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Immunohistochemical and clinicopathologic features of estrogen receptor-negative, progesterone receptor-positive, HER-2 negative breast carcinomas

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SUMMARY

OBJECTIVE: Currently, there is an ongoing debate whether progesterone receptor positive and estrogen receptor negative breast carcinomas represent a true distinct subtype of tumor or a mere immunohistochemical artifact. In this study, we conducted an immunohistochemistry panel with the antibodies TFF1, EGFR, and CK5 to reclassify this phenotype in a luminal or basal-like subtype.

METHODS: Tumors estrogen receptor -/progesterone receptor +, Her-2 – from a large population of breast cancer patients were selected to be studied. Immunohistochemistry with the antibodies TFF1, EGFR, and CK5 was performed. Tumors showing positivity for TFF1, regardless of EGFR and CK5 results, were classified as luminal-like carcinomas. Those lesions that were negative for TFF1, but were positive for EGFR and/or CK5, were classified as basal-like triple-negative carcinomas. When the three markers were negative, tumors were classified as undetermined. Clinical pathologic characteristics of patients and tumor recurrence were evaluated.

RESULTS: Out of 1188 breast carcinomas investigated, 30 cases (2.5%) presented the estrogen receptor -/progesterone receptor +/HER2- phenotype. Of them, 27 tumors (90%) were classified as basal-like triple-negative carcinomas, one as luminal-like (3.3%), and two as undetermined tumors (6.7%). The mean follow-up for the study group was 27.7 (2.7 to 50) months. Out of the 26 patients, 6 had cancer recurrence: 2 local and 4 systemic recurrences. The average time for recurrence was 17 (8 to 38) months.

CONCLUSION: Estrogen receptor -/progesterone receptor +/tumors exhibit aggressive behavior, similar to triple-negative tumors. An appropriate categorization of these tumors should be made to improve their therapeutic management.

KEYWORDS: Breast neoplasms. Immunohistochemistry. Receptors, estrogen. Receptors, progesterone. Carcinoma, basal cell.

INTRODUCTION

Breast cancers are currently classified into different subtypes based on gene expression signatures, but gene profiling still has a limited role in clinical practice[1]. Immunohistochemical surrogate markers have been used, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), to identify tumors with distinct clinicopathological characteristics, therapeutic responsiveness, and oncological outcomes[1-4].

ER is a well-defined prognostic factor, and its expression is related to a higher chance of a favorable response to anti-estrogen hormonal therapy. In contrast, the role of PR as a prognostic factor is still unknown. PR is positively regulated by the ER, and the presence of PR indicates a more intact ER pathway.

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Tumors with ER expression are classified as luminal-like and have a better prognosis than tumors without ER expression, such as pure HER2 positive and triple-negative tumors[4,5].

The existence of the ER-/PR+ carcinomas remains under debate between authors[6,7]. This subtype represents 3.4 to 7% of all cases. Some suggest this phenotype does not exist and may merely represent technical artifacts, resulting from a failure of the IHC[6]. In contrast, others support that ER-/PR+ tumors, although rare, represent a true distinct phenotype, with more aggressive biological behavior and worse oncological prognosis than double-hormone receptor-positive carcinomas[7]. A study conducted by Itoh et al. demonstrated that 65% of ER-negative and PR-positive tumors were really basal triple-negative, and only 20% were luminal according to genetic expression[8].

More recently, Yu et al.[9] also evaluated the molecular essence and clinical characteristics of ER–/PR+/HER2 negative breast tumors. They revealed that these tumors' clinicopathologic features and survival outcomes fell in between ER+/PR+ and ER–/PR– phenotypes, being similar to the ER–/PR– phenotype. Among the ER–/PR+ tumors, 30% were luminal-like, and 60% were basal-like carcinomas (BLC). For the first time in this subtype, the authors performed an immunohistochemical analysis combining three markers: trefoil factor 1 (TFF1), EGFR, and CK5. They demonstrated that this immunohistochemical method could be used as a surrogate of genetic testing.

These three markers have already been used to categorize basal-like breast tumors, with an IHC sensitivity of 76% and specificity of 100%[10,11]. TFF1 immunohistochemical expression of 10% or more is significantly associated with a luminal-like molecular profile, whereas CK5 and/or EGFR expression was associated with the basal-like subtype[9]. TFF1 is an estrogen-dependent protein, and its expression in breast tumor cells is related to ER expression and response to hormone therapy[12]. EGFR (or HER1) is a member of the family of transmembrane receptors of the epidermal growth factor, and it is involved in several cellular functions such as proliferation, differentiation, motility, survival, and tissue development. CK5 is part of the high molecular weight cytokeratins found in basal breast cells[13].

The present study is the first to reproduce the immunohistochemical panel proposed by Yu et al.[9] to determined IHC and clinicopathologic features of ER–/PR+/HER2– tumors in a large population of breast cancer patients.

METHODS

We performed a cross-sectional study, including all cases with histopathologic diagnosis of invasive breast carcinoma managed at our institution between 2012 and 2016. Tumors with the phenotype ER-/PR+/HER2 – were selected to be submitted to additional IHC for the proteins TFF1(pS2), CK5, and EGFR.

Immunohistochemical reactions were performed on the automation platform Benchmark® ULTRA (Ventana Medical Systems, Tucson, Arizona). The samples were cut into 3µm, and each slide received a positive control. Deparaffinization was performed with the equipment using reagent EZ PREP. The antigenic recovery was made with CC1 cap (cell conditioning 1), alkaline pH, for 64 minutes, at 95°C. The blocking of peroxidase was accomplished using reagent Ultra View Universal DAB Inhibitor, from the detection system.

The sections were incubated for 4 minutes at 36°C in the ready-to-use anti-PR monoclonal antibody (clone 1E2, Ventana). In the ready-to-use anti-ER antibody (clone SP1, Roche), the incubation occurred for 16 minutes at 37°C. The cuts remained for 32 minutes at 37°C in the TFF1 antibody (clone EPR3972, Abcam) in a 1:800 dilution. As for the CK5 antibody (clone EP1601Y, Cell Marque) in a dilution of 1:50, it stayed for 32 minutes at 42°C, and in the ready-to-use EGFR antibody (clone 5B7, Roche), the slides remained for 16 minutes at 37°C.

Reactions were detected with the Ultra View Universal DAB detection kit, using the diaminobenzidine (DAB) chromogen from the kit. The slides were counterstained with Mayer hematoxylin, differentiated with bluing reagent (Li2CO3+Na2CO3), and examined after dehydration and assembly.

In ER-/PR+ tumors, the nuclear staining was considered positive for ER and/or PR in at least 1% of tumor cells of any intensity[14]. The cut-off point for TFF1 positivity was 10% of staining observed in the neoplastic cellular cytoplasm, as described by Yu et al.[9]. The reaction was considered positive for CK5 and EGFR if ≥1% weak, moderate, or strong staining on the cell membrane and/or cytoplasm was observed[13]. The reading of the slides was made by a pathologist specialized in breast pathology. Tumors that were positive for TFF1, regardless of EGFR and CK5 results, were classified as luminal-like. Those without positive staining for TFF1, but showing positivity for EGFR and/or CK5, were considered triple negative BLC. When the three markers were negative, the results were classified as undetermined.

Clinicopathological features, such as age, race, histological type, tumor grade, lymphovascular invasion, tumor size, axillary involvement, percentage of Ki67, type of treatment, and disease-free survival were correlated with tumor subtype. The study began after the approval of the Committee of Ethics in Research from the HCPA (Approval Number: 1464984 - Date of approval: 03/26/2016).

The quantitative variables were described by the mean and standard deviation or median and range between percentiles, and categorical variables by absolute and relative frequencies. The relation between categorical variables was assessed with Fisher's exact test. Medians were compared using the Mann-Whitney test. To assess the association between numeric and ordinal variables, Spearman's correlation test was applied. Recurrence-free survival curves were estimated with the Kaplan-Meier method. Poisson regression analysis was applied to control confounding factors. Relative Risk (RR) and a confidence interval of 95% were used as the effect measure. The significance level adopted was 5% ($p \leq 0.05$), and the analyses were run in the SPSS program version 21.0.

RESULTS

Out of a total of 1188 breast carcinoma, 38 (3.19%) presented the ER-/PR+/HER2 – phenotype. Eight cases were excluded due to the unavailability of paraffin blocks for IHC. The 30 (2.5%) remaining available cases were submitted to additional IHC for the proteins TFF1 (pS2), CK5, and EGRF.

After analyses, 27 cases (90%) of the ER–/PgR+/HER2– tumors were classified as BLC, one as luminal-like (3.3%), and two as undetermined carcinomas (6.7%). Among the BLC cases, EGFR was positive in 24 (88.9%), and CK was positive in three (11.1%) cases. There was only a case considered positive for TFF1 with also presented positivity for EGFR (85% weak) and negativity for CK5. Two cases were negative for all three markers.

The patients' clinicopathological characteristics and the treatment regimen patients underwent are shown in Table 1 and 2, respectively.

The mean follow-up of the study group was 27.7 (2.7 to 50) months. Two patients lost follow-up right after diagnosis, and two lost follow-up at 2.7 and 7 months, respectively, with no tumor recurrence observed. Out of the 26 patients remaining, six had cancer recurrence: two local and four systemic recurrences. The average time for recurrence was 17 (8 to 38) months. The probability of disease-free survival at 3.1 years was 64%. Three (7.4%) patients died during the follow-up, two deaths were related to breast cancer recurrence.

The recurrences were correlated with different breast cancer subtypes. Two cases classified as inconclusive, presented systemic recurrences (RR 6,9; CI95% 2.4–14; p=0.046). Positivity for EGFR and CK5 was not related to prognosis (p=0.87 and p=0.64, respectively). Patients with tumors showing a higher number of cells with positive staining for EGFR and CK5 presented more recurrence than tumors without EGFR or CHK5 staining, but these data were not statistically significant (p=0.1 and p=0.058 respectively).

Table 1. Clinicopathological characteristics of the patients.

Characteristics	n=30
Age (years) – mean ± SD	56.7±12.5
Tumor Size (cm) – median	2,4 (1.8–3.8)
Lymph nodes	
N0 (%)	16 (57.1)
N1(%)	8 (25)
N2(%)	4 (10.7)
N3(%)	2 (7.1)
Histological subtype	
Invasive ductal carcinoma (%)	27 (83.3)
Inflamatory (%)	2 (6.7)
Medullar (%)	2 (6.7)
Metaplastic (%)	1 (3.3)
Lymphovascular invasion	
Yes (%)	12 (40)
No (%)	18 (60)
Histological grade	
2 (%)	11 (36.7)
3 (%)	19 (63.3)
Ki-67 (%)	
<14	1 (3.3)
≥14	29 (96.7)

SD: standard deviation.

Recurrence were no statistically associate with age (p=0.18), tumor size (p=0.35), lymph node involvement (p=0.61), tumor grade (p=1.00) and lymphovascular invasion (ILV) (p=0.16). Like radiotherapy, hormone therapy, and chemotherapy, none therapy modality was associated with recurrence (p>0.4).

A higher rate of complete pathologic response was observed in the basal-like subtype (44.4%).

DISCUSSION

We analyzed a large series of invasive breast carcinoma and identified 3.2% of the tumor phenotype ER-/PR+/HER2-. Although this subtype is rare, our results are consistent with other literature data, which described frequencies ranging from 1 to 5%[6-18].

Our study is the first to reproduce the immunohistochemical panel using TFF1, EGFR, and CK5 antibodies proposed by Yu et al.[9] In line with those authors, we identified the basal-like phenotype in most ER-/PR+/HER2- tumors. However, our

Table 2. Types of treatment.

Treatment	n=30 (%)
Type of surgery (n=29) – n(%)	
n°	3 (10.3)
BCS + SLN	9 (31.0)
BCS + ALND	6 (20.7)
Mastectomy + SLN	5 (17.3)
Mastectomy + ALND	6 (20.7)
Radiotherapy (n=28) – n(%)	
Yes	23 (82.1)
No	5 (17.9)
Chemotherapy (n=28) – n(%)	
Yes	20 (71.4)
Adjuvant	8 (40)
Neoadjuvant	11 (55)
Palliative	1 (5)
No	8 (28.6)
pCR (n=11) – n (%)	
Yes	4 (36.4)
No	7 (63.4)
Hormonal Therapy (n=28) – n (%)	
Yes	17 (60.7)
Tamoxifen	9 (52,9)
Anastrozole	8 (47.1)
No	11 (39.3)

SD: standard deviation; P: percentile; IDC: invasive ductal carcinoma; BCS: breast-conserving surgery; SLN: sentinel lymph node; ALND: axillary lymph node D: dissection; pCR: pathological complete response.

prevalence of BLC (90%) was even higher than previously reported (60%). This difference probably reflects biological variations in breast cancer between distinct populations[19].

It has been demonstrated that EGFR and CK5 had good accuracy in classifying triple-negative tumors as basal-like tumors[11]. The third antibody of the IHC panel was TFF1. It is an estrogen-sensitivity marker and, whenever positive, indicates that hormone therapy can be prescribed as effective adjuvant therapy[12]. Although only one tumor was positive for TFF1 in our series, 60% of our patients received either tamoxifen or anastrozole as part of their treatment. Therefore, the utility of these compounds in this specific clinical situation might be called into question and deserves further investigation. Almost 89.0% of the tumors were positive for EGFR, and more than half (55.6%) were positive for CK5. Previous studies show that both EGFR and CK5 are factors for poor prognosis[20,21]. In our study, patients with early disease recurrence had tumors with high expression of EGFR and CK5; however, the association was not statistically significant (p=0.4).

Basal-like carcinomas are known to be a very aggressive subgroup of tumors. They tend to develop distant metastases, particularly within the first five years after disease diagnosis, associated with poor oncological prognosis and high mortality[10]. Despite the limited length of follow-up in our study, cancer recurrence was detected in up to 23% of our patients, confirming the aggressive biological behavior of the ER-/PR+/HER2- tumors.

Our data seems to be following the study conducted by Fan et al.[16] They reviewed 3,966 breast cancers operated in China, between January 2005 to May 2008, finding 240 (6%) cases of ER-/PgR+/HER2- and 348 (8.8%) cases of triple-negative tumors. Although ER-/PgR+/HER2-carcinomas had a smaller tumor size (p=0.036) than triple-negative carcinomas, no significant differences were found between the two tumor groups in terms of relapse-free survival and overall survival. The authors concluded that ER-/PgR+/HER2- tumors should be regarded as a biologically and clinically distinct group of breast cancers, presenting an aggressive biological behavior.

Out of the 27 basal-like cases, 24 were invasive ductal carcinomas, two were medullar carcinomas, and one was metaplastic. The most common histological type in BLC is invasive ductal carcinoma, medullary, and metaplastic carcinomas[22,23]. All cases presented very high Ki67 values, with only one case presenting a <14% value. Sixty percent of cases presented grade 3, and the remainder were classified as grade 2 (40%), which reflected the association of BLC with high grade and high mitotic index tumors[10]. Lymph node involvement was identified in 38.5% of the cases, and tumor size had a median of 2.3 cm (0.6 to 5.8 cm), similar to a study conducted in 2017 by Li et al.[24], that demonstrated an association between advanced cancer stages and BLC tumors.

In our study, we identified a pCR rate of 44.4% in patients with the basal-like profile. Tumors with basal-like phenotype showed higher rates of pCR when compared to tumors with luminal phenotype. Chou et al.[25] demonstrated a response rate of 78% to chemotherapy with anthracyclines and taxanes in triple-negative breast carcinomas and a higher rate of pCR related with this subtype compared to luminal (45% versus 8%, respectively).

The median time for disease recurrence in our study was 17.8 months. Early disease recurrences are frequently observed in patients with tumors with the BLC phenotype. Li et al. described early disease recurrence, between one to three years after diagnosis, in patients with triple-negative tumors[25]. Also, the

rate of disease-free survival (64% in 3.1 years) was similar to the rate described in previous studies for basal-like tumors[22,24].

CONCLUSIONS

In conclusion, we showed that most ER-/PR+/HER2 breast tumors, which represent a rare and frequently misdiagnosed phenotype, can be classified as BLC. Further studies are warranted to elucidate the role of IHC in the management of this subgroup of tumors.

AUTHORS' CONTRIBUTIONS

RPN: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **DU:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **APD:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **JVB:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **G.R:** Writing – Review & Editing.

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