Original paper

Prognostic significance of androgen receptor expression in HER2-positive and triple-negative breast cancer

Betul Bolat Kucukzeybek[1], Ibrahim V. Bayoglu[2], Yuksel Kucukzeybek[2], Yasar Yildiz[2], Utku Oflazoglu[2], Murat K. Atahan[3], Halil Taskaynatan[2], Ahmet Alacacioglu[2], Seyran Yigit[1], Mustafa O. Tarhan[4]

[1]Department of Pathology, İzmir Katip Celebi University Ataturk Training and Research Hospital, İzmir, Turkey
[2]Department of Medical Oncology, İzmir Katip Celebi University Ataturk Training and Research Hospital, İzmir, Turkey
[3]Department of General Surgery, İzmir Katip Celebi University Ataturk Training and Research Hospital, İzmir, Turkey
[4]Department of Preventive Oncology, İzmir Katip Celebi University Ataturk Training and Research Hospital, İzmir, Turkey

Triple-negative breast cancer (TNBC) and HER2-positive breast cancer are more aggressive than other subtypes of breast cancer. Due to the limited number of treatment alternatives and the absence of target receptors in TNBC, and because of progression in the HER2-positive group despite targeted treatments, new treatment targets and therapeutic combinations are required. In this context, the present study aims to evaluate the prognostic importance of immunohistochemical androgen receptor (AR) expression in HER2-positive breast cancer and TNBC subtypes. AR nuclear staining density was evaluated immunohistochemically. A total of 111 operated patients with breast cancer were included in the study; 44 (39.6%) belonged to the HER2-positive breast cancer subgroup and 67 (60.4%) belonged to the TNBC subgroup. AR expression was 34.3% and 79.5% in TNBC and HER2-positive groups, respectively. The 5-year overall survival (OS) was 76% and 58% for the group with an AR-expression > 7.5% and AR-expression < 7.5%, respectively, in the TNBC subgroup ($p = 0.042$). In the HER2-positive patient group, the subgroups characterised by an AR-expression > 7.5% and AR-expression < 7.5% had 5-year OS rates of 57.6% and 63.5%, respectively ($p = 0.91$). Including the assessment of AR expression in the routine pathological examination will contribute to our understanding of the relevance of AR in the biology and prognosis of breast cancer.

Key words: breast cancer, triple-negative, HER2-positive, prognosis, androgen receptor.

Introduction

Breast cancer (BC) is the most common cancer and the second leading cause of cancer death among women [1]. Immunohistochemically, BC is classified based on the oestrogen receptor (ER), progesterone receptor (PR), and HER2 expression. HER2 overexpression and gene amplification are observed in 15-20% of invasive BCs. HER2 amplification is associated with poor prognosis. In patients with HER2 amplification, survival improves with various biological agents targeting this receptor; nevertheless, new therapeutic agents and combinations are still needed [2, 3]. Triple-negative breast cancer (TNBC) is characterised

by the lack of HER2 expression/gene amplification, ER expression, and PR expression. It accounts for 10-20% of all BCs. TNBC is more aggressive than other subtypes, presents a high risk of early relapse, with metastases mostly occurring in the first three years, and presents a worse survival compared with other subtypes [4, 5]. There is no treatment alternative except cytotoxic chemotherapy. Due to poor survival and the lack of a targeting receptor, new treatment agents and therapeutic targets are needed.

The androgen receptor (AR) is one of the pathways that are being studied concerning new therapeutic agents. Its clinical benefit for patients with metastatic triple-negative and AR-positive BC, demonstrated with the anti-androgen treatment applied to such patients, has led to the intensification of studies in this field [6].

The AR is a member of the nuclear steroid hormone receptor superfamily that includes ER, PR, glucocorticoid receptor, and mineralocorticoid receptor. They serve as transcription factors. When the ligand binds to the receptor, the receptor disconnects with chaperone proteins, leading to dimerisation. It passes to the nucleus and binds to androgen response elements, stimulating the transcription of androgen-responsive genes [7, 8]. Recently, as well as the genomic AR signalling pathway, non-genomic AR signalling pathways have been identified [9]. The success of AR-targeted new-generation anti-androgens in the treatment of prostate cancer has started to intensify the studies on AR in BC. AR expression is detected at a higher rate than ER and PR in BC. AR expression rates differ among BC subtypes. It is found at 23-35% in TNBC and 36-86% in HER2-positive subtype [10, 11, 12, 13, 14, 15, 16, 17, 18].

Although the carcinogenic, prognostic, and predictive roles of ER and PR for hormone therapy have been identified, there are ongoing studies on AR's prognostic and predictive effect, as well as its biological role in BC. Conflicting results were reported for the prognostic significance of AR expression in patients with TNBC. There are studies demonstrating the AR's expression association with good prognostic factors and survival, others report that it is associated with poor prognosis and survival, and another group of studies show that it is not associated with survival [13, 16, 17, 19, 20, 21, 22]. In a limited group of studies evaluating the prognostic significance of AR expression in BC cases presenting HER2 expression/amplification, no clear relationship has been defined between prognosis and AR expression [22, 23].

The present study aims to evaluate retrospectively the prognostic significance of immunohistochemical AR expression in the group of operated TNBC and HER2-positive BC cases treated with adjuvant chemotherapy or chemotherapy plus trastuzumab (the group presenting HER2 overexpression/amplification).

Material and methods

Patients and tissue samples

The present study includes operated patients with TNBC and HER2-positive BC, who had been diagnosed at Izmir Katip Çelebi University, Ataturk Training and Research Hospital, Department of Pathology, and who were followed up at the medical oncology clinic from 2006 to 2012. Immunohistochemically, those cases with ER and PR nuclear staining rate $< 1\%$, complete membranous staining for HER2 $< 10\%$ and/or without HER2/neu gene amplification by fluorescence *in situ* hybridisation method (HER2-neu/CEN17 < 2) were taken to be triple-negative. Again, immunohistochemically, those cases with HER2 complete membranous staining rate $> 10\%$ and/or with HER2/neu gene amplification by fluorescence *in situ* hybridisation method (HER2-neu/CEN17 > 2) were considered as HER2-positive. In the HER2-positive group, hormone receptor (HR) expression was also evaluated immunohistochemically. The patients with $\geq 1\%$ nuclear ER or PR expression were approved as HR-positive. Demographic and survival data for the patients were obtained from follow-up files of the medical oncology outpatient clinic. The staging was performed based on TNM. Clinical and pathological data, including the stage at diagnosis, tumour size, patient's age, lymph node status, the presence of lymphovascular invasion, recurrence or metastasis, use of adjuvant chemotherapy, histologic grade, and operation type, were attained from patient files. Disease-free survival (DFS) was defined as the time period from diagnosis until relapse/metastasis or the last follow-up date. Overall survival (OS) was defined as the period from the date of diagnosis to the time of death due to any cause. Patients' tumour containing blocks at the pathology laboratory were utilised for immunohistochemical AR staining. Nuclear AR staining density was assessed immunohistochemically. A value $> 1\%$ was taken to be positive. The patients who received neoadjuvant chemotherapy were excluded from the study.

Immunohistochemical method

Immunohistochemical staining for AR was performed on formalin-fixed and paraffin-embedded tissue using the streptavidin-biotin-peroxidase method. Tissue blocks containing representative tumour areas were selected for immunohistochemical stains. The expression of AR was evaluated by using a Dako Autostainer (DAKO, Santa Clara, CA, USA) and the DAKO Envision staining method. Sections were stained by using monoclonal mouse anti human AR antibody (Clone AR441 – DAKO, CA, USA). Normal prostate tissue was used as a positive control for

Table I. Patient-disease characteristics and AR expression rates

Characteristics	n = 111 (%)	AR-positive n (%)	AR-negative n (%)	p
Median age (range)	52 (29-80)	58 (52.3)	53 (47.7)	0.43
< 50		24 (46.2)	28 (53.8)	
> 50		34 (57.6)	25 (42.4)	
TNBC	67 (60.4)	23 (34.3)	44 (65.7)	0.029
Her2-positive	44 (39.6)	35 (79.5)	9 (20.5)	
Histological subtype				0.079
Invasive ductal carcinoma	91 (82)	50 (54.9)	41 (45.9)	
Invasive lobular carcinoma	10 (9)	7 (70)	3 (30)	
Mixt	3 (2.7)	1 (33.3)	2 (66.6)	
Other	7 (6.3)	0	7 (100)	
Stage at diagnosis				0.40
Stage I	10 (9)	3 (30)	7 (70)	
Stage II	63 (56.8)	34 (54)	29 (46)	
Stage III	38 (34.2)	21 (55.3)	17 (44.7)	
Surgical procedure				0.59
Breast-conserving surgery	49 (44.1)	23 (46.9)	26 (53.1)	
Modified radical mastectomy/ Mastectomy	55 (49.6)	32 (58.2)	23 (41.8)	
Other	7 (6.3)	3 (42.9)	4 (57.1)	
Lymph node status				0.69
Metastatic	69 (62.2)	40 (58)	29 (42)	
Non-metastatic	42 (37.8)	18 (42.9)	24 (57.1)	
Histological grade				0.013
I	12 (10.8)	8 (66.7)	4 (33.3)	
II	65 (58.6)	40 (61.5)	25 (38.5)	
III	34 (30.6)	10 (29.5)	24 (70.5)	
Ki-67 Labelling index				0.001
< 14	38 (34.2)	27 (71.1)	11 (28.9)	
14-30	19 (17.1)	14 (73.7)	5 (26.3)	
> 30	54 (48.6)	17 (31.5)	37 (68.5)	
pT1	20 (18)	9 (45)	11 (55)	0.33
pT2	72 (64.9)	41 (56.9)	31 (43.5)	
pT3	14 (12.6)	6 (42.9)	8 (57.1)	
pT4	5 (4.5)	2 (40)	3 (60)	
Menopausal status				0.86
Postmenopausal	59 (53.2)	34 (57,6)	25 (42.4)	
Premenopausal	52 (46.8)	24 (46,2)	28 (53.8)	
ER/PR Expression				0.015
Positive	32 (28.8)	27 (84.4)	5 (15.6)	
Negative	79 (71.2)	31 (39.2)	48 (60.8)	
Adjuvant chemotherapy				
Yes	106 (95.5)			
No	5 (4.5)			

AR – androgen receptor; DFS – disease-free survival; ER – oestrogen receptor; PR – progesterone receptor; TNBC – triple-negative breast cancer

Table II. Survival according to androgen receptor (AR) expression

Group	5-year DFS (%)		p
	AR < 7.5%	AR > 7.5%	
All patients	48.3	54.3	0.39
TNBC	46.4	68.4	0.89
HER2-positive	50	54.5	0.58
	5-year OS (%)		
	AR < 7.5%	AR > 7.5%	
All patients	61	69.2	0.35
TNBC	51	76	0.042
HER2-positive	63.6	57.6	0.91

AR – androgen receptor; DFS – disease-free survival; TNBC – triple-negative breast cancer

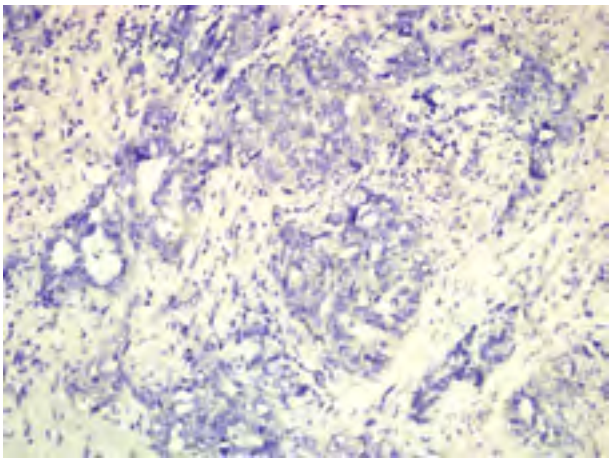

Fig. 1. Immunohistochemically negative staining of tumour cells for AR (original magnification 20×)

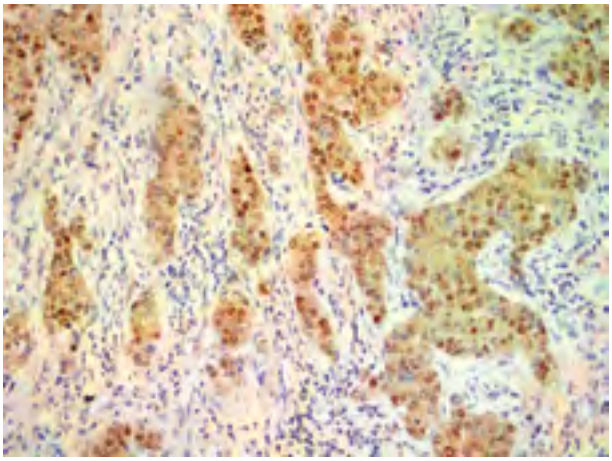

Fig. 2. Focal nuclear immunopositivity of the tumour cells for AR (original magnification 20×)

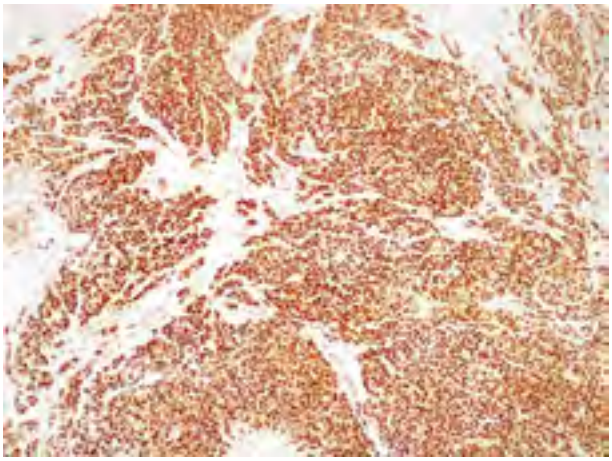

Fig. 3. Diffuse and strong nuclear immunopositivity of the tumour cells for AR (original magnification 10×)

AR staining. Two of the authors evaluated the staining intensity and staining density. Nuclear staining in tumour cells was considered positive. Besides AR, material concerning ER, PR, HER2, and Ki67 taken at the time of initial diagnosis were re-evaluated.

Statistical analysis

Statistical analyses were performed on SPSS package software, version 20. The authors utilised ROC curve analysis to calculate AR staining density cut-off value, Kaplan-Meier analysis for survival analyses, and log Rank-Breslow methods to compare two groups in terms of survival. The independent t-test was used to compare the staining density of two groups. One-way ANOVA and Kruskal-Wallis and χ^2 tests were performed to compare groups. The relationship of AR expression with other prognostic factors was evaluated with Spearman's Rho correlation test and Pearson's correlation test. $p \leq 0.05$ was taken to indicate statistical significance. The patients were evaluated in two groups based on the cut-off value obtained by ROC curve analysis.

Results

The present study evaluated patients with BC who were diagnosed at Izmir Katip Çelebi University, Atatürk Training and Research Hospital, Department of Pathology from 2006 to 2012, and who were followed up at the Medical Oncology clinic. The clinical and histopathological characteristics of the cases and their AR expression rates are shown in Table I. The study included a total of 111 operated patients with BC; 44 (39.6%) were diagnosed with HER2-positive BC and 67 (60.4%) had TNBC. All patients were female. The median age was 52 (29-80) years. Except for 5 patients, all of them received adjuvant chemotherapy including anthracycline/taxane. Thirty-three (75%) HER2-positive patients received adjuvant trastuzumab. Ninety-one (82%) patients had invasive ductal carcinoma, 10 (9%) had invasive lobular carcinoma, and three (2.7%) had mixed-type breast carcinoma histology. Of 7 patients (6.3%) classified as other histological subtypes, two had metaplastic carcinoma, two had invasive papillary carcinoma,

two had carcinoma with medullary features, and one was in carcinoma apocrine differentiation histology. Twelve (10.8%) patients were grade I, 65 (58.6%) were grade II, and 34 (30.6%) were grade III. Fifty-nine (53.2%) patients were post-menopausal, and 52 (46.8%) were pre-menopausal. Forty-nine (44.1%) patients underwent breast-conserving surgery, and 58 (49.6) had modified radical mastectomy or mastectomy. Sixty-nine (62.2%) patients presented lymph node metastasis at the time of diagnosis. Based on TNM staging system, 10 (9%) patients were stage I, 63 (56.8%) were stage II, and 38 (34.2%) were stage III. Through a follow-up of median 52 (12-124) months, 56 (50.5%) patients developed a relapse/metastasis. Forty-nine (44.1%) patients died during the follow-up period. Five-year DFS and 5-year OS were, respectively, 51.3% and 64.9% for all patients included in the study (Table II). Immunohistochemically, the AR expression was 52.3% for all patients included in the study (Figs. 1–3). Patients were divided into two groups based on AR expression by using the value 7.5 for the AR expression obtained through ROC curve analysis. The group with AR expression < 7.5% presented a 5-year disease-free survival rate of 48.3%, which was 54.3% for the group categorised by AR > 7.5%. The difference between two groups was not significant ($p = 0.39$). Five-year OS was 69.2% for the group with 'AR expression > 7.5%, which was 61% for the other group. The difference between two groups presented no significance ($p = 0.35$). Regarding the relationship between prognostic factors and AR expression for all patients included in the study, AR expression, and histologic grade, and AR expression and Ki-67 labelling index were correlated negatively, and it was statistically significant ($p = 0.013$, $R = –0.235$ and $p = 0.009$, $R = –0.299$). Androgen receptor expression was significantly associated with HR expression ($p < 0.001$, $R = 0.521$). Although nuclear grade, tumour diameter, lymph node metastasis, and stage at diagnosis presented a negative correlation, it was not significant ($p > 0.05$). There was non-significant positive correlation between age at diagnosis and androgen expression ($p = 0.37$). It was demonstrated that the presence of multiple foci or single focus at diagnosis ($p = 0.46$) and patients' menorrhoeal status ($p = 0.86$) were not related to the AR expression.

In the TNBC group, 33 (49.3%) patients presented a relapse/metastasis and 22 (41.8%) died during follow-up. The AR expression was 34.3% for the TNBC group. Divided based on AR expression, patients in the TNBC group with AR > 7.5% presented a 5-year DFS rate of 68.4%, which remained at 46.4% for the group with AR < 7.5%. The difference remained below the threshold of significance ($p = 0.89$). The 5-year OS rates for the same patient group was 76% and 58% for

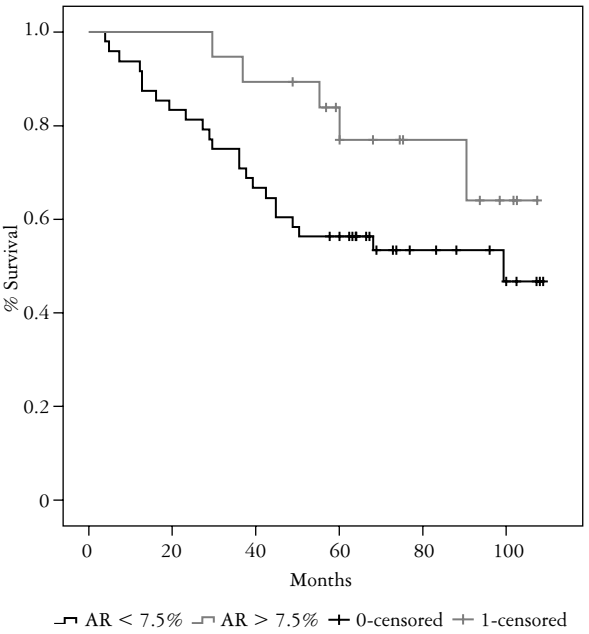

Fig. 4. Overall survival in the TNBC subgroup according to AR level ($p = 0.042$)

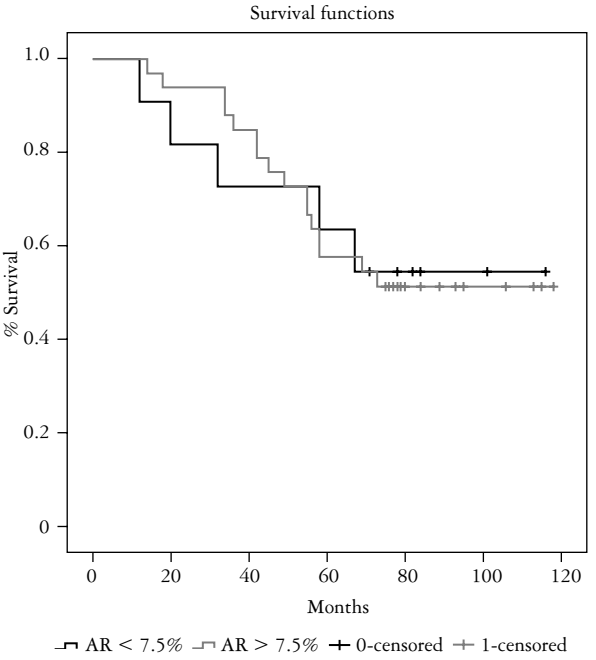

Fig. 5. Overall survival in the HER2-positive subgroup according to AR level ($p = 0.091$)

AR > 7.5% and AR < 7.5%, respectively. The survival difference between two groups was significant according to Breslow method ($p = 0.042$) (Fig. 4). The survival difference between two groups remained significant even after the exclusion of patients who did not receive adjuvant chemotherapy ($p = 0.044$). Regarding the relationship between prognostic factors and AR expression in the TNBC patient group, there was a significant negative correlation between AR expression and Ki-67 labelling

Table III. Androgen receptor expressions in the TNBC subgroup

TNBC	n = 67 (%)	AR-positive n (%)	AR-negative n (%)	p
Median Age (range)	50 (29-80)			0.156
< 50	33 (49.3)	7 (21.2)	26 (78.8)	
> 50	34 (50.7)	16 (47.1)	18 (52.9)	
Histologic grade				0.02
I	8 (11.9)	4 (50)	4 (50)	
II	35 (52.2)	14 (40)	21 (60)	
III	24 (35.8)	5 (20.8)	19 (79.2)	
Ki67 Labelling index				0.001
< 14	23 (34.3)	15 (65,2)	8 (34,8)	
14-30	10 (14.9)	5 (50)	5 (50)	
> 30	34 (50.7)	3 (8,8)	31 (91,2)	
Stage at diagnosis				0.43
Stage I	8 (11.9)	2 (25)	6 (75)	
Stage II	34 (50.7)	10 (29.4)	24 (70.6)	
Stage III	25 (37.3)	11 (44)	14 (56)	
pT1	11 (16.4)	3 (27.3)	8 (72.7)	0.565
pT2	46 (68.7)	18 (39.1)	28 (60.9)	
pT3	8 (11.9)	2 (25)	6 (75)	
pT4	2 (3)	0	2 (100)	
Lymph node status				0.55
Metastatic	29 (43.3)	9 (31)	20 (69)	
Non-metastatic	38 (56.7)	14 (36.8)	24 (63.2)	
Histological subtype				0.062
Invasive ductal carcinoma	51 (76.1)	17 (33.3)	34 (66.7)	
Invasive lobular carcinoma	7 (10.4)	5 (71.4)	2 (28.6)	
Mixt	3 (4.5)	1 (33.3)	2 (66.7)	
Other	6 (9)	0	6 (100)	

AR – androgen receptor; TNBC – triple-negative breast cancer

index, and between AR expression and histologic grade ($p = 0.001$, $R = –0.507$ and $p = 0.02$, $R = –0.283$, respectively). Androgen receptor expression was also negatively correlated with tumour diameter and stage at diagnosis, but none of them reached the level of significance ($p > 0.05$). There was no relationship between AR expression and other prognostic factors ($p > 0.05$) (Table III).

In the HER2-positive group, 23 (52.3%) patients developed relapse/metastasis and 21 (47.7%) died during follow-up. The AR expression was 79.5% for the HER2-positive group. Divided into two groups based on AR expression, 5-year DFS was, respectively, 54.5% and 50% for patients with AR > 7.5% and with AR < 7.5% in the HER2-positive group. The survival difference between two groups was not significant ($p = 0.58$) (Fig. 5). The same patient group presented 5-year OS rates of 57.6% and 63.6% for AR > 7.5% and AR < 7.5%. The difference between two groups remained below the threshold of significance ($p = 0.91$). In the HER-2 positive group, HR expression was established in 32 (72.7%) patients. In the HR-negative group, it was in 8 (66.7%) patients; in HR-positive group in 27 (84.4%) patients AR expression was identified. The difference between two groups was statistically significant ($p = 0.003$). In the HER2-positive group, AR expression and HR expression was significantly and positively correlated ($p = 0.021$, $R = 0.347$) There was no relationship between AR expression and other prognostic factors ($p > 0.05$) (Table IV).

Table IV. Androgen receptor expressions in the HER2-positive subgroup

HER2-Positive	n = 44 (%)	AR-positive n (%)	AR-negative n (%)	p
Median age (range)	53 (36-80)			0.15
< 50	19 (43.2)	17 (89.5)	2 (10.5)	
> 50	25 (56.8)	18 (72)	7 (28)	
ER/PR				0.021
Positive	32 (72.7)	27 (84.4)	5 (15.6)	
Negative	12 (27.3)	8 (66.7)	4 (33.3)	
ER				0.03
Positive	27 (61.4)	23 (85.2)	4 (14.8)	
Negative	17 (38.6)	12 (70.6)	5 (29.4)	
PR				0.031
Positive	28 (63.6)	23 (82,1)	5 (17.9)	
Negative	16 (36.4)	12 (75)	4 (25)	
Histologic grade				0.19
I	4 (9.1)	4 (100)	0	
II	30 (68.2)	26 (86.6)	4 (13.3)	
III	10 (22.7)	5 (50)	5 (50)	
Ki67 Labelling index				0.18
< 14	15 (34.1)	12 (80)	3 (20)	
14-30	9 (20.5)	9 (100)	0	
> 30	20 (45.5)	14 (70)	6 (30)	
Stage at diagnosis				0.52
Stage I	2 (4.5)	1 (50)	1 (50)	
Stage II	29 (65.9)	24 (82.8)	5 (17.2)	
Stage III	13 (29.5)	10 (76.9)	3 (23.1)	
pT1	9 (20.5)	6 (66.7)	3 (33.3)	0.38
pT2	26 (59.1)	23 (88.5)	3 (11.5)	
pT3	6 (13.6)	4 (66.7)	2 (33.39)	
pT4	3 (6.8)	2 (66.7)	1 (33.3)	
Lymph node status				0.27
Metastatic	31 (70.5)	26 (86.3)	5 (16.1)	
Non-metastatic	13 (29.5)	9 (69.2)	4 (30.8)	
Histological subtype				0.11
Invasive ductal carcinoma	40 (90.9)	33 (82.5)	7 (17.5)	
Invasive lobular carcinoma	3 (6.8)	2 (66.7)	1 (33.3)	
Other	1 (2.3)	0	1 (100)	

AR – androgen receptor; ER – oestrogen receptor; PR – progesterone receptor

Discussion

Breast cancer is a heterogeneous disease. After the 2000s, it started to be classified based also on gene expression profiles. HER2-enriched and basal-like subtypes are among the subtypes with the worst prognoses based on the gene expression profile. Immunohistochemically, the HER2-enriched subtype is generally ER-negative, PR-negative, and HER2-positive. HER2-enriched subtype and basal-like subtype comprise 20% and 10% of all BC cases, respectively. Seventy per cent of cases with basal-like subtype consist of TNBC [24, 25, 26, 27]. TNBCs are also a heterogeneous group. In 2011, this group was divided into 6 molecular subtypes, and later, in 2016, it was again divided into four molecular subtypes including

basal-like 1, basal-like 2, luminal androgen receptor (LAR), and mesenchymal. The LAR subtype comprises 16% of TNBCs. TNBC cases with LAR-like tumours are diagnosed at later ages than other subtypes, and they present higher Axillary lymph node involvement. The distant metastasis location is, in particular, the bone [28]. The LAR subtype is dependent on AR signalling. Although the AR expression is detected also in other molecular subtypes of TNBC, it is found to be highest in the LAR subtype [29, 30, 31].

The AR expression was 34.3% and 79.5% in TNBC and HER2-positive groups, respectively, in the present study. A significant difference was detected between two groups in terms of AR expression. In addition, in every HER2-positive group, a significant difference was determined in terms of AR expression between HR-positive and HR-negative groups. In a systematic review of 19 studies including a total of 7693 patients with BC, the AR expression was 31.8% in ER-negative tumours [10]. Collins *et al.* evaluated 2171 patients with invasive BC and found 59% AR expression in the HER2-positive group and 32% in the TNBC group [11]. Niemer *et al.* detected 63% AR expression in the HER2-positive subgroup and 10% in the TNBC subgroup [12]. In another study, Park *et al*. assessed AR expression in 413 patients diagnosed with BC and found AR expression to be 35% and 77% in the TNBC and HER2-positive subgroups, respectively. Androgen receptor expression was found to be associated with ER expression, lower histologic grade and smaller tumour size at diagnosis [14]. In a study by Qi *et al*., which evaluated AR expression in 980 patients with BC, the limited number of HER2-positive patients presented 86% AR expression in the HR-positive subgroup and 66% in the HR-negative subgroup [18].

The present study revealed a significant negative correlation between AR expression and histological grade and between AR expression and Ki-67 labelling index in the TNBC group. A longer OS was observed among the patients with high AR expression in the TNBC group. A positive correlation was detected between AR expression and HR expression in the HER2-positive patient group. There was no relationship between other prognostic factors and AR expression. Likewise, no correlation was detected between AR expression and survivals in the HER2-positive patient group. Our findings showed that AR expression was particularly associated with good prognostic factors in the TNBC group and that no significant correlation was found between AR expression and prognostic factors in the HER2-positive group. The relevant literature reports conflicting findings regarding the prognostic significance of AR expression in patients diagnosed with BC. In a study by McGhan *et al*., which evaluated 94 TNBC cases, AR expression was detected as 23%. AR expression was reported to be associated with advanced age and lymph node positivity. They reported no association between AR expression and survival [13]. McNamara *et al*. studied AR expression on TNBC cases and reported a negative correlation between AR expression and Ki-67 labelling index and a positive correlation between AR expression and tumour diameter, lymph node invasion, and distant metastasis [15]. Analysing 83 patients with TNBC, the authors observed AR expression in 27.7% of the patients. AR expression was found to be inversely correlated with histologic grade and Ki-67 labelling index. There was no association between AR expression and survival rates [17]. Choi *et al*. analysed 492 cases with TNBC and reported AR expression to be 17.7%. Androgen receptor expression was found to be associated with lower histological grade and advanced age. However, both univariate and multivariate analyses showed that AR expression was associated with decreased OS [21]. In a study conducted by Hu *et al*. on AR expression in patients with BC, it was demonstrated that AR expression caused an 83% increase in mortality in the TNBC subgroup. A small number of cases in the HER2-positive subgroup found 36% AR expression with no correlation of survival [22].

Aleskandarany *et al.* assessed AR expression in operated patients with BC and found 41.5% and 23.2% AR expression rates in HER2-positive and TNBC subgroups, respectively. When TNBC and HER2-positive subgroups were evaluated exclusively, AR expression was demonstrated not to be associated with breast-cancer-specific survival or distant-metastasis-free interval [23]. Gasparini *et al*. evaluated AR expression in patients with TNBC. AR expression was demonstrated to be linked to improved OS [16]. Tang *et al*. evaluated AR expression and its clinical significance in the TNBC patient group and observed AR expression in 12.6% of the cases. Androgen receptor expression was found to be associated with improved DFS, OS, menorrhoeal status, and lower histological grade [19]. In a study conducted by the German GeparTrio group on AR expression in BC cases treated with neoadjuvant chemotherapy, the AR expression was demonstrated to predict improved DFS and improved OS in the TNBC group. AR expression was not associated DFS and OS in the HER2-positive group [32]. Luo *et al*. evaluated AR expression in 137 TNBC cases and reported AR expression to be 27.7%. AR expression was observed to be associated with grade, nodal status, DFS, and OS [33]. In a study conducted by He *et al*. on 287 TNBC cases, AR expression was 25.8%. It was also associated with nodal status. Moreover, the multivariate analysis showed that AR expression was associated with improved DFS [20]. In a study in which Sas-Korczynska *et al*. evaluated male patients diagnosed with breast cancer, AR expression was shown to be connected with favourable OS [34].

Table V. Results of studies about AR in triple negative breast cancer and HER-positive breast cancer

References	Subgroup	AR expression (%)	Results
Vera-Badillo *et al.* 2014 [10]	ER-positive	74.8	Improved OS
	ER-negative	31.8	Similar DFS
Collins *et al.* 2011 [11]	Luminal A	91	AR expression is most common in the luminal A group
	Luminal B	68	
	HER-2 positive	59	
	Basal like	32	
Niemeier *et al.* 2010 [12]	ER-positive	95	Associated with ER-expression
	TNBC	10	
	HER2-positive	63	
McGhan *et al.* 2014 [13]	TNBC	23	Similar OS, increased lymph node metastases
Park *et al.* 2010 [14]	TNBC	7.3	Associated with ER expression, smaller tumour size, lower histological grade
	HER2-positive	21.9	
	ER-positive	69.8	
	PR-positive	79.4	
McNamara *et al.* 2013 [15]	TNBC		
Gasparini *et al.* 2014 [16]	TNBC	24.8	Improved OS
Mrklic I *et al.* 2013 [17]	TNBC	32.5	Similar DFS and OS
Qi *et al.* 2012 [18]	Luminal A	90	Associated with HR-expression, lower Ki-67 expression
	Luminal B, HER2-negative	80	
	Luminal B, HER2-positive	86	
	HER2-positive, HR-negative	66	
	TNBC	52	
Tang *et al.* 2012 [19]	TNBC	12.6	Improved DFS and OS
He *et al.* 2012 [20]	TNBC	25.8	Improved DFS
Choi *et al.* 2015 [21]	TNBC	17.7	Decreased OS
Hu *et al.* 2011 [22]			
Aleskandarany *et al.* [23]	Luminal A-B	64.1	Improved OS
	TNBC	41.5	
	HER2-positive	23.2	
Loibl *et al.* 2011 [32]	Luminal A	67.1	Improved DFS and OS (TNBC)
	Luminal B HER2-positive	53.7	Similar DFS and OS (HER2-positive)
	Luminal B HER2-negative	60.5	
	HER2-positive	58.5	
	TNBC	21.6	
Luo *et al.* 2010 [33]	TNBC	27.2	Improved DFS and OS (TNBC)
	non-TNBC	83.3	Similar DFS and OS (non-TNBC)
Sas-Korczynska *et al.* 2015 [34]	Male breast cancer	62.5	Associated with ER expression and longer OS

AR – androgen receptor; DFS – disease-free survival; ER – oestrogen receptor; DFS – disease-free survival; OS – overall survival; PR – progesterone receptor; TNBC – triple-negative breast cancer

Also, there are available studies suggesting that AR may serve as a prognostic and predictive marker. In the Gepartrio study, a lower pathological complete response rate was obtained with neoadjuvant chemotherapy among the AR-positive patient group [32]. Mehta *et al*. evaluated the effect of Paclitaxel, 5-fluorouracil, and cyclophosphamide on AR-positive TNBC and reported findings suggesting that AR might play a role in resistance against chemotherapeutics [35]. The studies related to AR were summarised in Table V.

Although the AR expression has been reported in varying degrees in BC subtypes, its role in carcinogenesis and cancer biology remains unclear. It is reported that the effect of androgens and AR may change depending on the type of cancer cells and the presence of other accompanying steroid hormone receptors. AR has been demonstrated to probably exert an anti-proliferative effect by antagonising ER in ER-positive tumours. It can suppress oestrogen-mediated tumour proliferation by competitively binding to the oestrogen response elements and co-activators. It can upregulate ER-β receptors [36, 37]. Various mechanisms are suggested for the role of AR in HER2-positive cancer cells. In HER2-positive tumours, AR is demonstrated to activate the Wnt/β-catenin pathway and cause HER3 upregulation, and thereby it can play a role in breast carcinogenesis [38]. Through HER2/HER3 heterodimers, AR can cause the activation of PI3K/AKT pathway, and it may lead to cell proliferation through MYC [39]. AR causes HER2 expression, which in turn leads to ERK activation. Then, cAMP response element binding protein activity and AR expression occur, resulting in a feedback loop [40]. It is reported that the androgens may exert a proliferative effect through AR in ER-negative AR-positive tumour cells. However, the role of AR in carcinogenesis is less clear in TNBC than in other subtypes. Toth-Fejel *et al*. demonstrated that anti-androgens inhibit proliferation of the ER-negative BC cell line by 22% and that the effect is inhibited by bicalutamide, which is an anti-androgen [41]. On the other hand, in their study conducted on ER-negative BC cell lines, Doane *et al*. detected that androgens exerted a proliferative effect through AR [42]. Amphiregulin, an EGFR ligand, is shown to be upregulated through AR in non-LAR TNBC cell lines. This suggests that the proliferation might have been increased through EGFR pathway. This proliferative effect is demonstrated to be blocked by the anti-androgen enzalutamide [29]. Lehman *et al*. reported that anti-androgens in LAR TNBC cell line caused an anti-proliferative effect [43]. In a study by Cuenca-Lopez *et al*., anti-androgen bicalutamide is shown to exert an anti-proliferative effect on TNBC cell lines. The same study detected a correlation between AR expression and EGFR expression, and between AR expression and PDGFR expression. Increased PI3k/Akt activation was observed in AR-positive TNBC [44]. All those findings suggest that EGFR, PDGR, and PI3K/akt signalling pathways may play a role in TNBC carcinogenesis through AR.

Given our findings and the data available on the limited number of HER2-positive patients in the literature, the prognostic importance of AR in HER2-positive patients has not been fully clarified. Despite the administration of HER2-receptor targeted treatments, patients continue to develop progression, and new therapeutic targets are required. There are clinical studies being designed regarding combined therapies targeting AR and HER2, which are also supported by preclinical studies (NCT02091960, NCT00755885). Although the literature presents conflicting results about the TNBC patient group, the majority of the literature and our study associate AR expression with good prognosis. The variation regarding the prognostic importance of AR, reported by the studies in the relevant literature, might be due to the heterogeneity of the TNBC group, heterogeneity of the cut-off value for AR positivity (> 10, > 5, > 1), heterogeneity of the primary antibody sensitivity, and heterogeneity of the antibody sources. The AR expression variation among the molecular subgroups of TNBC and the failure to classify the TNBC subjects according to molecular subtypes may also contribute to an account of this variation. Since the clinical effectiveness of new-generation anti-androgens targeting AR in prostate cancer was demonstrated, clinical studies have been initiated to evaluate AR as a target receptor in TNBC patient group as well, which is due to the absence of target receptors in TNBC, limited treatment options for TNBC, and positive preclinical studies showing the effectiveness of anti-androgens with TNBC cell lines (NCT02750358, NCT02689427, NCT02971761). The findings of clinical studies utilising AR in TNBC patient groups are promising. Gucalp *et al*. found a 19% clinical benefit rate as part of a phase II study in which they evaluated the effectiveness of bicalutamide in metastatic ER-negative and AR-positive (> 10%) patients [6]. A complete response was reported after bicalutamide in metastatic AR-positive TNBC previously treated with multiple-line chemotherapy [45]. Another phase II study evaluated the effectiveness of abiraterone in metastatic TNBC and reported a 6-month clinical benefit rate of 20% [46]. Enzalutamide's 24-week CBR in advanced AR-positive TNBC patients was found to be 29% [47].

There is no standardisation for the cut-off value of AR expression. In the present study, we took the cut-off value as 7.5 with ROC curve analysis. Including AR assessment in routine pathological examinations will help the efforts to clarify its clinical

and prognostic importance. It will also contribute to the planning of studies targeting AR signalling pathway. Lastly, it will facilitate the classification of TNBC/AR-positive and quadruple negative BC, which could be utilised in the future.

The authors declare no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
2. Lal P, Tan LK, Chen B. Correlation of HER-2 status with estrogen and progesterone receptors and histologic features in 3,655 invasive breast carcinomas. Am J Clin Pathol 2005; 123: 541-546.
3. Perez EA, Romond EH, Suman VJ, et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2-positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. J Clin Oncol 2014; 32: 3744-3752.
4. Lin NU, Vanderplas A, Hughes ME, et al. Clinicopathologic features, patterns of recurrence, and survival among women with triple-negative breast cancer in the National Comprehensive Cancer Network. Cancer 2012; 118: 5463-5472.
5. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007; 13: 4429-4434.
6. Gucalp A, Tolaney S, Isakoff SJ, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. Clin Cancer Res 2013; 19: 5505-5512.
7. Zhu X, Li H, Liu JP, et al. Androgen stimulates mitogen-activated protein kinase in human breast cancer cells. Mol Cell Endocrinol 1999; 152: 199-206.
8. Quigley CA, De Bellis A, Marschke KB, et al. Androgen receptor defects: Historical, clinical, and molecular perspectives. Endocr Rev 1995; 16: 271-321.
9. Foradori CD, Weiser MJ, Handa RJ. Non-genomic actions of androgens. Front Neuroendocrinol 2008; 29: 169-181.
10. Vera-Badillo FE, Templeton AJ, de Gouveia P, et al. Androgen receptor expression and outcomes in early breast cancer: A systematic review and meta-analysis. J Natl Cancer Inst 2014; 106: djt319.
11. Collins LC, Cole KS, Marotti JD, et al. Androgen receptor expression in breast cancer in relation to molecular phenotype: Results from the Nurses' Health Study. Mod Pathol 2011; 24: 924-931.
12. Niemeier LA, Dabbs DJ, Beriwal S, et al. Androgen receptor in breast cancer: Expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. Mod Pathol 2010; 23: 205-212.
13. McGhan LJ, McCullough AE, Protheroe CA, et al. Androgen receptor-positive triple negative breast cancer: A unique breast cancer subtype. Ann Surg Oncol 2014; 21: 361-367.
14. Park S, Koo J, Park HS, et al. Expression of androgen receptors in primary breast cancer. Ann Oncol 2010; 21: 488-492.
15. McNamara KM, Yoda T, Takagi K, et al. Androgen receptor in triple negative breast cancer. J Steroid Biochem Mol Biol 2013; 133: 66-76.
16. Gasparini P, Fassan M, Cascione L, et al. Androgen receptor status is a prognostic marker in non-basal triple negative breast cancers and determines novel therapeutic options. PLoS One 2014; 9: e88525.
17. Mrklić I, Pogorelić Z, Capkun V, et al. Expression of androgen receptors in triple negative breast carcinomas. Acta Histochem 2013; 115: 344-348.
18. Qi JP, Yang YL, Zhu H, et al. Expression of the androgen receptor and its correlation with molecular subtypes in 980 Chinese breast cancer patients. Breast Cancer 2012; 6: 1-8.
19. Tang D, Xu S, Zhang Q, et al. The expression and clinical significance of the androgen receptor and E-cadherin in triple-negative breast cancer. Med Oncol 2012; 29: 526-533.
20. He J, Peng R, Yuan Z, et al. Prognostic value of androgen receptor expression in operable triple-negative breast cancer: a retrospective analysis based on a tissue microarray. Med Oncol 2012; 29: 406-410.
21. Choi JE, Kang SH, Lee SJ, et al. Androgen receptor expression predicts decreased survival in early stage triple-negative breast cancer. Ann Surg Oncol 2015; 22: 82-89.
22. Hu R, Dawood S, Holmes MD, et al. Androgen receptor expression and breast cancer survival in postmenopausal women. Clin Cancer Res 2011; 17: 1867-1874.
23. Aleskandarany MA, Abduljabbar R, Ashankyty I, et al. Prognostic significance of androgen receptor expression in invasive breast cancer: Transcriptomic and protein expression analysis. Breast Cancer Res Treat 2016; 159: 215-227.
24. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000; 406: 747-752.
25. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003; 100: 8418-8423.
26. Prat A, Adamo B, Cheang MC, et al. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist 2013; 18: 123-133.
27. Pérez-Rodríguez G, Aranda-Moreno C, Olivares-Corichi IM, et al. The association of subtypes of breast cancer with tumour characteristics and reproductive factors in 1326 Mexican women. Contemp Oncol (Pozn) 2015; 19: 462-466.
28. Lehmann BD, Jovanović B, Chen X, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. PLoS One 2016; 11: e0157368.
29. Barton VN, D'Amato NC, Gordon MA, et al. Multiple molecular subtypes of triple-negative breast cancer critically rely on androgen receptor and respond to enzalutamide in vivo. Mol Cancer Ther 2015; 14: 769-778.
30. Yu KD, Zhu R, Zhan M, et al. Identification of prognosis-relevant subgroups in patients with chemoresistant triple-negative breast cancer. Clin Cancer Res 2013; 19: 2723-2733.
31. Jézéquel P, Loussouarn D, Guérin-Charbonnel C, et al. Gene-expression molecular subtyping of triple-negative breast cancer tumours: Importance of immune response. Breast Cancer Res 2015; 17: 43.
32. Loibl S, Müller BM, von Minckwitz G, et al. Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat 2011; 130: 477-487.
33. Luo X, Shi YX, Li ZM, et al. Expression and clinical significance of androgen receptor in triple negative breast cancer. Chin J Cancer 2010; 29: 585-590.
34. Sas-Korczynska B, Adamczyk A, Niemiec J, et al. Androgen receptor in male breast cancer. Pol J Pathol 2015; 66: 347-352.
35. Mehta J, Asthana S, Mandal CC, et al. Molecular analysis provides novel insights into androgen receptor signalling in breast cancer. PLoS One 2015; 10: e0120622.
36. Peters AA, Buchanan G, Ricciardelli C, et al. Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. Cancer Res 2009; 69: 6131-6140.
37. Macedo LF, Guo Z, Tilghman SL, et al. Role of androgens on MCF-7 breast cancer cell growth and on the inhibitory effect of letrozole. Cancer Res 2006; 66: 7775-7782.

38. Turashvili G, Bouchal J, Burkadze G, et al. Wnt signaling pathway in mammary gland development and carcinogenesis. Pathobiology 2006; 73: 213-223.
39. Ni M, Chen Y, Fei T, et al. Amplitude modulation of androgen signalling by c-MYC. Genes Dev 2013; 27: 734-748.
40. Chia KM, Liu J, Francis GD, et al. A feedback loop between androgen receptor and ERK signaling in estrogen receptor-negative breast cancer. Neoplasia 2011; 13: 154-166.
41. Toth-Fejel S, Cheek J, Calhoun K, et al. Estrogen and androgen receptors as comediators of breast cancer cell proliferation: providing a new therapeutic tool. Arch Surg 2004; 139: 50-54.
42. Doane AS, Danso M, Lal P, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. Oncogene 2006; 25: 3994-4008.
43. Lehmann BD, Bauer JA, Schafer JM, et al. PIK3CA mutations in androgen receptor-positive triple negative breast cancer confer sensitivity to the combination of PI3K and androgen receptor inhibitors. Breast Cancer Res 2014; 16: 406.
44. Cuenca-López MD, Montero JC, Morales JC, et al. Phospho-kinase profile of triple negative breast cancer and androgen receptor signaling. BMC Cancer 2014; 14: 302.
45. Arce-Salinas C, Riesco-Martinez MC, Hanna W, et al. Complete response of metastatic androgen receptor-positive breast cancer to bicalutamide: case report and review of the literature. J Clin Oncol 2016; 34: e21-e24.
46. Bonnefoi H, Grellety T, Tredan O, et al. A phase II trial of abiraterone acetate plus prednisone in patients with triple-negative androgen receptor positive locally advanced or metastatic breast cancer (UCBG 12-1). Ann Oncol 2016; 27: 812-818.
47. Traina TA, Miller K, Yardley DA, et al. Results from a phase 2 study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in advanced AR+ triple-negative breast cancer (TNBC). J Clin Oncol 2015; 33 (suppl): 1003.

Address for correspondence

Betul Bolat Kucukzeybek
Department of Pathology
İzmir Katip Celebi University Ataturk Training
and Research Hospital
35360 İzmir, Turkey
e-mail: bbkzeybek@yahoo.com