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Androgen Receptor as Prognostic Marker in Triple-Negative Breast Cancer Patients

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Abstract

Purpose: The purpose of this study was to assess the prognostic impact of androgen receptor (AR) expression in patients with triple-negative breast cancer (TNBC).

Methods/patients: 101 patients treated for primary TNBC without distant metastasis from 1999 to 2015 were identified from breast surgery database. Kaplan-Meier and Cox regression models evaluated disease-free survival (DFS) and overall survival (OS).

Results: AR expression was positive (IHC>1%) in 40% of patients. OS at 36 and 60 months was 86% and 80% in AR-negative patients, and 100% and 96% in AR-positive patients (log rank test 0.036). DFS at 36 and 60 months was 78% and 68% in AR-negative and 92% and 89% in AR-positive (log rank test 0.075).

Conclusions: Patients without AR expression have a significant correlation with poor outcome.

Keywords: Androgen receptor; Triple-negative breast cancer; Prognosis

Introduction

Breast cancer, the most common malignant disease among women in Spain [1], is a highly heterogeneous disease, classified into groups defined by molecular features with clear prognostic impact. Expression of estrogen receptor (ER), progesterone receptor (PR) and human epithelial growth factor receptor 2 (HER2) have been well established in multiple studies as predictive and/or prognostic markers, having led to a major shift in treatment approach.

Triple negative breast cancer (TNBC), a subgroup of breast cancer defined by absence of expression of ER, PR, and HER2, accounts for approximately 10% to 24% of all breast cancer. TNBC is associated with a younger age, advanced stage at diagnosis, high mitotic index, and BRCA1 mutations. TNBC is also associated with a more aggressive clinical behavior and poor prognosis. This kind of breast tumors shows higher risk of recurrence and death. The highest probability of relapse occurs in the first 5 years from the start of treatment and unlike luminal tumors, the incidence of recurrence decreases from this limit. The only systemic treatment approved in this type of tumors is chemotherapy, since therapeutic targets such as HER2 and hormone receptors are lacking.

In itself, TNBC is a heterogeneous disease since it includes a variety of diverse histologies (squamous, condroid, adenoid cystic carcinomas and secretory carcinomas) and a substantial proportion of ductal carcinomas with different molecular subtypes. Lehmann et al. were one of the first groups to use gene expression profiling to subclassify TNBC, identifying at least six different tumor molecular subtypes: basal-like types 1 and 2, immunomodulatory, mesenchymal, mesenchymal stem-like, and a luminal androgen receptor subtype. The last one appears to show a preserved androgenic signaling that could inhibit the progression of breast cancer. Although the precise mechanism remains unclear, some reports have suggested the use of AR as a possible molecular target, similar to ER expression in luminal breast tumors [2-7].

Two types of mammary epithelial cells express AR. Metaplastic apocrine cells and 5 to 30% of normal epithelial cells of both terminal duct lobular units and larger ducts, in the same way that they express ER and PR. In fact, AR is frequently coexpressed with ER, PR and/or HER2 (47-90%) among all types of breast cancer, with a frequency of 10% to 75% among TNBC (Figure 1) [2,6].

There have been several reports about the clinical significance of AR in TNBC, most of them having found a favorable prognosis with non-aggressive behavior (lower grade, lower mitotic score, less frequent metastasis and tumor recurrence) [8-13], but this observation is not uniform across the literature and controversies still exist. In some studies AR expression does not predict survival and in others AR expression even has been associated with worse OS, even though without significant effect on DFS [5,6,14-17].

The purpose of this study was to analyse the role of AR expression in patients with TNBC, and to demonstrate its prognostic relevance.

Materials and Methods

This was a retrospective cohort study carried out at Hospital Universitario Fundación Alcorcón (HUFA) (Madrid, Spain), a 448-bed facility with a defined geographical region of 33.7 km, and a 170,336 (2014) population of influence.

We examined data of electronic medical record files from 1547 women with newly diagnosed stage I to III breast cancer according to the 7th edition of the UICC/AJCC TNM (tumor, node, metastasis), between January 1999 and December 2015. From them, 119 cases were confirmed as TNBC: absence of ER and PR expression by immunohistochemistry (IHC), and HER2 staining of 0 or 1+ by IHC, and 2+ score with no gene amplification verified by fluorescence *in situ* hybridization (FISH). Cases clinically identified as metastatic disease were excluded, also 2 patients who refused surgery, and 6 more with non-ductal histology tumors, resulting in 101 patients included. Fifteen cases received neoadjuvant chemotherapy (pathological response Miller-Payne was recorded), 86 received adjuvant chemo, using different regiments according to the standards used at the time of diagnosis. All of the patients who have undergone conservative breast surgery received postoperative radiotherapy. The median follow-up time was 58.3 months. Study variables included family history of cancer, BRCA mutation, age at diagnosis, diabetes mellitus, grade, tumor size, lymph node status, Ki-67, pathologic response if neoadjuvant chemo had been administered, type of surgery, sentinel biopsy if done, systemic adjuvant therapy and radiotherapy, date of locoregional recurrence and/or distant metastasis, status at last follow up, and date and cause of death.

Two pathologists specialized in breast pathology, using the blind method, performed Immunohistochemical scoring. IHC was carried out on formalin-fixed paraffin-embedded tumor samples of the archived tumor sections, for determination of AR. The cut-off value for AR positivity was set at >1% of tumor cell nuclei stained positive (the same value adopted for ER and PR).

The Clinical Research Ethics Committee waived informed consent because no intervention was involved and no patient identifying information was included. The Ethical Committee of HUFA approved the study.

Demographic and histological characteristics of patients were described using absolute and relative frequencies in case of qualitative data, and quantitative variables were expressed as mean, standard deviation (SD), minimum, maximum and quartiles.

Overall survival (OS) and disease-free survival (DFS) were the primary outcomes. OS was defined as the time from the day of the primary surgery to the time of breast-related death and DFS was defined as the time from date of the primary surgery to disease relapse or progression date. OS and DFS functions were estimated with Kaplan-Meier methods and log-rank tests were performed to assess differences between groups. Univariate and multivariate cox regression models were adjusted to estimate hazard ratios (HR).

All statistical tests were 2-sided with a type 1 error of 0.05 and probability values of <0.05 were considered statistically significant. STATA (Version14.0; Stata Corp LP, College Station, TX) and SPSS 17 performed survival analysis.

Results

Between 1999 and 2015, 101 female TNBC patients without metastasis at diagnosis underwent surgery at our institution. Fifteen (14.8%) corresponded to neoadjuvant chemotherapy and 86 (62.2%) to adjuvant chemotherapy. The age range was 30 to 93 y (median age was 60 y); Patients and tumor characteristics are summarized in Table 1.

Forty of 101 (39.6%) TNBC expressed AR. In analyzing the relationship between the status of the androgen receptor and the different clinicopathological characteristics, we found statistically significant differences in Ki-67 and histologic grade, with higher Ki-67 and grade in AR-negative tumors. In fact, median of Ki-67 in AR-negative tumors was 50 compared to 30 in the group of AR-positive tumors. The group of negative AR tumors presented a higher percentage of G3 tumors (90%).

In addition, AR-negative patients were younger, with worse clinical stage and more metastatic lymph nodes than AR-positive. Among 21 patients who had disease relapse, only 4 had AR-positive TNBC.

When we evaluated OS and DFS at 36 and 60 months of follow up, AR-negative patients had worse prognosis, finding significant association in OS (p=0.034): OS at 36 and 60 months was 86% and 80% in AR-negative patients, and 100% and 96% in AR-positive patients. However non-significant association was found between the two groups in DFS (p=0.075): DFS at 36 and 60 months was 78% and 68% in AR-negative patients, and 92% and 89% in AR-positive (Tables 2 and 3 and Figure 2).

AR-positive status had a protective effect for OS, HR=0.15 (CI 95%: 0.02-1.16, p=0.068), and for DFS, HR=0.41 (CI 95%: 0.15-1.13, p=0.085), but not statistically significant. Other predictive factors analysed were advance disease stage, tumor diameter ≥ 2 cm, and higher histological grade. Of them, only positive axillary lymph node metastasis had a statistically significant effect, with poorer OS. HR of AR status in DFS, adjusted by positive axillary lymph and tumor diameter ≥ 2 cm was 0.49 (CI 95% 0.18-1.36, p=0.171) (Table 4).

		Total	AR		p-value
			No	Yes	
		101	61 (60.4%)	40 (39.6%)	
Age	Mean	58.07 ± 14.22	56.7 ± 15.45	60.15 ± 12	0.439
	Range	30-93	30-85	35-93	
Ki67	Median (p25-p75)	40 (25-70)	50 (30-70)	30 (9-50)	0.001
	Range	3/90	5/90	3/90	

Diabetes	NO	92 (91.1%)	57 (93.4%)	35 (87.5%)	0.477
	YES	9 (8.9%)	4 (6.6%)	5 (12.5%)	
Grade	G2	19 (18.8%)	6 (9.8%)	13 (32.5%)	0.008
	G3	82 (81.2%)	55 (90.2%)	27 (67.5%)	
Stage_clinical_c	I	38 (37.6%)	19 (31.1%)	19 (47.5%)	0.253
	II	48 (47.5%)	32 (52.5%)	16 (40%)	
	III	15 (14.9%)	10 (16.4%)	5 (12.5%)	
Tumor size	≤ 2 cm	59 (59.6%)	33 (55%)	26 (66.7%)	0.297
	>2 cm	40 (40.4%)	27 (45%)	13 (33.3%)	
Lymph node	NO	65 (64.4%)	35 (57.4%)	30 (75%)	0.071
	Yes	36 (35.6%)	26 (42.6%)	10 (25%)	
Neoadjuvant	No	86 (85.1%)	48 (79.6%)	38 (95%)	
	Yes	15 (14.9%)	13 (21.3%)	2 (5%)	
ypT_if_neoadjuvant	yT0	3 (25%)	2 (18.2%)	1 (100%)	
	ypT1mi	1 (8.3%)	1 (9.1%)		
	ypT1a	2 (16.7%)	2 (18.2%)		
	ypT1b	3 (25%)	3 (27.3%)		
	ypT1c	2 (16.7%)	2 (18.2%)		
	ypT3	1 (8.3%)	1 (9.1%)		
ypN_if_neoadjuvant	ypN0	10 (83.3%)	9 (81.8%)	1 (100%)	
	ypN1a	1 (8.3%)	1 (9.1%)		
	ypN2a	1 (8.3%)	1 (9.1%)		
Pathological_response_Miller_Payne	G1	1 (6.7%)	1 (7.7%)		
	G3	4 (26.7%)	4 (30.8%)		
	G4	6 (40%)	6 (46.2%)		
	G5	4 (26.7%)	2 (15.4%)	2 (100%)	
Type_of_surgery	Conservative	51 (50.5%)	30 (49.2%)	21 (52.5%)	
	Mastectomy	50 (49.5%)	31 (50.8%)	19 (47.5%)	
Sentinel_biopsy	Negative	43 (89.6%)	26 (89.7%)	17 (89.5%)	
	Micrometastases	3 (6.3%)	1 (3.4%)	2 (10.5%)	
	Macrometastases	2 (4.2%)	2 (6.9%)		
Adjuvant_Radiotherapy	YES	67 (64.6%)	45 (72.4%)	22 (52.6%)	
	NO	34 (35.4%)	16 (27.6%)	18 (47.4%)	

Table 1: Relationship between different clinicopathological features and AR expression.

	Total	**AR**	**p-value**

			No	Yes	
		101	61 (60.4%)	40 (39.6%)	
Disease relapse	No	80 (79.2%)	45 (73.8%)	35 (87.5%)	0.096
	Yes	21 (20.8%)	16 (26.2%)	5 (12.5%)	
Locoregional_Recurrence	No	90 (89.1%)	54 (88.5%)	36 (90%)	1
	Yes	11 (10.9%)	7 (11.5%)	4 (10%)	
Distant_Mets_metacronic	No	85 (84.2%)	48 (78.7%)	37 (92.5%)	0.063
	Yes	16 (15.8%)	13 (21.3%)	3 (7.5%)	
Liver_mets	Yes	4		4	
Lung_mets	Yes	10		10	
SNC_mets	Yes	5	4	1	
Lymph_nodes_mets	Yes	7	6	1	
Bone_mets	Yes	6	5	1	
Peritoneal mets	Yes	1		1	
Soft_tissue_mets	Yes	7	5	2	
Status_last_follow_up	Death other causes	2 (2%)		2 (5%)	0.024
	Death cancer dependent	10 (9.9%)	9 (14.8%)	1 (2.5%)	

Table 2: Disease relapse, metastases and death.

	AR	Time	Fail	Survivor function	95% conf. int.	
DFS	No	36	12	78.00%	64.30%	86.90%
		60	16	67.80%	52.20%	79.20%
	Yes	36	4	92.10%	77.50%	97.40%
		60	5	88.80%	72.70%	95.70%
OS	No	36	7	85.80%	72.40%	93.00%
		60	9	80.30%	65.10%	89.30%
	Yes	36	0	100%		
		60	1	96.20%	75.70%	99.50%

Table 3: DFS and OS at 36 and 60 months of follow up.

Disease free survival	Univariate Cox Regression Analysis			
	Hazard ratio (HR)	95% CI for HR		p-value
AR	0.41	0.15	1.13	0.085
Clinical stages II and III	1.76	0.68	4.55	0.241
Tumor size ≥ 2 cm	1.94	0.8	4.65	0.146
Lymph node status positive	2.42	1.02	5.74	0.046
Grade G3	5.07	0.68	37.83	0.113

Table 4: Univariate analysis with respect to disease free survival.

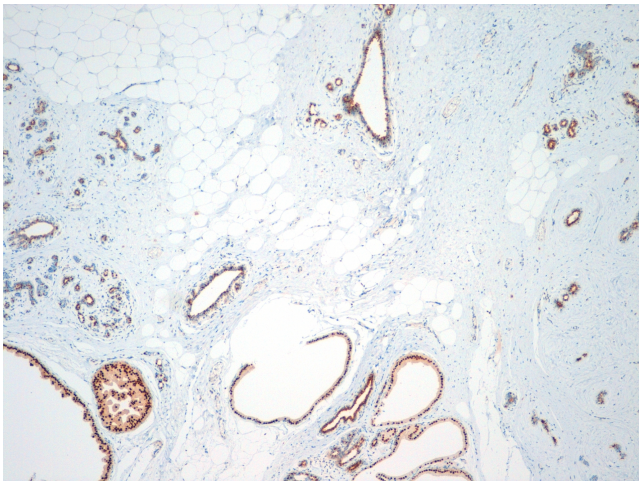

Figure 1: Low-power view showing immunostaining for androgen receptor (AR), demonstrated in the nuclei of both ductal and lobular epithelial cells, and diffusely expressed in metaplastic apocrine cells.

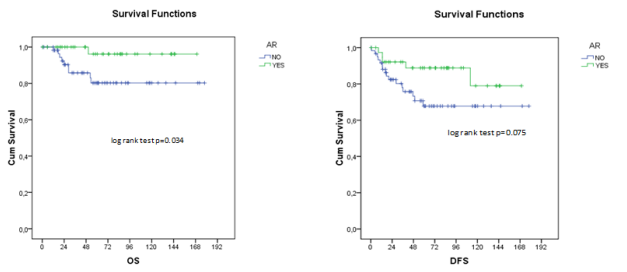

Figure 2: OS and DFS based on AR expression in TNBC. AR-negative had poor prognosis in OS, but no significant difference in DFS.

Discussion

Among breast cancer, TNBC is a diverse group of cancers with poor survival. In recent studies, there have been various attempts to determine significant clinical variables/biomarkers in TNBC to allow a better therapeutic strategy.

AR expression on breast cancer has been long acknowledged, and its function is still unclear. The role of the androgen-regulated pathway in breast cancer may be different depending on ER expression: when the tumor is ER-positive, there is competition between AR and ER, because of antiproliferative effects due to cross talk between the signaling pathways of the steroid receptor. However, in TNBC the opposite occurs: there are high levels of aromatase that convert androgens into estrogens assuming the role of the latter. With conflicting preclinical evidence, the precise biological role of AR in TNBC remains to be elucidated [16].

The prevalence of AR in TNBC is ranging from 13.7% to 75%, depending on the threshold for positivity used in the assessment of the AR in various studies. In Spain, Gonzalez et al. analysed the AR expression in 111 breast cancer patients, finding that AR is commonly expressed (75%), but they didn't differentiate between TNBC and non-TNBC [15]. We found 40% of AR-positive TNBC patients.

In some studies, AR does not predict survival in TNBCs [8,16,17]. However, we found that patients with AR-negative were younger, with higher KI-67, positive nodal status, and higher tumor grade. At a median follow up of 58 months, the AR expression was a significant prognostic factor for OS, also for DFS, although without significance. Our results are in line with similar observed in other studies, which found AR expression in TNBCs associated with a better OS and DFS, as well as other favorable tumor characteristics such as lower grade, lower mitotic score, less frequent metastasis and tumor recurrence. In fact, three studies specifically investigating TNBC were included in a meta-analysis, which showed that AR expression was associated with worse OS but had no significant effect on DFS. These studies demonstrate that the prognostic significance of AR in TNBCs is yet to be clarified [16].

Possible limitations of this study could be the small sample size, and the cut-off for ER, PR, and HER2 positivity, which has changed among the years (reduced from 10% to 1% in the case of hormone receptors, and from 30% to 10% in the case of HER2). In addition, this variability could be not only by differences in criteria used, but because after 2013 it is well known that a longer cold ischemia decreases quality of the inmunohistochemical techniques used. Other possible limitation could be the heterogeneity of the chemotherapy, as not all patients received the same regimen.

We also did not exclude classic apocrine carcinoma (>90% cells have abundant pink cytoplasm and nuclei with prominent nucleoli) which could have a poorer prognosis that could negate the positive prognostic influence of AR in others TNBC. Furthermore, the responses to targeting AR therapeutically can differ based on the origin of a tumor in apocrine cells *vs.* luminal cells [16]. So an important consideration for future studies would be to separate apocrine carcinomas that are AR-positive from luminal AR-positive tumors to determine the possible impact of morphologic apocrine differentiation on the ultimate response.

Although there are heterogeneity and potential targetability within TNBC, at this time chemotherapy is the only treatment option for either early or advanced disease. The first clinical studies using antiandrogen agents, performed in the 1980s, did not show any meaningful result. Recently, Gucalp used bicalutamide, an oral minimally toxic AR antagonist in 26 AR-positive metastatic TNBC patients [9]. Enzalutamide is another novel targeted AR inhibitor with encouraging efficacy in advanced AR-positive TNBC and now other antiandrogen agents are under investigation in AR-positive TNBC tumors (Orterenol, VT-464) [16].

Clearly, the identification of novel targets for therapy among the TNBCs is of major interest, so new clinically applicable biologic markers for TNBC need to develop in order to identify the patients with poor prognosis, and alternative treatment options are necessary.

Although the College of American Pathologists (CAP) along with the American Society of Clinical Oncology (ASCO) still do not recommend the routine assessment of AR, we think it is reasonable to routinely assess for expression of AR if not in all breast carcinomas, at least in TNBC to avail the patients of the potential benefits of AR as a future therapeutic target.

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