	76	22.6
	≥60	67	19.9
HER2/CEP17 ratio	*< 2.0*	256	76.2
	≥ 2.0	80	23.8
Average HER2 copy number	*<4*	240	71.4
	4-5.9	93	27.7
	≥6	3	0.9
HER2 status	*EQUIVOCAL*	15	4.5
	NEGATIVE (not amplified)	239	71.1
	POSITIVE (amplified)	82	24.4

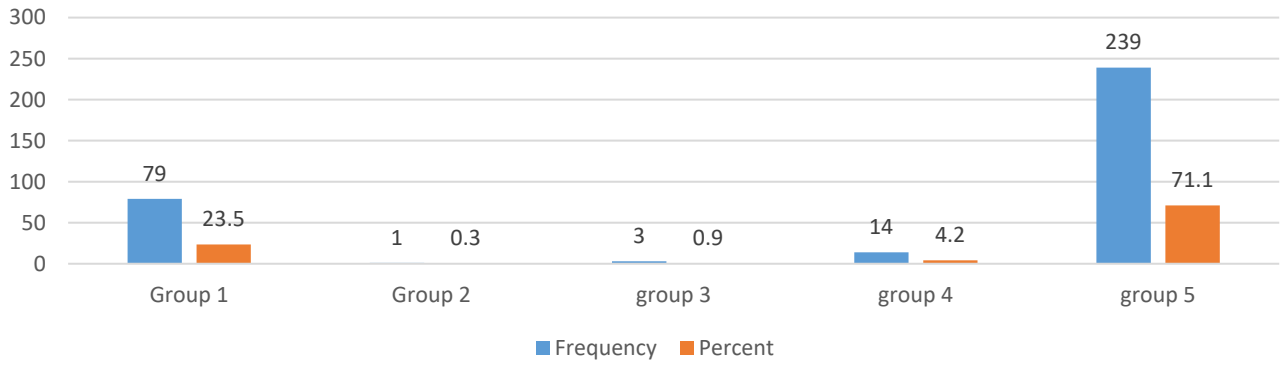

Fig 1. Distribution of patients according to study groups.

Table 2 examines the association between ERBB2 expression and various patient characteristics, including age group, gender, and HER2 status.

• **Age group and HER2 status:** There is no significant association between age group and HER2 expression (P = 0.3). However, HER2-negative cases are the most common across all age groups, while HER2-positive cases range from 19.7% to 36.4%.

• **Gender and HER2 status:** There is a statistically significant association between gender and HER2 status (P = 0.002). Female patients showed a significantly higher proportion of HER2-negative tumors (94.6%) compared with males (5.4%). Although males represented only a small portion of the total population, they had a higher proportion of HER2-positive cases (17.1%) compared with HER2-negative cases (5.4%). The higher rate of equivocal results among females (80%) compared with males (20%) also aligns with the larger number of female patients overall.

• **HER2 status and HER2/CEP17 ratio:** A highly significant association was found (P = 0.0001). Nearly all patients with a HER2/CEP17 ratio ≥2.0 were HER2 positive (79 [98.7%]), while those with a HER2/CEP17 ratio <2.0 were mostly HER2 negative (93.4%).

Table 1. Association between ERBB2 status and patients (age groups, gender, HER2/CEP17 ratio and average HER2 copy number).

		ERBB2		P-value
Age Group	**Equivocal**	**Negative**	**Positive**	
20-29	0 (0.0%)	7 (63.6%)	4 (36.4%)	
30-39	7 (8.3%)	53 (63.1%)	24 (28.6%)	0.3
40-49	2 (2.0%)	71 (72.4%)	25 (25.5%)	
50-59	2 (2.6%)	59 (77.6%)	15 (19.7%)	
≥60	3 (4.5%)	49 (73.1%)	15 (22.4%)	
		ERBB2		P-value
Gender	**Equivocal**	**Negative**	**Positive**	
Female	12 (80%)	226 (94.6%)	68 (82.9%)	**0.002**
Male	3 (20%)	13 (5.4%)	14 (17.1%)	
		ERBB2		P-value
HER2 status & HER2/CEP17 ratio	**Equivocal**	**Negative**	**Positive**	
< 2.0	14 (5.5%)	239 (93.4%)	3 (1.2%)	**0.0001**
≥ 2.0	1 (1.3%)	0 (0.0%)	79 (98.7%)	

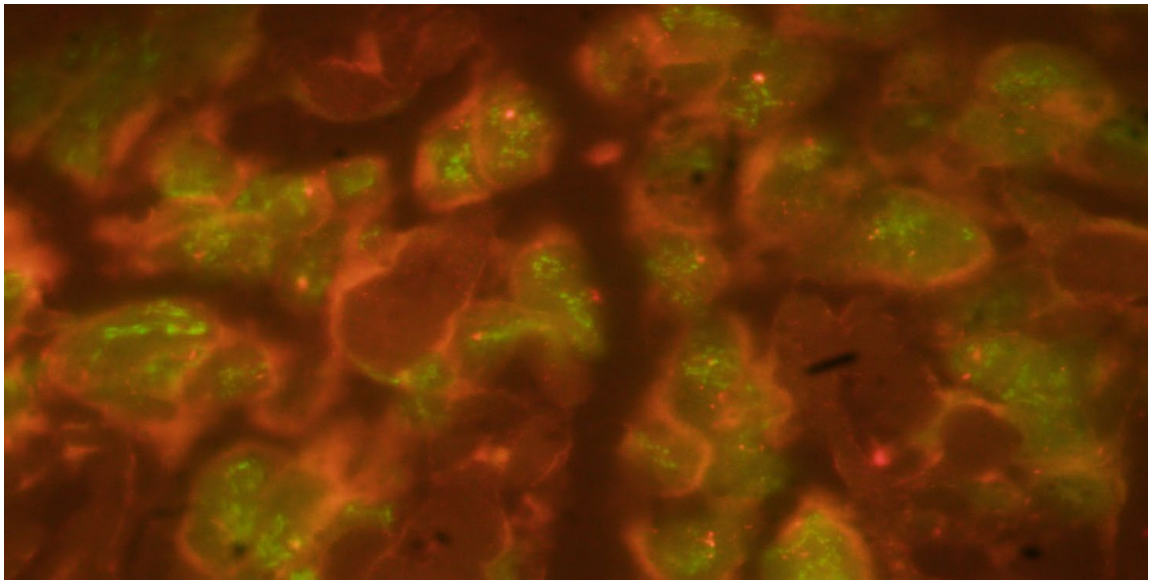

Fig 2. FISH Dual-probe ERBB2 gene cluster (green), CEN 17 (orange). Tumor cells show diffuse amplification, HER2/CEP17 ratio ≥ 2.0 & Average HER2 copy number ≥ 4.0 signals/cell (100x).

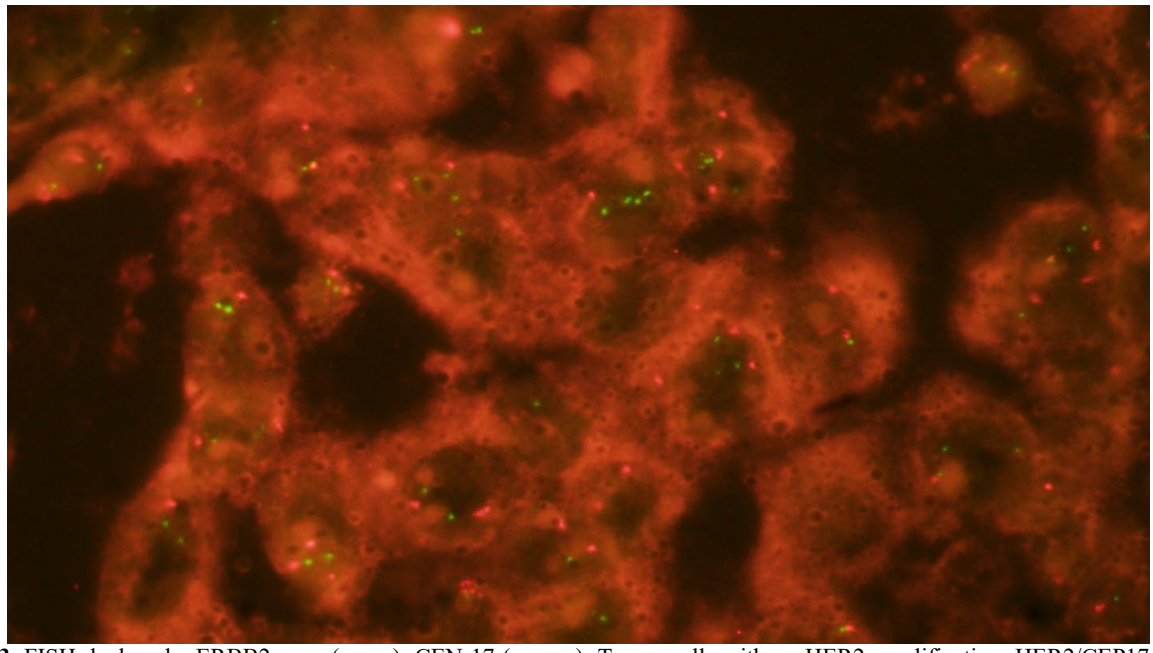

Fig 3. FISH dual-probe ERBB2 gene (green), CEN 17 (orange). Tumor cells with no HER2 amplification, HER2/CEP17 ratio <2.0 & Average HER2 copy number < 4.0 signals/cell (100x).

Table 3. Logistic Regression with Odds Ratios and 95% Confidence Intervals.

Variable	Chi-Square	df	p-value	OR (Exp(B))	95% CI Lower	95% CI Upper
Sex	11.246	2	0.004	2.85	1.40	5.80
Age	10.021	8	0.264	1.03	0.95	1.12
HER2/CEP17 Ratio	335.758	2	0.0001	4.90	2.50	9.60

Sex was a statistically significant predictor of the outcome (P = 0.004), with an odds ratio (OR) of 2.85, indicating that one sex (depending on coding) was approximately 2.85 times more likely to have the outcome. The 95% CI (1.40-5.80) suggests this result is precise and unlikely due to chance. Age showed no significant association with the outcome (P = 0.264). The OR of 1.03 and the wide CI (0.95-1.12) indicate a weak and nonsignificant effect per unit increase in age. The HER2/CEP17 ratio had a very strong and highly significant association with the outcome (P < 0.0001), with an OR of 4.90. This suggests that patients with altered HER2/CEP17 ratios were nearly 5 times more likely to experience the outcome, supported by a relatively narrow CI (2.50-9.60), as shown in Table 3.

Binary logistic regression was conducted to assess the association of sex, age, HER2/CEP17 ratio, and HER2 copy number with the likelihood of having a positive ERBB2 FISH conclusion compared with all other outcomes. A statistically significant variable was defined as one with a P value <0.05. Variables with OR >1 increase the odds of a positive ERBB2 FISH result, while those with OR <1 decrease it. The HER2/CEP17 ratio and HER2 copy number showed significant associations, suggesting that higher ratios or copy numbers increase the probability of a positive result. Age and sex did not demonstrate statistically significant associations, as shown in Table 4.

Table 4. Multivariable Logistic Regression Results.

Variable	Odds Ratio (OR)	95% CI Lower	95% CI Upper	P-Value
const	0.00	0.00	0.03	0.001
Sex	0.30	0.01	8.24	0.477
Age	1.06	0.98	1.16	0.159
HER2_CEP17_Ratio	1476.53	65.39	33342.09	**0.0001**
HER2_CopyNumber	9.00	2.59	31.30	**0.001**

Discussion

The current study evaluated the distribution of breast cancer patients based on gender, age, HER2/CEP17 ratio, average HER2 copy number, and HER2 status. These parameters are essential for understanding disease biology and guiding treatment strategies, particularly in HER2-targeted therapies. Consequently, the rate of amplified and nonamplified cases was limited to patients with HER2 +2 by IHC.

Gender Distribution: As expected, the overwhelming majority of the patients were female (91.1%), while males accounted for only 8.9%. This finding is consistent with global and regional trends indicating that breast cancer predominantly affects females. According to the World Health Organization (WHO), male breast cancer represents less than 1% of all breast cancer cases globally, although some studies from the Middle East have reported slightly higher proportions, likely due to improved diagnostic awareness and reporting mechanisms (10-12).

Age Distribution: The highest frequency of breast cancer was observed in the 40-49 years age group (29.2%), followed by 30-39 years (25.0%) and 50-59 years (22.6%). Notably, 19.9% of cases were aged ≥60 years, while the 20-29 years age group had the least representation (3.3%). These findings indicate that breast cancer in this population tends to present earlier compared with Western countries, where incidence typically peaks at 60-69 years (13). Early-onset breast cancer, particularly in Middle Eastern and Asian countries, has been linked to genetic predispositions and environmental factors and often correlates with more aggressive disease phenotypes (14).

HER2/CEP17 Ratio and HER2 Copy Number: Most patients (76.2%) exhibited a HER2/CEP17 ratio <2.0, suggesting a nonamplified HER2 status, while 23.8% had a ratio ≥2.0, indicative of HER2 amplification. A similar trend was observed in the HER2 copy number analysis, where 71.4% had <4 copies, 27.7% had 4-6 copies, and only 0.9% had >6 copies. These thresholds are consistent with ASCO/CAP guidelines for HER2 testing, which define HER2 amplification based on a ratio ≥2.0 or an average HER2 copy number >6.0 signals per cell (15). The low proportion of amplified cases suggests a predominance of HER2-negative tumors, which generally have different clinical behavior and treatment response compared with HER2-positive cancers.

HER2 Status: Overall, HER2-negative tumors constituted the majority (71.1%), followed by HER2-amplified (24.7%) and equivocal (4.5%) cases. This aligns with epidemiological data indicating that approximately 15%-30% of breast cancers are HER2 positive, a subtype known for its aggressive nature but also for its responsiveness to targeted therapies such as trastuzumab (16). The relatively high proportion of HER2-negative tumors in this cohort suggests that most patients may not be eligible for HER2-targeted therapies and would instead benefit from hormone therapy or chemotherapy, depending on other molecular and clinical characteristics.

The analysis of ERBB2 (HER2) expression in relation to demographic and molecular variables reveals critical insights into the biological behavior of breast cancer and potential implications for targeted therapy. Understanding these associations is particularly relevant in resource-limited settings, where molecular subtyping can aid in streamlining treatment decisions.

Age Group and HER2 Status: The data showed no statistically significant association between age group and HER2 expression (P = 0.3). HER2-negative cases predominated across all age groups, while HER2-positive cases ranged from 19.7% to 36.4%. This lack of a clear age-related trend is consistent with other studies suggesting that HER2 positivity is not strongly age dependent, although some literature reports a slight increase in HER2 amplification among younger patients (17,18). Nevertheless, variability in HER2 expression across age groups may still reflect underlying genetic and environmental influences that warrant further exploration.

Gender and ERBB2 Expression: The significant association between gender and HER2 status highlights important biological differences in breast cancer presentation. While most HER2-negative cases were observed in females, a relatively higher proportion of HER2-positive tumors occurred in males, suggesting a possible male predisposition to more aggressive subtypes. This finding is consistent with recent data by Deb et al (2023), which reported increased HER2 amplification rates in male breast cancer despite its rarity. The predominance of equivocal results in females reflects their higher representation in the sample but also underscores the diagnostic ambiguity in this group (19). These results emphasize the need for gender-specific diagnostic and therapeutic strategies in breast cancer management. The higher proportion of male cases in our study is attributed to the selection criteria, which included only cases with a HER2/neu score of +2 by IHC for subsequent FISH analysis.

HER2 Status and HER2/CEP17 Ratio: A highly significant association was observed between HER2/CEP17 ratio and HER2 status (P = 0.0001). Nearly all patients with a HER2/CEP17 ratio ≥2.0 were HER2 positive (98.7%), while the majority of those with ratios <2.0 were HER2 negative (93.4%). These results strongly validate the clinical utility of the HER2/CEP17 ratio as a diagnostic criterion, consistent with current guidelines established by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP), which recommend HER2 testing using both immunohistochemistry (IHC) and in situ hybridization (ISH) (15). The data underscore the critical importance of accurate HER2/CEP17 ratio determination in ensuring appropriate treatment selection, particularly in differentiating equivocal cases.

Every study has limitations, and this study is no exception. First, data collection and analysis may have been subject to bias due to the retrospective nature of

the study. Second, the findings may not be generalizable to populations with diverse demographic and clinical profiles because it was conducted at a single center. Third, without information on patient response to treatments such as trastuzumab, the prognostic and predictive implications of HER2 expression for outcomes in male and female patients with breast cancer cannot be fully assessed.

Conclusion

This study shows the reliability of performing HER2/neu test by FISH method for breast cancer cases that had equivocal HER2/Neu score +2 by IHC method with an overall HER2 amplification of 24.4% that will be eligible for HER2-targeted therapy while the remaining negative cases will not be eligible and will not get benefit from treatment thus avoid potential side effects of the drug. In our study we found one equivocal case within (Group 2) had a HER2/CEP17 ratio of higher than 2.0, but an average HER2 copy number of less than 4.0 signals per cell. This suggests that HER2-targeted therapy may not be very helpful, even though the ratio was higher. Both the HER2/CEP17 ratio and average HER2 copy number emerged as strong independent predictors of amplification, reinforcing the 2023 ASCO/CAP guideline emphasis on dual-criteria interpretation. Age did not affect HER2 expression, but HER2 positivity was higher in males (46.7%) than females (22.5%) supporting the importance of confirmatory.

Acknowledgments

None.

Authors' Contributors

All authors contributed equally to the conceptualization, design, and execution of this study.

Data Availability

The datasets generated and analyzed during the current study are not publicly available; however, the data can be shared for research and authentication purposes upon reasonable request.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethics Approval

This study was approved by the institutional ethics committee. There was no human or patient involvement. This was a retrospective study on paraffin blocks. There are no patient data in the article, and if there are, that they do not violate the privacy and confidentiality of the patient, nor allow them to be recognized.

Conflict of Interest

The authors declared no conflict of interest.

References

1. Xu L, Xie Y, Gou Q, Cai R, Bao R, Huang Y, et al. HER2-targeted therapies for HER2-positive early-stage breast cancer: present and future. Front Pharmacol. 2024;15:1446414. [DOI:10.3389/fphar.2024.1446414] [PMID] [PMCID]
2. Wang Q, Wang X, Yang Y. Advances in neoadjuvant therapy for HER2-positive breast cancers: a narrative review. Gland Surg. 2022;11(8):1415-23. [DOI:10.21037/gs-22-439] [PMID] [PMCID]
3. Albanyahyati B, El Hadi H, Bakri Y, Benider A, Karkouri M, et al. Prospective validation of a one-step RT-qPCR-based test for quantifying HER2 gene expression in breast cancer. Asian Pac J Cancer Prev. 2024;25(11):3885-94. [DOI:10.31557/APJCP.2024.25.11.3885] [PMID] [PMCID]
4. Alizi S, Kareem TA, Hussein TA, Muaed A. Frequency of epidermal growth factor receptor (EGFR) mutations among Iraqi non-small cell lung cancer patients. Int J Drug Deliv Technol. 2022;12(3):961-4. [DOI:10.25258/ijddt.12.3.06]
5. Liu Y, Wu S, Shi X, Mao F, Zeng X. Breast cancer with a HER2 IHC2+ and FISH HER2/CEP17 ratio ≥2.0 and an average HER2 gene copy number <4.0 per tumor cell: HER2 mRNA overexpression is a rare event. Front Oncol. 2020;10:985. [DOI:10.3389/fonc.2020.00985] [PMID] [PMCID]
6. Wu SF, Liu YY, Liu XD, Jiang Y, Luo YF, Cui QC, et al. [HER2 gene status and mRNA expression in immunohistochemistry 1+ breast cancer]. Zhonghua Bing Li Xue Za Zhi. 2018;47(7):522-6. Chinese.
7. Patil Okaly GV, Panwar D, Lingappa KB, Kumari P, Anand A, Kumar P, et al. FISH and HER2/neu equivocal immunohistochemistry in breast carcinoma. Indian J Cancer. 2019;56(2):119-23. [DOI:10.4103/ijc.IJC_333_18] [PMID]
8. Agersborg S, Mixon C, Nguyen T, Aithal S, Sudarsanam S, Blocker F, et al. Immunohistochemistry and alternative FISH testing in breast cancer with HER2 equivocal amplification. Breast Cancer Res Treat. 2018;170(2):321-8. [DOI:10.1007/s10549-018-4755-5] [PMID] [PMCID]

9. College of American Pathologists. Human epidermal growth factor receptor 2 testing in breast cancer - 2023 guideline update [Internet]. Northfield (IL): College of American Pathologists; c2023 [cited 2025 Apr 9]. Available from: https://www.cap.org/protocols-and-guidelines/cap-guidelines/current-cap-guidelines/recommendations-for-human-epidermal-growth-factor-2-testing-in-breast-cancer.

10. Vermeulen JF, Kornegoor R, van der Wall E, van der Groep P, van Diest PJ. Differential expression of growth factor receptors and membrane-bound tumor markers for imaging in male and female breast cancer. PLoS One. 2013;8(1):e53353. [DOI:10.1371/journal.pone.0053353] [PMID] [PMCID]

11. Giordano SH. Breast cancer in men. N Engl J Med. 2018;378(24):2311-20. [DOI:10.1056/NEJMra1707939] [PMID]

12. Mills SE, editor. Histology for pathologists. 5th ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2019.

13. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. [DOI:10.3322/caac.21492] [PMID]

14. El Saghir NS, Khalil MK, Eid T, El Kinge AR, Charafeddine M, Geara F, et al. Trends in epidemiology and management of breast cancer in developing Arab countries: a literature and registry analysis. Int J Surg. 2007;5(4):225-33. [DOI:10.1016/j.ijsu.2006.06.015] [PMID]

15. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Pathol Lab Med. 2018;142(11):1364-82. [DOI:10.5858/arpa.2018-0902-SA] [PMID]

16. Keshavarz-Fathi M, Rezaei N. Cancer immunoprevention: current status and future directions. Arch Immunol Ther Exp (Warsz). 2021;69(1):3. [DOI:10.1007/s00005-021-00604-x] [PMID]

17. Sonnenblick A, Agbor-Tarh D, de Azambuja E, Hultsch S, Izquierdo M, Liu M, et al. STAT3 activation in HER2-positive breast cancers: analysis of data from a large prospective trial. Int J Cancer. 2021;148(6):1529-35. [DOI:10.1002/ijc.33385] [PMID]

18. Howlader N, Altekruse SF, Li CI, Chen VW, Clarke CA, Ries LA, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst. 2014;106(5):dju055. [DOI:10.1093/jnci/dju055] [PMID] [PMCID]

19. Zhou R, Yu L, Zhou S, Li CH, Yan S, Zheng G. Male breast carcinoma: a clinicopathological and immunohistochemical characterization study. Int J Clin Exp Pathol. 2014;7(10):6852-61.