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**Research Article**

# CORRELATION BETWEEN ESTROGEN, PROGESTERONE RECEPTOR, HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 WITH GRADE AND SUBTYPE IN EARLY DIAGNOSIS OF BREAST CANCER

**[1,*]Mohy el din Abd el Fattah,[2]Nashwa Nagy El-Khazragy and [3]Neama dahee Hassanen**

[1]Department of Organic chemistry, Suez Canal University, Faculty of science chemistry department, Egypt
[2]Department of Clinical Pathology/Hematology, Faculty of Medicine-Ain Shams University, Egypt
[3]Suez Canal university, Fqaculty of Science , Chemistry department,, Egypt

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**Abstract**

The current study was conducted on 100 individuals at Global Research Labs in Egypt. Included patients selected from the Ain Shams Internal Medicine hospital, oncology department in the period from March 2018 to March 2019. Patients were diagnosed as Breast Cancer before receiving any treatment in form of Chemotherapy or Radio-therapy. They were diagnosed on the basis of histopathology and radiological picture such as CT scan. The age of the patients ranged from 30 to 75 years. Majority of tumors were predominantly of histopathological grade two. By Immunohistochemistry 77% were ER+/PR+, 40% were Her2/neu positive and 8% were triple negatives. A significant association was seen between histologic grade and hormone receptor status. Microscopic review of 100 consecutive human breast biopsy and mastectomy specimens were correlated with estrogen and progesterone receptor and HER 2 content of the tissue, by subtype and degree of differentiation. Of the 4 grades of differentiation; the less differentiated Grade III tumors showed significantly lower levels of estrogen and progesterone receptors in infiltrating ductal and lobular carcinoma. While grade ll tumors showed significantly higher levels of ER, PR & HER 2 receptors.

**Keywords:** Breast carcinoma, Histologic grading, Hormone receptor status.

## INTRODUCTION

Breast cancer is the most frequent malignant tumor and the most common cause of cancer-related death among women in the developed countries (American Cancer Society, 2013; Ferlay *et al.,* 2013). Breast cancer is increasing in the developing countries, including Egypt, where it ranks at the first cancer in women in 2020 according to international agency for research on cancer WHO. Breast cancer is the fifth leading cause of death from cancer worldwide; it affects 1 in every 8 women. Also it is the most common cancer type among women. The mortality rate has been significantly reduced in recent years because of its early diagnosis and the advanced methods of treatment; however, By using screening methods such as ultrasound and mammography, and by continuously training women to conduct self-examination, Various predictive and prognostic factors affect tumor progression (Moutafoff *et al.,* 2011; Mahmood *et al.,* 2015). Predictive factors are distinguished from prognostic factors in that the latter can be measured and are associated with the nature of the disease, whereas the former determine the response to treatments (Mahmood *et al.,* 2015). Some factors are both prognostic andpredictive, including estrogen receptor (ER) and progesterone receptor (PR) status, and human epidermal growth factor receptor (HER2/neu) overexpression. Prognostic factors include the type of tumor, number of involved lymph nodes at the time of tumor diagnosis, size of the tumor, tumor grade, Ki67 status (cellular marker for proliferation), and the patient's age (Ariga *et al.,* 2005; Baulies *et al.,* 2014). Numerous studies have been conducted on these prognostic factors and their relationships with one another; however, the studies have reported disparate results. (Baulies *et al.,* 2014) breast cancer is a hormone-dependent disease, and thus, resulting from the mitogenic effects of estrogen and progesterone (Anderson *et al.,* 2002).

***Corresponding Author:** ***Mohy el din Abd el Fattah,*** Department of Organic chemistry, Suez Canal University, Faculty of science chemistry department, Egypt.

Gene expression profiling of human tumors has provided a new paradigm for classifying breast carcinomas, predicting response to treatment, and risk of recurrence. Estrogen receptor (ER), human epidermal growth factor 2 (HER2) receptor, and proliferation-related genes are the main drivers of classification in many of the gene expression profiling tests for breast cancer. However, ER, progesterone receptor (PR), and HER2 receptor status remain essential in determining the need and type of adjuvant therapy. These biomarkers are routinely tested for in all invasive breast carcinomas; ER testing is also performed on cases of ductal carcinoma in situ (DCIS). (Benjamin Calhoun *et al.,* 2015). The occurrence of ER and PR expression is associated with histological type of breast cancer. Lobular and tubular cancers are characterised by a high incidence of oestrogen and progesterone receptors. In these types of cancers, receptors are present in a greater percentage of cases than in other carcinomas (Nadji *et al.,* 2005; Lower *et al.,* 2005). The same dependence has been demonstrated for the progesterone receptor (Nadji *et al.,* 2005). Many authors agree that the ER expression is in inverse relation to the size of the primary tumor (Nadji *et al.,* 2005; Stonelake *et al.,* 1994). A similar relationship was described for the progesterone receptor (Stonelake *et al.,* 1994; Ogawa *et al.,* 2004), but not all authors confirm this relationship (Chow *et al.,* 2000). Expression of estrogen receptors is also associated with age and menopausal status. estrogen receptor is more frequently detected in breast cancers in postmenopausal women than in premenopausal women (Takashima *et al.,* 2001; Chow *et al.,* 2000) and more frequently in older women than younger ones (Bozcuk *et al.,* 2001). Expression of ER and PR is not constant and changes with disease progression (Branković-Magić *et al.,* 2011). Typically, the number of cells expressing ER and/or PR progressively decreases with disease progression (Allemani *et al.,* 2004); an example of this is the inverse relationship between expression of ER and the size of the primary tumour. Many authors agree that the prognosis is better in the case of

patients whose tumours exhibit ER and/or PR expression than in patients whose cancers do not show such expression (Tsutsui *et al.,* 2002). But opinions on the value of oestrogen and progesterone receptors as prognostic factors.The ER has well-established prognostic and predictive values (Davies *et al.,* 2011; Rastelli, 2008), while the PR has a controversial additional predictive value (Olivotto *et al.,* 2004; Fuqua *et al.,* 2005). The presence or not of ER and PR helps determine a possible relapse of breast cancer (Davies *et al.,* 2011). The hormonal receptor status allows to distinguish four subgroups of breast cancers: ER+PR+, ER+PR-, ER-PR+, and ER-PR- (Hefti *et al.,* 2013). This classification helps to decide hormonal treatment for ER/PR positive patients and chemotherapy for the ER/PR negative patients (Barlett *et al.,* 2011). Expression of estrogen receptors (ER) and progesterone receptors (PR) is a very powerful and useful predictor. Because the response rate to hormonal treatment in breast cancer is associated with the presence of ER and PR, assessment of receptor expression profile allows clinicians to predict breast cancer response to hormonal treatment (Ferlay *et al.,* 2013).

The higher the content of ER and PR in breast cancer, the greater the likelihood of response to hormonal therapy (Elledge *et al.,* 2000; Hawkins, 2000). In patients with advanced breast cancer, in those classified as ER(–)/PR(–) the response rate to hormonal treatment is 10%,in the group of ER(–)/PR(+) patients it is 32%, in patients with ER(+)/PR(–) it is 40%, and in patients with ER(+)/PR(+) it is 73% (American Cancer Society, 2013). HER2 (human epidermal growth factor receptor 2) receptor is a membrane tyrosine kinase and when activated affects cell proliferation and survival. The *HER2* oncogene is located on chromosome 17q12. *HER2* amplification is the primary pathway of HER2 receptor over expression and is a major driver of tumor development and progression in a subset of breast cancers. *HER2* is amplified in about 15% to 20% of breast cancers. The overexpressed HER2 receptor is a valuable therapeutic target. The 2007 ASCO guidelines mandate that HER2 should be evaluated in every invasive breast cancer, either at the time of diagnosis or recurrence to guide therapy. Currently HER2 testing is carried out by several methods. It is crucial to standardize testing techniques to accurately assess HER2status. The aim of this review on HER2 in breast cancer is to discuss the important aspects of HER2 biology, its significance in breast cancer, and the current standards for its detection. (Wolff *et al.,* 2007).Therefore the immunohistochemical evaluation of ER, PR and HER2 are routine clinical practice in the diagnosis and treatment of breast cancer management worldwide. The current research is essential to update the immunohistochemical activity of ER/PR in primary localized breast cancers. Herein, the aim of this study was to evaluate the expression level of ER, PR and HER2 receptors their distribution, and their correlation with classic clinicopathologic prognostic parameters (age, menopausal status, histologic type, and grade) to enhance the breast cancer patients' medical care. The present study will contribute to classify patients into different subgroups based on their hormonal receptor status in order to determine the better treatment strategies for women with breast cancer in Egypt.

## MATERIAL AND METHODS

The current study was conducted on 100 individuals at Global Research Labs. they classified into two subgroups according to age groups younger and older than 50. The breast cancer cases constitute 100 patients; samples collected from patients after diagnosis has been confirmed by histopathology based on immune-histochemical analysis as well as Computerized axial tomography (CT scan), mammography and Magnetic resonance imaging (MRI). The patients were compared to All included patients selected from the Ain Shams Internal Medicine hospital, oncology department in the period from March 2018 to March 2019. After taking the approval of research ethics committee of Faculty of medicine, Ain Shams University, Patients were diagnosed as Breast Cancer before receiving any treatment in form of Chemotherapy or Radio-therapy.

All patients will be subjected to the following:

- Detailed history from each patient, with special reference to present and past family history.
- Full patients Clinical and laboratory data will be collected from patient data sheets.
- Sample collection: 3 ml of whole blood will be collected by vein puncture in a gel Vacutainer tube. The collected samples will be centrifuged at 4000 rpm for 10 minutes at room temperature; serum samples will be stored at at -80ºc for laboratory tests.
- Laboratory Tests:
- Tumor markers: Carcinoembryonic antigen (CEA) and Cancer antigen (CA-15.3) will be measured using chemiluminescence technique (*Abbott Laboratories; Germany*).
- Histopathology: histopathological examination on Formalin fixed Paraffin blocks will be held for diagnosis and scoring.

**Sample collection and preparation**

3 ml of whole blood was collected by venipuncture from all enrolled subjects in gel vacutainer, centrifuged at 4000 rpm for 20 minutes and stored at -20$^{o}$ C until analyzed. Immunohistochemistry: Estrogen receptors (ER), Progesterone Receptors (PR) and Human Epidermal Receptor-2 (HER2) will be examined in tissue using specific polyclonal antibodies TB was formalin-fixed within 4–8 hrs for 6–48 hrs (minimum 6 hrs). IHC was performed, following epitope retrieval, with a polymer based detection system (Envision plus, Dako, Carpinteria, CA) using mouse monoclonal antibodies for ER and PR (Dako, Carpinteria, CA), ER (1D5; 1:50), PR (PgR636; 1:400), and Herceptin kit (HercepTest, Dako, Carpinteria, CA) according to the manufacturer's instructions. For ER and PR, antigen retrieval is performed as follows: sections were deparaffinized and rehydrated with deionized water. They were then heated in citrate buffer (pH 6.0), using an electric pressure cooker for 3 minutes at 12–15 pounds per square inch (PSI) approximately at 120°C, and cooled for 10 minutes prior to immunostaining. All slides were loaded on an automated system (DAKO Autostainer) and exposed to 3% hydrogen peroxide for 5 minutes, incubated with primary antibody for 30 minutes, with labeled polymer (Envision® + dual link) for 30 minutes, 3,3′-diaminobenzidine (DAB) as a chromogen for 5 minutes, and hematoxylin as counterstain for 5 minutes. These incubations were performed at room temperature. Between incubations sections were washed with Tris-buffered saline (TBS). Cover-slipping was performed using the Tissue-Tek SCA (Sakura Finetek USA, Inc, Torrance, CA) coverslipper. Positive controls of known positive tissues (endometrium and breast) and negative controls with primary antibody replaced with TBS are run with

the patient/study slides. Nuclear staining in more than 10% of tumor cells was considered positive for ER and PR. Most testing labs use a special staining process that makes the hormone receptors show up in a sample of breast cancer tissue. The test is called an immunohistochemical staining assay, or Immuno Histo Chemistry (IHC). Not all labs use the same method for analyzing the results of the test, and they do not have to report the results in exactly the same way. So you may see any of the following on your pathology report:

- A percentage that tells you how many cells out of 100 stain positive for hormone receptors. You will see a number between 0% (none have receptors) and 100% (all have receptors).
- An Allred score between 0 and 8. This scoring system is named for the doctor who developed it. The system looks at what percentage of cells test positive for hormone receptors, along with how well the receptors show up after staining (this is called "intensity"). This information is then combined to score the sample on a scale from 0 to 8. The higher the score, the more receptors were found and the easier they were to see in the sample.
- The word "positive" or "negative."

Different labs have different cutoff points for calling the cancer either "hormone-receptor-positive" or "hormone-receptor-negative." For example, if less than 10% of your cells or fewer than 1 in 10 stain positive, one lab might call this a negative result. Another lab might consider this positive, even though it is a low result. Research studies have shown that even cancers with low numbers of hormone receptors may respond to hormonal therapy. A score of "0" generally means that hormonal therapy will not be helpful in treating the breast cancer. When the score is 0, the cancer is called hormone-receptor-negative. Talk with your doctor to make sure that your test is done by a laboratory with a great deal of experience in hormone receptor testing.

The more tests the lab does, the more accurate your results are likely to be. If you receive a negative test result, ask for a complete explanation as to why the cancer is considered hormone-receptor-negative. Talk to your doctor about the criteria that were used to determine the negative status and whether the results should be looked at again. Immunohistochemistry (IHC) detects HER2 protein overexpression using monoclonal or polyclonal antibodies that bind to the protein. Currently in the United States, there are two Food and Drug Administration (FDA) approved methods for HER2 assessment: HerceptTest™ (DAKO, Glostrup Denmark). Accurate HER evaluation is determined not only by choice of antibody but also by other determinants such as tissue fixation, fixation time, and determination of thresholds for reporting positive results. According to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines the optimal tissue handling requirements include: the time from tissue acquisition to fixation should be as short as possible; the formalin fixation in buffered formalin should range from 6–48 hours; and, the time to fixation and duration of fixation should be recorded for each sample. Additional IHC internal validation and quality assurance procedures, as well as external proficiency assessment are recommended. HER2 testing results by IHC fall into three categories, positive, equivocal and negative (Table 3). Each of these results triggers different patient management.

## RESULTS

### Demographic data

In the present study female patients with breast carcinoma were aged between 3rd and 7th decade of life. The youngest was 25 years and oldest 76 years. Left breast (50%) was marginally more affected than right side (49%) of breasts and in a single case both breasts (1%) were affected. The commonest grade was grade 2 accounting to 54% followed by grade 3 and 1 with 27% and 19 % respectively.

**Table 1. Histopathological grading of breast carcinoma**

| Grade of The Tumor | Frequency | (%) |
|---|---|---|
| 1 | 19 | 19.0 |
| 2 | 54 | 54.0 |
| 3 | 27 | 27.0 |
| Total | 100 | 100.0 |

Table 2 represents the distribution of different predisposing factors that may contributes to development on breast cancer, they include: age, menopausal status, Diabetes Mellitus and hypertension and positive family history for cancer, higher frequency of breast cancer group had negative family history for cancer (80%), age >50 years (56%), Hypertensive (68%) and diabetic (70%). Premenopausal status was predominant (52%) among the breast cancer group. The demographic data are illustrated in table 2.

**Table 2. Demographic Characteristics of the breast cancer group**

| Variable | Statistics | Statistics |
|---|---|---|
| Age [years] | mean±SD | |
| Breast cancer | Range | 50.2±11.2[30-76] |
| Age group | | |
| ≤50 | N[%] | 50% |
| >50 | | 50% |
| Menopausal Status | | |
| Pre-menopausal | N[%] | 52% |
| Post-menopausal | | 48% |
| Family History | | |
| Negative | N[%] | 80% |
| Positive | | 20% |
| Diabetes Mellitus | | |
| Negative | N[%] | 70% |
| Positive | | 30% |
| Hypertension | | |
| Negative | N[%] | 68% |
| Positive | | 32% |
| HCV infection | | |
| Negative | N[%] | 68% |
| Positive | | 32% |

**Table 3. Clinico-pathological Characteristics of the breast cancer group**

| Variable | Statistics | Statistics |
|---|---|---|
| Histopathological type | | |
| Invasive ductal carcinoma | N[%] | 92 |
| Others[medullary, sarcomas] | | 8 |
| Histopathological grade | | |
| Grade I | | 19 |
| Grade II | N[%] | 54 |
| Grade III | | 27 |
| Estrogen receptors | | |
| Negative | N[%] | 31 |
| Positive | | 69 |
| Progesterone Receptors | | |
| Negative | N[%] | 31 |
| Positive | | 69 |
| Her2 Receptors | | |
| Negative | N[%] | 60 |
| Positive | | 40 |

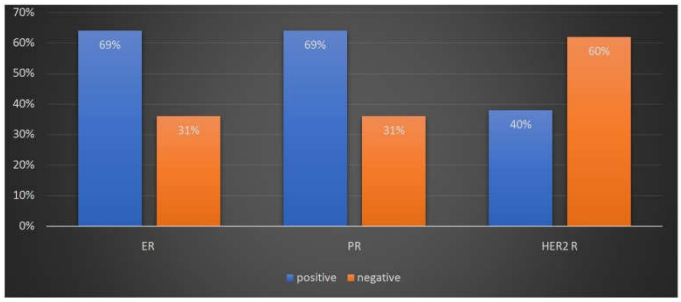


**Figure 1. Clinico-pathological Characteristics of the breast cancer group**

**Table 4. IHC Hormone Receptor Status in breast carcinoma**

| Hormone Receptor Status | Frequency | % |
|---|---|---|
| ER+/PR+ | 55 | 55 |
| ER+/PR- | 16 | 16 |
| ER-/PR+ | 7 | 7 |
| ER-/PR- | 22 | 22 |
| HER2/neu+ | 40 | 40 |
| HER2/neu- | 60 | 60 |
| Triple positive | 23 | 23 |
| Triple negative | 6 | 6 |

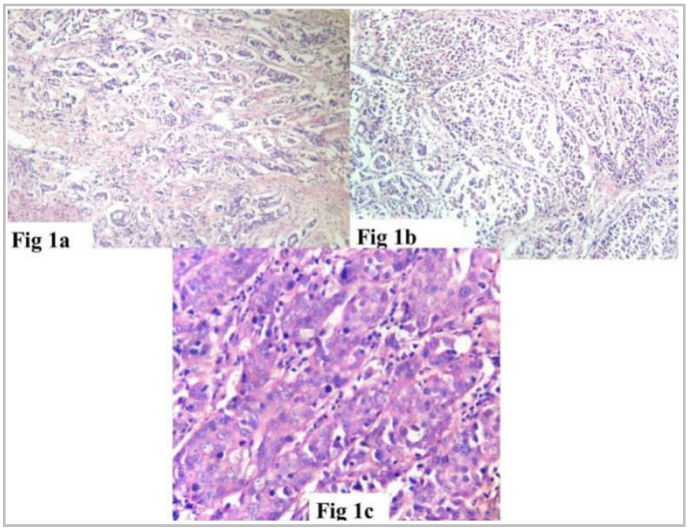


**Figure2. H&E showing (a) grade 1 (10x) (b) grade 2(10x) (c) grade 3(40x)**

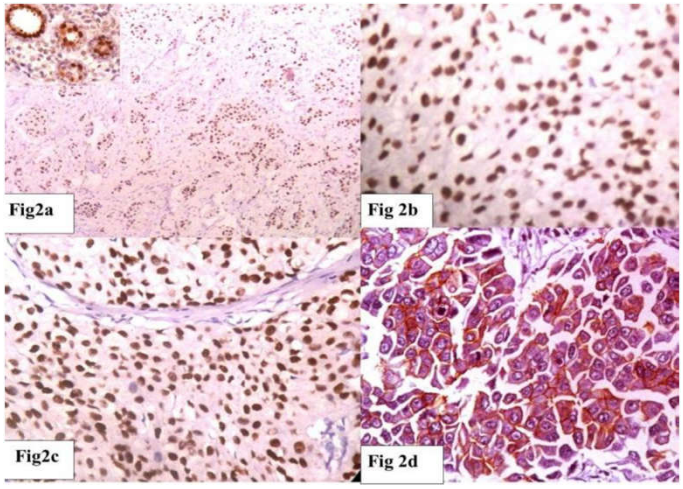


**Figure3. IHC photographs showing nuclear stain positivity for estrogen receptor (a) Allred score-8 (10x)Inset showing internal control, (b) Allred score-8 (40x) (c) Allred score-8 (40x)**

And (d) IHC cytoplasmic membrane stain positivity for Her2/neu receptor-score-3+ (40x)

**Table 5. Association of IHC hormone receptor status with AGE of the patients with breast carcinoma**

| IHC hormone receptor status | <50 YEARS (%) | >50 YEARS (%) |
|---|---|---|
| ER+/PR+ | 40 | 25 |
| ER+/PR- | 2 | 2 |
| ER-/PR+ | 2 | 2 |
| HER2/neu+ | 23 | 17 |
| HER2/neu- | 34 | 26 |
| Triple positive | 14 | 10 |
| Triple negative | 1 | 5 |

**Table 6. Association of IHC hormone receptor status with Grade of the tumor**

| IHC hormone receptor status | GRADE 1(%) | GRADE 2(%) | GRADE 3(%) |
|---|---|---|---|
| ER+/PR+ | 8 | 53 | 5 |
| ER+/PR- | 4 | 3 | 1 |
| ER-/PR+ | 2 | 4 | 3 |
| ER-/PR- | 5 | 2 | 1 |
| HER2/Neu+ | 2 | 38 | 0 |
| Triple positive | 2 | 22 | 0 |
| Triple negative | 0 | 4 | 2 |

**Table 7. Association of IHC hormone receptor status with Histopathological type of carcinoma**

| IHC hormone receptor status | DCIS % (Ductal Carcinoma In Situ) | LVI % (Lymphovascular invasion) |
|---|---|---|
| ER+/PR+ | 47 | 19 |
| ER+/PR- | 2 | 0 |
| ER-/PR+ | 0 | 0 |
| HER2/Neu+ | 25 | 9 |
| Triple positive | 15 | 5 |
| Triple negative | 2 | 4 |

## DISCUSSION

Breast cancer (BC) is the most prevalent cancer type in women and a leading cause of cancer mortality in the world. Breast cancer is a very heterogeneous disease, and its histological classification is mainly based on the expression of hormonal receptors such as estrogen receptor (ER), progesterone receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2). (Lakhani *et al.,* 2012) With respect to gene expression, BC is classified into five molecular subtypes including luminal ER positive (luminal A and luminal B), HER2 enriched, basal-like (also known as triple-negative breast cancer), and normal breast-like subtype (Sørlie, 2004). The current study was conducted on 100 individuals at Global Research Labs. They classified into two subgroups according to age groups younger and older than 50. The breast cancer cases constitute 100 patients; samples collected from patients after diagnosis has been confirmed by histopathology based on immune-histochemical analysis as well as Computerized axial tomography (CT scan), mammography and Magnetic resonance imaging (MRI). Clinico-pathological Characteristics of the Breast cancer group: The breast cancer group was categorized according to histopathological features of the tumor into different subgroups, the invasive ductal carcinoma constitutes the majority of the studied patients, approximately 92%. An 54% of the tumors were of grade II, 69% were positive for Estrogen receptors, the same percentage was for Progesterone receptors, and however, only 40% were positive for Her2 receptors. Different predisposing factors that may contributes to development on breast cancer, they include: age, menopausal status, Diabetes Mellitus and hypertension and positive family history for cancer, higher frequency of breast cancer group had negative family history for cancer (80%), age

>50 years (50%), Hypertensive (68%) and diabetic (70%). Premenopausal status was predominant (52%) among the breast cancer group. After testing, results will be divided into categories that describes the breast cancer. Most breast cancers are hormone-receptor-positive.

- ER+: About 69% of breast cancers are estrogen-receptor positive.
- ER+/PR+: About 65% of estrogen-receptor-positive breast cancers are also progesterone-receptor-positive. This means that the cells have receptors for both hormones, which could be supporting the growth of the breast cancer.
- ER+/PR-: About 4% of breast cancers are estrogen-receptor-positive and progesterone-receptor-negative. This means that estrogen, but not progesterone, may be supporting the growth and spread of the cancer cells.
- ER-/PR+: About 4% of breast cancers are estrogen-receptor-negative and progesterone-receptor-positive. This means that the hormone progesterone is likely to support the growth of this cancer. Only a small number of breast cancers test negative for estrogen receptors but positive for progesterone receptors. More research is needed to better understand progesterone-receptor-positive breast cancers.
- ER-/PR-: If the breast cancer cells do not have receptors for either hormone, the cancer is considered estrogen-receptor-negative and progesterone-receptor-negative (or "hormone-receptor-negative"). About 5% of breast cancers fit into this category.

HER2 + : About 40% *HER2*-positive breast cancer means the individual tested is likely to have a tumor that is aggressive, will respond poorly to endocrine treatment, and will be resistant to standard chemotherapy. The person may be considered a candidate for HER2-targeted therapy, such as trastuzumab, lapatinib or pertuzumab (American Cancer Society)

HER2 - : About 60% If the tumor is *HER2*-negative, then

HER2-targeted therapy isn't expected to be effective and the individual tested will avoid unnecessary side effects from treatment that is unlikely to help. (American Cancer Society)

- Triple negative: About 6%breast cancer cells don't have estrogen or progesterone receptors and also don't make too much of the protein called HER2. and because they don't have too much HER2, drugs that target HER2 aren't helpful, either. Chemotherapy can still be useful. (American Cancer Society)
- Triple positive: About 23% cancers are ER-positive, PR-positive, and HER2-positive. These cancers can be treated with hormone drugs as well as drugs that target HER2.

Any positive test result whether just for estrogen receptors, just for progesterone receptors, or both means that the breast cancer is considered "hormone-receptor-positive." Hormonal therapy may help to slow or stop the growth of hormone-receptor-positive breast cancers by lowering your body's estrogen levels or blocking the effects of estrogen. These medications also may reduce the risk that the cancer will come back (recur). If your cell sample tests positive, hormonal therapy should be in the treatment plan. If the breast cancer is hormone receptor-negative (ER- and PR-), hormonal therapy is unrecommend in this case. But many other effective treatments are available. Breast carcinoma is a heterogenous disease. Carcinomas lacking expression of estrogen, progesterone, and HER2/neu receptors by immunohistochemistry and *Her2* amplification are designated as triple negative. This group of carcinomas comprises approximately 10% to 20% of all breast carcinomas and is characterized by an aggressive nature with shorter rates of disease-free and overall survival. This aggressive behavior is further compounded by the lack of available targeted therapies. Patients receive cytotoxic chemotherapy regimens. Although tumors are initially sensitive to this therapy, drugs are toxic and ineffective in maintaining long-term response thereby providing limited benefit. Much effort is being spent on this group of cancers for the identification of appropriate molecular targets, an effort that is proving challenging due to the presence of marked heterogeneity, both at the morphologic and molecular levels. An understanding of the advances in this field is crucial for developing targeted therapies and tailored patient management protocols. IHC-based classification of both ER/PR and Her2 status provides prognostic and therapeutic information not achievable from either alone. Prior classifications separating breast cancer into one of two categories based on ER expression alone is less discriminatory in terms of prognosis, and the additional subclassification based on Her2 expression provides enhanced and important therapeutic guidance. Breast cancer has also sometimes been dichotomized into triple negativity or other (Carey *et al.,* 2007). 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 connotations.

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 (Carey *et al.,* 2007). ER/PR+, Her2+ tumors virtually always have a high recurrence score (Fan *et al.,* 2006). Recently it was shown in a retrospective analysis that ER/PR+, Her2− tumor may benefit less from taxanes in the adjuvant setting (Hayes *et al.,* 2007). We have classified breast cancer using IHC into 4 global subtypes out of the 8 possible subtypes commonly used by other authors (Nguyen *et al.,* 2008). We believe 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). Patients with ER+/PR+ tumors had a lower recurrence rate than those with ER+/PR− tumors (Howell *et al.,* 2005). 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 (Howell *et al.,* 2005). Also, the relative resistance by ER+/PR− tumors was not observed in the BIG 1–98 trial which is the largest study of an aromatase inhibitor as up-front adjuvant therapy for early breast cancer (Coates *et al.,* 2007). Studies that have been classified as using more than 4 subtypes are plagued by these controversies and those inherent in small sample size and multiplicity of variables (Francis *et al.,* 2006; Brouckaert *et al.,* 2009).

**Conclusion**

We support IHC classification as a clinical tool as ER/PR and Her2 testing is widely available at a reasonable cost, 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.

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