

PROGNOSTIC ROLE OF ANDROGEN RECEPTOR EXPRESSION IN PATIENTS WITH METASTATIC TRIPLE NEGATIVE BREAST CANCER

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At present, there are no valid prognostic biomarkers in patients with triple negative breast cancer (TNBC) except well known clinical factors such as tumor size, lymph node status, differentiation grade and proliferation rate. **Aim:** To evaluate the prognostic role of androgen receptor (AR) expression in patients with TNBC. **Materials and Methods:** The effect of the AR expression level in tumor tissue on overall survival depending on clinical, histological and immunohistochemical characteristics of the tumor was evaluated in 116 patients with metastatic TNBC. **Results:** The independent prognostic value of AR expression in patients with TNBC of different stages was shown. The median overall survival was higher in patients with AR-positive tumors compared with AR-negative tumors (57 months vs. 27 months, $p < 0.0001$). Five-year survival since diagnosis in the group with AR-positive TNBC was $47.6 \pm 8.3\%$ vs $20.0 \pm 5.3\%$ in the group with AR-negative TNBC ($p < 0.05$). **Conclusions:** The results of our study indicate a favorable impact of AR expression on the overall survival of TNBC patients.

Key Words: triple negative breast cancer, androgen receptor, clinical prognosis, overall survival.

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Androgen receptor (AR) is steroid hormone receptor that functions as a classical ligand-activated intracellular transcription factor [1, 2]. This factor controls genes that are involved in different, sometimes even opposite processes in the cell stimulating or suppressing cell proliferation and apoptosis, which may depend on the activated signaling pathways. AR expression is present in normal breast tissue and most molecular subtypes of breast cancer (BC). ARs are localized in epithelial cells as well as estrogen and progesterone receptors but are absent in the myo-epithelium and the breast stroma [2, 3]. The ultimate role of androgens in the development and progression of the BC has not been established. The theory of the androgen importance in the BC pathogenesis is based on the presence of AR in 70–90% of primary breast tumors and in 75% of distant metastases [4, 5]. The presence of AR in the tumor depends on the molecular subtype of the BC and is observed in almost 90% of luminal A cases, in 70–90% of luminal B cases, in 60% of HER2-positive subtype and 10–50% of triple negative BC (TNBC) [5].

In recent years, the greatest interest of the researchers has been focused on the study of AR in TNBC, as a possible potential target for treatment. Indeed, in the well-known molecular genetic classification by Lehmann [6], “AR-positive” TNBC is presented as a separate subtype — luminal with ARs.

It should be noted that the prognostic and predictive role of AR in TNBC remains unclear. The study of Kim et al. [7] showed that in patients with TNBC the presence of AR expression is associated

with better progression-free survival (HR 0.42, 95% CI 0.27–0.64). In the study of Choi et al. [8] it was found that AR expression may be an unfavorable prognostic factor in a subgroup of patients with pT1 TNBC, but its prognostic value in patients with metastases and tumor $\geq$ pT2 has not been proven. In a meta-analysis of 13 clinical trials performed by Wang et al. [9] with the participation of 2826 patients with TNBC, AR-positive tumors were detected in 24.4% of patients, and the association of AR expression with postmenopausal status of patients, high tumor differentiation grade (G1-G2) and an increased risk of lymph node metastasis has been established.

The aim of the study was to evaluate the prognostic role of AR expression in patients with TNBC.

MATERIALS AND METHODS

The study of AR expression in tumor tissue was performed in 116 female patients with TNBC. The patients received combined therapy according to the existing standards: surgical treatment, neoadjuvant polychemo-therapy (PCT) using anthracycline-containing regimens, radiation therapy. In a case of disease progression, PCT was performed by the paclitaxel, carboplatin regimen as the first line of treatment, and monotherapy with vinorelbine, gemcitabine or capecitabine as the second line of palliative chemotherapy. All patients signed an informed consent on participation in the study. This research was approved by Ethics Committee of the National Cancer Institute of the Ministry of Health of Ukraine.

Morphological and immunohistochemical (IHC) studies were performed in the Department of Pathology of the National Cancer Institute. IHC study was performed on deparaffinized sections prepared from paraffine blocks of surgically removed tumors using AR mouse monoclonal antibody, clone AR441DBS (Abcam, USA), and monoclonal mouse anti-human

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Abbreviations used: AR — androgen receptor; BC — breast cancer; IHC — immunohistochemical; HR — hazard ratio; OS — overall survival; PARP1 — poly(ADP-ribose) polymerase 1; PCT — polychemo-therapy; TNBC — triple negative breast cancer.

Ki-67, clone MIB-1 (Dako, Denmark). For detection of IHC reaction, EnVisionTM FLEX imaging system (Dako, Denmark) was used. The results of the IHC reaction were assessed by a semi-quantitative method, by counting the number of positively stained cells — labeling index (expressed in percents). Tumors in which AR expression was detected in more than 10% of tumor cells were considered AR-positive.

The statistical analysis of the primary data was performed using the methods of descriptive statistics and univariate and multivariate Cox proportional-hazards model, the significance of data differences was assessed by Fisher coefficient. The overall survival (OS) of patients was determined by Kaplan — Meier method.

RESULTS AND DISCUSSION

We have analyzed a relation of AR expression to the clinical characteristics of patients with TNBC, such as the stage at the time of diagnosis (according to the TNM classification, 6th edition), the biological characteristics of the tumor (differentiation grade, proliferative activity (Ki-67); and the age and menstrual status of the patients.

The age of patients at the time of inclusion in the study ranged from 27 to 76 years (mean 51.7 ± 9.5 years). Among 116 patients with TNBC, positive AR expression in tumor tissue was detected in 44 (38%) patients, while in the remaining 72 (62%) cases AR expression was not detected (AR-negative). It should be noted that AR-positive tumors were more common in older patients. The mean age of patients with AR-negative TNBC was (49.5 ± 9.7) years vs (56.1 ± 7.5) years in patients with AR-positive tumors ($p = 0.002$). Moreover, we found a direct correlation between patient's age and AR expression status (Spearman's $\rho = 0.357$; 95% CI 0.186–0.507; $p = 0.0001$).

The association between clinical and biological characteristics of the tumors and the AR expression is presented in the Table. In patients with TNBC, positive AR expression was more common in menopausal patients (86.4% vs 13.6%, $p = 0.001$).

It should be noted that in patients with AR-negative TNBC, the disease was more often diagnosed at the

later stages ($p = 0.005$), and axillary lymph node involvement was more common than in patients with AR-positive TNBC ($p = 0.01$). However, there were no statistically significant differences between the AR-positive and AR-negative groups in relation to tumor differentiation grade (see the Table).

In our study, no significant difference by the index of proliferative activity (Ki-67) between the groups with different AR status was found — ($49.1 \pm 19.6\%$) in AR-negative tumors vs ($49.6 \pm 18.6\%$) in AR-positive tumors.

We investigated the relation of AR expression to the OS of patients with TNBC by Kaplan — Meier method. The median OS was higher in patients with AR-positive tumors compared with AR-negative tumors (57 months vs 27 months, $p < 0.0001$). The five-year survival from the moment of diagnosis in patients with AR-positive TNBC was also higher compared to patients with AR-negative tumors ($47.6 \pm 8.3\%$ vs $20.0 \pm 5.3\%$; $p < 0.05$). The results showed (Fig. 1) that the AR expression significantly affected the survival of patients with TNBC according to the log-rank test, $p < 0.0001$. Hazard ratio (HR) for the death of patients with AR-positive TNBC (as compared to AR-negative tumors) in non-stratified group analyzed by Cox proportional-hazards model was calculated as 0.34 [95% CI (0.22–0.55)] ($p = 0.0001$).

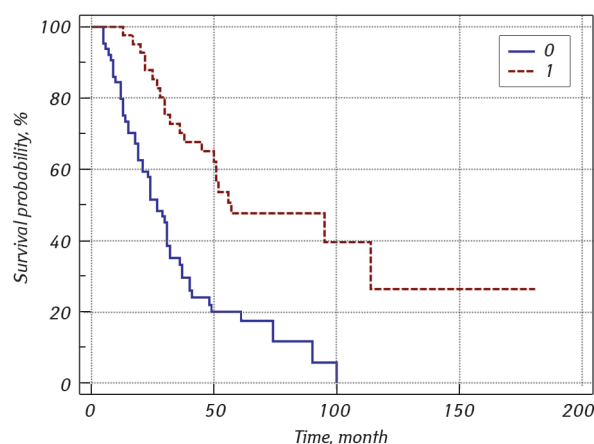


Fig. 1. OS of patients with TNBC. Group 0 — AR-negative. Group 1 — AR-positive, log-rank test, $p < 0.0001$

Table. Clinical and biological characteristics of tumors of patients with TNBC depending on the AR expression

Index		AR-negative group, n=72 (% in parenthesis)	AR-positive group, n=44 (% in parenthesis)	p
Menstrual function	Preserved	37 (51.4)	6 (13.6)	0.001
	Menopause	35 (48.6)	38 (86.4)	
Stage	I	4 (5.6)	6 (13.6)	0.005
	IIA	16 (22.2)	17 (38.6)	
	IIB	16 (22.2)	6 (13.6)	
	IIIA	11 (15.3)	8 (18.2)	
	IIIB	3 (4.2)	5 (11.4)	
	IV	22 (30.6)	2 (4.5)	
Differentiation grade	G-2	14 (20)	12 (28.6)	0.42
	G-3	56 (80)	30 (71.4)	
Primary tumor size T	T1	8 (11.1)	9 (20.9)	0.41
	T2	41 (56.9)	23 (53.5)	
	T3	14 (19.4)	5 (11.6)	
	T4	9 (12.5)	6 (14)	
Axillary lymph node involvement	N0	19 (26.4)	22 (51.2)	0.01
	N1	35 (48.6)	8 (18.6)	
	N2	17 (23.6)	12 (27.9)	
	N3	1 (1.4)	1 (2.3)	

Analysis of the bivariate Cox proportional-hazards model in the non-stratified group showed that the detection of the disease at the later stages increased the risk of death by an average of 1.5 times (HR 1.49; 95% CI 1.25–1.77) ($p < 0.0001$) (Fig. 2).

The prognostic value of AR expression was also assessed taking into account the stratification by the stages of the disease using Kaplan — Meier method (Fig. 3, 4). As shown in Fig. 3, the stage of the disease at the time of diagnosis had a statistically significant effect on the OS of patients with AR-negative TNBC ($p < 0.0001$). The study of the impact of the disease stage on the OS of patients with AR-positive tumors (Fig. 4) revealed no significant effect according to the log-rank test ($p = 0.61$).

To date, more data indicate the important role of the AR signaling pathway in the origin and progression of BC of various molecular subtypes, including TNBC. AR expressing TNBC cell lines often carry PI3KCA mutations, making them sensitive to PI3K/mTOR inhibitors. The above observations form the basis for the hypothesis that the cross-interaction of these two signaling pathways promotes the growth of TNBC cells [11, 12]. It should be noted that AR in TNBC interact with cell cycle regulators, such

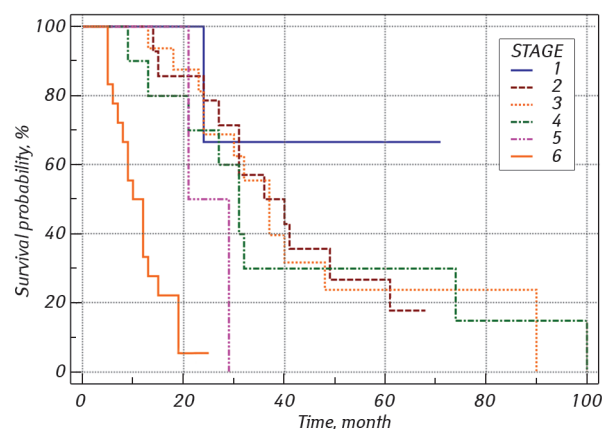


Fig. 2. OS of patients with TNBC depending on the stage of the disease at the time of diagnosis. Clinical stages of the BC: 1 — I; 2 — IIA, 3 — IIB, 4 — IIIA, 5 — IIIB, 6 — IV

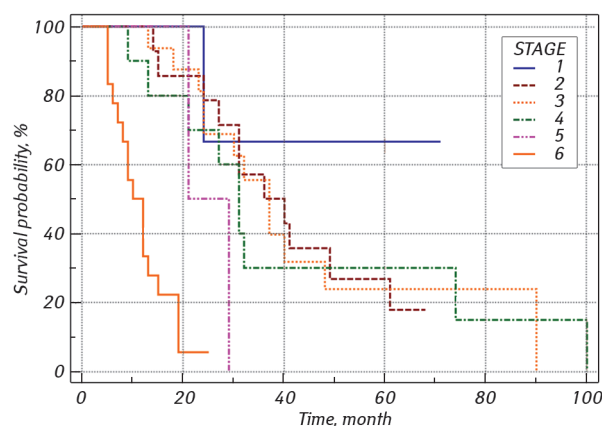


Fig. 3. OS of patients with AR-negative TNBC depending on the stage of the disease at the time of diagnosis. log-rank test, $p < 0.0001$. Clinical stages of the BC: 1 — I; 2 — IIA, 3 — IIB, 4 — IIIA, 5 — IIIB, 6 — IV

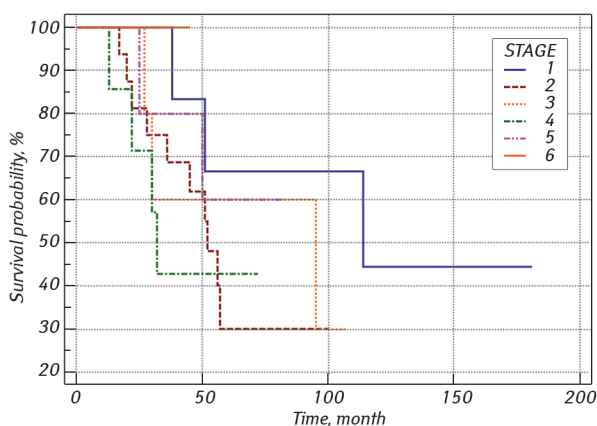


Fig. 4. OS of patients with AR-positive TNBC depending on the stage of the disease at the time of diagnosis. log-rank test, $p = 0.61$. Clinical stages of the BC: 1 — I; 2 — IIA, 3 — IIB, 4 — IIIA, 5 — IIIB, 6 — IV

as the poly-ADP-ribose-polymerase 1 (PARP1) and BRCA1. BRCA1 and PARP1 act as co-activators of the AR signaling pathway and stimulate the transcription of AR genes thereby promoting tumor growth. Preclinical data have shown that PARP1 inhibitors, by preventing the repair of DNA damage, reduce cell migration and invasion, as well as the activity of AR in TNBC with pronounced AR expression [13, 14].

In this study, we have analyzed the prognostic role of AR in 116 patients with TNBC. The positive expression of AR in tumor tissue was found in 38% patients, which is consistent with the data of other researchers [1, 2, 4, 9]. According to one of the largest studies of AR expression in different molecular subtypes of BC [10], it was found that 77% of invasive BC and 32% of basal-like tumors are characterized by pronounced AR expression. In the study of Gong et al. [15], positive AR expression was detected in 42.6% of TNBC. It should be noted that in our study tumors with positive AR expression were more common in older patients. The obtained results are consistent with the data of Asano et al. [16]. In our study, positive expression of AR in the tumor was more common in menopausal patients. It should be noted that in patients with AR-negative TNBC, tumors were more often diagnosed at the later stages, and axillary lymph node involvement in this group was more common than in patients with AR-positive BC. The results are consistent with the latest meta-analysis published by a research group of Wang in 2016 [9]. When analyzing the data of 2826 patients with TNBC, who participated in 13 clinical trials in the period from 2007 to 2015, the authors found that positive expression of AR was more common in menopausal patients [9].

In our study, no significant difference by the index of proliferative activity (Ki-67) between the groups with different AR status was found. These data do not coincide with the data of the above meta-analysis [9]. Other studies have also found no statistically significant relationship between the expression of AR, Ki-67 and tumor differentiation grade [16].

We conducted a comparative analysis of the relation of AR expression to the OS of patients with TNBC, and found that the risk of BC-related death is significantly reduced in the patients with AR-positive tumors. According to the literature, patients with positive AR expression had a significantly lower risk of recurrence compared with other subgroups of TNBC patients [17, 18]. In addition, meta-analysis by Wang et al. [9] confirmed that women with AR-positive TNBC had a 20% lower risk of recurrence compared with the patients with AR-negative TNBC. While the results for recurrence-free survival rates were consistent in all meta-analyses [9, 17, 18], both Qu et al. [17] and Wang et al. [9] found no relationship between AR status and OS rates. However, in a study of Kim et al. [18], an improvement in OS of patients with AR-positive TNBC was shown. The results of a recent prospective study by Asano et al. [16] also indicated better OS of patients with AR-positive TNBC.

To sum up, the evidence base and the results of our study indicate a favorable prognostic value of AR expression in TNBC. Despite a significant body of research, OS data are still inconsistent and further study is required.

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