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Original Article

Evaluation of Breast Cancer Subtypes Based on ER/PR and Her2 Expression: A Clinicopathologic Study of Hormone Receptor Status (ER/PR/HER2-neu) in Breast Cancer

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Abstract:

Breast cancer stands first in the incidence of malignancy in women. Enormous studies have been conducted worldwide regarding hormone receptor status in breast cancer. The study was done in the department of pathology in Khulna Medical College, Khulna to compare the clinicopathologic features with four breast cancer subtypes defined by immunohistochemistry (IHC) expression of estrogen receptor (ER) or progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2): ER/PR+, Her2+; ER/PR+, Her2−; ER/PR−, Her2+; and ER/PR−, Her2− and to evaluate hormone receptor status in breast cancer to estimate a patient's response to endocrine therapy and their prognosis for better clinical outcomes. It is a retrospective observational study from 1st January, 2015 to 31st December, 2017. A total 378 invasive breast cancer subjects who underwent diagnostic tests for hormone receptors status were included in this study. Clinical and pathologic features and survival of the four subtypes were compared. Data of oestrogen, progesterone and human epidermal growth factor receptor 2 expression statuses was analyzed. Overall record of 378 patients was studied of whom 43% were identified to have positive hormone receptor status. The age of the patients ranged from 24 to 86 years with 65% in 25-50 years, 30.8% in 51-75 years and 4.08% in 76-100 years. Fiftyeight percent were diagnosed as Stage III, 37% Stage II and 5.3% Stage IV. Those diagnosed with oestrogen receptor (positive status) were 10.7%, human epidermal growth factor receptor 2 over-expression 8.7%, oestrogen/progesterone hormone receptor positivity 51% and 23.4% patients were positive for all the three receptors. The triple negative subtype has the worst overall and disease free survival.

Key words: Breast cancer, Estrogen/progesterone receptor, Human epidermal growth factor receptor 2 (HER2/neu), Immunohistochemistry, Triple negative

Introduction:

Over the last few decades there have been outstanding advances in breast cancer management leading to earlier detection of disease and the development of more effective treatments resulting in significant declines in breast cancer deaths and improved outcomes for women living with the disease[1,2] . Breast cancer is no longer seen as a single disease but rather a multifaceted disease comprised of distinct biological subtypes with diverse natural history, presenting a varied spectrum of clinical, pathologic and molecular features with different prognostic and therapeutic implications. Consensus regarding the definitive prognostic/predictive analysis has yet to be reached, but significant progress continues to be made in the ongoing search for a specific and reproducible method of identifying successful treatment utilizing biological markers. Recent attention has been directed at molecular classifications of breast cancer[3-11]. While molecular and genetic testing is very elegant, prognostic and predictive, it is expensive and not yet widely available. Also, despite the prognostic information provided by the molecular test, current reports of assay results impart specific guidance of response to targeted and proven therapy; for example, endocrine and trastuzumab therapy for tumors expressing estrogen receptor(ER)/progesterone receptor (PR) or human epidermal growth factor receptor 2 (Her2) proteins, respectively. The immunohistochemistry (IHC) classification provides both therapeutic and prognostic information. In this study breast cancer is classified into four groups based on IHC profile ER/PR and Her2/neu expression, positive (+) and/or negative (–). The groups are:

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ER/PR+, Her2+ = ER+/PR+, Her2+;
ER/PR+, Her2+; ER+/PR, Her2+

ER/PR+, Her2 = ER+/PR+, Her2;
ER/PR+, Her2; ER+/PR−, Her2−

ER/PR, Her2+ = ER/PR, Her2+

ER/PR, Her2 = ER/PR−, Her2−

The IHC classification correlates well with intrinsic gene expression microarray categorization: ER/PR+, Her2+ with Luminal B; ER/PR+, Her2− with Luminal A; ER/PR−, Her2+ and ER/PR−, Her2− with triple negative/basal-like tumors[4]. Apart from lending itself to subtype analyses of tumor when fresh tissue is not available, the IHC classification has prognostic and therapeutic implications, is inexpensive and readily available.

Materials and Methods:

Female subjects of age ranged from 24 to 86 years with mean age 62.7 years having invasive primary breast cancer were included in this study in the department of pathology in Khulna Medical College, Khulna with first date of diagnosis between January 1, 2015 and December 31, 2017. The proforma was designed to collect information regarding various parameters: age, gender, marital status, cancer site, tumor characteristics (morphology, grade and size, ER/PR and Her2 expression), stage of cancer at diagnosis, nodal disease status and location, margin status, specifics of treatment, recurrence, date and location of recurrence, and length of survival. World Health Organisation (WHO) classification of breast tumours[5] was used. Carcinomas were graded according to the modified Bloom and Richardson method[6]. ER/PR results were obtained from the registry having been processed in Pathology Laboratory of Khulna Medical College. The ER assay clone used was 1D5, the PR assay clone was PgR636 and the detection system was a polymer. IHC staining permits the detection and localization of ER/PR within sections from formalin-fixed, paraffin-embedded tissues. Staining of >20% of tumor cell nuclei is considered positive. Staining of 5% to 19% of tumor cell nuclei is considered borderline. Staining of <5% of tumor cell nuclei is considered negative (table I). For the purpose of this study both borderline and overtly positive results were considered positive.

Table I: ER/PR and Her2 scoring system and criteria

The ER/PR scoring system and criteria	
Scoring system	
0	Negative for receptor
1+	Borderline
2+	Positive for receptor (Intermediate)
3+	Positive for receptor (Strong)
Criteria	
0	0% nuclear staining
1+	<10% nuclear staining
2+	10% to 75% nuclear staining
3+	>75% nuclear staining
Her2 scoring system and criteria	
Scoring system	
0	Negative
1+	Negative
2+	Weak positive
3+	Positive
Criteria	
0	Negative: No staining is observed, or membrane staining is <10% of the tumor cells.
1+	Negative: A faint /barely perceptible membrane staining is detected in >10% of the tumor cells. The cells are only stained in part of the membrane.
2+	Weak positive: A weak to moderate complete membrane staining is observed in >10% of the tumor cells.
3+	Positive: A strong complete membrane staining is observed in >10% of the tumor cells.

Her2/neu results were obtained from the medical record. The clone used was a polyclonal (Her2 HercepTest Kit) and the detection system used was a polymer. This protocol considers a test result of 0 to 1+ as negative and 3+ as positive. For the current study a Her2/neu result of 2+ is considered a negative result. The results of this study were calculated by standard statistical formula.

Results:

Final analysis included 378 invasive breast cancer patients. The mean age of all subjects was 62.7 years. Baseline characteristics of subjects including tumor subtype are presented in table II. Of 378 subjects, 39(10.2%) were ER/PR+, Her2+, 260 (68.9%) were ER/PR+, Her2−, 28 (7.5%) were ER/PR−, Her2+, and the remaining 51 (13.4%) were classified as triple negative.

Table II: Baseline characteristics by tumor subtype

	ER/PR+, Her2+ (n=39)	ER/PR+, Her2 (n=260)	ER/PR, Her2+ (n=28)	ER/PR, Her2 (n=51)
	58.9±14.6	64.4±13.2	59.9±12.7	58.1±14.7
Age (years)	15 (38.8%)	165 (63.5%)	10 (34.1%)	23 (45.4%)
II	20 (50.9%)	82 (31.5%)	12 (43.5%)	22 (43.4%)
III	4 (10.3%)	13 (5.0%)	6 (22.4%)	6 (11.2%)
Ductal	31 (79.3%)	179 (68.6%)	24 (83.5%)	42 (82.2%)
Lobular	4 (9.5%)	39 (15.0%)	1 (3.6%)	2 (4.6%)
Ductal and lobular	2 (6.0%)	23 (8.8%)	1 (3.6%)	1 (2.6%)
Inflammatory	1 (2.5%)	1 (0.4%)	3 (10.6%)	1 (2.6%)
Others	1 (2.5%)	19 (7.4%)	1 3.6%)	4 (7.9%)
Well differentiated	2 (6.0%)	75 (28.9%)	1 (3.6%)	2 (4.0%)
Moderately differentiated	16 (41.4%)	117 (44.9%)	6 (20.0%)	6 (12.5%)
Poorly differentiated	19 (49.1%)	56 (21.5%)	22 (77.7%)	39 (76.3%)
Missing	1 (2.5%)	12 (4.6%)	1 (3.6%)	4 (7.2%)
Positive	17 (44.0%)	72 (27.7%)	12 (41.2%)	16 (32.2%)
Negative	19 (49.1%)	165 (63.5%)	15 (51.8%)	32 (63.8%)
Not examined	3(6.9%)	23 (8.8%)	2 (7.1%)	2 (4.0%)
Chemotherapy	26 (68.1%)	86 (32.9%)	24 (83.5%)	37 (73.7%)
Radiotherapy	23 (58.6%)	168 (64.5%)	20 (70.6%)	35 (68.4%)
Hormone replacement therapy	35 (90.5%)	216 (82.8%)	5 (17.7%)	8 (16.5%)

Differences in baseline characteristics between the four subtypes are presented in table II. Subjects with ER/PR+, Her2− subtype were more likely to be older, have early stage breast cancer, present with small tumor and have a well/moderately differentiated histological grade. They were less likely to be lymph node positive, have a lobular tumor type, and be treated with chemotherapy.

Table III: Five-year overall and disease-free survival by tumor subtype, ER/PR and Her2 status

Subtype	**Overall survival**	**Disease**-free survival
Subtype		
ER/PR+, Her2+	88.7%	83.2%
ER/PR+, Her2 "	90.3%	86.8%
ER/PR, Her2+	78.8%	66.0%
ER/PR, Her2 −	79.0%	73.5%
ER/PR status		
ER/PR+	90.1%	86.4%
ER/PR −	79.0%	70.8%
Her2 status		
Positive	84.6%	75.9%
Negative	88.5%	84.7%
Overall	87.8%	83.1%

Overall and disease-free survival by tumor subtype is analysed. In the subjects with the triple negative subtype, ER/PR−, Her2− had the worst overall survival and worst disease-free survival when compared with subjects with ER/PR+, Her2− subtype (table VI).

Table IV: Hazard ratios for overall and disease-free survival by tumor subtypes after adjusting for age, stage, histological grade, chemotherapy treatment and lymph node

Subtype	**Overall sur**vival	**Disease-free survival**
Subtype		
ER/PR+, Her2 −	1.00	1.00
ER/PR+, Her2+	1.03	1.03
ER/PR, Her2+	1.34	1.54
ER/PR, Her2−	1.75	1.83
ER/PR status		
ER/PR+	1.00	1.00
ER/PR −	1.57	1.94
Her2 status		
Positive	1.00	1.00
Negative	0.98	1.29

Among the 260 subjects with ER/PR+, Her2− subtype, 175 received no chemotherapy and 56 received chemotherapy. Those who received chemotherapy had significantly better overall and disease-free survival benefits when compared with subjects who did not receive chemotherapy. No significant differences in overall and disease-free survival benefits were observed in other subtypes. This may be due to the small number of subjects in the other subtype groups.

Discussion:

One of the most important parameters in breast cancer management and patient survival is the hormone status and responsiveness of tumour to hormone. In developed countries many studies have been carried out to evaluate the hormone receptors and HER2-neu status. In United States (US) and Europe numerous studies have been used to demonstrate and evaluate differences in hormone receptor status and histology by race and ethnicity among women[7-12]. The relationship between tumour size and lymph node involvement is clinically well known and is found to be the most powerful indicator for poor prognosis in breast cancer patients[8]. Young age at diagnosis as an independent predictor of poor survival, has been revealed by numerous studies conducted in Europe and the US[7,8,9]. It was seen that young women with breast tumours had a tendency to have larger tumour sizes, more positive lymph nodal status, more negative hormone receptors status, higher tumour grades at diagnosis than the older women[10]. Our results reveal statistically significant differences in clinical and pathologic features and outcomes between subtypes. Using the most common subtype (ER/PR+, Her2−) as a reference, the triple negative subtype (ER/PR−, Her2−) had the worst overall survival, and worst disease-free survival. Breast cancer has also sometimes been categorized into triple negativity or other[11]. This classification is informative but simplistic and may be misleading by grouping the ER/PR−, Her2+ with ER/PR+, Her2+ and ER/PR+, Her2−. This was borne out in our results, where the ER/PR+, Her2+ had statistically equivalent survival to the referent ER/PR+, Her2− subtype, and in practice, both types have better prognostic and therapeutic significance. However, the ER/PR−, Her2+ point estimates were more similar to the triple negative values. Also, recent studies have suggested that within the ER/PR+ subtypes, the clinical and pathologic response to chemotherapy varies with the ER/PR+, Her2+ subtype defined by both hormone receptor and Her2 expression showing better response to chemotherapy[12]. ER/PR+, Her2+ tumors virtually always have a high recurrence score[13]. Recently it was shown in a retrospective analysis that ER/PR+, Her2− tumor may benefit less from taxanes in the adjuvant setting[14]. In this study breast cancer is classified into 4 global subtypes by using IHC out of the 8 possible subtypes commonly used by other[15]. It is believed that, this classification is practical, simple, informative, clinically useful, and quite discriminative between the subtypes. The other four groups will emerge if we differentiate based on PR expression (ER+/PR+ vs. ER+/PR− tumors). The independent prognostic and predictive role of PR expression irrespective of ER has been a subject of great controversy as demonstrated by the report from adjuvant trial comparing the efficacy of tamoxifen with that of the aromatase inhibitor anastrazole, showing overall that patients with ER+/PR+ tumors had a lower recurrence rate than those with ER+/PR− tumors[16]. The observation from the same study that patients with ER+/PR− tumors respond nearly as well to anastrozole as those with ER+/PR+ tumors suggests that the ER signaling pathway is functional in many ER+/PR− tumors, consistent with the well-known fact that the PR gene is regulated by the estrogen pathway[17]. The IHC-based classification systems are still useful in clinical practice, especially when fresh tissue is not available, and has been shown to correlate well with intrinsic classification using gene expression microarrays: ER/ PR+, Her2+ with Luminal B; ER/PR+, Her2− with Luminal A; ER/PR−, Her2+ (ER−/Her2+) and ER/PR−, Her2− with triple negative/basal-like tumors[14,17]. It is worth noting that there is substantial intralaboratory and interlaboratory variation in ER results because fixation, antigen retrieval, and staining methods may differ among laboratories[18]. For this classification to be more helpful, ongoing efforts should also be directed at standardization of current testing and development of more reliable and reproducible testing for ER/PR and Her2/ neu expression[12.15,18]. In our current analysis we have not considered the semi-quantitative information from IHC in terms of ER/PR or Her2 levels of expression on clinical outcomes largely because we do not have adequate sample for such analysis. We believe such sub setting within the subtypes may be unreliable, with regards to the message highlighted in this study, due to inadequate sample size. This study of Bangladeshi population reports shows the distribution of subtypes as different from that seen in a predominantly European and American population where ER/PR−, Her2− is more prevalent (39% premenopausal versus 14% postmenopausal American women versus 16% European women of all ages)[19]. Also of note, 80% of our subjects are ER+ accounting in part for the overall 5-year survival of 87.8% and 5-year disease-free survival of 83.1%. A large percentage of our patients demonstrate favorable features such as small tumor size (<2 cm; 71%), negative nodal status (61%), and low to moderate histologic grade (59%). An investigation of all subgroups showed benefit from chemotherapy, but after controlling for age, tumor size, and lymph node status, the sample size was not sufficient to make a

strong assertion except in the ER/PR+, Her2− subgroup. In the ER/PR+, Her2− subgroup (260 subjects), 175 patients did not receive chemotherapy and 86 patients did receive chemotherapy. Chemotherapy conferred overall and disease-free survival advantages. This study supports other studies[3.13.19] which have shown both the triple negative and Her2+/ER− subtypes to have poorer clinical, pathologic and molecular prognoses. The triple negative group has the worst overall and disease-free survival. For the triple negative group the disease-free survival is less than four years, but it is also less than three years for the Her2+/ER−. We lack targeted therapies for triple negative breast cancer and this continues to direct the focus of ongoing research[15-20]. Despite the enormous effort and funding channeled towards molecular diagnostics, there is still relevance for IHC, especially when performed by developing countries. Although molecular arrays have been around for approximately a decade, new therapeutic target proteins are not being identified, thus the predictive value of the assays are relatively global or limited to known targets such as ER/PR protein or the Her2 gene. Also, despite multiple and different gene sets used in most of the molecular testing, there is significant agreement in the outcome predictions for individual patients by these tests[20] suggesting that they are probably tracking a common set of biologic phenotypes which are heavily weighted toward ER/PR and Her2 gene pathways. Finally, the superiority of molecular technology over IHC testing is theoretical and based on the premise that molecular technology provides quantization and reproducibility. This presumptive theory is the basis for some ongoing studies but is yet to be proven.

Conclusion:

Our study showed the triple negative subtype (ER/PR−, Her2−) has the worst overall and disease-free survival compared to the other subtypes. We support IHC classification as a clinical tool as it is a clinically-used, therapeutically informative classification of breast cancer based on immunophenotype/biologic phenotypes, and is prognostic as well as somewhat predictive. Additional ongoing efforts should be directed at standardization of current testing methods and development of more reliable and reproducible testing.

References :

1. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007;13:4429-34.

2. Ravdin PM, Cronin KA, Howlader N, Berg CD, Chlebowski RT, Feuer EJ, et al. The decrease in breast-cancer incidence in 2003 in the United States. N Engl J Med 2007;356:1670-74.

3. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006;295:2492-502.

4. Chang HY, Nuyten DS, Sneddon JB, Hastie T, Tibshirani R, Sørlie T,et al. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc Natl Acad Sci U S A 2005;102:3738-743.

5. Dolled-Filhart M, Rydén L, Cregger M, Jirström K, Harigopal M, Camp RL, et al. Classification of breast cancer using genetic algorithms and tissue microarrays. Clin Cancer Res 2006;12:6459-468.

6. Liu R, Wang X, Chen GY, Dalerba P, Gurney A, Hoey T, et al. The prognostic role of a gene signature from tumorigenic breast-cancer cells. N Engl J Med 2007;356:217-226.

7. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M,et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817-826.

8. Sørlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418-423.

9. Sørlie T, Perou CM, Fan C, Geisler S, Aas T, Nobel A, et al. Gene expression profiles do not consistently predict the clinical treatment response in locally advanced breast cancer. Mol Cancer Ther 2006;5:2914-918.

10. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999-2009.

11. Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 2005;365:671-79.

12. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25:118-145.

13. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13:2329-34.

14. Fan C, Oh DS, Wessels L, Weigelt B, Nuyten DS, Nobel AB, et al. Concordance among gene-expression-based predictors for breast cancer. N Engl J Med 2006;355:560-69.

15. Hayes DF, Thor AD, Dressler LG, Weaver D, Edgerton S, Cowan D, et al. Cancer and Leukemia Group B (CALGB) Investigators. HER2 and response to paclitaxel in node-positive breast cancer. N Engl J Med 2007; 357:1496-506.

16. Nguyen PL, Taghian AG, Katz MS, Niemierko A, Abi Raad RF, Boon WL, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and Her-2 is associated with local and distant recurrence after breast-conserving therapy. J Clin Oncol 2008;26:2373-88.

17. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, et al. ATAC Trialists' Group. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet 2005;365:60-62.

18. Coates AS, Keshaviah A, Thürlimann B, Mouridsen H, Mauriac L, Forbes JF, et al. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. J Clin Oncol 2007;25:486-92.

19. Francis G, Beadle G, Thomas S, Mengersen K, Stein S. Evaluation of oestrogen and progesterone receptor status in Her-2 positive breast carcinomas and correlation with outcome. Pathology 2006;38:391-98.

20. Brouckaert O, Pintens S, Van Belle V, Van Huffel S, Camerlynck E, Vergote I, et al. Short-term outcome of primary operated early breast cancer by hormone and Her-2 receptors. Breast Cancer Res Treat 2009;115:349-58.