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Androgen receptor expression and outcome of neoadjuvant chemotherapy in triple-negative breast cancer

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Abstract. – OBJECTIVE: Triple-negative breast cancers (TNBC) include a heterogeneous group of diseases, characterized by the lack of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) expression. TNBC that shows an overexpression of the androgen receptor (AR) defines the phenotype known as "luminal androgen receptor" (LAR), while the absence of the AR defines a "quadruple negative breast cancer" (QNBC). Several reports have associated AR positivity with a lower response to neoadjuvant chemotherapy (NAC), while divergent data have been reported about the impact of AR positivity on survival. The aim of this study was to retrospectively review our series of patients with TNBC tested for AR and submitted to NAC and compare pathologic complete response (pCR) rates in patients with a LAR phenotype or with QNBC.

PATIENTS AND METHODS: The clinical records of all patients with TNBC tested for AR that underwent NAC at our Institution from January 1, 2015 to June 30, 2019 were reviewed. Histopathological features as well as ER, PgR, Ki67, HER2 values, clinical and pathological stage, and results of BRCA gene expression profiling were registered for all patients.

RESULTS: Of the 145 TNBC patients treated by NAC, 20 (13.8%) had a LAR phenotype, while 125 (86.2%) had a QNBC. Overall, a pCR was achieved in 52 patients (35.8%). Patients with LAR phenotype had a lower rate of pCR as compared to patients with QNBC phenotype (25% *vs.* 37.6%). High Ki67 values (>50%) were observed less frequently in patients with a LAR phenotype (50% *vs.* 76.8% in QNBC).

CONCLUSIONS: Our data seem to confirm that the LAR phenotype is associated to lower rates of pCR after neoadjuvant chemotherapy; routine assessment of AR expression in addition to classical biomarkers in patients with TNBC could help to better personalize treatment.

Key Words:
Breast cancer, Triple negative breast cancer, Neoadjuvant chemoterapy, Androgen receptor.

Introduction

The basic features of Triple-Negative Breast Cancers (TNBC) consist of the absence of estrogen receptor (ER), progesterone receptor (PgR) expression, and the lack of human epidermal growth factor receptor 2 (HER2) gene amplification.

TNBC represents 15-20% of the different breast cancer subtypes. Patients with TNBC have a considerably higher risk of relapse and death due to a more aggressive behavior of the disease and the lack of novel targeted therapies[1,2].

In 2011, Lehmann et al[2] at first classified TNBC in six molecular subtypes. But in 2015 TNBC have been classified in four main tumor-specific subtypes by the analysis of gene expression profiles from 21 breast cancer data sets: Basal Like 1 (BL1), Basal Like 2 (BL2), Mesenchymal (M), and Luminal Androgen Receptor (LAR)[3,4].

The BL1 subtype is characterized by an elevated cell cycle and DNA damage response gene signatures determining an accelerated cell proliferation. Possible therapeutic approaches could include target antimitotic agents as platinum salt and poly (ADP-ribose) polymerase (PARP) inhibitors[4].

The BL2 subtype is characterized by the expression of epidermal growth factor receptor (EGFR), TP63, MET with activation of glycolysis and gluconeogenesis pathways.

The M subtype includes the enrichment of different biological routes as cell motility, ex-

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tracellular matrix interaction, epithelial-to-mesenchymal transition (EMT), and growth factor signaling as phosphoinositide 3-kinase pathway in a catalytic subunit (PIK3CA). In terms of histology, these tumors are mostly metaplastic carcinomas and could potentially respond to tyrosine kinase (TKI) and mTOR inhibitors[3-5].

Finally, the LAR subtype is characterized by luminal gene expression and is driven by the androgen receptor (AR); on histology, LAR subtypes are closely associated to apocrine tumors.

Only 10-25% of TNBC show an expression of the androgen receptor (LAR subtype)[6]. All the remaining TNBC do not express the AR and are defined as quadruple negative breast cancer (QNBC)[6-8].

Actually, no guideline requires routine determination of androgen receptor in TNBC.

The LAR subtype seems to be associated with a lower pathologic complete response (pCR) to neoadjuvant chemotherapy (NAC) but also with a better disease-free survival (DFS) and overall survival (OS) than QNBC; as novel treatments are being proposed for this subtype, routine assessment of AR expression could help to better personalize the treatment of patients with AR+TNBC[9-15].

The aim of the present study was to investigate the clinical relevance of LAR by verifying its correlation with pCR in TNBC patients undergoing NAC[15-17].

Patients and Methods

Among 233 consecutive patients with TNBC assessed for AR in our multidisciplinary Breast Unit, between January 1, 2015 and June 30, 2019, 145 underwent NAC.

These 145 patients constitute the object of the present study. All patients were female. All patients were screened for BRCA 1-2 mutation. Clinical data were collected from a retrospective review of the medical records. AR was evaluated by immunohistochemistry (IHC, Ventana Medical Systems, Oro Valley, AZ, USA) (Figure 1), and a 1% cutoff was used as appropriate threshold for AR positivity[15]. A pathologist expert in breast diagnosis (A.M.) reviewed all the immunohistochemical slices to confirm AR expression.

Clinical and pathological characteristics of the 145 patients are summarized in Table I (Clinical features of NAC-TNBC patients according to AR status).

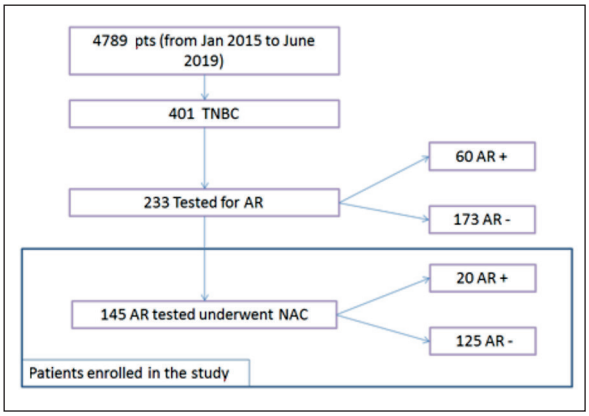

Figure 1. Patients selection.

All cases were discussed in a multidisciplinary "Tumor Board". NAC was used both in operable tumors, to increase the chances of breast-conserving surgery and in locally advanced tumors (IIIB, IIIC and inflammatory carcinoma) not candidate to surgery as a first treatment[18]. Therapeutic regimens included anthracyclines (epirubicin, 100 mg/m^2) and cyclophosphamide (500 mg/m^2; triweekly for 4 cycles) and taxanes (docetaxel,

Table I. Clinical features of NAC-TNBC patients according to AR status.

Characteristic	AR+	AR-
Total patients	20	125
Mean age at diagnosis	58	49
Menopausal status		
Premenopausal	7	69
Postmenopausal	13	56
cT		
cT1	2	27
cT2	15	67
cT3	3	20
cT4	0	11
cN		
cN0	8	33
cN+	12	92
Ki67 status		
Ki67 > 50%	10	96
Ki67 < 50%	10	29
pCR		
pCR	5	47
no-pCR	15	78
BRCA 1-2		
Mutation	4	29
Wild-type	16	96

TNBC, triple negative breast cancer; pCR, pathological complete response; cT, tumor size classification; NAC, neoadjuvant chemotherapy; cN, node classification.

70 mg/m^2; triweekly for 4 cycles); or carboplatin (100 mg/m^2; weekly for 12 cycles)[19]. The effectiveness of NAC was evaluated according to the current guidelines on response criteria in solid tumors. Patients were defined as having a pCR when the pathology report showed no residual invasive cancer in the excised breast specimen and the lymph nodes. Also, patients in which the pathologic exam revealed only *in situ* carcinoma in the absence of an invasive component were considered as having a pCR[16,20].

Statistical Analysis

Statistical analysis was performed using the SPSS (version 24.0, IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA). Estimates of OS and DFS were produced by cumulative incidence, using the Kaplan-Meier method. The oncological results of the global sample were calculated at 30 months.

Results

Twenty of the 145 patients (13.8%) were AR+ (LAR) and 125 (86.2%) AR-(QNBC). In the LAR subgroup, the mean age at the time of diagnosis was 58 years compared to 49 years in the QNBC subgroup. The LAR phenotype was more frequent in post-menopausal women.

LAR patients showed lower Ki67 rates than patients with QNBC: Ki67 rates >50% were seen in 10/20 (50%) of LAR patients as compared to 96/125 (76.8%) of patients with QNBC.

Overall, a pCR was documented in 52/145 patients (35.8%): 25% (5/20) of patients in the LAR subgroup and 37.6% (47/125) of patients in the QNBC subgroup (Figure 2).

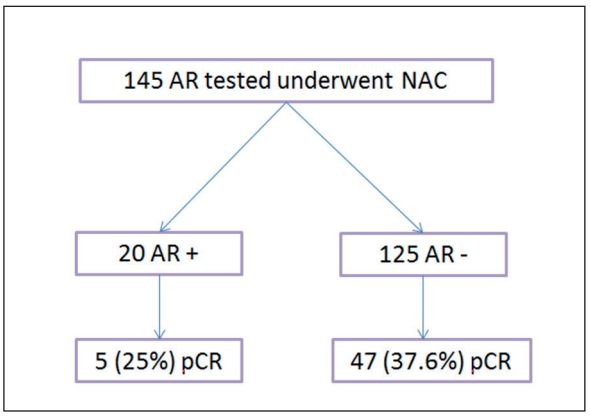

Figure 2. pCR rate in TNBC and QNBC.

Table II. Clinical features of patients with TNBC according to pCR.

Characteristic	pCR	no pCR
Total patients	52	93
Mean age at diagnosis	47	49
Menopausal status		
Premenopausal	32	47
Postmenopausal	20	46
cT		
cT1	11	15
cT2	29	54
cT3	8	12
cT4	4	12
cN		
cN0	23	23
cN+	29	70
Ki67 status		
Ki67 > 50%	25	81
Ki67 < 50%	27	12

TNBC, triple-negative breast cancer; pCR, pathological complete response; cT, tumor size classification; NAC, neoadjuvant chemotherapy; cN, node classification.

Clinical features of patients with TNBC according to pCR are shown in Table II (clinical features of patients with TNBC according to pCR).

BRCA mutations were detected in 33/145 patients, with similar rates between LAR and QNBC (20.0% *vs.* 23.3%, respectively). A pCR was achieved in 80% (4/5) of the LAR BRCA+ group as compared to 32% (15/47) in the QNBC BRCA+ group.

In our series a not statistically significant worse outcome was shown for the LAR group in terms of OS and DFS (Figure 3).

Discussion

TNBC is commonly used as a big tree term for a histologic group of tumors, genetically heterogeneous. All tumors lacked expression of ER, PR, and HER2. However, this "negative definition" means that TNBC instead represents a constellation of molecularly, morphologically, and behaviorally diverse entities. TNBCs may be stratified into clinically meaningful phenotypes (considering stromal components) to tailor optimal treatments.

The concept that AR modulates the growth and progression of breast cancer is currently undeniable. AR might act alone or in combination with other effectors participating in intracellular sig-

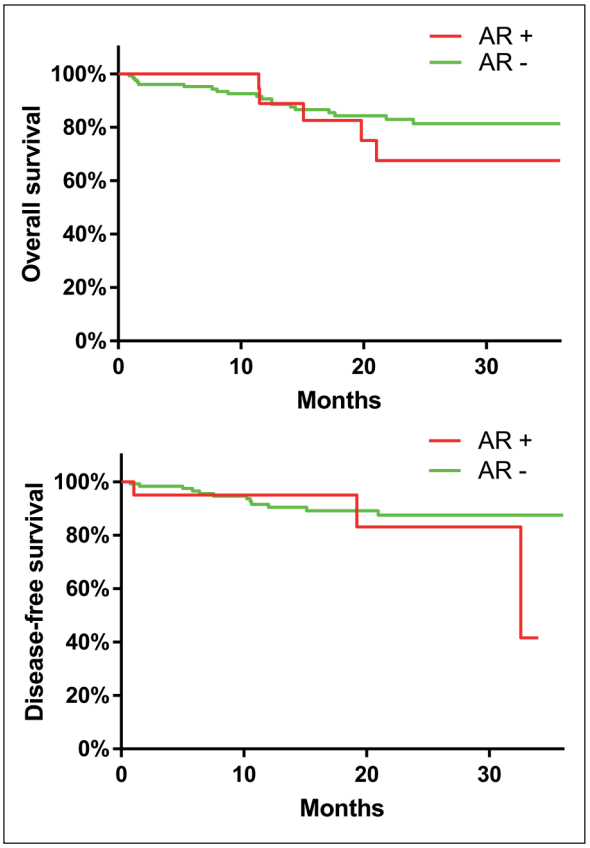

Figure 3. Overall Survival (OS) and Disease-Free Survival (DFS) in patients AR+ or AR-.

naling pathways. In TNBC, AR is hypothesized to mimic ER signaling, initiating transcriptional activation that promotes cell growth through the involvement of the transcription factor FOXA1[17].

Jiang et al[21] reported that mutations in the AR/FOXA1 pathway could result in abrogation of AR-related signaling, resulting in improved sensitivity to standard chemotherapy and better overall survival.

LAR subtype has been reported to be more chemoresistant than QNBC[22]. Santonja et al[23] reported lower rates of pCR in LAR patients as compared to all other TNBC subtypes (14.3% of pCR in LAR *vs.* 41.9% in the remaining subtypes combined, p=0.077).

In our series, a pCR was obtained in 25% (5/20) of patients with LAR subtype as compared to 37.6% (47/125) of patients with QNBC.

The lower proliferative rates observed on average in the LAR subtype (50% *vs.* 76.8% in QNBC) could explain the lower rate of pCR[24].

Germline BRCA mutation can also affect pCR rates in patients with TNBC (46% pCR in BRCA mutation carriers *vs.* 22% in non-carriers)[21,25]. In our series, 57.6% (19/33) of patients that showed a BRCA germline mutation achieved a pCR with significant differences between LAR subtype and QNBC (80% *vs.* 32%).

With regard to the prognostic impact of AR positivity, there are conflicting data in the literature. Several reports have associated AR positivity with a lower response to neoadjuvant chemotherapy (NAC), while divergent data have been reported about the impact of AR positivity on survival[26-28].

Three meta-analyses have indicated AR positivity as a favorable prognostic factor, with longer DFS in AR-positive *vs.* AR-negative breast cancer patients. Qu et al[29] reviewed 12 studies, including 5270 patients with breast cancer, finding a rate of AR positive patients of 65.2%. Looking at the DFS hazard ratio, a lower risk of recurrence emerged in AR positive tumors. Whang et al[30] quantified the risk of recurrence in AR positive patients as 20% lower than AR negative patients. In terms of OS no statistically significant differences were identified in these studies.

But a recent meta-analysis by Xu et al[31] did not confirm the positive correlation between AR expression and DFS, OS, distant-disease-free survival (DDFS), and relapse-free survival (RFS) in TNBC.

In our series, we found no significant differences in OS and DFS in the two groups. Moreover, recent studies[6,32] have indicated that assessment of AR could help to personalize therapeutic strategies in TNBC: in low-risk TNBC (AR+EGFR-) addition of an anti-androgen (i.e., NCT02689427), could open the way to a de-escalation of chemotherapy while in high-risk TNBC (AR-EGFR+) chemotherapy remains the mainstream treatment.

AR expression could also influence the radiosensitivity of TNBC, although preliminary evidence suggests that bicalutamide might restore the effect of therapeutically directed ionizing radiation in these patients[6,32].

Conclusions

Even with the bias of a limited number of cases, our study indicates that patients with a LAR subtype have a lower response to standard chemotherapy. Routine assessment of AR expression in addition to classical biomarkers in patients with TNBC could help to better personalize treat-

ment. Therefore, when offering NAC regimens in patients with LAR subtypes, the risk of unsatisfactory responses should be discussed with the patient. More extensive studies are still needed to validate these results in large patient cohorts and controlled prospective clinical trials.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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