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Breast Cancer Human Epidermal Growth Factor Receptor 2 mRNA Molecular Testing Compared to Immunohistochemistry with Correlation to Neoadjuvant Therapy Response

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Abstract

BACKGROUND: Breast cancer is the most common cancer type among women worldwide. Human epidermal growth factor receptor 2 (HER-2) is amplified in 10–34% of breast carcinomas and offers a therapeutic option from HER2-targeted therapy. Hence, HER2 is tested routinely in all breast cancer patients using immunohistochemistry (IHC) and *in situ* hybridization. Yet, some pitfalls do exist due to tumoral heterogeneity, inter and intrapersonal variations. mRNA expression assays can provide an alternative method for accurately measuring HER-2 avoiding these limitations.

AIM: Comparing results of mRNA gene expression analysis for HER2 with IHC results and correlating it with the therapy response.

MATERIALS AND METHODS: One hundred breast cancer core biopsies were tested for HER-2 using IHC and the same blocks were sectioned and tested for mRNA gene expression for HER2 by the Xpert breast cancer STRAT4 device.

RESULTS: Concordance rate between mRNA expression and IHC for HER-2 was 93% with Kappa measurement showing perfect agreement ($\kappa = 0.81$, 95% CI, $p < 0.0005$).

CONCLUSION: The study reveals high concordance between HER2 measurement using IHC and mRNA analysis. Molecular testing can provide an effective standardized method for HER-2 measurement in breast cancer patients.

Introduction

Breast cancer is the most common malignancy in women and is the first cause of cancer deaths in women [1]. Management and prognosis of breast cancer are dependent on many factors as tumor histology, grade and stage, as well as the protein markers, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67 [2].

HER2 is a proto-oncogene encoding epidermal growth factor receptor with tyrosine kinase activity, located on chromosome 17 at q21. Amplified HER2 gene with overexpression of its protein is identified in 10–34% of invasive breast carcinomas [3].

Amplification of HER2 is considered as a poor prognostic factor linked to a higher recurrence rate and poor survival. It also predicts the response to anthracycline-based chemotherapies [4]. The most eminent role for HER2 evaluation is assessing the likelihood of the patient to benefit from HER2-targeted therapies such as trastuzumab, lapatinib, and pertuzumab [5]. As HER2-targeted therapy is considerably expensive and only effective in HER2-overexpressed/amplified breast cancers, accurate measurement of HER2 expression is crucial in breast cancer management [3].

Immunohistochemistry (IHC) is the classical method for the evaluation of HER2 expression [6]. Yet with IHC, subjective variability may exist due to issues such as differences in antibody used and methods of interpretation [7].

Assays for mRNA expression provide an alternative accurate approach for measuring HER2 amplification that can avoid this subjective variability. Hence, we aimed to compare mRNA expression assay for HER2 using RT-qPCR-based diagnostic platform called the GeneXpert®, (Cepheid, Sunnyvale, CA, USA) with standard IHC and correlating this with the neoadjuvant therapy response.

Materials and Methods

Retrieval of cases

This prospective study included 100 breast cancer patients, whom were candidates for neoadjuvant therapy selected in the time period between March 2019 and March 2020. The specimens were collected from the Pathology Department, Kasr El Ainy Hospital, Cairo University, Egypt. The authors obtained individualized patient informed consents then the approval of the ethical committee in the faculty of Medicine, Cairo University. The cases were followed till the regimen of the therapy was completed and surgical excisions were done, and then evaluated histopathologically for therapy response.

Histopathological and immunohistochemical examination

The paraffin blocks of the tumor tissue were serially sectioned at 4 μm thickness, stained with routine Hematoxylin and Eosin stains for pathological examination according to the WHO recommendations [8].

HER2 IHC was done using Ventana PenchMark XT by primary antibodies anti-HER-2/neu (4B5). UltraView Universal DAB is the Detection Kit used. HER-2 interpretation was done according to the ASCO/CAP guidelines 2018 [9].

Molecular biomarker testing

Expression of mRNA by automated real-time quantitative polymerase chain reaction (RT-qPCR) was achieved using the Xpert® Breast Cancer STRAT4 and its diagnostic platform, the GeneXpert®, (Cepheid, Sunnyvale, CA, USA).

The GeneXpert is an automated system integrating sample purification, nucleic acid amplification, and target sequence detection. The system consists of an instrument, barcode scanner, computer, and pre-loaded software for running tests and viewing the results.

The formalin-fixed paraffin-embedded (FFPE) tissue was first macrodissected and scraped from the tumor rich area after examining the H and E section then placed into a 1.5 mL tube, to be admixed with 1.2 mL of FFPE lysis reagent and 20 μL of proteinase K. Then the samples were incubated at 80 C for 30 min. After that 1.2 ml of ethanol was added. Finally, The sample lysate (about 520 μL) was shifted to the STRAT4 cartridge and placed into a GeneXpert module for RNA extraction, purification, and RT-qPCR analysis.

All reagents required for sample preparation and RT-PCR analysis are preloaded in the cartridge. Nucleic acids in the lysate are captured on a filter, washed, and eluted by sonication. The purified nucleic acid is mixed with dry RT-PCR reagents, and the solution is transferred to the reaction tube for RT-PCR and detection. Time to result is approximately 75 min in the GeneXpert.

Results

This study included 100 cases of breast carcinoma. Their age of them ranged from 25 to 88 years with a mean of 47 ± 11.31 years. Among the studied cases, 81% were invasive mammary carcinoma (NST), whereas 14% were invasive lobular carcinoma (ILC) and only 5% were other special types. Concerning grade, 71% of the cases were grade II and 29% were grade III. Regarding cT stage, 11% were T1, 69% were classified as T2, 14% were T3, and 6% were T4.

Concordance between IHC and molecular testing of mRNA

Expression of HER2 protein by IHC showed 27 positive cases and 73 negative cases (Table 1). While by HER2 mRNA showed 24 positive cases and 76 negative cases, with an overall concordance rate between the mRNA expression of HER2 results compared with IHC results 93%. The Kappa measure of agreement showed perfect agreement ($\kappa = 0.81$, 95% CI, $p < 0.0005$). The sensitivity (positive percent agreement) was 81.5% while the specificity (Negative percent agreement) was 97.3%. The positive predictive value was 91.7%, whereas the negative predictive value was 93.4%.

Response to therapy

According to AJCC "y" Classification system, patients showed the following ypTM after neoadjuvant therapy:

- For ypT: 28 cases were T0, 30 cases were T1, 26 cases were T2, 6 cases were T3 and one case was T4
- For ypN: 42 cases were N0, 19 cases were N1, 21 cases were N2 and nine cases were N3
- For M: 9 cases were metastatic (no surgery done).

According to Chevallier Method, Of these 100 studied cases, 28 cases reached a pathological complete response (pCR), 55 cases showed only partial response (pPR), 17 cases showed no response (pNR) (8 cases showed stable or progressive disease and 9 cases were metastatic).

HER2 was significantly correlated with the therapy response ($p = 0.031$) where HER2 positive

cases showed response to therapy (either pCR or pPR, 96.3%), more than HER-2 negative cases (78.2%).

Tumor grade showed a statistically significant correlation with the response to neoadjuvant therapy, (p = 0.036), where the pCR was higher among grade III tumors (46.4%) than grade II (20.8%). No significant correlation was seen with the different histological types (p = 0.164), but we noticed that ILC cases showed more resistance to treatment where, the pNR rate was higher among ILC (35.7%) than in invasive ductal carcinoma (IDC) patients (13.6%) (Table 2).

Table 1: Concoradance between mRNA expression and IHC

HER2 mRNA	HER2 IHC		Total
	Positive	Negative	
Positive			
Count	22	2	24
% within HER2 mRNA	91.7	8.3	100.0
% within HER2 IHC	81.5	2.7	24.0
Negative			
Count	5	71	76
% within HER2 mRNA	6.6	93.4	100.0
% within HER2 IHC	18.5	97.3	76.0
Total			
Count	27	73	100
% within HER2 mRNA	27.0	73.0	100.0
% within HER2 IHC	100.0	100.0	100.0

HER2: Human epidermal growth factor receptor 2, IHC: Immunohistochemistry.

Table 2: Correlation of therapy response with HER2

Pathological and immunological characteristics	Response		No response (pNR) (%)	p-value
	pCR (%)	pPR (%)		
HER-2				
Positive	9 (33.3)	17 (63)	1 (3.7)	0.031* (response vs. No response)
Negative	19 (26)	38 (52.1)	16 (21.9)	
Histopatholo-gical Grade				
Grade 2	15 (20.8)	44 (61.1)	13 (18.1)	0.036*
Grade 3	13 (46.4)	11 (39.3)	4 (14.3)	
Histopatholo-gical Type				
IDC	24 (29.6)	46 (56.8)	11 (13.6)	0.164
ILC	4 (28.6)	5 (35.7)	5 (35.7)	
Special Types	0 (0)	4 (80)	1 (20)	

*Statistically Significant, HER2: Human epidermal growth factor receptor 2, ILC: Invasive lobular carcinoma, IDC: Invasive ductal carcinoma, pCR: Pathological complete response, pPR: Partial response, pNR: No response.

Discussion

The mean age in our study was 47 years (ranging between 25 and 88 years). A comparable mean of age (49 years) and nearly the same age range (25–79 years) were detected in Constantinou *et al*., 2018 [10]. Furthermore, it was close to the mean age (45.4 years) of patients in Olfatbakhsh *et al*., 2018 [11] study on breast cancer patients who received neoadjuvant chemotherapy.

As regards the histological types of examined cases, 81 (81%) were NST, 14 (14%) cases were ILC and 5% other special types. Kizy *et al*., 2018 [12] reported a comparable incidence of duct carcinoma NST (74%), the lobular (12%). Also Kumarapeli *et al*., 2019 [13] in a study conducted on breast cancer, showed nearly the same incidences, 73.6% for duct carcinoma NST and 13% for lobular carcinoma.

In our study, Grade II cases (71%) were more than Grade III (29%). Lower incidence of high-grade cases was seen in Constantinou *et al*., 2018 [10] (20.5% poorly differentiated/grade III tumors). While in Ho-Yen *et al*., 2014 [14] study, high grade tumors accounted for 51% of the cases and it was higher also in Zagouri *et al*., 2014 [15] (incidence of high grade tumors reached 59%).

In our study, there was a high overall concordance rate between the mRNA results compared with IHC results for HER2 93% and the Kappa measure of agreement showed perfect agreement (κ = 0.81). The sensitivity (positive percent agreement) was 81.5% while the specificity (Negative percent agreement) was 97.3%.

A close figure to our results was mentioned in Wasserman *et al*., 2017 [16], with a concordance rate 91.25%, specificity of 94%, and slightly higher sensitivity (87%). Also Denkert *et al*., 2019 [17] in their study showed a closer concordance rate to ours (94.6%) with a similar Kappa agreement (0.81) and slightly higher specificity (94%). A higher concordance rate was seen in Fillipits *et al*., 2021 [18] and Mugabe *et al*., 2021 [19] (98.2% and 97.8%), respectively. While in Janeva *et al*., 2021 [20], A lower concordance rate was seen (87%) and lower Kappa agreement (0.66).

Wu *et al*., 2018 [21] showed results that is well matched with ours, where concordance rate was 95.7%, kappa 80% and specificity 95.8 and only higher sensitivity rate 95.5%. Also in Gupta *et al*., 2018 [22] both the sensitivity and specificity were near to ours 0.85 and 0.98. Bel *et al*., 2019 [23] showed nearly the same agreement for HER2 between two modalities 92%.

Lower results seen in some studies as in Janeva *et al*., 2021 [20] can be partially explained by the usage of archived blocks (about 20 months old). These stored blocks can show some mRNA degradation, but our study was prospective, whereas the patients are chosen then the samples are collected and tested.

Second, the type of biopsy used for testing may explain the higher results seen in some studies, as in Mugabe *et al*., 2021 [19], where they used some surgical specimens, this can readily affect the results, especially when using surgical excision specimens with extensive ductal carcinoma *in situ*. This was avoided in our study as we used core needle biopsy specimens and further examining all the samples by standard H and E pathology sections, determining, locating and scraping the exact invasive tumor tissue and excluding any normal tissue or non-invasive tumor.

Furthermore, we tried harder to choose samples that contain enough invasive tumor tissue and excluding the few indeterminate cases from our analysis. Furthermore, the usage of different clones for IHC and subjective variability in interpretation can also offer some explanation.

Regarding the post-neoadjuvant systemic therapy response, 28% reached pCR, 55% showed only partial response, 8% showed stable or progressive disease while 9% was metastatic. Comparing this to Silver *et al*., 2010 [24] showed different results, only 17.8% showed complete response, 50% showed partial response, and 32.2% showed either stable or progressive course. This difference can be explained by the small sample in Silver *et al*., study (only 28 patients). Furthermore, Zhao *et al*., 2015 [25] and Olfatbakhsh *et al*., 2018 [10] showed lower rate of complete response among their study groups, (14% and 19%), respectively.

Conclusion

This study showed a high concordance between results of HER2 assessment using RT-qPCR-based diagnostic platform and IHC, concluding that mRNA gene expression analysis can be a rapid and accurate modality for evaluating HER2 overexpression in breast cancer patients.

References

1. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends-an update. Cancer Epidemiol Biomarkers Prev. 2016;25(1):16-27. https://doi.org/10.1158/1055-9965.EPI-15-0578
   PMid:26667886
2. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, *et al*. Tailoring therapies-improving the management of early breast cancer: St Gallen international expert consensus on the primary therapy of early breast cancer 2015. Ann Oncol. 2015;26(8):1533-46. https://doi.org/10.1093/annonc/mdv221
   PMid:25939896
3. Ahn S, Woo JW, Lee K, Park SY. HER2 status in breast cancer: Changes in guidelines and complicating factors for interpretation. J Pathol Transl Med. 2020;54(1):34-44. https://doi.org/10.4132/jptm.2019.11.03
   PMid:31693827
4. Tandon AK, Clark GM, Chamness GC, Ullrich A, McGuire WL. HER-2/neu oncogene protein and prognosis in breast cancer. J Clin Oncol. 1989;7(8):1120-8. https://doi.org/10.1200/JCO.1989.7.8.1120
   PMid:2569032
5. Patel A, Unni N, Peng Y. The changing paradigm for the treatment of HER2-positive breast cancer. Cancers (Basel). 2020;12(8):2081. https://doi.org/10.3390/cancers12082081
   PMid:32731409
6. Cancello G, Maisonneuve P, Rotmensz N, Viale G, Mastropasqua MG, Pruneri G, *et al*. Prognosis and adjuvant treatment effects in selected breast cancer subtypes of very young women (< 35 years) with operable breast cancer. Ann Oncol. 2010;21(10):1974-81. https://doi.org/10.1093/annonc/mdq072
   PMid:20332136
7. Roepman P, Horlings HM, Krijgsman O, Kok M, Bueno-de-Mesquita JM, Bender R, *et al*. Microarray-based determination of estrogen receptor, progesterone receptor, and HER2 receptor status in breast cancer. Clin Cancer Res. 2009;15(22):7003-11. https://doi.org/10.1158/1078-0432.CCR-09-0449
   PMid:19887485
8. Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ, editors.WHO Classification of Tumors of the Breast. 4th ed. IARC: Lyon; 2012.
9. ASCO/CAP guidelines 2018 for HER2 Testing; 2018. Available from: https://www.asco.org/sites/new-www.asco.org/files/contentfiles/practice and-guidelines/documents/2018-her2-testing-summary-table.pdf. [Last accessed on 2021 Sep 25].
10. Constantinou C, Papadopoulos S, Karyda E, Alexopoulos A, Agnanti N, Batistatou A, *et al*. Expression and clinical significance of claudin-7, PDL-1, PTEN, c-Kit, c-Met, c-Myc, ALK, CK5/6, CK17, p53, EGFR, Ki67, p63 in triple-negative breast cancer-a single centre prospective observational study. *In Vivo*. 2018;32(2):303-11. https://doi.org/10.21873/invivo.11238
    PMid:29475913
11. Olfatbakhsh A, Tafazzoli-Harandi H, Najafi S, Hashemi EA, Sari F, Mokhtari Hesari P, *et al*. Factors impacting pathologic complete response after neoadjuvant chemotherapy in breast cancer: A single-center study. Int J Cancer Manag. 2018;11(5):e60098. Available from: https://www.sites.kowsarpub.com/ijcm/articles/60098.html. https://doi.org/10.5812/ijcm.60098
12. Kizy S, Huang JL, Marmor S, Blaes A, Yuan J, Beckwith H, *et al*. Distribution of 21-gene recurrence scores among breast cancer histologic subtypes. Arch pathol Lab Med. 2018;142(6):735-41. https://doi.org/10.5858/arpa.2017-0169-OA
    PMid:29528718
13. Kumarapeli AR, Bellamy W, Olgaard E, Massoll N, Korourian S. Short-duration rapid chilling of mastectomy specimens does not interfere with breast cancer biomarker and molecular testing. Arch Pathol Lab Med. 2019;143(1):92-8. https://doi.org/10.5858/arpa.2017-0377-OA
    PMid:29932859
14. Ho-Yen CM, Jones JL, Kermorgant S. The clinical and functional significance of c-Met in breast cancer: A review. Breast Cancer Res. 2015;17(1):52. https://doi.org/10.1186/s13058-015-0547-6
    PMid:25887320
15. Zagouri F, Bago-Horvath Z, Rössler F, Brandstetter A, Bartsch R, Papadimitriou CA, *et al*. High MET expression is an adverse prognostic factor in patients with triple-negative breast cancer. Br J Cancer. 2013;108(5):1100-5. https://doi.org/10.1038/bjc.2013.31
    PMid:23422757
16. Wasserman BE, Carvajal-Hausdorf DE, Ho K, Wong W, Wu N, Chu VC, *et al*. High concordance of a closed-system, RT-qPCR breast cancer assay for HER2 mRNA, compared to clinically determined immunohistochemistry, fluorescence *in situ* hybridization, and quantitative immunofluorescence. Lab Invest. 2017;97(12):1521-6. https://doi.org/10.1038/labinvest.2017.93
    PMid:28892092
17. Denkert C, Link T, Jank P, Just M, Hanusch C, Brasch F, *et al*. Comparison of an Automated Cartridge-Based System for mRNA Assessment with Central Immunohistochemistry in the Neoadjuvant GeparX Trial. Available from: https://www.researchgate.net/profile/Paul-Jank/publication/333407035. [Last accessed on 2021 Sep 25].
18. Filipits M, Rudas M, Singer CF, Fitzal F, Bago-Horvath Z, Greil R, *et al*. ESR1, PGR, ERBB2, and MKi67 mRNA expression in postmenopausal women with hormone

receptor-positive early breast cancer: Results from ABCSG trial 6. ESMO Open. 2021;6(4):100228. https://doi.org/10.1016/j.esmoop.2021.100228

PMid:34371382

19. Mugabe M, Ho KE, Ruhangaza D, Milner D, Rugwizangoga B, Chu VC, *et al*. Use of the Xpert breast cancer STRAT4 for biomarker evaluation in tissue processed in a developing country. Am J Clin Pathol. 2021;156(5):766-76. https://doi.org/10.1093/ajcp/aqab016

PMid:34050358

20. Janeva S, Parris TZ, Nasic S, de Lara S, Larsson K, Audisio RA, *et al*. Comparison of breast cancer surrogate subtyping using a closed-system RT-qPCR breast cancer assay and immunohistochemistry on 100 core needle biopsies with matching surgical specimens. BMC Cancer. 2021;21(1):439. https://doi.org/10.1186/s12885-021-08171-2

PMid:33879115

21. Wu NC, Wong W, Ho KE, Chu VC, Rizo A, Davenport S, *et al*. Comparison of central laboratory assessments of ER, PR, HER2, and Ki67 by IHC/FISH and the corresponding mRNAs (ESR1, PGR, ERBB2, and MKi67) by RT-qPCR on an automated, broadly deployed diagnostic platform. Breast Cancer Res Treat. 2018;172(2):327-38. https://doi.org/10.1007/s10549-018-4889-5

PMid:30120700

22. Gupta S, Mani NR, Carvajal-Hausdorf DE, Bossuyt V, Ho K, Weidler J, *et al*. Macrodissection prior to closed system RT-qPCR is not necessary for estrogen receptor and HER2 concordance with IHC/FISH in breast cancer. Lab Invest. 2018;98(8):1076-83. https://doi.org/10.1038/s41374-018-0064-1

PMid:29858579

23. Bel C. Comparaison de L'évaluation Des Récepteurs Hormonaux, du Ki67 et de Her2 Par le Kit STRAT4 (RT-qPCR) et L'immunohistochimie Dans le Cancer du Sein. Available from: https://www.hal.uca.fr/dumas-02383163v1. [Last accessed on 2021 Sep 25].

24. Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li Q, *et al*. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. J Clin Oncol. 2010;28(7):1145-53. https://doi.org/10.1200/JCO.2009.22.4725

PMid:20100965

25. Zhao Y, Dong X, Li R, Ma X, Song J, Li Y, *et al*. Evaluation of the pathological response and prognosis following neoadjuvant chemotherapy in molecular subtypes of breast cancer. Onco Targets Ther. 2015;8:1511-21. https://doi.org/10.2147/OTT.S83243

PMid:26150728