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Predictive Value of Histologic Characteristics on Hormone Receptor and HER-2 Status of Patients with Invasive Breast Carcinoma, No Special Type, in an Academic Medical Center

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ABSTRACT

Objective. This study aims to assess the predictive value of histologic characteristics in determination of hormone receptor (ER/PR) and HER-2/Neu status in patients with invasive breast carcinoma of no special type (NST).

Methodology. A 4-year review of histopathology and immunohistochemistry reports of women diagnosed with invasive carcinoma NST, was done. Multiple logistic regression was used to determine the association between histologic characteristics and ER and PR status, while multinomial multiple logistic regression was used to determine the association between histologic characteristics and HER-2 status, and that between ER and PR expression, and HER-2 immunoreactivity. All analyses included age, pathologic tumor size, lymph node stage, and lymphovascular space invasion as covariates.

Results. A total of 137 cases were included in the study. Architectural grade is a significant positive predictor of equivocal HER-2 status (P=0.026). Nuclear grade is a significant negative predictor of ER status (P=0.031). Elston score and Nottingham histologic grade showed no significant association with hormone receptor and HER-2 status. ER status demonstrated no significant association with HER-2 expression, but PR status appears to be a significant negative predictor of a strongly positive HER-2 status (P=0.035). Lymph node stage seems to be a significant positive predictor of an equivocal HER-2 status.

Conclusion. Histologic characteristics can predict ER, PR, and HER-2 status, and interactions between expression of these markers provide some insights regarding the complex genetic interactions in the pathogenesis of breast cancer, and its translation into different histologic phenotypes.

Key words: breast carcinoma, histology, immunohistochemistry

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INTRODUCTION

Current practices in the diagnostic workup of breast cancer include histopathologic examination of biopsy and mastectomy specimens, and determination of estrogen receptor (ER), progesterone receptor (PR), and HER-2/Neu (C-erb B2) status via immunohistochemistry (IHC), and Fluorescence in situ hybridization (FISH), as confirmatory test for HER-2 status, should the results be equivocal.[1,2]

Although the use of IHC has been increasing in the Philippines, it is still not widely available, especially in technologically challenged institutions. In some centers where IHC is available, the cost of the test continues to be a major impediment to its use. In both cases, diagnostic workup often stops at routine histopathologic examination. In such cases where IHC could not be performed or could not be availed, there is a pressing need to maximize the utility of the information written on a routine histopathology report, and possibly use it to predict hormonal receptor and HER-2 status in patients with breast cancer.

Response of different grades of breast carcinomas to hormonal therapy has been observed as early as 1950s, demonstrating the possible correlation of tumor grade with the expression of hormone receptors.[3] The increasing use of IHC as part of diagnostic workup of breast cancer paved the way to studying the pattern of hormone receptor and HER-2 expression across histologic grades of breast carcinoma.

In general, low-grade tumors express ER and/or PR, and increasing tumor grade is associated with a negative ER and/or PR phenotype.[4–10] There is conflicting evidence as regards HER-2 immunoreactivity in relation to tumor grade, but high-grade tumors are observed to be associated with HER-2 overexpression.[7] Age seems to influence ER/PR expression, in that younger patients are generally ER/PR negative, and older patients are generally ER/PR positive,[5,7,10] while immunoreactivity to HER-2 appears to decrease with age.[5,10] An inverse relationship seems to exist between ER/PR and HER-2 immunoreactivity.[5,10] Interestingly, there is an apparent association between hormone receptor and HER-2 status and presence of axillary lymph node metastases, in that ER/PR-positive tumors are associated with a negative lymph node status,[8] while HER-2-positive tumors are associated with a positive lymph node status.[5] In the last decade, there is paucity of data regarding the correlation of the components of the Nottingham histologic grading system and hormone receptor and HER-2 expression.

The main objective of this study is to determine if histologic characteristics can predict hormone receptor and HER-2/Neu status of patients with invasive breast carcinoma of no special type (NST) in a local setting. Furthermore, the study aims to determine the relationship between ER, PR, and HER-2 expression in the said histologic type of breast cancer.

METHODOLOGY

This is an observational, analytic, retrospective study approved by the De La Salle Medical and Health Sciences Institute-Center for Clinical Epidemiology and Biostatistics (DLSMHSI-CCEB) on November 14, 2017, and DLSMHSI-Independent Ethics Committee (DLSMHSI-IEC) on January 18, 2018, and conducted at the department of Laboratory Medicine, De La Salle University Medical Center (DLSUMC), from October 9, 2017 to June 25, 2018.

Female patients who underwent modified radical mastectomy in DLSUMC within the January 1, 2014 to December 31, 2017 with a final histopathologic diagnosis of invasive ductal carcinoma or invasive carcinoma, NST were included in the study; provided that they met the following inclusion criteria: 1. the invasive carcinoma must not have a mixed component (e.g. invasive carcinoma with mucinous component, mixed invasive ductal and lobular carcinoma); 2. there must be no other malignant lesions or pathology indicating the presence of such malignant lesions, accompanying the invasive carcinoma (e.g. ductal and/or lobular carcinoma in situ, Paget disease of the nipple); the presence of benign lesions (e.g. fibrocystic changes, intraductal hyperplasia, and fibroadenoma) does not preclude inclusion in the study; and 3. the patient must have undergone IHC for ER, PR, and HER-2 in DLSUMC, using samples from core needle biopsy, excision biopsy, or sections of the tumor from modified radical mastectomy; the aforementioned procedures should also have been performed in DLSUMC. The last criterion was included to ensure adherence to the preanalytic guidelines of handling breast specimens stated in the ASCO/CAP guidelines for IHC testing of ER, PR, and HER-2 for breast cancer.[1,2] (See Appendix)

A minimum total sample size of 121 was computed using the method described by Peduzzi et al.,[11] with K as the maximum number of predictor variables included in the analysis (K = 7), and p as the smallest among the proportions of ER- and PR-positive cases (0.68 and 0.58, respectively), and HER-2-negative cases (0.64). Values of p were taken from literature.[12]

Histopathology report forms of the patients included in the study were reviewed, and the Nottingham histologic grade, Elston-Ellis score, and its components were noted. Four trained pathologists assessed the histologic characteristics of all cases using the Nottingham histologic grading system.[13] Primary tumor (pT) and lymph node stage (pN) were determined using the AJCC 8th edition cancer staging manual for breast cancer.[14]

IHC was performed, following epitope retrieval, with a polymer based detection system (EnVision+, Dako, Carpinteria, CA) using monoclonal rabbit antibodies for ER-α (Clone EP1, Ready-to-Use (RTU)), monoclonal mouse antibodies for PR (Clone PgR 636, RTU) (Dako, Carpinteria, CA), and Herceptin kit (HercepTest, Dako, Carpinteria, CA). IHC reports of included patients were reviewed, and the ER, PR, and HER-2 status were noted. A trained pathologist assessed all the cases following the ASCO/CAP guidelines for IHC testing of ER, PR, and HER-2 for breast cancer.[1,2]

Multivariate logistic regression analysis was used to assess the effect of histologic characteristics on ER and PR status, with the age, lymphovascular space invasion, T, and N as covariates. Multivariate multinomial logistic regression analysis was employed to assess the effect of histologic characteristics and covariates on HER-2 status. Multivariate multinomial logistic regression was used to determine the relationship between HER-2 and ER/PR status; the significant covariates that were included in the model are: age, lymphovascular space invasion, Elston-Ellis score, pT, and pN. Statistical analysis was performed at 95% level of significance, using STATA 14.2 (College Station, Texas, USA).

RESULTS

A total of 137 cases of women diagnosed with invasive carcinoma, no special type (NST), were included in the study. Table 1 summarizes the pertinent characteristics of the included cases. The mean age of patients with invasive carcinoma NST is 55.11 years. Most of the cases are Nottingham histologic grade 2 (moderately differentiated), and have an Elston-Ellis score of 6. In terms of histologic characteristics, majority of the cases have an architectural

Table 1. Patient characteristics in the study

Patient characteristics	Mean	SD
Age (years)	55.11	11.55
Nottingham histologic grade[a]	n	%
1	24	17.52
2	105	76.64
3	8	5.84
Lymphovascular invasion		
Negative	120	87.59
Positive	17	12.41
Elston score		
3	1	0.75
4	3	2.24
5	20	14.93
6	82	61.19
7	20	14.93
8	6	4.48
9	2	1.49
Histologic characteristics[a]		
Architectural grade		
1	2	1.49
2	28	20.90
3	104	77.61
Nuclear pleomorphism		
1	4	2.99
2	113	84.33
3	17	12.69
Mitotic count		
1	115	85.82
2	10	7.46
3	9	6.72
Pathologic stage[b]		
Tumor size		
T1	13	9.56
T2	84	61.76
T3	26	19.12
T4	13	9.56
Lymph node stage		
N0	33	24.09
N1	29	21.17
N2	58	42.34
N3	17	12.41
Immunohistochemical phenotype[c]		
ER		
Negative	35	25.55
Positive	102	74.45
PR		
Negative	43	31.39
Positive	94	68.61
HER-2		
Negative	82	59.85
Equivocal	28	20.44
Strongly positive	27	19.71

a - Classification as per Elston and Ellis.[13]
b - Classification as per AJCC 8th edition Cancer staging manual for breast cancer.[14]
c - Classification as per American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines.[1,2]

grade of 3, nuclear score of 2, and mitotic count score of 1. As per pathologic stage, most of the cases are T2 and N2. In terms of immunohistochemical phenotype, majority of the cases are ER-positive, PR-positive, and HER-2-negative.

Multiple logistic regression was performed to determine the effect of histologic characteristics on ER and PR status, while multinomial multiple logistic regression was used to determine the effect of the said predictors on HER-2 expression. The regression coefficients and the pertinent statistics are shown in Table 2. Architectural grade demonstrated no significant effect on ER and PR status; however, it appears to be a significant positive predictor of HER-2 immunoreactivity, although only at the equivocal level ($P=0.026$). Nuclear pleomorphism is a significant negative predictor of ER immunoreactivity ($P=0.031$). Mitotic count is not a predictor of hormone receptor and HER-2 status.

Similar analyses were employed to identify the effect of Elston-Ellis score and Nottingham histologic grade on hormone receptor and HER-2 status. The regression coefficients and pertinent statistics are summarized in Table 3. Elston-Ellis score and Nottingham histologic grade are not predictors of hormone receptor and HER-2 immunoreactivity.

Multinomial logistic regression was performed to assess the effect of ER and PR status on HER-2 expression. The regression coefficients and pertinent statistics are shown in Table 4. ER immunoreactivity is not a predictor of HER-2 status, while PR immunoreactivity appears to be a significant negative predictor of a strongly positive HER-2 status ($P=0.035$).

Lymph node stage, while included in the models as covariate, appears to be a significant positive predictor of HER-2 immunoreactivity, albeit at an equivocal level. The regression coefficients and pertinent statistics including P values, where lymph node stage has served as covariate for each model are summarized in Table 5.

DISCUSSION

The Nottingham histologic grading system accounts three histologic characteristics – architectural grade, nuclear pleomorphism, and mitotic count, to classify breast cancers as to three histologic grades. The study suggests that increasing nuclear grade is associated with a negative ER status, and increasing architectural grade is associated with HER-2 expression. There are limited studies correlating the components of the Elston score with hormone receptor status. Increasing nuclear grade points to a more anaplastic morphology and indicates that a tumor is actively dividing. There is less time to assume the normal phenotype of mammary ductal epithelial cells to express steroid hormone receptors. Also, ER-independent breast cancers usually rely on other genetic mechanisms for growth, and are associated with high-grade histology; and these may be possible explanations behind the association.[15] There is also scarce data as regards correlation between the components of the Elston score and HER-2 expression. Increasing architectural grade indicates rapid division of cells that result in formation of tumor nests, clusters, and sheets, rather than formation of tubular structures characteristic of normal mammary ductal epithelial cells. HER-2 is an oncogene that drives cellular proliferation, and HER-2-enriched breast cancers are usually high-grade.[15,16] The nature of HER-2 as a driver of cellular proliferation may explain the correlation. In a limited-resource setting, breast cancer patients with high nuclear and architectural grade on routine histology, should prioritize determination of hormone receptor and HER-2 status via IHC, because there is high likelihood of a negative ER phenotype and HER-2 expression in these cancers, which determines treatment options for these patients.

Table 2. Effect of histologic characteristics on hormone receptor and HER-2 immunoreactivity[a]

Histologic characteristic	Marker		Coef	SE	z	P	95% CI	
Architectural grade	ER		-0.755	0.588	-1.280	0.199	-1.906	0.397
	PR		-0.683	0.500	-1.370	0.172	-1.664	0.297
	HER-2[b]	Equivocal	**1.807**	**0.812**	**2.220**	**0.026**	**0.215**	**3.399**
		Positive	0.522	0.550	0.950	0.342	-0.555	1.600
Nuclear pleomorphism	ER		**-1.150**	**0.532**	**-2.160**	**0.031**	**-2.193**	**-0.108**
	PR		-0.996	0.523	-1.910	0.057	-2.020	0.028
	HER-2[b]	Equivocal	-0.864	0.706	-1.220	0.221	-2.248	0.520
		Positive	-0.562	0.640	-0.880	0.380	-1.817	0.693
Mitotic count	ER		0.248	0.427	0.580	0.562	-0.590	1.085
	PR		0.415	0.430	0.970	0.335	-0.428	1.257
	HER-2[b]	Equivocal	-0.151	0.498	-0.300	0.761	-1.126	0.824
		Positive	-0.120	0.444	-0.270	0.787	-0.991	0.751

a - Logistic regression model includes age, lymphovascular space invasion, tumor size, and lymph node stage as covariates
b - Multinomial logistic regression model has negative HER-2 immunoreactivity as base outcome

Table 3. Effect of elston score and overall histologic grade on hormone receptor and HER-2 immunoreactivity[a]

Parameter	Marker		Coef	SE	z	P	95% CI	
Elston-Ellis score	ER		-0.406	0.250	-1.620	0.105	-0.896	0.085
	PR		-0.314	0.231	-1.360	0.174	-0.768	0.139
	HER-2[b]	Equivocal	0.143	0.281	0.510	0.612	-0.408	0.693
		Positive	-0.030	0.277	-0.110	0.915	-0.573	0.514
Nottingham histologic grade	ER		-0.282	0.473	-0.590	0.552	-1.209	0.646
	PR		-0.214	0.430	-0.500	0.618	-1.058	0.629
	HER-2[b]	Equivocal	0.988	0.594	1.660	0.096	-0.177	2.153
		Positive	0.138	0.258	0.530	0.593	-0.368	0.644

a - Logistic regression model includes age, lymphovascular space invasion, tumor size, and lymph node stage as covariates
b - Multinomial logistic regression model has negative HER-2 immunoreactivity as base outcome

Table 4. Effect of hormone receptor immunoreactivity on HER-2 immunoreactivity[a]

Marker	HER2	Coef	SE	z	P	95% CI	
ER	Equivocal	0.586	0.627	0.940	0.350	-0.642	1.815
	Positive	-0.884	0.500	-1.770	0.077	-1.864	0.097
PR	Equivocal	0.182	0.548	0.330	0.740	-0.891	1.255
	Positive	-1.021	0.484	-2.110	0.035	-1.970	-0.072

a - Multinomial logistic regression model includes age, lymphovascular space invasion, tumor size, and lymph node stage as covariates, and has negative HER-2 immunoreactivity as base outcome

Table 5. Effect of lymph node stage on equivocal HER-2 immunoreactivity

Predictor variable[a]	Coef	SE	z	P	95% CI	
Histologic characteristics	0.554	0.253	2.190	0.028	0.058	1.049
Elston score	0.519	0.242	2.140	0.032	0.044	0.994
Overall histologic grade	0.539	0.241	2.230	0.026	0.066	1.012
ER immunoreactivity	0.520	0.245	2.120	0.034	0.040	1.001
PR immunoreactivity	0.508	0.245	2.080	0.038	0.029	0.988

a - Model includes architectural grade, nuclear pleomorphism, and mitotic count as principal predictor variables, and includes age, lymphovascular space invasion, tumor size, and lymph node stage as covariates.

The results of the study show that Elston score and Nottingham histologic grade do not predict hormone receptor and HER-2 status, which is conflicting with studies done previously.[4–10] In an attempt to eliminate complex interactions between variables, a simple logistic regression model that includes only the dependent variable (ER, PR, and HER-2 status) and the main predictor variable (Elston score and Nottingham histologic grade) was run, and results were inconsistent (not shown). The association may become apparent with improved statistical power. The findings demonstrate the importance of IHC for ER, PR, and HER-2 in managing patients with breast cancer, since Elston score and histologic grade cannot predict such. In a limited-resource setting, clinicians must always advise patients to allot funds for these tests in order to determine amenability to hormonal and anti-HER-2 drugs, and to properly prognosticate their patients.

The study suggests that PR expression is associated with a negative HER-2 status. A similar relationship is expected for ER expression, albeit the model showed otherwise. A simple model including HER-2 as dependent variable, and ER as main predictor variable was run, and it showed that ER expression is also a negative predictor of a strong HER-2 status ($P=0.037$). The association may become apparent with improved statistical power. This finding is congruent with that of studies done previously.[5,10] The pathogenesis of breast cancer is complex, but current evidence suggests that ER-positive breast cancers harbor distinct genetic abnormalities (16q deletions and 1q gains) that are generally not observed in ER-negative breast cancers, implying that the molecular pathogenesis of ER-positive breast cancers is different from ER-negative breast cancers. As previously mentioned, ER-independent breast cancers rely on different genetic mechanisms for growth; one of these being HER-2.[15] This may explain the

inverse relationship between ER and HER-2 expression. PR expression can be regulated by estrogen bound to ER or by estrogen-independent mechanisms, and the estrogen-ER-dependent expression of PR may explain the same inverse relationship between PR and HER-2.[17] Considering the results of the study, PR may be dropped from the usual IHC panel of breast cancer in a limited-resource setting, but there is evidence that ER(+), PR(-) breast cancers tend to respond poorly to Tamoxifen than those that are ER(+), PR(+).[16] Such a finding underscores the importance of PR status in management of patients with breast cancer, and it is optimal to determine the status of all three markers in all breast cancer patients, if possible.

Interestingly, the results of the study show that increasing lymph node stage is an independent predictor of HER-2 expression, which is consistent with the findings of a previous study.[5] Breast cancers expressing HER-2 are associated with high-grade histology, aggressive clinical behavior, and decreased survival, which may be attributed to the nature of HER-2 as a potent driver of cellular proliferation.[16] While this study demonstrates that advancing lymph node stage is predictive of HER-2 expression, in practice, clinicians still need to advise patients as regards the importance of HER-2 testing via IHC and its confirmation via FISH, to determine amenability to treatment with Trastuzumab, which is an expensive drug.

The study is time-bound, which limits the number of cases included in the study, which, in turn limits statistical power. Extension of the duration of the study includes more patients and may improve statistical power, which may make some relationships apparent. Another limitation is the complexity of analysis of HER-2 because of three outcomes that can be dichotomized with confirmatory testing via FISH, which is not available in our institution. The possibility of discordance in histopathology and IHC of core needle biopsy and mastectomy specimens may also be a possible limitation. The unavailability of IHC for both core needle biopsy and mastectomy specimens is secondary to variations in clinical practice in consideration of patient-related factors. Nevertheless, one recent study demonstrated that histopathology and core needle biopsy IHC were known to have high concordance rates with those of mastectomy specimens.[18] In order to minimize its potential effect, in future studies, we recommend doing IHC for both core needle biopsy and mastectomy specimens if patient and institutional factors permit.

CONCLUSION

The findings of this study demonstrate the predictive value of histologic characteristics on hormone receptor and HER-2 status in breast cancers, as well as the relationship between expression of hormone receptors and HER-2. These gave insights as regards the complex genetic mechanisms that are responsible in the development of breast carcinoma, and their influence on its histology. Because of the current limitations of this study, we still recommend that all three markers should be assessed in all breast cancer patients, even in a limited-resource setting, to optimize prognostication and management, and to properly channel the patient's limited funds to more appropriate diagnostic and therapeutic procedures.

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STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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APPENDIX

Preanalytic guidelines in handling breast specimens for IHC testing of ER, PR, and HER2 for breast cancer (adapted from ASCO/CAP guideline recommendations, 2010 and 2014)[1,2]

1. The time from tumor removal to fixation (cold ischemia time) should be kept to 1 hour or less.
2. The ideal fixative to be used is 10% neutral buffered formalin (NBF). The specimen should be fixed with an adequate volume of fixative (i.e. at least ten-fold greater than specimen volume).
3. The time of tissue fixation should be at least 6 hours but no greater than 72 hours.
4. Sections made more than 6 weeks are not recommended for HER-2 analysis.