Breast Cancer Receptor Profiles in Jamaica: A 6-year Analysis

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ABSTRACT

Objective: To determine the breast cancer IHC receptor status for tests performed at the UWI from January 2002 to December 2007, and to investigate for an association between receptor profile and patient age, tumour grade and stage.

Methods: The UWI breast cancer IHC receptor database was examined to determine receptor profile, patient age, tumour histology, grade, size and lymph node status.

Results: 1,383 breast cancer cases were tested for ER and HER 2 status during the study period; PR testing was not performed. Receptor profiles were: ER+/HER2- (50.2%), ER-/ HER2- (28.1%), ER+/HER2+ (15.3%) and ER-/ HER2+ (6.4%). Across all age groups, ER+/HER2- was the most frequent profile (45-52%) and ER-/ HER2- was second most frequent (27-34%). There was no statistically significant association between receptor profile and age (p = .079). Amongst Grade III tumours, ER-/HER2- was most prevalent profile (44.6%); ER+/HER2- was most prevalent for Grade I and Grade II tumors (60.7% and 48.8% respectively). There was a statistically significant association between receptor profile and tumour grade (p=< .001). There was no statistically significant association between receptor profile and tumour stage (p =.359).

Conclusions: The prevalence of ER/HER2-negative breast cancer was 28%, in keeping with TNBC prevalence in African-American populations. There was a statistically significant association between receptor profile and tumour grade (p< .001) (most Grade III tumours were ER-/HER2-), in keeping with the biologically aggressive behavior of TNBC.

Keywords: Breast cancer receptors, immunohistochemistry, Jamaica

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INTRODUCTION

Breast cancer is the most common malignancy among Jamaican women, with an age-standardized incidence rate (ASIR) of 43.1 per 100 000 (1). Breast cancer prognostication has traditionally been derived from tumour-related histopathological variables of size, histological grade and nodal status. The Nottingham Prognostic Index is a well-validated prognostic system that uses these criteria to determine prognosis following surgery for breast cancer (2). While these pathological criteria remain relevant, breast cancer molecular profiling has become increasingly important in prognostication. Biomarkers such as the oestrogen receptor (ER), progesterone receptor (PR) and Her 2-neu oncogene (HER2) are not only important prognostic markers, but are also predictive of response to targeted therapies (3). The triad of ER, PR and HER2 status has therefore become a mandatory part of therapeutic decision-making in breast cancer management (4), with the predictive value of these biomarkers influencing the choice of systemic therapy.

In Jamaica, breast cancer receptor status testing is centralized to the Immunohistochemistry (IHC) Laboratory in the Department of Pathology, University of the West Indies (UWI), which undertakes testing for the entire island. The laboratory receives requests for IHC breast receptor studies from the University Hospital of the West Indies (UHWI), the teaching hospital with which the UWI is affiliated, the National Public Health laboratory (the government laboratory facility), regional pathology laboratories (e.g. Mandeville Regional Hospital, Cornwall Regional Hospital) and several private pathology laboratories.

The UWI Department of Pathology IHC breast receptor database was established in 2002 and records results of tests performed on tumor tissue from patients with breast cancer. In addition, the database contains records from the histopathology report for each patient, including age, tumour histological type, grade, size, and lymph node status (if nodal sampling was performed).

In this report we will present results from the breast cancer IHC breast receptor database for tests performed during the 6-year period following its establishment. During this period, breast IHC breast receptor testing was limited to ER and HER2 (PR testing was not offered), hence only results of ER and HER2 are available and will be presented.

METHODS

We reviewed the results of all IHC studies for the assessment of breast cancer expression of ER and HER2, performed at the UWI between January 2002 and December 2007. The biopsy specimens were from patients diagnosed at the UHWI as well as cases referred from other hospitals. The samples included breast core biopsies and mastectomy specimens. The IHC breast receptor database was examined to determine the ER/ HER2 receptor profiles for all breast cancer specimens submitted for receptor testing during the study period, to record patient age, tumour histological type and grade, as well as tumour size and lymph node status if nodal sampling was performed.

Protocol for immunostaining for oestrogen receptor and HER-2-Neu

The fixative for all internal and external cases was 10% phosphate-buffered formalin. The most appropriate block for performance of IHC studies was chosen after review of the H&E-stained slides. Paraffin sections were cut at 2-4 microns, mounted on slides coated with poly-L-lysine solution, dried and then deparafinnised in xylene and rehydrated in decreasing strengths of ethanol to deionised water then rinsed in phosphate buffered saline. Blocking of non-specific background binding was carried out using Universal Blocking Reagent. Incubations were performed with primary monoclonal antibodies: Heat Shock Protein 27 (Biogenex, Cat. No, AM171-5M) and

HER-2-Neu (Biogenex, Cat. No. AM 134-5M). Antigen / Antibody interaction was developed using anti-mouse immunoglobulin and conjugated streptavidin as a substrate for HSP and diaminobenzidine for HER-2/Neu. The primary monoclonal antibody was omitted in the negative control and replaced by phosphate buffered saline.

Scoring System

Oestrogen Receptor:

Positivity for oestrogen receptor was scored on a scale of I to III based on the number of cells with bright red cytoplasmic staining as follows: Grade I - few positive cells, Grade II - moderate number of positive cells and Grade III – many positive cells. The absence of any cell staining was interpreted as a negative result.

HER 2- Neu:

HER2 receptor status was tested with the HercepTest®, with scores reported on a scale of 0 to 3+ as follows: Score 0 (no membrane staining, or less than 10%); 1+ (faint membrane staining in more than 10% of tumour cells); 2+ (weak to moderate complete membrane staining in more than 10% of tumour cells); 3+ (strong complete membrane staining in more than 10% of tumour cells) (5). HER2 protein overexpression was assessed as negative for a score of 0 or 1+, and positive for a score of 3+. Scores of 2+ required confirmatory testing with fluorescent in situ hybridization (FISH) for final determination. A positive result was defined as more than six HER2 gene copies per nucleus or a FISH ratio (HER2 gene signals to chromosome 17 signals) of more than 2.2; a negative result was a FISH result of less than four HER2 gene copies per nucleus, or FISH ratio of less than 1.8 (6).

Univariate analyses with descriptive summary statistics are presented for relevant variables. Chi-square tests for association between different receptor profiles and patient age,

tumour grade and tumour stage was done using Stata version 13.0. The UHWI/UWI/Faculty of Medical Sciences Ethics Committee approved this study.

RESULTS

Immunohistochemical studies for the assessment of expression of ER and HER2 were performed on 1,383 breast cancer cases during the period 2002-2007. Table 1 summarizes the clinicopathological data for these patients. Median age was 52 years (range 20-94 years, IQR 43-63). IHC receptor profiles were: ER+/HER2- (50.2%), ER-/ HER2- (28.1%), ER+/HER2+ (15.3%) and ER-/ HER2+ (6.4%) (Table 2). Histopathological staging details, including tumor size and nodal status were available for 860 patients and are presented in Table 3. The majority of tumours were greater than 2cm in size (T2 or greater), and 67% had lymph node metastases. The aggregate pTNM Stage was derived for the 439 cases with both tumor size and nodal status available and was: Stage I (5%), Stage II (50%) and Stage III (45%).

Correlative analysis results are presented in Tables 4-6. Across all age groups, ER+/HER2- was the most frequently seen profile (45-52%), with the ER-/ HER2 - profile being the second most frequently seen (27-34%). There was no statistically significant association between receptor profile and age (Chi-squared test statistic = 15.4453; df = 9; p-value = .079).

Tumour grade was recorded for 658 cases. Amongst Grade III tumours, the most commonly seen receptor profile was ER-/HER2- (44.6%). For both Grade I and Grade II tumors, the ER+/Her2- profile was most common (60.7% and 48.8% respectively). There was a statistically significant association between receptor profile and tumour grade (Chi-squared test statistic = 32.7394; df = 6; p-value < .001).

The majority of ER-/ HER2- tumours were Stage II (51%) or Stage III (44%). Most ER+/ HER2- tumours were also Stage II (53%) or Stage III (41%). There was no statistically significant association between receptor profile and tumour stage (Chi-squared test statistic = 6.6078; df = 6; p-value =.359).

DISCUSSION

This 6-year analysis of breast receptor profiles from our IHC database represents the first study of the IHC receptor profiles of breast cancer from multiple centers across Jamaica. Previous studies of patients with breast cancer treated at the University Hospital of the West Indies (7,8) have demonstrated rates similar to that which we found in this larger multi-center study group, in which cases from the entire island underwent testing at a centralized IHC testing facility. The rate of ER positivity in our study was 66%, compared to 62% (7) and 63% (8) in previous reports, while 22% of cases were HER2 positive, similar to previous reports of 20% HER2 positivity in patients treated at the UHWI (7).

The triple-negative phenotype (ER, PR and HER2 negative) is a surrogate for basal-like breast cancer, and is known to be more aggressive and associated with a relatively poorer prognosis (9). In population-based studies, basal-like breast cancer has been shown to have a higher prevalence in African American compared with non-African American patients (10), comprising 26% and 16% of cases in African American and non–African American women respectively. Stead et al showed a 3-fold higher risk of triple negative breast cancer (TNBC) (95% CI 1.6, 5.5; p = 0.0001) in black compared with white women. In their study, the overall rate of TNBC was 20%; 30% of tumours in black women were triple negative, compared with 11 to 13% of tumours in

other women (11). There have been few studies examining the hormone receptor profile in African patients. In a study of 120 cases out of Kenya, Bird et al noted that 24% had ER- positive tumours, 34% were ER- and/or PR-positive, 10% were ER-negative but PR-positive, and 66% were negative for ER and PR (12).

The Jamaican population is diverse in ethnicity, with a strong Afro-centric background. Based on this we postulated that rates of TNBC may be relatively high in our population. As previously noted, PR testing was not done during the study period, limiting our ability to comment on the prevalence of triple-negative breast cancer (TNBC). Despite this limitation, we found a prevalence of 28% for ER/HER2-negative disease, which is in keeping with TNBC prevalence in the aforementioned North American studies. Despite the African influence on Jamaican ethnic background, it is interesting that our breast cancer hormone expression profile is more similar to that seen in African-Americans in North American studies.

Since the period under study, PR testing has been implemented, in keeping with guideline recommendations that determination of tumor ER/PR and HER2 status should be done on all breast cancers (13). As we continue to add to our database with more complete information on PR, we will re-visit these prevalence rates.

We were unable to show a statistically significant association between receptor profile and patient age, which was anticipated based on the known propensity of basal-like and TNBC for younger age groups (10). Aggressive tumour features such as high histological grade are characteristic of TNBC, and our study showed a significant association between receptor profile and tumour grade (p-value < .001).

HER2-overexpressing breast cancer, accounting for 22% of cases in this study, is a disease-subset associated with more aggressive behaviour, with significantly shortened disease-free

survival and overall survival (14). Recognition of the poor prognosis of HER2-positive breast cancer (15) has driven research and development of several anti-HER2 agents. Trastuzumab, a monoclonal antibody targeting the extracellular domain of HER2 protein, was shown to increase the clinical benefit of first-line chemotherapy in metastatic HER2 over-expressing breast cancer (16). This drug was approved by the US FDA for the adjuvant treatment of HER2-positive breast cancer based on the significant increases in both disease free and overall survival with its addition to standard adjuvant chemotherapy. In the combined analysis of two landmark Phase III trials, the NSABP B-31 and the NCCTG N9831, adding trastuzumab to chemotherapy resulted in a significant improvement in DFS (HR 0.48, 95% CI 0.39-0.59, $p<0.0001$) and a 33 percent reduction in the risk of death ($p=0.015$) (17). Based on our data, trastuzumab therapy may be indicated for up to 22% of our breast cancer patients. In our experience however, only a limited number of eligible patients are able to receive this treatment primarily due to high drug cost. With such a potential gain in survival, a cost-benefit analysis is encouraged to support implementation of a trastuzumab subsidy by the national insurance scheme (National Health Fund).

CONCLUSION

The prevalence of ER-negative/HER2-negative breast cancer in Jamaica (28%) is in keeping with TNBC prevalence in African-American populations in North American studies. Inclusion of PR testing as part of breast IHC receptor testing will help us better define the prevalence of TNBC, and treatment strategies aimed at this aggressive subset of breast cancer must be pursued locally. There was a statistically significant association between receptor profile and tumour grade ($p=<$

.001). Over 20% of breast cancers studies over-expressed HER2, and efforts aimed at increasing accessibility of targeted anti-HER2 therapy are encouraged.

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Table 1: Clinicopathological characteristics of patients with breast cancer IHC testing performed 2002-2007

	Total (n = 1383)
Age:	
Range	20 – 94
Median (IQR)	52 (43 – 63)
Age group:	
40 and under	240 (17.3%)
41-60	730 (52.8%)
61-70	214 (15.5%)
71 and over	199 (14.4%)
Sex:	
Female	1377 (99.6%)
Male	6 (0.4%)
Specimen type:	
Biopsy	708 (51.2%)
Mastectomy	675 (48.8%)
Histology:	
In situ ductal	110 (8.0%)
In situ lobular	4 (0.3%)
Infiltrating ductal	1148 (83.0%)
Infiltrating lobular	108 (7.8%)
Mixed ductal/ lobular	13 (0.9%)
Tumour grade (BRS):	
I	208 (15.1%)
II	486 (35.1%)
III	257 (18.6%)
Not graded	432 (31.2%)

Table 2: Receptor profiles results for breast cancer IHC testing during period 2002-2007

Receptor profile	**No of patients (%)**
ER+/Her2+	212 (15.3%)
ER+/Her2-	694 (50.2%)
ER-/ Her2+	88 (6.4%)
ER-/ Her2-	389 (28.1%)
Total	**1383**

Table 3: Histopathological staging for breast cancer cases with IHC testing (2002-2007)

	n = 860
Tumor size, cm:	
Range	0 – 15
Median (IQR)	3 (2 – 5)
Lymph Nodes examined, No:	
Range	0 – 22
Median (IQR)	4 (0 – 10)
Tumour size (TNM)	
T0	44 (5.1%)
T1	162 (18.8%)
T2	444 (51.6%)
T3	210 (24.4%)
Nodal status (TNM) (n = 458)	
N0	154 (33.6%)
N1	150 (32.8%)
N2	112 (24.5%)
N3	42 (9.2%)
TNM Stage (n = 439)	
I	22 (5.0%)
IIA	111 (25.3%)
IIB	110 (25.1%)
IIIA	154 (35.1%)
IIIC	42 (9.6%)

Table 4: Receptor profile according to patient age

	No of patients within each Age range (%)			
Receptor profile	**40 and Under**	**41-60**	**61-70**	**71 and over**
ER+/Her2+	23 (17.0%)	70 (15.9%)	14 (9.4%)	16 (11.7%)
ER+/Her2-	61 (45.2%)	202 (46.0%)	77 (51.7%)	61 (44.5%)
ER-/ Her2+	14 (10.4%)	35 (8.0%)	8 (5.4%)	5 (3.7%)
ER-/ Her2-	37 (27.4%)	132 (30.1%)	50 (33.6%)	55 (40.1%)
TOTAL	**135**	**439**	**149**	**137**

Chi-squared test statistic = 15.4453; df = 9; p-value = .079

Table 5: Receptor profile according to tumour grade

	No of patients within each Tumour Grade (%)		
Receptor profile	**I**	**II**	**III**
ER+/Her2+	13 (8.4%)	52 (15.9%)	19 (10.9%)
ER+/Her2-	94 (60.7%)	160 (48.8%)	65 (37.1%)
ER-/ Her2+	3 (1.9%)	27 (8.2%)	13 (7.4%)
ER-/ Her2-	45 (29.0%)	89 (27.1%)	78 (44.6%)
TOTAL	**155**	**328**	**175**

Chi-squared test statistic = 32.7394; df = 6; p-value < .001 (excluding "Not Graded")

Table 6: Receptor profile according to tumour stage

	No of patients within each Tumour Stage (%)		
Receptor profile	**I**	**II**	**III**
ER+/Her2+	2 (9.1%)	22 (9.9%)	31 (15.8%)
ER+/Her2-	11 (50.0%)	101 (45.7%)	77 (39.3%)
ER-/ Her2+	0 (---)	17 (7.7%)	18 (9.2%)
ER-/ Her2-	9 (40.9%)	81 (36.7%)	70 (35.7%)
TOTAL	**22**	**221**	**196**

Chi-squared test statistic = 6.6