ORIGINAL ARTICLE

Association between androgen receptor status and prognosis in triple negative breast cancer

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Summary

Purpose: Triple negative breast cancer (TNBC) is a heterogeneous disease group with a higher recurrence risk and poorer prognosis. In this study, we aimed to investigate the frequency and prognostic value of androgen receptor (AR) expression in tissues of TNBC patients.

Methods: A total of 84 TNBC patients treated between 2000 - 2015 in Hacettepe University Cancer Institute were included and their medical records were analyzed retrospectively. The available paraffin blocks were assessed immunohistochemically to determine AR expression. Tumors with ≥1% nuclear staining were considered AR-positive, while the ones with <1% staining were considered AR-negative. We analyzed the association between AR expression, and clinical-pathologic characteristics and prognosis in TNBC.

Results: Of the 84 TNBC patients, 25 (29.8%) were AR-positive. The frequency of grade 3 tumors was lower among AR-positive TNBC tumors compared to AR-negative tumors

(40 vs 86.4%, p<0.001). In the AR-positive group, invasive ductal carcinoma (IDC) was less prevalent compared to AR-negative group (56 vs 86.4%, p<0.002). However, there were not statistically significant differences between AR positive and negative groups in terms of overall survival (OS) and disease free survival (DFS) (p=0.449, p=0.733, respectively). We found that grade 3 tumors were less frequent in AR-positive TNBC in our study. Nonetheless, we did not detect statistically significant difference in terms of overall survival and disease free survival between AR positive and negative TNBC.

Conclusion: Routine evaluation of AR could contribute to further studies that may enlighten the role of AR targeting therapies in TNBC.

Key words: androgen receptor, prognosis, triple negative breast cancer

Introduction

TNBC is characterized with low expression of the estrogen receptor (ER), progesterone receptor (PR), as well as lack of human epidermal growth factor receptor 2 (HER2) overexpression [1,2]. TNBC constitutes approximately 20% of breast cancers worldwide and has unfavorable prognosis, exhibiting a more aggressive course compared to other BC subtypes [3]. Currently, no well-defined targeted therapy in TNBC exists yet [4,5].

AR is a member of the nuclear steroid receptor family and overexpressed both in normal breast tissue and breast cancer cells [6]. AR is a promising novel therapeutic target and its expression varies substantially from a subtype to another. AR is considered to be present in all major BC subtypes at an estimated rate of 53-80% diverse from ER and PR [7-9]. AR is expressed in luminal A (91%) and luminal B (68%) at higher rates, while it is expressed

less frequently (32%) in basal-like cancer [10]. Despite being expressed in various subtypes of TNBC, AR is expressed in the luminal androgen receptor (LAR) subtype at highest rates [11]. However, the prognostic value of AR expression is unclear and outcomes of different studies are conflicting. While AR-positive TNBC patients are reported to have better prognosis than AR-negative TNBC patients in some trials [12,13], some others indicate that AR positivity is associated to poor prognosis [14,15]. In this study, we aimed to investigate the prevalence and prognostic value of AR expression in TNBC.

Methods

The medical records of 2832 breast cancer (BC) patients followed at Hacettepe University Cancer Institute between 2000-2015 were retrospectively reviewed. Of these patients, the records of 430 BC patients with pathologically proven TNBC were evaluated. A total of 84 patients with histopathologically confirmed TNBC were eligible for the study. Patients whose paraffin blocks were not available in our center, whose diagnosis was not confirmed in our hospital, patients with metastatic disease at presentation, and the ones with missing data were excluded. Patients were staged according to TNM classification. TNBC were defined as tumors having <1% expression of ER and PR determined by immunohistochemistry (IHC), and being 0-1+ by IHC, or 2+ and fluorescence *in situ* hybridization (FISH)-negative according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines [1]. All of the patients had received neoadjuvant or adjuvant chemotherapy depending on stage, relapse risk, and clinicopathological characteristics in line with the National Comprehensive Cancer Network (NCCN) guideline. All patients with breast conserving surgery (BCS) received adjuvant radiotherapy. DFS was defined as the interval between the date of surgical removal of the primary tumor and the date at which a relapse was detected or the date of the last follow-up without disease relapse. OS was defined as the period of time between histopathological diagnosis and death or last control date.

Immunohistochemistry protocol

Tissue microarray (TMA) blocks were prepared from formalin-fixed paraffin-embedded tissues. Immunohistochemistry (IHC) staining was performed on TMA sections. Androgen receptor antibody clone 2F12 (1:25, Novocastra) was optimized with known positive controls according to datasheets. The sections were placed in a Bond Max Automated Immunohistochemistry Vision Biosystem Leica (Leica Biosystems, Newcastle, UK) according to the following protocol: First, tissues were deparaffinized and pre-treated with the Epitope Retrieval Solution 1 at 90°C for 30 min. After washing steps, peroxidase blocking was carried out for 5 min using the Bond Polymer Refine Detection Kit DC9800 (Leica Biosystems, Newcastle, UK). Tissues were again washed and then incubated with the primary antibody for 15 min. The incu-

bation with Post Primary reagent (Leica Microsystems) for 8 min was followed by washing with Bond Wash solution for 6 min. Bond Polymer (Leica Microsystems) placement on the slides for 8 min was followed by DAB (Diaminobenzidine tetrahydrochloride) as a chromogen for 8 min at ambient temperature. This was followed by hematoxylin counterstaining for 7 min and mounting of the slides. Immunohistochemical evaluation was performed according to the percentage and intensity of tumor cells with brown nuclear staining. The intensity of staining was graded semi quantitatively as mild, moderate and strong. Tumors with nuclear staining of 1% and greater were accepted AR-positive, while tumors with <1% staining were considered AR-negative [15,16].

Statistics

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 17, SPSS, Inc, Chicago, IL). The distribution of the data was analyzed using normality tests. Because of the non-normal distribution of the data, the Mann-Whitney U test was used for the comparison of two groups. Chi-square and Fisher exact test were used for the comparison of categorical variables. OS and DFS of the groups were calculated by the Kaplan–Meier method, and the log-rank test was used to compare survival rates. A p value < 0.05 was considered to show statistical significance.

Results

A total of 84 TNBC patients were included. The AR positivity rate was 29.8% (25/84). The median age of patients was 51 years (25-81). The distribution of histopathological types was as follows: invasive ductal carcinoma (IDC) in 65 patients (77.4%), invasive lobular carcinoma (ILC) in 2 patients (2.4%), medullary carcinoma in 3 patients (3.6%), apocrine carcinoma in 6 (7.1%), metaplastic carcinoma in 5 (6%), and adenosquamous carcinoma in 3 patients (3.6%).
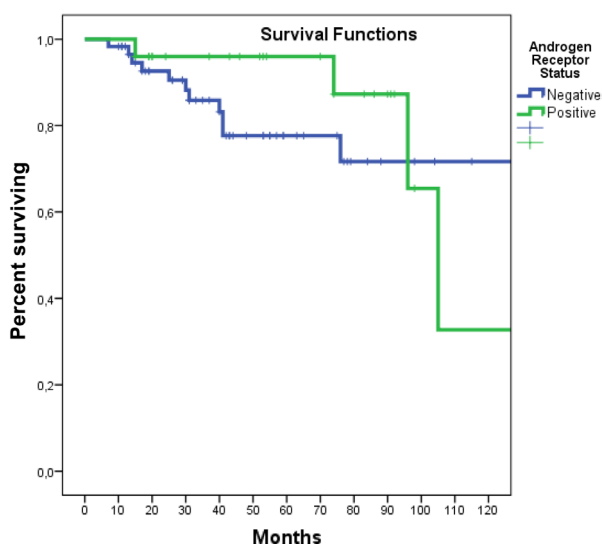
Looking at the histological types according to AR positivity, there were 14 IDCs (56%), 2 ILCs (8%), 6 apocrine carcinomas (24%), 1 metaplastic carcinoma (4%), 2 adenosquamous carcinomas (8%) in the AR-positive group, whereas there were 51 IDCs (86.4%), 3 medullary carcinomas (5.1%), 4 metaplastic carcinomas (6.8%), and 1 adenosquamous carcinoma (1.7%) in the AR-negative group (p=0.002). As for the histological grades of tumors, 10/25 (40%) of AR-positive tumors were grade 3, while 51/59 (86.4%) AR-negative tumors were grade 3 (p<0.001).

There were not statistically significant differences in terms of age, stage, tumor size, lymph node metastasis, lymphovascular invasion (LVI), adjuvant chemotherapy, neoadjuvant chemotherapy, and adjuvant radiotherapy. The comparison of AR-positive and AR-negative patients are shown in Table 1.

Table 1. The comparison of AR-positive and AR-negative TNBC patients

	AR (+) (n = 25) n (%)	AR (-) (n = 59) n (%)	p value
Age, years, (mean ± SD)	49.8 ± 12.8	54.8 ± 11.5	NS
Histologic type			0.002
IDC	14 (56)	51 (86.4)	
Non-IDC types	11 (44)	8 (13.6)	
Stage			NS
I	7 (28)	7 (11.9)	
II	10 (40)	38 (64.4)	
III	8 (32)	14 (23.7)	
Histological grade			<0.001
I	2 (8)	1 (1.7)	
II	13 (52)	7 (11.9)	
III	10 (40)	51 (86.4)	
Tumor size (cm)			NS
≤2	7 (28)	13 (22.0)	
>2	18 (72)	46 (78.0)	
Metastatic LN			NS
(+)	12 (48)	25 (42.4)	
(-)	13 (52)	34 (57.6)	
LVI			NS
(+)	5 (20)	7 (11.9)	
(-)	20 (80)	52 (88.1)	
CT			NS
Adjuvant	23 (92)	46 (77.9)	
Neoadjuvant	2 (8)	13 (22.1)	
RT			NS
(+)	13 (52)	33 (55.9)	
(-)	12 (48)	26 (44.1)	
Type of surgery			NS
MRM	19 (76)	46 (77.9)	
BCS	6 (24)	13 (22.1)	

AR: androgen receptor, IDC: invasive ductal carcinoma, LN: lymph nodes, LVI: lymphovascular invasion, CT: chemotherapy, MRM: modified radical mastectomy, BCS: breast conserving surgery, NS: non significant

Figure 1. Overall survival across AR-positive and AR-negative patients (p=0.449).
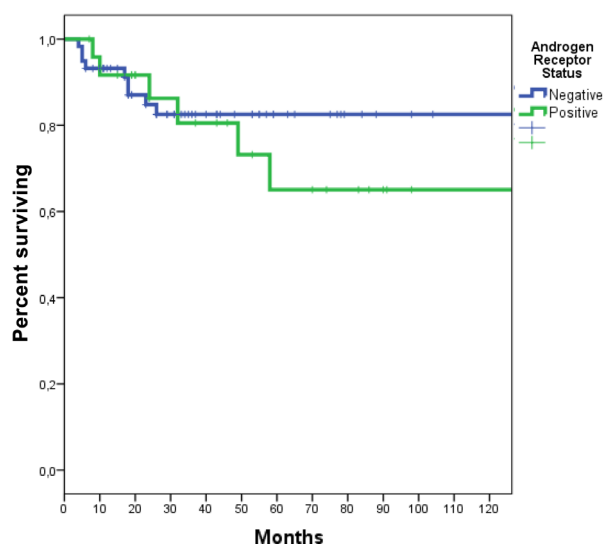
Figure 2. Disease-free survival across AR-positive and AR-negative patients (p=0.733).

According to histological types, AR positivity rate was 21.5% (14/65) in the IDC and 57.8% (11/19) in non-IDC histologic types (p=0.002). The median OS was 105.1 months in the AR-positive TNBC patients and 171.9 months in the AR-negative TNBC patients (p=0.449, Figure 1). The median DFS was 24.5 months in the AR-positive TNBC patients and 18.2 months in the AR-negative TNBC patients (p=0.733, Figure 2).

Discussion

AR positivity rate was 29.8% in TNBC in our study. We found that grade 3 tumors were less frequent in AR-positive TNBC but there was not statistically significant difference between AR-positive and AR-negative TNBC patients in terms of OS and DFS. Additionally, we analyzed the AR positivity according to histological types and detected a lower rate of AR positivity in IDC tumors compared to non IDC histologic types. These results indicate that AR expression may be associated with low grade tumors, may display different prevalence in various histological types and lacks prognostic value in TNBC.

TNBC has a more aggressive course with unfavorable prognosis. In contrast to other BC subtypes related to absence of hormone receptors and HER2 overexpression, no targeted therapy options exist currently. The cytotoxic drugs are the main treatment tools [13]. Therefore, novel therapeutic options and new biomarkers to predict response to these therapies are required. Although the presence of AR expression in BC is known, the knowledge regarding its clinical significance is not clear. The prevalence of AR expression ranges widely from 6.6 to 75% in the literature [16-22]. The heterogeneity in literature studies results may have stem from distinct features of the populations analyzed, number of participants enrolled, methodology of testing, the primary antibodies to detect AR, and cut off values to define AR positivity (≥1% , >5% or >10% etc) [23]. In a meta-analysis on TNBC patients by Changjun et al., 2826 patients from 13 trials were evaluated and the rate of AR positivity was reported to be 24.4% [24]. In another study by Asano et al. [25], the rate of AR positivity was noted to be 29.5% in 190 Japanese TNBC patients. In a study on Chinese TNBC patients in which cut off value of AR positivity was determined as 10%, the rate of AR expression was reported to be 31.4% [26]. In the current study, we determined the cut off value of ≥1% for AR positivity and we found an AR positivity rate of 29.8%. Therefore, it can be concluded that the prevalence of AR expression in the Turkish TNBC patients is compatible with the literature.

In another study from Turkey evaluating the AR expression immunohistochemically based on the percentage of stained tumor cells, the mean value of the distribution of AR expression in TNBC was reported to be 89.5% [27].

TNBC is a substantially heterogeneous disease with distinct intrinsic molecular subtypes. It may display different prognostic course depending on the molecular subtype. Several trials have been performed to reveal prognostic or therapeutic markers in TNBC patients according to subtypes. In a genomic profiling study by Burstein et al., four stable subtypes of TNBC tumors were identified: luminal androgen receptor (LAR), basal-like immunosuppressed, mesenchymal, and basal-like immune-activated [28]. The LAR subtype that is enriched of hormonally regulated pathways depends on AR signaling [13], while AR expression may be seen in multiple molecular subtypes of TNBC, the highest rate of AR expression seen in the LAR subtype [11]. Apart from unselected TNBC, the LAR, a novel subtype of the non-basal subgroup with diverse prognosis creates opportunity for development of targeted therapies [29].

Despite the clearly defined predictive and prognostic value of ER and PR, the prognostic value of AR and its relationship with clinicopathological characteristics has not been ascertained yet. Results of the studies regarding this issue are conflicting. AR positivity was reported to be related to lower clinical stage, lower histological grade, lower mitotic score, and better prognosis in some studies [12,14,17,22,25,30]. A meta-analysis including 2826 TNBC patients revealed that tumor grade was lower but lymph node metastasis was more frequent in AR-positive TNBC. AR-positive patients were found to have longer DFS while no statistically significant difference was shown in terms of OS compared to AR-negative ones [24]. In another study by Xiao-Qing et al., 360 operable TNBC patients were included. AR positivity was reported to be positively correlated with both DFS and OS. However, grade 3 tumors were found to be more prevalent in AR-positive patients than AR-negative patients [26]. In other studies the lack of AR expression was reported to be related to increased risk of recurrence and distant metastasis in lymph node positive TNBC [31,32]. Nevertheless, other trials have yielded opposite results that AR expression does not have impact on prognosis or it is associated with worse outcome [15,18,22,33,34]. In a study by McGhan et al., it was stated that AR-positive TNBC had increased tendency for lymph node metastasis. There was no significant difference between AR-positive and negative TNBC in terms of locoregional recurrence, OS and DFS [18]. In

another study on TNBC patients, Choi et al. noted that AR expression was a predictor of unfavorable OS and DFS in the lymph node negative subgroup. Furthermore, they reported that AR expression was correlated with lower histologic grade [15]. In our study, we found that grade 3 tumor prevalence was significantly lower in AR-positive TNBC. OS and DFS did not differ significantly between AR-positive and negative TNBC patients. When analyzed according to histologic types, the rate of AR positivity was significantly lower in the IDC compared to non-IDC histologic types. Another remarkable result of our study is that all of 6 apocrine carcinomas were positive for AR. Choi et al. also reported that AR expression was correlated with apocrine histology [15]. The limitation of the study results regarding the prognostic significance of AR may rely on the diversity of the cut off values, methodology of testing, number of participants, adjuvant treatments, and follow-up periods.

Currently there are new studies in TNBC patients about agents targeting AR. In a single arm phase II study by Gücalp et al., the efficacy of AR antagonist bicalutamide was investigated in 26 ER/PR negative, AR-positive metastatic BC patients. Although bicalutamide did not induce objective response in any patient, 6-month Clinical Benefit Rate (CBR) (defined as the total number of patients who show a complete response (CR), partial response (PR), or stable disease (SD), > 6 months) was 19%, and median progression free survival (PFS) was 12 weeks [35]. In another study by Traina et al. [36], enzalutamide, a new potent AR inhibitor, was used in AR-positive TNBC. A total of 118 patients were enrolled in this single-arm phase II study. Nuclear AR staining greater than 0%, was considered positive. The primary end point was determined as CBR at 16 weeks. CBRs at the 16th week were 25% vs 33% in the intent-to-treat (ITT) population and the evaluable subgroup was defined as patients with AR expression of ≥10%. Median PFS and OS in the evaluable subgroup were 3.3 months and 17.6 months respectively, higher than the ITT population. Other remarkable results of this study were the detection of CR in 2 patients and PR in 5 patients [36].

As a conclusion, AR expression is associated with low grade tumors, albeit without prognostic significance. In TNBC, routine testing of AR may lead to more extensive studies and contribute to the development of novel therapeutic options targeting AR.

Conflict of interests

The authors declare no conflict of interests.

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