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Expression of androgen receptors in triple negative breast carcinomas

Ivana Mrklić[a,*], Zenon Pogorelić[b], Vesna Ćapkun[c], Snježana Tomić[a]

[a] Department of Pathology, Forensic Medicine and Cytology, Split University Hospital Centre, University of Split, School of Medicine, Spinčićeva 1, Split, Croatia
[b] Department of Pediatric Surgery, Split University Hospital Centre, University of Split, School of Medicine, Spinčićeva 1, Split, Croatia
[c] Department of Nuclear Medicine, Split University Hospital Centre, University of Split, School of Medicine, Spinčićeva 1, Split, Croatia

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ABSTRACT

Triple negative breast cancer (TNBC) consists of a group of tumors with poor prognosis, owing to aggressive tumor biology and lack of targeted therapy. The aim of this study was to assess the immunostaining for androgen receptors (ARs) in the group of TNBC, in addition to basal-like (BL) immunophenotype, BL morphology and conventional clinicopathological factors and to demonstrate its prognostic relevance in this group of tumors. The study included 83 patients. Slides were stained immunohistochemically for estrogen and progesterone receptors, HER2, CK5/6, CK14, EGFR, Ki-67 and AR. Of the 83 TNBC samples, 32.5% showed positive immunostaining for AR, 66.3% had BL immunophenotype, and 48.2% had BL morphology. Positive AR immunostaining was inversely correlated with higher clinical stage, higher mitotic score, higher histological grade and higher proliferation index measured by Ki-67. Significantly more AR negative tumors were observed among the tumors with BL immunophenotype and BL morphology. There was no significant association between positive AR immunostaining and disease free survival or overall survival. More than one third of TNBC were AR-positive, and this represents a potential opportunity for novel targeted treatment in the group of breast tumors for which therapeutic options are currently limited.

Introduction

Triple negative breast cancer (TNBC) defined by estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) negativity consists of a group of tumors with poor prognosis. Owing to lack of expression of ER, PR and HER2, specific targeted therapy is ineffective, and chemotherapy is currently the only modality of available systemic therapy. Recent studies have associated TNBC with more aggressive clinical behavior (van de Rijn et al., 2002; Lacroix et al., 2004; Nielsen et al., 2004; Rouzier et al., 2005), distinct metastatic pattern, with more frequent lung and brain metastases (Hicks et al., 2006; Fulford et al., 2007), and poorer prognosis despite good response to conventional chemotherapy regimens (Rouzier et al., 2005; Carey et al., 2007; Rakha et al., 2007). Recently, considerable effort has been made to subclassify TNBC into different prognostic groups, to select patients who are candidates for more aggressive therapy regimens, but further subclassification according to combination of valid biomarkers, available in clinical practice is required.

Although the role of ER and PR in promoting breast cancer has been well documented, the role of androgen receptors (ARs) in breast cancer is still uncertain. Positive AR immunostaining was found in approximately 70% of invasive breast carcinomas (Kuenen-Boumeester et al., 1996; Moinfar et al., 2003; Gonzalez et al., 2008; Park et al., 2010; Hu et al., 2011; Qi et al., 2012). It has been indicated that ARs are expressed in a significant number of TNBC and that they might play a role as a prognostic marker and therapeutic target in this subgroup of tumors (Kuenen-Boumeester et al., 1996; Ogawa et al., 2008; Park et al., 2010; Hu et al., 2011).

In the present study we investigated AR expression in the TNBC group, in addition to basal-like (BL) immunophenotype defined with basal cytokeratins and epidermal growth factor receptor (EGFR), BL morphology and conventional clinicopathological factors, to demonstrate its prognostic relevance in this group of tumors.

Materials and methods

Patients

The case records of 1849 patients with breast cancer, who underwent surgery at the Department of Surgery, Split University

Abbreviations: ARs, androgen receptors; BL, basal-like; DFS, disease-free survival; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; MAPK, mitogen-activated protein kinase; OS, overall survival; TNBC, triple negative breast cancer.

* Corresponding author at: Department of Pathology, Forensic Medicine and Cytology, Split University Hospital Centre, Spinčićeva 1, 21000 Split, Croatia.
E-mail address: ivana.mrklic@mefst.hr (I. Mrklić).

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Hospital Centre, Croatia, between January 2003 and December 2009, were retrospectively reviewed. Based on pathology documentation, 124 tumors were identified that lacked immunostaining for ER, PR and HER2. Out of 124 TNBC, 83 patients that did not receive preoperative chemotherapy and had available paraffin biopsy blocks were included in the further analysis and for those tumors ER, PR and HER2 status were re-evaluated. Clinical history information was collected through the breast cancer database of the Department of Oncology and Radiotherapy, Split University Hospital Centre.

Forty-six (55.4%) patients were treated with mastectomy, and 26 (31.3%) with quadrantectomy, following axillary lymph node dissection. For eleven (13.3%) patients with TNM stage III or IV, only biopsy was performed. All patients that underwent breast conserving surgery subsequently received postoperative radiotherapy. Systemic adjuvant chemotherapy was administered to all the patients, and none of them were treated by hormonal therapy or HER2 targeting therapy.

Complete follow-up was available for 81 patients. The mean follow-up was 43 months (range 2–95). The disease-free survival (DFS) was defined as the interval from the date of the primary surgery to the first locoregional recurrence or distant metastases. The overall survival (OS) was defined as the time from the date of the primary surgery to the time of the breast cancer-related death.

All histological and immunohistochemical (IHC) tumor slides were evaluated by two pathologists (S.T. and I.M.) and graded according to the guidelines proposed by Elston and Ellis (1991). The histological types were determined according to WHO (Ellis et al., 2003). Tumor staging was based on TNM classification (Sobin et al., 2009).

Histopathology and immunohistochemistry

Tissue samples were fixed in 10% buffered formalin, and embedded in paraffin wax for routine histological examination. 4-μm thick sections were cut from a paraffin block of each specimen and attached to slides for IHC. The slides were stained with hematoxylin and eosin (H & E) with additional immunostaining for ER (1:200, M7047, Dako, Glostrup, Denmark), PgR (1:100, M3569, Dako), and HER2/neu (HercepTest assay, K75207, Dako), CK5/6 (1:100, M7237, Dako), CK14 (1:25, L106436, Novocastra, Leica Microsystems, Newcastle on Tyne, UK), EGFR (1:40, M3563, Dako), Ki-67 (1:200, M7240, Dako), and androgen receptors (1:75, M3562, Dako). Immunoassays were performed using a Dako autostainer (Dako Cytomation, Glostrup, Denmark). HER2 status was evaluated by IHC (Hercept Test, Dako) or by chromogenic in situ hybridization (CISH; SPOT-Light® HER2 CISH Kit, Invitrogen/Zymed, Camarillo, CA, USA). The tests were scored according to ASCO/CAP guideline recommendations (Wolff et al., 2007). ER and PR were considered positive if there were at least 1% positive invasive tumor nuclei in the sample (Hammond et al., 2010). AR was also considered positive if there were at least 1% positive invasive tumor nuclei in the sample (Fig. 1). CK5/6, CK14 and EGFR were considered positive if ≥10% tumor cells showed positive membranous immunostaining, and BL immunophenotype was defined by positivity of one or more basal cell markers: CK5/6, CK14 or EGFR (Ho-Yen et al., 2012). BL morphology was considered positive if characteristic features such as syncytial growth pattern, high mitotic index, large central acellular zone comprising necrosis, pushing borders, dense lymphocytic infiltrate at the periphery of the invasive component and the presence of metaplastic and medullary elements were present (Reis-Filho and Tutt, 2008). Ki-67 was determined by counting 1000 tumor cells using the Olympus Image Analyser (40× objective), at the hot spots and at the periphery of the invasive component, and is expressed as the percentage of positive cells among a total number of 1000 malignant cells (Dowsett et al., 2011).

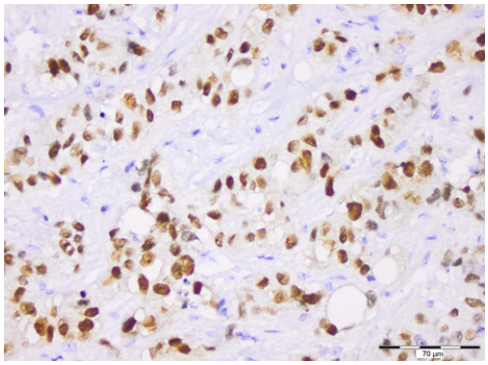

Fig. 1. Immunohistochemical staining of AR in TNBC. Scale bar = 70 μm.

Statistical analysis

Obtained data were analyzed using Statistics for Windows Release 12.0 (Statsoft, Tulsa, OK, USA). All p values of less than 0.05 were considered to indicate statistical significance. All statistical tests were two-sided, with the confidence interval of 95%. Correlations between categorical variables were studied using chi-square test. For univariate analysis, survival time was analyzed by the Kaplan–Meier method, and the log-rank test was used to assess differences among groups. For disease-free survival and overall survival, survival time was censored at death if the cause was not breast cancer or if the patient was alive without relapse on March 1, 2011 (the date of outcome data collection). For multivariate analysis Cox proportional hazard regression model was used to examine all factors found to be predictive of survival in univariate analysis simultaneously. The optimal cut-off values for Ki-67 and mitotic score were selected by using receiver operating characteristic (ROC) method, by minimizing the sum of the observed false-positive and false-negative errors with bootstrapping methodology (Efron and Tibshirani, 1986), with the outcome as referral value.

Results

Out of 83 TNBC, 60 (72.28%) were histologically identified as invasive ductal carcinomas NOS, 13 (15.66%) metaplastic carcinomas, 2 (2.4%) invasive lobular carcinomas, 3 (3.61%) invasive mixed carcinomas, 3 (3.61%) medullary carcinomas and 2 (2.4%) apocrine carcinomas. Out of 83 TNBC, 27 (32.5%) showed positive immunostaining for androgen receptors. Characteristics of 83 triple negative tumors examined in the study are summarized in Table 1.

The optimal cut-off point for Ki-67 was 61%, with sensitivity 56% and specificity 85% (area 0.657, SE 0.060, 95% CI: 0.539–0.776, $p = 0.020$). The optimal cut-off point for mitotic score was 21, with sensitivity 64% and specificity 86% (area 0.672, SE 0.061, 95% CI: 0.551–0.792, $p = 0.011$).

Analysis of the relationship between AR immunostaining and clinicopathological parameters revealed that positive immunostaining was inversely correlated with higher clinical stage ($p = 0.025$), higher mitotic score ($p = 0.003$), higher histological grade ($p = 0.038$) and higher proliferation index measured by Ki-67 ($p = 0.014$). Significantly more AR negative tumors were observed among the tumors with BL immunophenotype ($p = 0.05$) and BL morphology ($p = 0.004$). No significant difference between the groups with and without positive AR immunostaining in age at diagnosis, size of the tumor, histological subtype and vascular invasion was identified (Table 2).

Univariate survival analysis revealed that positive immunostaining for AR was not associated with DFS (LR = 0.38, $p = 0.537$),