Original paper

Androgen receptor in male breast cancer

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We present the androgen receptor (AR) status in 32 breast cancers diagnosed in male patients. Androgen receptor expression was found in 62.5% tumors and it was more frequent (85% of cases) in estrogen-positive tumours. The analyses of its impact on treatment results showed that AR immmunopositivity is a prognostic factor for overall survival, and AR immunonegativity is also correlated with worse prognosis (distant metastases developed more frequently and earlier).

Key words: androgen receptor, male breast cancer.

Introduction

Male breast cancer is a very rare malignant neoplasm with an incidence rate of 0.1-1% [1, 2, 3]. The biological factors of male breast cancer are similar to those present in female variant, however, some differences were noted [4, 5, 6]. Male breast cancer, in comparison to female variant, is characterised by more advanced stage, well-differentiated histology (G1, G2), and higher frequency of hormone receptors expression [2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14]. Moreover, it is characterised by worse prognosis [4, 5, 6, 15]. Available literature data and the results of our earlier study show that nodal involvement and hormone receptor status are the most important prognostic factors in male patients with breast cancer [5, 9, 11, 13, 14, 15, 16, 17]. Our observations showed a correlation between hormone receptor (estrogen receptor [ER] / progesterone receptor [PgR]) immunonegativity and poor prognosis (relative risk was 4.12 and 6.94 for overall and disease-free survival, respectively) [16]. Androgen receptor (AR), which plays a role in the inhibition of estrogen-dependent signalling, is a potential prognostic marker in breast cancer [2, 18, 19, 20, 21]. Some clinical data suggest that AR positivity would modify clinical outcomes in breast cancer patients [22, 23, 24].

The aim of the present study was to assess: (i) AR status with regard to the frequency of its immunopositivity, (ii) correlation between AR status and clinicopathological parameters, and (iii) the impact on overall and disease-free survival.

Material and methods

Patients

Between 1950 and 2010, 17320 patients with breast cancer were treated at the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Krakow. Among them there were 81 (0.5%) male patients; detailed characteristics of this group was presented in our earlier papers [16, 25, 26]. The immunohistochemical (IHC) analysis of AR expression was performed on tumor samples collected from 32 patients (the same group which was tested earlier); in these cases an adequate amount and good

quality of tissue in paraffin blocks was available. The consent to perform this retrospective analysis was given by the Bioethical Committee of the Regional Medical Chamber in Krakow.

The mean age of the patients was 62.7 ±12.5 years (range: 34-84, median: 63 years). Surgery was the primary treatment modality in all patients. Mastectomy was performed in 31 patients (96.8%) and 1 patient (3.2%) underwent tumorectomy with axillary lymphadenectomy. The following adjuvant therapy was applied: postoperative radiotherapy (12 patients, 37.5%), chemotherapy (10 patients, 31.3%) and hormonotherapy (18 patients, 56.3%); in some cases multimodal treatment was performed in adjuvant setting. The clinicopathological and biological characterisics of 32 cases of male breast cancer are presented in Table I.

Immunohistochemistry

The expression of AR was assessed on formalin-fixed paraffin-embedded tissue sections, which were cut at 4 μm and mounted on SuperFrost; Plus (Menzel-Gläser, Germany) slides and then deparaffinized and hydrated through a series of xylenes and alcohols.

After antigen unmasking (Target Retrieval Solution, pH = 6.1 DakoCytomation Denmark A/S, Glostrup, Denmark: 50 min, 96°C), slides were incubated for 30 min. in 0.3% H_2O_2 diluted in methanol. Non-specific binding of antibodies was blocked during 5 min. incubation with UltraVision Protein Block (Thermo Scientific, Fremont, USA). After incubation with primary antibody (diluted 1 : 50), BrightVision detection system (Immunologic, Duiven, The Netherlands) and DAB (Vector Laboratories, Inc., Burlingame, USA) were applied. Finally, hematoxylin was used for nuclear counterstaining. Each step of the staining procedure was followed by washing in Tris-Buffered Saline and Tween 20 (TBST). The details of other immunohistochemical stainings were presented elsewhere [16].

We did not obtain IHC results for all proteins in all cases because of inadequate amount of tumour tissue that hindered obtaining reliable results.

IHC evaluation

The presence of AR was evaluated exclusively in the invasive component of the tumours. Immunopositivity of AR was classified as previously described [27] – more than 50% tumour cells with weak staining or any percentage with moderate/strong staining. The details of other immunohistochemical evaluations were presented elsewhere [16].

Statistical methods

All calculations were performed by STATISTICA v.10 software (StatSoft, Inc. Tulsa, OK, USA) and the significance level for all the tests was set as $\alpha = 0.05$. Differences between two groups (AR immunonegativity and AR immunopositivity) were tested using the Mann-Whitney *U*-test (for continuous variables) and Pearson's χ^2 test (for categorized variables). The probability of overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method. The log-rank test was applied to assess the influence of AR status on the treatment results (OS and DFS).

Results

The androgen receptor (AR) immunopositivity was noted in 20 cases (62.5%), while in 6 tumours immunonegativity was observed. Examples of AR staining results are shown in Fig. 1.

The comparison of clinicopathological characteristics according to AR status is presented in Table I.

Androgen receptor immunonegativity was found in all carcinomas without ER expression, while 85%

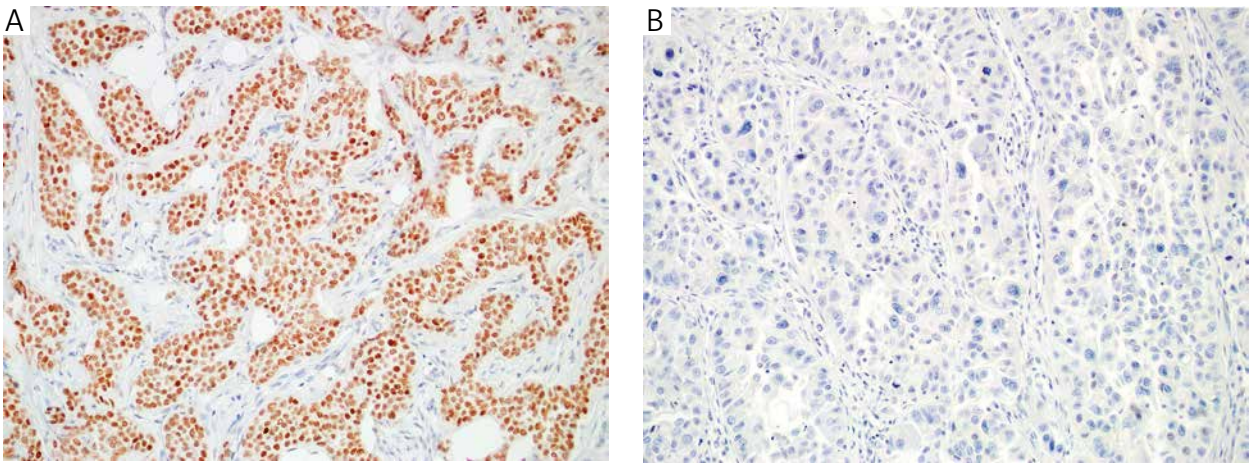

Fig. 1. Strong (A) and absent (B) androgen receptor (AR) expression in male breast cancer cells

Table I. Clinicopathological and molecular characteristics of 32 male patients with breast cancer

Parameters	Whole group	Androgen receptor status		p
	N = 32 (100%)	immunopositive N = 20 (62.5%)	immunonegative N = 6 (18.8%)	
Tumor stage				
T1	4 (12.5%)	3 (15%)	1 (16.7%)	0.198*
T2	4 (12.5%)	4 (20%)	0	
T3	1 (3.1%)	0	1 (16.7%)	
T4	12 (37.5%)	7 (35%)	2 (33.3%)	
Tx	11 (34.4%)	6 (30%)	2 (33.3%)	
Tumor grade				
G1	8 (25%)	6 (30%)	1 (16.7%)	0.598*
G2	17 (53.1%)	9 (45%)	3 (50%)	
G3	5 (15.6%)	3 (15%)	2 (33.3%)	
Gx	2 (6,3%)	2 (10%)	0	
Nodal status				
pN0	15 (46.9%)	9 (45%)	2 (33.3%)	
pN+	17 (53.1%)	11 (55%)	4 (66.7%)	0.119*
ER$				
ER–	2 (6.3%)	0	2 (33.3%)	
ER+	22 (68.8%)	17 (85%)	4 (66.7%)	0.013*
ER/PgR$				
ER– and PgR–	2 (6.3%)	0	2 (33.3%)	
ER+ or PgR+	25 (78.1%)	18 (90%)	4 (66.7%)	0.010*
Basal/mesenchymal markers#				
no basal/mesenchymal markers expression	9 (28.1%)	6 (30%)	3 (50%)	
expression of at least one basal/mesenchymal marker	14 (43.8%)	11 (55%)	3 (50%)	0.526*
MIB-1 LI (mean value ± SD)	35.3 ±15.4	33.1 ±13.7	41.6 ±19.3	0.424**
Distant metastases	11 (34.4%)	6 (30%)	3 (50%)	0.366*
Time (mean ± SD) to distant recurrence [months]	54 ±70.5	80 ±88	10 ±17.3	0.092**

ER: estrogen receptor, PgR: progesterone receptor, +: immunopositivity, –: immunonegativity, MIB-1 LI: MIB1 labelling index, SD: standard deviation
 # cytokeratin 5/6, P-cadherin, smooth muscle actin (SMA), vimentin, epidermal growth factor receptor (EGFR);
** p-value calculated using Pearson's chi-squared test (χ^2) test; ** p-value calculated using the Mann-Whitney U-test, $ mismatches in the number of cases are the consequence of different number of immunohistochemical results for each marker*

of tumors with AR positivity showed ER expression (p = 0.013; Table I). AR positive tumours, as compared to negative ones, were well differentiated (G1 in 30% vs. 16.7%) with smaller proliferation activity (MIB-1 LI 31.1% vs. 41.6%) and lower stage (T1-2 in 35% vs. 16.7%). However, the above-presented differences were not statistically significant.

The median follow-up period was 46 months (range: 1-302 months; medium: 72.9 months). Figure 2 presents OS and DFS according to AR status. It was observed that patients with carcinomas characterised by AR positivity presented favourable OS (p = 0.045, Fig. 2A) and DFS (p = 0.062, Fig. 2B). The estimated 5-year OS and DFS rates for AR positivity was 62.9% and 77.4%, while for AR negativity – 33.3% and 25%, respectively. The absolute improvement of OS and DFS at 5 years was 29.6% and 52.4%, respectively, but only in case of OS it was statistically significant (p = 0.045).

This positive influence of AR immunopositivity on treatment outcome was confirmed by the results of an analysis of distant metastases occurrence. In AR-posi-

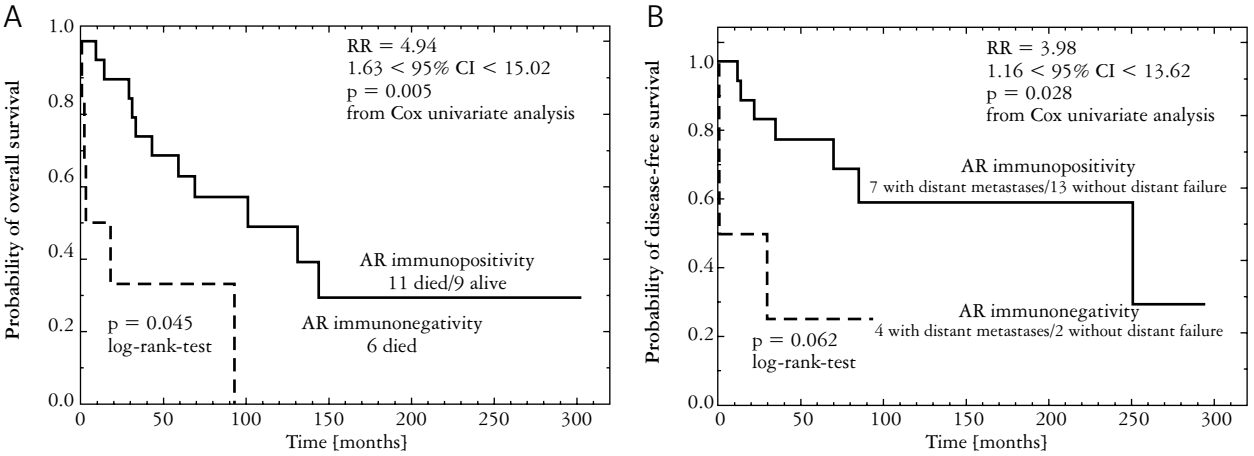

Fig. 2. Overall survival (A) and disease-free survival (B) of male patients with invasive ductal breast cancer stratified by androgen receptor (AR) expression

tive group distant metastases developed in 6/20 cases (30%), in comparison to 3/6 (50%) in AR-negative group. The anatomical localisations of distant failure in relation to AR status (positivity vs. negativity) were as follows: bones (2 vs. 3 cases), lungs (4 vs. 1 cases) and skin (1 case vs. 0). Distant metastases developed later (mean period: 80, range: 16-251 months) in cases with AR positivity than in cases with AR negativity (mean period: 10, range: 1-30 months). However, these differences were not statistically significant ($p = 0.092$).

Discussion

Androgen receptor is a mediator of androgen action in androgen-response tissues (prostate and breast). AR plays an essential role in the development and maintenance of normal glandular organization and integrity. Aberrant AR responses can lead to the disruption resulting in tumorigenesis [28]. AR positivity is detected in approximately 70-80% of all breast cancer cases and it is associated with favourable outcomes, especially in patients with ER-positive tumors [2, 19, 29, 30, 31, 32].

In our material, AR positivity was noted in 62.5% of analysed cases and was related to favourable overall and disease-free survival. The absolute benefit at 5 years was 29.6% for OS and 52.4% for DFS, but only differences in OS were statistically significant. Similar results were noted by other authors, who reported that absolute differences in probability of OS at 3 and 5 years were 6.7% and 13.5%, respectively, while for DFS – 8.8% and 20.7%, respectively [22]. However, these results were observed in female patients. Because of low incidence of male breast cancer and the lack of publications in this area, we referred to literature concerning female breast cancer. In one of the studies it was found that AR negativity predicted the occurrence of distant metastases [23]. On the other hand, Wenhui *et al.* noted adverse effect of AR positivity and its correlation with poor clinical outcome (both overall and disease-free survival) [33]. Vera-Badillo *et al.* hypothesized that AR positivity is a good prognostic marker in ER-positive tumors and a poor prognostic marker in ER-negative tumors [22]. However, the aforementioned hypothesis was not confirmed in meta-analysis.

We noted significant correlation between AR positivity and expression of other steroid receptors (estrogen, progesterone). In our series, 85% of tumors with AR positivity were ER immunopositive. Other researchers, similarly to us, noted this relationship. In their study, 74.8% of ER-positive tumors showed AR expression [22]. Moreover, similarly to us, other studies reported that high expression of AR was not correlated with T stage, histological grade and the status of other hormone receptors [34]. Other authors observed that AR to ER ratio may influence breast cancer response to hormonal therapy [31]. High AR to ER ratio (≥ 2) is related to increasing risk of failure after tamoxifen application. Additionally, this ratio correlates with lymph node involvement and with lower value of proliferation index (MIB-1 LI), and it is an independent predictor of DFS [31].

Furthermore, AR expression negatively correlates with expression of Ki-67 [24], TP53 [35] and with lack of expression of cytokeratine 5/6 [34] and E-cadherin [32]. These results are consistent with the fact that AR-positive tumors are well-differentiated [31], while AR-negative ones metastasise more frequently [23].

Our observations were similar, however, the differences did not reach statistical significance. We noted that AR positivity (in comparison with AR negativity) characterised well differentiated carcinomas (G1: 30% vs. 16.7%) and tumours with lower proliferation index (mean value of MIB-1 LI: 33.1% vs. 41.6%). Moreover, less patients with AR-positive tumours developed distant metastases (30% vs. 50%), which also occurred later (80 months *vs.* 10 months

after treatment) than in patients with AR-negative carcinomas.

Other clinical observations showed that AR negativity was associated with shorter DFS and OS, especially in case of triple negative breast cancer [32, 35, 36]. These results suggest that AR expression could be a useful prognostic marker in triple negative tumors and it may be considered as one of the markers of this subtype [23, 37].

Conclusions

Androgen receptor in male breast cancer is expressed: (i) frequently (by 62.5% of tumors), and (ii) more often in tumours with estrogen receptor positivity (in 85% of ER+ carcinomas). Moreover, its expression is a positive prognostic marker for overall and disease-free survival.

The authors declare no conflict of interest.

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