Expression of Basal-like Biomarkers in Triple Negative Invasive Breast Carcinoma in Puerto Rico

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Objective: Routine Progesterone and Estrogen hormone receptor proteins and human epidermal growth factor receptor 2 (HER-2) analysis on invasive breast carcinomas provide therapeutic and prognostic values, revealing significant subgroups: luminal A, luminal B, HER-2 and the "triple negative" tumors. The aim of this study was to determine the expression of basal cytokeratins and Epidermal Growth Factor Receptor in "triple negative" invasive breast carcinomas in Puerto Rico women.

Methods: All invasive breast carcinoma cases received from 2008 to 2010 were included. Assessment of tumoral expression of Estrogen Receptor, Progesterone Receptor and HER-2 was performed. The cases were divided into groups based on their molecular categories and analyzed according to the age. "Triple negative" tumors were further analyzed according to their expression of Epidermal Growth Factor Receptor and cytokeratins 5/6 and 14.

Results: From 717 cases reviewed, 487 cases of invasive breast carcinoma were included. The molecular categories were 66%, 10%, 9% and 15% for the luminal A, luminal B, Her-2 and "triple negative" groups, respectively. No significant difference (p= 0.64) was observed between the molecular categories and the age of the patients. Assessment of basal cytokeratins and Epidermal Growth Factor Receptor expression was performed on 41 "triple negative" tumors; 71% expressed at least one basal cytokeratin or Epidermal Growth Factor Receptor and 29% were negative to all markers.

Conclusion: Prevalence and relation between the molecular categories and the expression of basal cytokeratins in "triple negative" tumors in our population is comparable to other published data. [*P R Health Sci J 2015;34:89-92*]

Key words: Breast carcinoma, Basal, Triple-negative, Molecular categories

The "triple negative" invasive breast carcinoma are tumors negative for Estrogen receptor (ER), Progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) oncoprotein analysis. They represent approximately 10- 17% of all breast carcinomas and are more frequent in young African, African American and Latino women (1-6). These tumors have been known to be clinically aggressive, and therapeutic options are limited because they are not amenable to hormone therapy or HER-2 targeted therapy (1). The "triple negative" tumors may be subdivided in two categories: basal-like and non-basal-like (3, 7-9).

The purpose of this study was to determine the expression of basal cytokeratins and Epidermal Growth Factor Receptors (EGFR) in "triple negative" invasive breast carcinomas in Puerto Rico women. The cases were subdivided based on the different molecular categories, including luminal A, luminal B, HER-2 and the "triple negative" tumors, and a frequency analysis was performed. Each group was analyzed based on the molecular categories and age.

Materials and Methods

This is a retrospective study which evaluated all cases of invasive carcinoma of breast received in the UPR-School of Medicine Immunoperoxidase Laboratory, from 2008 to 2010, for which ER and PR hormonal receptors and HER-2 oncoprotein analysis were performed for clinical assessment. Information of patients was obtained by reviewing the final pathology reports. Cases with benign pathologic findings, Ductal or Lobular Carcinoma In Situ, and cases with incomplete

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analysis of HER-2 oncoprotein and equivocal results, were excluded from the analysis.

The primary pathologist sent the pertinent Hematoxylin and Eosin (H&E) stained slides, paraffin tissue blocks and the corresponding pathology request form, which includes demographical data (name, age and sex) of patient, a brief clinical history, pathologic diagnosis (if available) and specimen type, fixative solution and duration of fixation. Some cases also included a copy of the diagnosis report given by the primary pathologist, which provided additional information, such as the tumor size, histological type, grade, among other parameters.

The paraffin tissue blocks were used to prepare the slides for the immunohistochemistry analysis. The slides were immunostained for ER, PR and HER-2, along with the appropriate external tissue control. Monoclonal antibodies were used for ER (SP1, prediluted, Ventana Medical Systems, Tucson, AZ), PR (1E2, prediluted, Ventana Medical Systems, Tucson, AZ) and HER-2 (4B5, prediluted, Ventana Medical Systems, Tucson, AZ). The assessment of the expression of ER, PR and HER-2 was performed by a main pathologist, who examined all the microscopic slides. Control tissue results were as expected. If no clear-cut results were obtained, a second pathologist reviewed the case and a consensus result was given. The final pathology report was performed following the applicable guidelines of ASCO-CAP, published in 2007 (10).

Negative results for ER and PR were based on immunohistochemical staining of less than 1% of the tumoral cells. If a tumor had more than 10% of staining, it was considered positive for both, ER or PR status. Tumoral cells with nuclear staining for ER and PR between 1- 10% were classified as low positive. HER-2 negativity was based on no immunoreactivity or faint weak immunoreactivity staining of less than 10% of the tumoral cells. Positivity for HER-2 was defined as uniform and homogeneous membrane staining of more than 30% of the tumoral cells. If only 10- 30% of the tumoral cells stained for HER-2 or there was incomplete and non-uniform membrane staining, the HER-2 was categorized as equivocal. In such cases, Fluorescent In Situ Hybridization was recommended for final HER-2 status.

An accession number was assigned to each patient and the information recorded on a study form, including: the patients' age, sex, specimen type, diagnosis, ER, PR, and HER-2 expression status, and if additional immunohistochemical stains or information were available, the data was recorded in a "comment section". Coded information was entered into a computer file for analysis. A quality control process was conducted to minimize data entry errors and to identify outliers.

The tumors were further divided in the corresponding molecular categories as summarized in Table 1. Recently, the Luminal B subtype have also been defined as ER+, PR+/-, Her2+ (10) or ER+, PR+/-, Her2-, Ki-67>14% (11), but for the purposes of this study we used positivity of all markers as the subtype definition. In "triple negative" cases, additional immunohistochemical analyses were performed, using several markers that have been reported as useful in defining the basal-like phenotype, including EGFR (3C6, prediluted, Ventana Medical Systems, Tucson, AZ), CK 5/6 (D5 & 16B4, prediluted, Ventana Medical Systems, Tucson, AZ) and CK14 (LL002, prediluted, Ventana Medical Systems, Tucson, AZ).

Table 1. Immunophenotyping for molecular category using ER, PR and HER-2 status.

	Luminal A	Luminal B	HER-2	"Triple Negative"
ER	+	+	-	-
PR	+	+	-	-
HER-2	-	+	+	-

The cases were prospectively divided into different groups according to their respective molecular categories and analyzed according to the age of the patients. The "triple negative" tumors were further analyzed according to the expression of EGFR and basal cytokeratins.

To describe the study population, categorical variables were summarized using frequency distribution. Differences between breast cancer molecular subtypes with regard to demographic and clinical characteristics were examined using Pearson chi-square. The Fisher's exact test was used when expected cell counts were less than 5. A p-value of less than 0.05 was considered to be statistically significant, under the null hypothesis. All statistical analyses were performed with STATA software version 11.1 (Stata Corp LP, College Station, TX).

This study was approved by the institutional review board of the University of Puerto Rico- Medical Sciences Campus.

Results

A total of 717 breast cases were reviewed at our institution during the study period, and 487 cases of invasive breast carcinoma, evaluated for clinical purposes, were included. Of the 717 cases, 230 cases were excluded from the study since they presented benign pathologic findings (n=154), Ductal or Lobular Carcinoma In Situ (n=54), or incomplete analysis of HER-2 oncoprotein (n=12). Further analysis was performed among the 487 cases of breast carcinoma. The prevalence for the molecular subtypes was 66% (n=320) for luminal A, 10% (n=48) for luminal B, 9% (n=46) for HER-2 and 15% (n=73) for the "triple negative" group. Breast carcinoma cases are described in Table 2.

Table 2. Frequency of molecular breast subtypes for invasive breast carcinoma.

Molecular subtype	Frequency	%
Luminal A	320	66
Luminal B	48	10
Her-2	46	9
Triple Negative	73	15

From the 73 "triple negative" cases, in only 41 cases the paraffin blocks were available for assessment of basal biomarkers. Seventy-one percent (n=29) of the "triple negative" cases showed expression of at least one basal cytokeratin (CK5/6 or CK14) or EGFR. Nineteen out of the 41 cases (46%) were positive for EGFR, 21 cases were positive for CK5/6 (51%) and 15 (37%) cases were positive for CK14. The remaining 29% (n=12) "triple negative" cases were negative to all basal biomarkers. Twelve percent (n=5) of the "triple negative" cases were positive for all basal markers.

Comparing the percent distribution of the "triple negative" cases with the remaining molecular subgroups, there is a slightly higher percentage of "triple negative" cases in women less than 40 years old. However, there is no statistical significance difference between the molecular categories and age groups (Chi2$_{df}$=0.90, p=0.64). The findings are summarized in Table 3.

Table 3. Summary of molecular breast subtypes according to age groups.

Age at time of diagnosis (years)	"Triple Negative" subtype	Other subtypes	Total
	Number (%)	Number (%)	Number (%)
<40	6 (8)	23 (6)	29 (6)
40-64	41 (56)	249 (60)	290 (59)
≥65	26 (36)	142 (34)	168 (35)
Total	73 (100)	414 (100)	487 (100)

Discussion

Breast cancer is one of the most common malignant tumors in women. Management of the patients depends on clinical and pathologic features. Additionally, routine hormone receptor protein and HER-2 oncoprotein analysis on invasive breast carcinomas in the past decade provide therapeutic, predictive and prognostic values, and has revealed clinically significant subgroups (1). The best characterized of these have been designated luminal A, luminal B, HER-2 and the so-called "triple negative" tumors (2).

The basal-like breast subtype was initially recognized by gene expression profiling studies (1, 12, 13) and histologically recognized by high Nottingham grade, geographic necrosis, good circumscription, and mild to moderate host lymphocytic response (1,14). Basal-like carcinomas are characteristically triple negative and show expression of basal type cytokeratin (CK 5/6, CK14 and CK17), EGFR, vimentin, and p53 (1, 14-19). Often a panel of basal type cytokeratins and EGFR is used in triple negative tumors to identify basal-like carcinomas (1). Gene expression studies have consistently identified basal-like carcinomas to have poor prognosis (1, 13, 20-23).

Maambo and Ioffe reported a molecular subtype frequency of 15-25% for the HER-2 group and 10-20% for the "triple negative" group (24). In our study, the frequency for the luminal subtype was 76%, 9% for the HER-2 and 15% for the "triple

negative" subtype, which show a slightly lower frequency for the HER-2 subtype, although the equivocal HER-2 results that were positive by FISH were not included in the analysis since we did not have feedback of the FISH results.

The basal-like breast carcinomas constitute at least 85% of the "triple negative" tumors (1). Based on our analysis, 71% of the "triple negative" cases showed expression of at least one basal cytokeratin or EGFR. Forty-six percent of the basal-like cases were positive for EGFR, 51% for CK5/6 and 37% for CK14. Twelve percent (n=5) of the "triple negative" cases were positive for all basal markers.

Bertucci et al (25) showed that 71% of "triple negative" tumors were of basal subtype by gene expression profiling (26). Nielsen et al (15) demonstrated that the immunophenotype of this type of cancer is characterized by expression of CK5/6 (61%), EGFR (72%), vimentin (94%), CK8/18 (83%) and p53 mutations (50%).

The age distribution of "triple negative" breast cancer cases was similar to the age distribution of other molecular subtypes (p=0.64). These tumors occur in both pre and postmenopausal patients; however, identifying basal-like carcinoma in a young premenopausal woman may suggest the presence of hereditary breast or ovarian carcinoma syndrome (1, 22). Although currently there are not specific chemotherapeutic drugs available to treat these patients, new data are emerging, hence, it is important to recognize these tumors as therapeutic options improve (1, 23, 24).

Among the limitations of this study are the low number of cases, the availability of paraffin blocks for basal markers evaluation in "triple negative" tumors. The lack of Her-2 FISH results in Her-2 equivocal cases as well as the lack of additional clinical informations may also limit the results of this study.

Conclusion

In this study we classified the invasive breast carcinoma cases based on the different molecular categories and a frequency analysis was performed. We also determined the expression of basal cytokeratins and EGFR in "triple negative" invasive breast carcinomas in Puerto Rico. Each group was analyzed based on the molecular categories and age. Prevalence of the molecular categories and the expression of basal cytokeratins in our population are comparable to other ethnic groups, as previously described in the literature. We found no statistical significance difference between the molecular categories and age of patients. Identification of the invasive breast cancer subtype in our population can improve our understanding of the disease, allowing the development of early detection strategies and help provide personalized prevention and guidance of the therapeutic approach.

Resumen

Objetivo: El análisis rutinario de receptores hormonales de estrógeno y progesterona y el receptor de factor de crecimiento

epidermal 2 (HER-2, por sus siglas en ingles) en carcinomas invasivos de mama tienen un valor terapéutico y pronóstico, revelando subgrupos significativos: luminal A, luminal B, HER-2 y triple negativos. El propósito de este estudio es determinar la expresión de citoqueratinas basales y el receptor del factor de crecimiento epidermal en carcinomas invasivos, triple negativos, de mama en mujeres en Puerto Rico. Métodos: Todos los casos de carcinoma invasivos recibidos en el laboratorio desde el 2008 al 2010 se incluyeron en el estudio. Se determinó la expresión tumoral de receptores de estrógeno, progesterona y HER-2. Los casos se dividieron en grupos basados en sus respectivas categorías moleculares y se analizaron de acuerdo a la edad. Los tumores triple negativos se analizaron de acuerdo a la expresión del receptor del factor de crecimiento epidermal, citoqueratinas 5/6 y 14. Resultados: De 717 casos revisados, 487 casos de carcinomas invasivos se incluyeron en el estudio. Las categorías moleculares fueron: 66%, 10%, 9% y 15% para los grupos luminal A, luminal B, HER-2 y triple negativos, respectivamente. No se observaron diferencias significativas (p= 0.64) entre las categorías moleculares y la edad de los pacientes. La evaluación de las citoqueratinas basales y el receptor del factor de crecimiento epidermal se realizo en 41 casos de tumores triple negativos, 71% expresaron al menos una citoqueratina basal o receptor del factor de crecimiento epidermal y 29% fueron negativos a todos los marcadores. Conclusión: La prevalencia y relación entre las categorías moleculares y expresión de citoqueratinas basales en tumores triple negativos en nuestra población es comparable a otros datos previamente publicados.

Acknowledgment

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