

CLINICAL STUDIES

Prevalence of Steroid Receptors and HER 2/neu in Breast Cancer Biopsies of Women Living in Puerto Rico.

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Objective. The purpose of this study is to determine the prevalence rate of estrogen and progesterone receptors and HER 2/neu in the breast cancer biopsies analyzed in the Laboratory of Immunohistochemistry of the University of Puerto Rico School of Medicine in the year 2000. This data may serve as a reference point for future studies of the epidemiological aspects of breast cancer among women living in Puerto Rico.

Background. Determination of estrogen receptor (ER) and progesterone receptor (PR) on biopsy specimens of breast carcinoma prior to treatment is standard practice in the management of breast carcinoma. ER and PR are used to identify patients who are likely to respond to endocrine therapy. The prevalence of ER, PR and Her2/neu among USA women is 77%, 55% and 10-34%, respectively. One of the major clinical roles for testing HER 2/neu expression is to determine eligibility for treatment with Trastuzumab.

Methods. Retrospective analysis of 309 breast cancer

biopsies was done. Paraffin embedded blocks of breast cancer tissue biopsies were received from different hospitals and Pathology Laboratories located throughout the island specifically for routine analysis of steroid receptor (ER/PR) and/or HER 2/neu expression. Immunostaining was performed in a Ventana Medical Systems® automated instrument.

Results. Positive nuclear staining for ER and PR were seen in 65.9% (203/308) and 51.8% (159/307), respectively. In the HER2/neu test, 27.8% (46/165) gave a strong and complete membranous staining (score 3+).

Conclusions. There is a lower prevalence of estrogen receptor in the breast cancer biopsies of women living in Puerto Rico than their USA counterparts, but similar prevalence of progesterone receptor status and HER 2/neu protein over expression. Condensed Abstract

Key words: Breast cancer, Puerto Rican women, Steroid receptor, HER 2/neu

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females living in Puerto Rico. It is also the most commonly diagnosed cancer site among females in the USA. The incidence in breast cancer in Puerto Rico in 1989 was 51.4 per 100,000, lower than the probability for US white women, 110.8 per 100,000, but the risk of developing and dying of breast cancer is increasing at a faster pace among Puertorrican women than among USA

women (1). Several features of breast cancer are prognostic indicators and could influence the management of breast carcinoma. The major prognostic indicators are tumor stage, tumor type, histological grade and hormone receptor status. Determination of estrogen and progesterone receptors on biopsy specimens of breast carcinoma prior to treatment is standard practice in the management of breast carcinoma (2).

The normal mammary epithelium contains receptors for estrogen and progesterone. The interaction between the hormones and their receptors stimulates epithelial differentiation and proliferation. Estrogens are thought to stimulate cell proliferation in normal breast epithelium and hormone responsive cancers by increasing the production of growth factors and other regulatory molecules. The estrogen receptor (ER) and progesterone receptor (PR) are intracellular receptors that can be measured directly in tumor tissue. These receptors are polypeptides that bind their respective hormones, translocate to the nucleus and induce specific gene

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expression. When the hormone binds its respective receptor, the DNA binding domain is modified, and transcription is initiated.

Steroid receptor status (ER and PR) is recommended to be measured on every breast cancer, since the hormone status has been proven to be useful in clinical patient management (2). ER and PR are used to identify patients who are likely to respond to endocrine therapy (3). Immunohistochemistry (IHC) has been correlated with other methods of determining receptor status (4). IHC has become the preferred method for determining the ER and PR status in breast cancer biopsies (5,6). Advantages of the IHC method include availability of paraffin embedded tissue and direct counting of true percentage of tumoral cells that are positive in small tissue biopsies. It is also a fast and economic method, that can be included as part of a routine diagnostic service.

The analysis of breast cancer specimens for alterations in HER 2/neu (c-erbB2 gene) expression has become a common practice in surgical pathology. The major clinical role is to determine eligibility for treatment with Trastuzumab (Herceptin[®], Genetech Inc, South San Francisco, Cal). The protooncogene c-erb-B2 belongs to the tyrosine kinase oncogene family. It encodes for the transmembrane protein p185 (HER 2/neu) and its over expression is measured in about one third of breast cancers (7). Normal cells and the majority of breast cancer cells carry two copies of the c-erb-B2 gene on chromosome 17 and express low levels of p185. Using an antibody against HER 2/neu, we can identify its over expression in the tumor.

In 1987, Slamon et al. addressed the possible prognostic significance of c-erb-B2 gene amplification in 187 breast cancer patients (8). This study showed a strong correlation between c-erb-B2 amplification with shortened disease free survival and overall survival in the subset of 86 node positive patients. Several studies have tried to replicate these findings with mixed results. Studies based on IHC detection of HER 2/neu protein over expression concur that this marker may add to the prediction of overall survival (8, 9). Some authors have reported that there is a negative correlation between HER 2/neu and steroid receptor status, but there have been mixed results (10, 11).

Most of the work measuring gene expression has been done by IHC because it is convenient to use paraffin embedded tissue. Also, HER 2/neu over expression can be directly viewed in the malignant cells in the tissue sample. IHC is an economic and fast method. Commercial kits for both FISH and IHC testing have been approved recently by the FDA and are commercially available.

The purpose of this study is to determine the prevalence rates of ER, PR and HER 2/neu in the breast cancer biopsies analyzed in the Laboratory of Immunohistochemistry of

the University of Puerto Rico School of Medicine in the year 2000. This data may serve as a reference point for future studies of the epidemiological aspects of breast cancer among women living in Puerto Rico.

Materials and Methods

Retrospective analysis of 309 breast cancer biopsies received at the laboratory of immunohistochemistry of the Department of Pathology and Laboratory Medicine of the University of Puerto Rico School of Medicine was done. Paraffin embedded blocks of breast cancer biopsies were received from different hospitals and pathology laboratories located throughout the island specifically for routine steroid receptor (ER/PR) and/or HER 2/neu expression analysis. The patients' age ranged from 25 to 93 years. Four 5 µm sections were cut from the paraffin block of the tissue biopsy of each patient. One was stained with Hematoxylin and Eosin for confirmation of the diagnosis. The other sections were deparaffinized in xylene and hydrated in progressive alcohols, immersed in buffered citrate and heated in a pressure cooker within a microwave oven for 18 minutes (antigen retrieval). Immunostaining was performed in a Ventana Medical Systems[®] immunohistochemical instrument with automated steps and staining timers. The manufacturer's diaminobenzidine (DAB) detection kit was used, including biotin labeled secondary antibodies against mouse immunoglobulins, avidin labeled horseradish peroxidase and DAB as localization reagents. Specific controls for strong positive and weak staining of HER 2/neu were used in each run. Positive staining of ER and/or PR required nuclear coloration in over 10% of the neoplastic cells. Positive staining of HER 2/neu required dense and complete coloration of the cell membrane in over 10% of the malignant cells. The antibodies used were mouse monoclonal antibodies from Ventana Medical Systems[®]: Clone 6F11 (ER), Clone PGP-1A6 (PR) and Clone CB11 (HER 2/neu).

Two pathologists examined the stained tissue slides, tumors were considered positive for expression of ER or PR if more than 10% of the cancer cells showed nuclear staining. For HER 2/neu marker, tissues were given a score of 0, 1+, 2+ or 3+. These scores were defined by no membranous staining (0), incomplete membranous staining in more than 10% of the cells (1+), complete membranous staining in more than 10% of the cells of weak to moderate intensity (2+) and complete membranous staining in more than 10% of the cells of strong intensity (3+). In this study, only a score of 3+ was considered over expression of HER 2/neu.

The associations between ER, PR and HER 2/neu positivity were analyzed with the chi-square test using

the computer program Minitab®. A p-value of less than 0.05 was considered statistically significant.

Results

The positive staining pattern of steroid receptors is shown in Figure 1. Positive nuclear staining for ER and

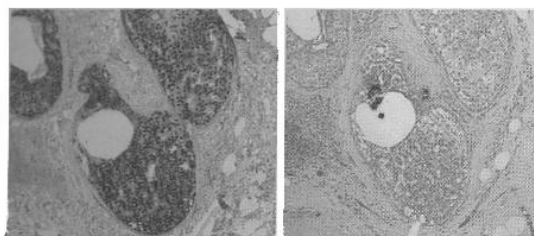


Figure 1. Steroid staining. The picture on the left shows an hematoxylin & eosin staining of breast carcinoma. The picture on the right shows a breast carcinoma positive for steroid receptors, strong (brown) nuclear staining in more than 10% of tumoral cells. The surrounding tissue is negative for steroid receptors.

for PR were seen in 65.9% (203/308) and 51.8% (159/307), respectively (Table 1). Of all the cases, 48.7% (149/306) were positive for both receptors. Negative results for

Table 1. ER, PR and HER 2/neu prevalence in invasive breast carcinoma.

Marker	Positive	Negative	Total
ER*	203 (65.9%)	105 (34.1%)	308
PR†	159 (51.8%)	148 (48.2%)	307
HER 2/neu	47 (27.8%)	119 (72.2%)	165

* Estrogen receptor

† Progesterone receptor

both receptors were seen in 31.4% (96/306), leaving 19.9% (61/306) that were positive for only one of the steroid receptors (ER or PR). ER expression correlated positively with PR expression ($p < 0.001$) (Table 2).

Table 2. Distribution of receptor expression in invasive breast carcinoma.

Receptor	Cases (n)	%
ER*(+)PR†(+)	149	48.7
ER*(+)PR†(-)	52	17.0
ER*(-)PR†(+)	9	2.9
ER*(-)PR†(-)	96	31.4
Total	306	100

p-value < 0.001

* Estrogen receptor

† Progesterone receptor

HER2/neu test was done in 165 biopsies of invasive breast carcinoma. Of these, 46 (27.8%) gave a strong and complete membranous staining (score 3+). (Figure 2, Table 1) There was no association of HER2/neu over expression with ER ($p = 0.098$), but there was a slight inverse association with PR ($p = 0.037$) (Table 3).

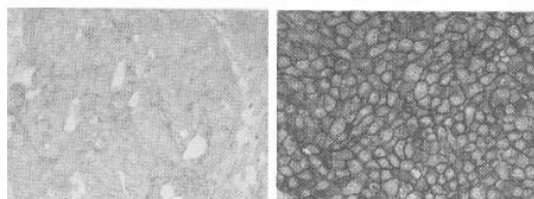


Figure 2. HER 2/neu staining of breast carcinoma. The photo on the left shows a HER 2/neu negative breast carcinoma. The right is a positive staining because it has complete and strong membranous staining of more than 10% of tumoral cells.

Table 3. Relation between HER 2/neu and steroid receptors.

Markers	N	%
HER 2/neu (-)ER* (-)	26	14.4
HER 2/neu (-)ER (-)	24	13.3
HER 2/neu (+)ER (-)	85	47.2
HER 2/neu (-)ER (-)	45	25.0
Total	180	
p-value = 0.098		
HER 2/neu (+)PR† (+)	19	10.7
HER 2/neu (+)PR (-)	31	17.2
HER 2/neu (-)PR (-)	72	40.0
HER 2/neu (-)PR (-)	58	32.2
Total	180	
p-value = 0.037		

* Estrogen receptor

† Progesterone receptor

The prevalence of steroid receptors was examined from patients of different regions of Puerto Rico. Only the regions of San Juan and Mayagüez had a significant number of cases for analysis. Seventy-six biopsies of invasive breast carcinoma were referred from patients living in San Juan. Of these, 49 were positive for ER (64.5%) and 42 for PR (55.3%). Seventy-two biopsies were referred from Mayagüez, of which 47 cases were positive for ER (65.3%) and 37 were positive for PR (50.7%). There is no significant statistical difference between these two groups. ($p > 0.5$) (Table 4)

Thirty biopsies diagnosed as intraductal breast carcinoma were also examined and 56.7% (17/30) were ER+, 53.3% (16/30) were PR+ and only 13 cases were positive

Table 4. Steroid receptors in San Juan and Mayagüez

Steroid receptor	San Juan	Mayagüez
ER*	64.5% (49/76)	65.3% (47/72)
PR†	55.3% (42/76)	50.7% (37/72)

p-value > 0.500

* Estrogen receptor

† Progesterone receptor

for both ER and PR. Only 20 cases were examined for HER2/neu expression; of these, 30.0% (6/20) were positive (3+). (Table 5)

Table 5. Steroid receptors and HER 2/neu intraductal breast carcinoma.

Receptor	Positive	Negative	Total
ER*	17 (56.7%)	13 (43.3%)	30
PR†	16 (53.3%)	14 (46.7%)	30
Her2/neu	6 (30%)	14 (70%)	20

* Estrogen receptor

† Progesterone receptor

Discussion

Breast carcinoma has several prognostic indicators that can be used in assessing the management of patients. Among these prognostic indicators are tumor markers, such as hormone receptor status, which have been proven to be useful in clinical management, and HER 2/neu status.

We have determined a prevalence of 65.9% for ER (with a confidence interval of 95%: 60.6-71.2) and of 51.8% for PR (C.I. 95%: 46.2-57.4) among invasive breast cancer biopsies of women living in Puerto Rico. Our ER prevalence of women living in Puerto Rico is lower than USA women, who have an average prevalence of 77% (p=0.0008). The PR prevalence of USA women is 55%, which is similar to the PR prevalence of our study group (p=0.1251). When comparing our results with those found in another study done on women living in Puerto Rico, biopsied at San Pablo Hospital (13), there is a difference in the prevalence of steroid receptors (p<0.01). Peredo's study found 72% ER positivity and 58.2% PR positivity, which are similar to the results seen in USA studies. Differences between both studies done on Puerto Rican women could be explained by differences in methodology, including among others, antibodies used, antigen retrieval methods and interpretation of a positive test. Peredo's study used a cutoff of 5% to consider a positive result.

It is expected that the frequency of estrogen receptors to be related to the frequency of progesterone receptors because the production of progesterone receptor depends on estrogenic stimulus and the presence of estrogen receptors in the cell (13, 14). Our study found a strong

positive correlation among both steroid receptors (p<0.001). The specific distribution of ER and PR is important because the response to hormone therapy is dependent on this distribution. Cancers that are ER+/PR+ are responsive to hormone therapy, while those that are ER-/PR- are non-responsive. Response to anti-hormonal therapy is not as predictable in cancers with mixed ER/PR profile. Some investigators believe that breast carcinoma with mixed expression of ER-/PR+ type does not represent a separate profile, but belongs to the ER+/PR+ type because of false negative results in the estrogen receptor test (3). This would mean that over two thirds of the patients in our study would probably respond to anti-hormonal therapy.

Prevalence ER and PR status in biopsies from patients living in San Juan and Mayagüez were not statistically different, even though the biopsies from San Juan were fixed in formalin and some from Mayagüez were fixed in Histochoice. The number of biopsies from patients living in other regions of the island was too small to analyze differences in ER and PR status.

Differences have been reported for the positivity of ER and HER 2/neu over expression between invasive and intraductal carcinomas (15). The number of biopsies diagnosed as intraductal breast carcinoma in this study was too small to confidently analyze the prevalence of ER, PR and HER 2/neu of women living in Puerto Rico.

In the subgroup of patients studied for HER2/neu, we found an over-expression of the tumor marker in 27.9% (C.I. 95%: 21.0-34.6) of the analyzed patients. This result is comparable with others in the United States that have found a prevalence of HER 2/neu of 10-34% in breast carcinoma (16). There is a statistical difference (p=0.004) with the results of the group of Puerto Rican women done by Peredo et al. (19.6%) (13). This may be due to differences in methods, because they included 2+ as well as 3+ as positive for over expression and they used a different antibody (DAKO). There was also a difference in the population studied: our population included recurrences as well as newly diagnosed cancer patients, while Peredo's study only included newly diagnosed patients. In their study, they divided the patients in two groups, those with positive nodal involvement and those negative for metastases. The patients with nodal involvement showed a higher HER 2/neu over expression (35%), which is similar to our study. This finding is consistent with other studies in which nodal involvement correlated with higher HER 2/neu over expression and may explain differences in the results between studies. We did not find any statistically significant associations between HER 2/neu and ER (p=0.098), but there was a slight positive association with PR (p=0.037).

In conclusion, we found a lower prevalence of estrogen receptor in the breast cancer biopsies of women living in Puerto Rico than their USA counterparts, but similar prevalence of progesterone receptor status and HER 2/ neu protein over expression.

Resumen

El propósito de este estudio es determinar la tasa de prevalencia de receptores de estrógeno y progesterona y de Her2/neu en 309 biopsias de cáncer de mama de mujeres residentes en Puerto Rico, recibidas en el año 2000. Se tiñeron mediante inmunoperoxidasa usando el instrumento automatizado de Ventana Medical Systems®, para determinar la presencia de los receptores y la sobre expresión de Her2/neu. La prevalencia del receptor de estrógeno es 65.9% (203/308) y la prevalencia del receptor de progesterona es 51.8% (159/307). Un total de 96 casos fueron negativos para ambos receptores, correspondiendo al 31.4% de las biopsias. Se identificó una correlación positiva entre ambos receptores ($p < 0.001$). La prevalencia de sobre expresión de Her2 neu corresponde a 27.8% (46/165). La prevalencia del receptor de estrógeno es inferior a la de la mujer norteamericana. La prevalencia del receptor de progesterona y sobre expresión de Her2 neu es similar a la mujer norteamericana.

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