Learning Experience In Immunohistochemical Reporting Of Breast Cancer At A Rural Tertiary Hospital In India: A Comparison In Initial And Reviewed Reporting Of ER,PR, HER2 Status

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Citation

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Abstract

Aim- To compare between the initial reporting and reviewed reporting of estrogen receptor, progesterone receptor and Her2-neu receptor status.Methods- Information regarding ER/PR and Her2 expression of cases with available stored slides was reviewed and analyzed concurrently by 2 pathologists. The levels of significance were analyzed by Fischer test using SPSS 16.0 software.Results- There was statistically significant difference between initial reporting and reviewed reporting of Her-2 neu receptor whereas no significance was seen in the initial and reviewed reporting of ER and PR status.

INTRODUCTION

ESTROGEN AND PROGESTERONE RECEPTORS

It has long been recognized that some human breast cancers are hormone dependent in that their growth depends on a continued supply of female sex hormones. [1] Estrogen is important in regulating the differentiation and proliferation of breast epithelial cells and interacts with estrogen receptor in the nucleus. Prolonged exposure has been shown to be an important risk factor for cancer. PR expression in normal breast epithelium is regulated by ER. Presence of ER and PR in invasive carcinoma correlates positively with survival and is an important prognostic factor[2, 3].The determination of ER and PR levels in breast cancer has become part of the standard work-up by the pathologist.

There are mainly 2 types of hormone assays: the biochemical assay and the immunohistochemical assay. Immunohistochemical assay – Monoclonal antibodies are used to localize the presence of steroid hormone receptors on frozen and paraffin sections. Both methods have been shown to have high concordance rates,[4, 5] although some studies indicate that the immunohistochemical assay is more predictive for prognosis than the biochemical assay[5].

Some workers feel that the ER is best used in combination with other tumor characteristics such as nuclear grade, in which case the predictive power together is greater than that of either alone[6]. King, Fischer and Lesser et al have shown that ER and PR positivity correlates significantly with low grade tumors [7, 8, 9].

Parl et al suggest that ER and PR status is a better prognostic indicator when used along with tumor grading[10]. In concordance with their findings, Berger et al[11] have found that prognosis associated with poorly differentiated ER negative tumors is worse than that of ER positive tumors of equal histopathologic grade.

Pertshuk et al[12] have shown ER positivity to correlate well with well differentiated, grade I tumors. No such association was studied for progesterone receptors. In addition, they suggest that PR status is a better indicator of survival and disease-free interval than ER status for women at the same stages of disease. Molino et al[13] have found no correlation between receptor status and grade and suggest that the receptor status is indicative of prognosis only in patients with 4 or more positive nodes and in the stage T1 and premenopausal groups. Nixon et al[14] have demonstrated a positive correlation between patients with higher grade tumors and ER negativity.

C-ERBB-2 ONCOGENE

Initially considered to be genetic material from a retrovirus that results in malignant transformation when incorporated into normal cells, the majority of HER-2 oncogenes are now thought to be activated homologues of protooncogenes that have persisted in normal cell[15]. HER-2/neu, also known as c-erbB-2 is a member of the erbB oncogene family, and is related, but distinct from, epidermal growth factors[16].

The expression of this protein has been associated with a poor histologic grade,[15,17] the spread to axillary nodes, and the number of nodes involved;[16] a negative association between c-erbB-2 expression and a ER and PR has been noted.[18] In axillary node-positive patients, there is a significant correlation between amplification of HER-2 protein and shorter disease –free and overall survival.[19] HER-2 oncogene amplification correlates with the absence of estrogen and progesterone receptors.[18] Amplification of c-erbB-2 strongly correlates with each element (architectural pattern, nuclear atypia, and number of mitotic figures) of histologic grade.[20] Even if a primary tumor does not express c-erbB-2 protein, subsequent metastases may express the protein; in contrast, if a primary tumor expresses c-erbB-2 protein, this capacity is retained in all subsequent tumor metastases. Amplification of c-erbB-2 has been noted among in situ carcinomas as well. Numerous subsequent studies found that either HER2 gene amplification or protein expression predicted poor prognosis[15]. There is also some evidence of a relationship between HER2 and response of cancers to chemotherapy and endocrine therapies[17].

Following the development of a humanized monoclonal antibody against HER2 (transtuzumab), the reasons for establishing the HER2 status of breast cancers changed, since it is a prerequisite for transtuzumab's clinical use. Transtuzumab was originally licensed for the treatment for the treatment of patients with metastatic disease who had HER-2 positive cancers. More recently several prospective randomized trials have shown that adjuvant transtuzumab reduces the risk of recurrence and mortality in patients with HER2 positive early stage breast cancer[17, 21].

The principal testing methods used are immunohistochemistry and/or in situ hybridization using either fluorescence (FISH) or a chromagen. Immunohistochemistry-monoclonal antibodies/polyclonal anti-sera are used to analyze HER-2. In situ hybridization-fluorescently labeled probes complementary to centromeres of either HER2 gene or chromosome 17 are used.[17]

Expression of these immunohistochemical receptors is associated with a characteristic clinical outcome and in the present day, the breast cancers are classified and prognosticated by its molecular/immunohistochemical expression. The treatment regimen too is customized for the patient according to expression of molecular markers.

Hence the aim of the study is to compare between the initial reporting and reviewed reporting of estrogen receptor, progesterone receptor and Her2-neu receptor status

SUBJECTS AND METHODS

Settings and design-This is a comparative study of a retrospective cohort of breast cancers registered during the period January 1, 2006 to December 31, 2007 in the Department of Pathology at Kasturba Hospital, Manipal and was approved by the Manipal Institutional Ethics Committee.

STUDY POPULATION

Subjects with invasive primary breast cancer were extracted from the medical records department, Kasturba hospital, Manipal using ICD-O-3 codes with the date of diagnosis between January 1, 2006 and December 31, 2007. The registry identified 217 breast cancer subjects of which 65 without complete ER/PR and Her2 data were excluded.

The following information regarding cancer characteristics (morphology, grade, size, ER/PR and Her2 expression), were obtained from case records. Information regarding ER/PR and Her2 expression of cases with available stored slides was reviewed and analyzed concurrently by 2 pathologists.

RESOURCES

IMMUNO-HISTOCHEMICAL STAINING

Four micrometer sections attached on poly-lysine coated and incubated overnight at 37□□□C slides were dewaxed in xylene, rehydrated in graded ethanol, distilled water and covered with 10mM citrate buffer (pH 6). They were then incubated for 30 min with primary monoclonal anti-bodies against Her2 (DAKO, clone 250, 1:100, antigen retrieval: 2 min pressure cooker), ER (DAKO, clone SP1, 1:50, antigen retrieval: 2 min pressure cooker) and PR (DAKO, clone PgR636, 1:50, antigen retrieval: 2 min pressure cooker), followed by incubation with biotin-labeled secondary antibodies. The streptavidin-peroxidase complex was

visualized using di-aminobenzidine as a chromogenic substrate. For each run of staining, a positive control slide was prepared from breast carcinoma known to be positive for the proteins studied.

ER/PR

The best-preserved and best-stained areas of the sections were assessed. Nuclear staining was assessed for ER and PR. Quick score was determined by adding the scores for the proportion of cells stained and the intensity of staining, which ranged from 0 to 8.

Figure 1

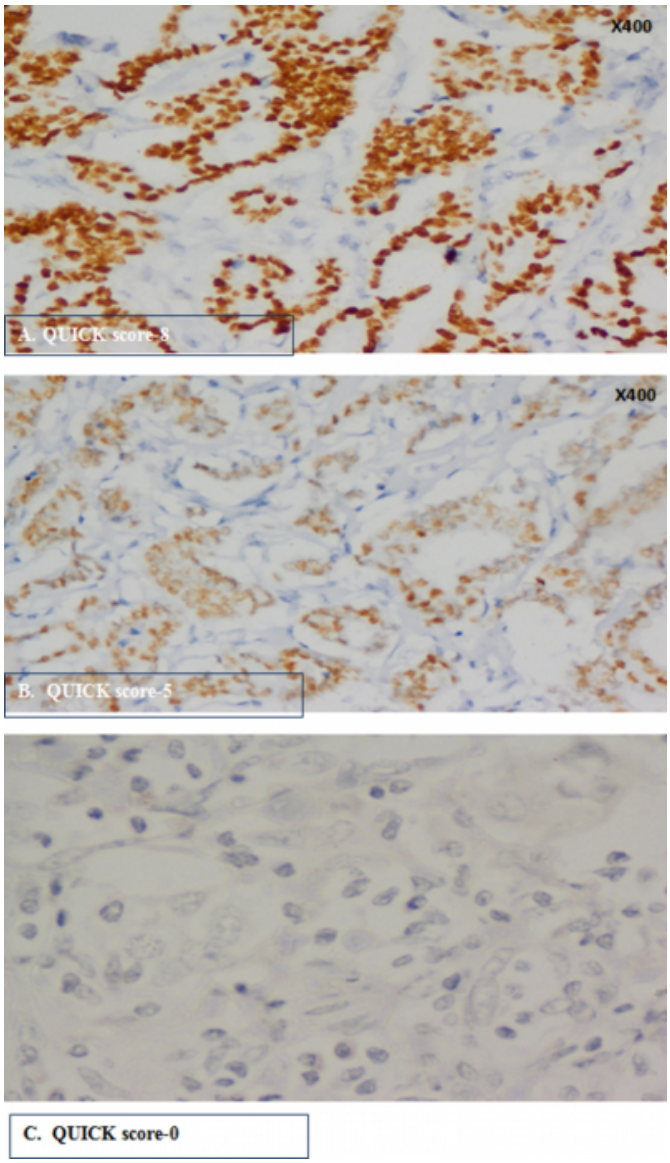
Table 1: Quick score for ER/PR

Score for proportion of stained cells	Score for intensity of staining
0-no nuclear staining	0-no staining
1-<1% nuclear staining	1-weak staining
2-1 to 10 nuclear staining	2-moderate staining
3-11 to 33% nuclear staining	3-strong staining
4-34-66% nuclear staining	
5-67-100% nuclear staining	

For the purpose of this study, even a single cell with weak staining intensity was considered positive and no single cell with no staining was considered negative.

Figure 2

Fig 1: Hormone receptor - QUICK scoring



HER2-NEU

The best-preserved and best-stained areas of the sections were assessed. Only membrane staining was assessed for Her2-neu.

Figure 3

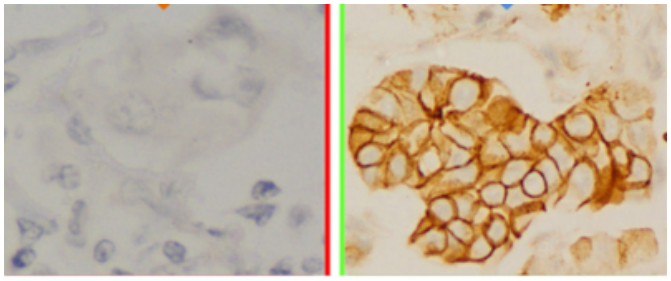
Table 2: Her2 protein assessment

Score to report	Her2 protein assessment	Staining pattern
0	Negative	No staining is seen or membrane staining is <10% of invasive tumor cells
1+	Negative	Faint/barely perceptive membrane staining detected in >10% of invasive tumor cells
2+	Equivocal	Weak to moderate complete membrane staining in >10% of invasive tumor cells or <30% with strong complete membrane staining
3+	Positive	Strong complete membrane staining in >30 % of invasive tumor cells

For the current study, a Her2-neu result of 2+ is considered negative result unless verified by fluorescent in-situ hybridization (FISH).

Figure 4

Fig 2: HER2- neu receptor status



A. Her2neu- negative

B. Her2neu – positive 3+

The retrieved slides were assessed for the ER, PR, Her2-neu status according to above mentioned criteria concurrently by 2 pathologists.

Statistics- determination of level of significance by Fischer test using SPSS 16.0 software.

RESULTS

Figure 5

Table 3: Comparison between the initial reporting and reviewed reporting of estrogen receptor status

n=113

	+ ve	-ve
Initial ER assessment	69	44
Reviewed ER assessment	71	42

p value 0.8911

Figure 6

Table 4: Comparison between the initial reporting and reviewed reporting of progesterone receptor status

n=117

	+ ve	**-ve**
Initial PR assessment	66	51
Reviewed PR assessment	65	52

p value 1.0

Figure 7

Table 5: Comparison between the initial reporting and reviewed reporting of Her2-neu receptor status

n=111

	+ ve	-ve
Initial Her-2 new assessment	37	74
Reviewed Her-2 neu assessment	20	91

p value 0.001

There was statistically significant difference between initial reporting and reviewed reporting of Her-2 neu receptor whereas no significance was seen in the initial and reviewed reporting of ER and PR status.

DISCUSSION

ER status reports was available in 147 cases and 59.9% of the breast cancers were reported positive for estrogen receptor. However only slides from 113 breast cancer patients were available for review, out of which 62.8% were estrogen positive. There was 7.1% discordance in the initial and reviewed reporting of ER positivity, with the discordance rate around the same value in the order of 7.0% between initial and reviewed reporting of ER negativity in breast cancers.

There was no statistically significant difference between

initial reporting and reviewed reporting of estrogen receptor status, however the subtle difference between the both is possibly due to lack of expertise in the initial phase, subjective difference in reporting of estrogen receptor and revision in recommendations for reporting estrogen receptor status.

Initially >5% staining of tumor cell nuclei was considered positive, while the new recommendation is to consider even a single cell with weak staining intensity as positive and no single cell with no staining as negative. Further in few cases the background staining and positive staining of the necrotic areas were reported as positive for estrogen receptor initially.

There is a statistically significant difference in the initial reporting and outside reporting of estrogen receptor status, the possible explanation for false positive ER status in our lab may be due to over interpretation of background staining and non-specific staining in the necrotic areas.

It is highlighted that while ER positive and PR positive tumors are likely to respond to estrogen deprivation, these steroid receptors are far from perfect as response predictors. It has been suggested that a more recently described product of the estrogen regulated gene pS2 might be a better predictor[22, 23].

PR status reports was available in 146 cases and 53.4% of the breast cancers were reported positive for progesterone receptor. However only slides from 117 breast cancer patients were available for review, out of which 55.5% were progesterone positive.

The concordance rate of initial and reviewed reporting of progesterone positive tumors was as high as 95.4% with a false positivity of 7.7% while the concordance rate of initial and reviewed reporting of progesterone negative tumors was 92.3%. There was 7.7% discordance in the initial and reviewed reporting of PR positivity and 4.6% discordance rate in the initial and reviewed reporting of PR negativity in breast cancers.

There was no statistically significant difference between initial reporting and reviewed reporting of progesterone receptor status, the subtle difference between the both as the case of estrogen receptor reporting is due to lack of expertise, subjective difference in reporting of progesterone receptor and revision in recommendations for reporting progesterone receptor status.

Information regarding Her-2 neu status was available for 143 cases, 52.4% were negative for Her2-neu receptor, 13.3% were weakly positive (2+) and 34.3% were strongly positive for Her2-neu receptor. Since IHC slides from all 143 cases were not available, review was done on available 111 immunohistochemical slides. 67% were negative for Her2-neu receptor whereas 14.3% were weakly positive for Her2-neu receptor and only 18.8% were reported strongly positive for Her2-neu receptor.

There was statistically significant difference between initial reporting and reviewed reporting of Her-2 neu receptor, the vast difference between the both is due to lack of expertise, subjective difference in reporting of Her2-neu receptor assessment and revision in recommendations for reporting Her-2 neu receptor status. During initial reporting false positive cases were 17 in number with no false negative cases.

Initially >10%complete membrane staining of tumor area was considered positive while the new recommendation is to consider >30% complete membrane staining of tumor cells as positive. Further in few cases the background staining, nuclear staining and positive staining of the necrotic areas were reported as positive for Her-2 neu receptor. In a small subset of cases, those with normal duct positivity for Her2-neu receptor were also reported, but since normal ducts must not express this protein, on review these cases were graded as negative for Her-2 neu protein. Normal ductal epithelium expresses Her-2 neu receptor on the luminal border. Hence when antigen retrieval is done by microwave method, there is over retrieval of normally present receptors, giving an increase in false positive results.

Over expression of Her-2 neu receptors is associated with aggressive biologic behavior and clinical outcome. Antibody based therapy with transtuzumab (Herceptin), can be effective in metastatic breast cancer when taken alone or in combination with chemotherapeutic agents[24]. Disagreement in Her-2 expression scoring implies that some patients who are in need of transtuzumab treatment will not receive it, and patients who are insensitive to the effects of transtuzumab will be treated with it[25]. Therefore, combined detection of Her-2.neu expression and amplification by immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) is used to supplement each other.

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