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Research Article
Breast

HER2/neu status detection in breast carcinoma: Is FISH the preferred approach over IHC

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Background: HER2/neu amplification is found in 15-30% of all breast cancer patients, which can be detected by either immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). The concordance of FISH and IHC analysis for HER2/neu amplification remains limited, especially with studies in the Indian population. There is a need to further classify this information as HER2/neu positive patients often have a worse disease prognosis and require anti-HER2/neu therapy. **Methods:** In a retrospective study 149 patients with invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC), who underwent both IHC and FISH testing for HER2/neu amplification were analysed to determine the concordance between the two tests in this population. **Results:** Out of 149 patients reviewed, 58 had equivocal results on IHC, 52 patients had negative results and 39 patients had positive results on IHC. Analysis of the 91 non-equivocal IHC cases and their FISH results demonstrated an inter-rater reliability of Kappa= 0.606 (p <0.0005) 95% CI (0.445, 0.767). Of the 52 patients with negative IHC scores, 13 (25%) were found to be positive on FISH testing for HER/neu amplification. This represents a substantial number of patients who otherwise would not have received anti-HER2/neu therapy. **Conclusions:** The present results indicate that FISH testing for HER2/neu status should be done on all breast cancer patients whenever possible, irrespective of IHC score status so that appropriate treatment decisions can be made. The higher sensitivity and specificity of FISH testing can reduce the number of both false positive and false negatives seen with immunohistochemistry testing in the present study.

Keywords: Breast malignancy, HER2/neu status, Fluorescence In Situ Hybridization

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Background

Breast cancer remains the second most common cancer in Indian women and the leading cause of cancer in urban populations in India with limited data on HER2/neu amplification [2]. The status of HER2/neu gene amplification and over expression is critical in determining the treatment for breast cancer patients, especially for evaluating the role of anti-HER2/ neutherapy.

HER2/neu amplification has been associated with a poor prognosis due to the activation of the growth-signaling pathway that leads to tumor cell proliferation.

Trastuzumab and other anti-HER2/neu therapies have been proven to be an effective treatment for HER2/neu positive breast cancer patients [3]. Screening programmes for HER2/ neu expression in breast carcinoma patients has also been proven to reduce the cost of therapy [4]. In 2013, the American Society of Clinical Oncology/ College of American Pathologists (ASCO/CAP) updated the guidelines for testing HER2/neu amplification in breast cancer.

A study on the impact of these modified cut-offs revealed a 2% increase in overall HER2/neupositivity at their institution [5]. Current guidelines recommend testing for HER2/neuamplification for all invasive breast cancers with fluorescence *in situ* hybridization that are equivocal on immunohistochemical analysis [3]. However data on India's prevalence of HER2/ neuamplification and concordance between IHC and FISH testing modalities remains limited. In last few years, all breast cancer patients at our center were counselled to undergo FISH testing for HER2/ neuamplification irrespective of the IHC status.

The aim of the present study was to further clarify the concordance of FISH and IHC testing for HER2/ neupositive patients following diagnosis of IDC or ILC.

Methods

Setting: The study was conducted at the department of Pathology and Medical oncology, Kasturba medical college Manipal between 01/01/2015 to 12/31/2017.

Duration of study: 3 years

Type of study: Retrospective study

Sampling methods: 149 breast cancer patients who underwent both FISH and IHC testing to determine HER2/neuamplification were analyzed

Inclusion criteria: Breast cancer patients in whom both IHC and FISH test was performed to detect HER2/neu status

Exclusion criteria: Breast cancer patients in whom both IHC and Her2/neu was not performed to detect Her2/neu status.

Data collection procedure: The data was retrieved by electronic and paper medical records. Records were also reviewed for estrogen and progesterone receptor positivity, Ki-67 cellular proliferation marker, histological grade and subtype of breast cancer.

Method: Immunohistochemistry of the HER-2/neu protein was performed on 4mm thick paraffin embedded tissue sections placed on poly-L-Lysine coated slides. The slides are fixed at 37^{0}C for overnight. Next day after de-paraffinisation, blocking of endogenous peroxidase and hydration, antigen retrieval was performed by MERS (Multiple epitope retrieval system). The slides were then cooled and washed with distilled water and treated with phosphate buffer solution. HER-2/neu immunostaining was performed using rabbit anti-human c-erbB-2 oncoprotein as a primary antibody CDako, (Copenhagen, Denmark) at 1:300 dilutions for 30 minutes.

The slides were washed again with phosphate buffer solution. Dako envision (secondary & tertiary antibody) was then applied for 30 minutes to bind the primary antibody and washed in PBS. DAB (Di-amino benzidine) chromogen was added to create a visible brown reaction at the antigen site followed by a wash in distilled water to stop the reaction. The slides were then counterstained with MAYER's hematoxylin. The slides were dehydrated with alcohol and fixed and mounted in DPX.

FISH amplification was determined according to ASCO-CAP 2013 guidelines.

Data analysis: The correlation between Her2/neu status with IHC and FISH was calculated using Cohen's Kappa coefficient method.

Scoring system: HER2/neu staining was scored as 0, 1+, 2+ and 3+. The score of 0 was given for no staining or <10% of tumor cells. The score of 1+ was given for faint/barely perceptible membrane staining, in which the membrane was stained partly.

The score of 2+ was given for weak to moderate complete membrane staining and 3+ for strong complete membrane staining. The scores of 0 and 1+ was classified as negative expression, 3+ score was called as positive expression and 2+ score was called equivocal. The prepared slides were scored in a blinded fashion by two pathologists according to the manufacturer's suggested criteria. The immunostaining was read in a semi quantitative manner and graded as per scoring system.

Ethical consideration and permission: The study was approved by the Institutional Review Board of OO (IEC 278/2017) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Results

Of the 149 identified breast cancer patients who underwent both IHC and FISH testing, 58 had equivocal results on IHC and were excluded from the inter-rater reliability analysis. Of the remaining 91 non-equivocal cases, 52 patients tested negative on IHC for HER2/neu amplification (score of 0 or 1+).

Out of the 52 IHC negative patients, 13 (25%) patients were found to be positive for HER2/neu amplification with FISH testing. Thirty-nine (39) of the 91 non-equivocal patients tested positive on IHC for HER2/neuamplification (score 3+). Out of these 39 patients, 5 (12.8%) had negative results on subsequent FISH testing (Table 1).

Table 1: Immunohistochemistry and FISH correlation for HER2/neu status in 149 patients*

FISH	Immunohistochemistry			Total
	Negative	Equivocal	Positive	
Positive	13 (8.7)	28 (18.8)	34 (22.8)	75
Equivocal	0	2 (1.3)	0	2
Negative	39 (26.2)	28 (18.8)	5 (3.4)	72
Total	52	58	39	149

*Data expressed as number of patients (percentage of total patients)

As FISH testing is usually only recommended with equivocal IHC 2+ scores, 13 patients in the present study (8.7%) would not have received the necessary anti-HER2/neu therapy without confirmatory FISH testing. Analysis of the 91 non-equivocal IHC cases and their FISH results demonstrated an inter-rater reliability of Kappa= 0.606 ($p < 0.0005$) 95% CI (0.445, 0.767).

Although defined under the category of substantial agreement, our Kappa value of 61% agreement is not promising when it comes to critical anti-HER2/neu treatment for our patients and these patients require FISH testing for confirmation.

Discussion

Receptor over expression has been reported in many tumors such as breast, lung, gastrointestinal, ovarian, colorectal and others. HER2/neu over expression can be seen in 15-30% of all breast cancers, making targeted therapy of this cell surface receptor a key in treatment of many malignancies. Trastuzumab, a monoclonal antibody targeting the HER2 receptor has proven to extend disease-free survival in breast cancer patients. [6] Accurate testing of HER2/neu tyrosine kinase receptor positivity is essential for breast cancer patients as targeted therapy is now available for those who test positive for receptor amplification. While many tests exist, positivity is largely determined by immunohistochemistry and fluorescence in situ hybridization techniques.

A recent update from the American Society of Clinical Oncology/College of American Pathologists suggests using both in situ hybridization along with immuno-histochemistry review as part of the interpretation of receptor positivity [7].

Many studies have cited poor concordance between these two tests and suggest using FISH as a reliable method to determine patients in need of trastuzumab or other anti-HER2/neu therapy [8], [9]. Gokhale S et al in his study had similar results compared to the present study, where the discordance between IHC and FISH was highest among the IHC 0 and 1+ compared to IHC 3+ [10].

Contrary to our results Kakar et al reported a high concordance rate of 88% with IHC 3+ staining but a low concordance rate of 35% with IHC 2+ staining when compared to FISH testing [8]. Similar results of higher concordance among IHC 0 and 1+ compared to IHC 3+ was observed in studies by Dybdal N et al and Lebeau A et al [11], [12]. Immunohistochemistry is an affordable method to consider for most patients especially with the high patient volume seen at our institution and limited resources. The IHC testing method does rely on subjective interpretation by pathologists and can lead to inconsistent results with in situ hybridization.

Even after blinded scoring from two pathologists at our institution, 8.7% results were false negative for HER2/neu receptor amplification by immunohistochemistry testing. Without subsequent FISH testing, these patients would not have received anti-HER2/neu therapy which is necessary to modify disease-free survival. Many also suggest using in situ hybridization to follow in patients with equivocal immunohistochemistry results, however our high rate of both false positive and negatives may demonstrate a need to test all patients with in situ hybridization to determine accurate HER2/neu positivity [1]. Recent studies indicate that a small percentage of patients classified as 1+ on immunohistochemistry are positive on in situ hybridization testing (FISH) [13]. Similar to the present study, patients without subsequent FISH testing would have been misclassified. The recent 2018 ASCO focussed update provided more interpretation guidelines but testing guidelines for HER2/neu positivity still remain up to the interpretation of the clinician [14].

The limitations of IHC are reduced sensitivity due to antigen alteration caused by standard fixation methods and inter observer variability despite introduction of standard reference samples. IHC results are also affected by prolonged warm / cold ischemia time. These could be some of the reasons for false negativity of IHC in the present study [15]. In order to avoid under diagnosis and inappropriate treatment by omitting HER2/neu positive patients, FISH has to be considered as a regular testing option [16]. More studies on the concordance of IHC and FISH need to be conducted in populations and hospitals in similar conditions to determine the cost-benefit for additional in situ hybridization testing to clarify a patients' amplification status [17]. Despite having a Cohen's kappa value of 0.606 defined as substantial agreement, 13 patients would not have received the necessary chemotherapy. Further studies investigating accurate measures of HER2/neu gene amplification will be monumental for determining appropriate patient treatment.

Limitations of the study: Small number of patients, Retrospective study.

Conclusions

Her2/neu status by FISH method should be adapted in all patients with breast cancer irrespective of IHC status as false negative IHC will devoid the breast cancer patients with a potentially curative treatment.

What does this study add to existing knowledge?

The present study has demonstrated that the concordance rate between IHC and FISH methods in determining HER2/neu status was not very high and around 25% of the false negative IHC patients would not have received anti HER2/neu therapy if only IHC testing was performed without FISH. Hence all the patients with breast cancer should be advised to undergo FISH test in determining HER2/neu status.

Author's contribution

Dr. Chethana Babu K. Udupa & Attiya P. Harit: Concept and design

Dr. Karthik S. Udupa: Definition of intellectual content

Attiya P. Harit; Gopika S.R: Literature search

Attiya P. Harit & Dr. Chethana Babu K. Udupa: Manuscript preparation

Dr. Karthik S. Udupa, Jackson R Randolph & Andrew Galligan: Manuscript editing and manuscript review

IRB Permission: The study was approved by the Institutional Review Board of OO (IEC 278/2017) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained

Reference

01. Fornier M, Risio M, Van Poznak C, Seidman A. HER2 testing and correlation with efficacy of trastuzumab therapy. Oncology (Williston Park). 2002;16(10)1340-51. *[Crossref][PubMed][Google Scholar]*

02. Nandakumar A, Ramnath T, Chaturvedi M. The magnitude of cancer breast in India- a summary. Ind J Surg Oncol. 2010;1(1)8-9. *doi: 10.1007/s13193-010-0004-z[Crossref][PubMed][Google Scholar]*

03. Bethune GC, Pettit AS, Veldhuijzen van Zanten D, Barnes PJ. Well-differentiated invasive breast cancers with equivocal HER 2 immunohistochemistry- what is the yield of routine reflex in-situ hybridization testing?. Histopathol. 2017;70(6)966-974. *doi: 10.1111/his.13160 [Crossref][PubMed][Google Scholar]*

04. Brunelli M, Manfrin E, Bria E, Massari F, Tortora G, Brunello E et al. HER2/neu gene determination in women screened for breast carcinoma- how screening programs reduce the skyrocketing cost of targeted therapy. Antican Res. 2013;33(9)3705-10. *[Crossref][PubMed][Google Scholar]*

05. Varga Z, Noske A. Impact of modified 2013 ASCO/ CAP guidelines on HER2 testing in breast cancer- One year experience. PLoS One. 2015;10(10)e0140652. *doi: 10.1371/journal.pone.0140652 [Crossref][PubMed][Google Scholar]*

06. Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers- overexpression and therapeutic implications. Mol Biol Int. 2014;852748. *doi: 10.1155/2014/852748 [Crossref][PubMed][Google Scholar]*

07. Wolff AC, Hammond ME, 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. J Clin Oncol. 2013;31(31)3997-4013. *doi: 10.1200/JCO.2013.50.9984 [Crossref][PubMed][Google Scholar]*

08. Kakar S, Puangsuvan N, Stevens JM, Serenas R, Mangan G, Sahai S, et al. HER-2/neu assessment in breast cancer by immunohistochemistry and fluorescence in situ hybridization- comparison of results and correlation with survival. Mol Diagn. 2000;5(3)199-207. *doi: 10.1054/modi.2000.16690 [Crossref][PubMed][Google Scholar]*

09. Tubbs RR, Pettay JD, Roche PC, Stoler MH, Jenkins RB, Grogan TM. Discrepancies in clinical laboratory testing of eligibility for trastuzumab therapy- apparent immunohistochemical false-positives do not get the message. J Clin Oncol. 2001;19(10)2714-21. *doi: 10.1200/JCO.2001.19.10.2714 [Crossref][PubMed][Google Scholar]*

10. Gokhale S, Gatalica Z, Mohammad A, Rampy AI, VelagaletiGopalrao VN. FISH for HER-2/neu in breast cancer- Standardization makes the difference!. Indian J Cancer. 2004;41(4)152–158. *[Crossref][PubMed][Google Scholar]*

11. Dybdal N, Leiberman G, Anderson S, McCune B, Bajamonde A, Cohen RL, et al. Determination of HER2 gene amplification by fluorescence in situ hybridization and concordance with the clinical trials immunohisto-chemical assay in women with metastatic breast cancer evaluated for treatment with trastuzumab. Breast Cancer Res Treat. 2005;93(1)3–11. *doi: 10.1007/s10549-004-6275-8 [Crossref][PubMed][Google Scholar]*

12. Lebeau A, Deimling D, Kaltz C, Sendelhofert A, Iff A, Luthardt B, et al. HER-2/neu analysis in archival tissue samples of human breast cancer- Comparison of immunohistochemistry and fluorescence in situ hybridization. J Clin Oncol. 2001;19(2)354–363. *doi: 10.1200/JCO.2001.19.2.354 [Crossref][PubMed][Google Scholar]*

13. Wu SF, Liu YY, Liu XD, Jiang Y, Luo YF, Cui QC et al. HER2 gene status and mRNA expression in immuno- histochemistry 1+ breast cancer. Chin J Pathol. 2018;47(7)522-6. *doi: 10.3760/cma.j.issn.0529-5807.2018.07.008 [Crossref][PubMed][Google Scholar]*

14. Wolff AC, Hammond ME, Allison KH, Harvey BE, Mangu PB, Bartlett JM 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. J Clin Oncol. 2018;36(20)2105-2122. *doi: 10.1200/JCO.2018.77.8738 [Crossref][PubMed][Google Scholar]*

15. Pauletti G, Dandekar S, Rong H, Ramos L, Peng H, Seshadri R, et al. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer- a direct comparison of fluorescence in situ hybridization and immunohistochemistry. J Clin Oncol. 2000;18(21) 3651-64. *doi: 10.1200/JCO.2000.18.21.3651 [Crossref][PubMed][Google Scholar]*

16. Solomon JP, Dell'Aquila M, Fadare O, Hasteh F. Her2/neu status determination in breast cancer- a single institutional experience using a dual-testing approach with immunohistochemistry and fluorescence in situ hybridization. Am J Clin Pathol. 2017;147(4)432-437. *doi: 10.1093/ajcp/aqw224 [Crossref][PubMed][Google Scholar]*

17. KalalIravathy Goud SD, Vijayalaxmi K, Babu SJ, Vijay AR. Evaluation of HER-2/neu status in breast cancer specimens using immunohistochemistry (IHC) & fluorescence in-situ hybridization (FISH) assay. Indian J Med Res. 2012;135(3)312-7. *[Crossref][PubMed][Google Scholar]*