Breast Cancer Research and Treatment (2019) 176:699–708
https://doi.org/10.1007/s10549-019-05252-6

EPIDEMIOLOGY

Tumor characteristics and outcome by androgen receptor expression in triple-negative breast cancer patients treated with neo-adjuvant chemotherapy

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Received: 10 April 2019 / Accepted: 22 April 2019 / Published online: 20 May 2019

Abstract

Purpose To assess clinical pathological characteristics and outcome of triple-negative breast cancers (TNBC) by androgen receptor (AR) protein expression.

Methods We retrospectively evaluated AR by immunohistochemistry on core-needle biopsy, (CNB) and residual disease (RD) in a consecutive institutional series of TNBC patients treated with neo-adjuvant chemotherapy (NACT) between 2000 and 2017. We investigated univariate associations between AR-expression on CNB (using different cut-offs), clinical pathological variables, and pathologic complete response (pCR). Next, we used multiple correspondence analysis (MCA) to investigate the relationships between AR on CNB and standard clinical and pathological variables, including stromal tumor infiltrating lymphocytes (sTILs). Finally, we investigated the prognostic value of AR-expression on CNB and RD using the Fine and Gray model.

Results We included 71 patients; median follow-up was 6.7 years. Considering the ≥ 1% cut-off, AR was present in 32% on the CNB and 14% on RD. AR-low (1-34% positive tumor cells) patients were associated with younger (premenopausal) age and AR-high (≥ 34% positive tumor cells) with older (postmenopausal) age. AR on CNB did not correlate with other features nor was it predictive for pCR or prognostic for metastatic outcome, regardless of the used cut-off. The MCA suggested that body mass index (BMI) affects the predictive role of AR-low and -high for pCR differently. AR-loss on RD was prognostic for a better 5-year distant disease-free survival (DDFS) as compared to RD with retained AR-expression (61.6% (95% CI 44.26–79.14) and 25.0% (95% CI 3.94–87.21), respectively; $p = 0.01$).

Conclusion Low and high AR-expression on CNB of TNBC were correlated with age and menopausal status but qualitative AR was not predictive for pCR. AR-loss on RD was prognostic for DDFS in TNBC patients treated with NACT.

Keywords Triple-negative breast cancer · Androgen receptor · Predictive · Prognostic · Metastasis · Neo-adjuvant chemotherapy

Abbreviations

AR	Androgen receptor
BCSS	Breast cancer-specific survival
CNB	Core-needle biopsy
Cos2	Squared cosine
DAB	3,3′-Diaminobenzidin tetrahydrochloride
DDFS	Distant disease-free survival
ER	Estrogen receptor
FFPE	Formalin-fixed paraffin embedded
FISH	Fluorescent in situ hybridization
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
LPBC	Lymphocyte-predominant breast cancer
MCA	Multiple correspondence analysis
NACT	Neo-adjuvant chemotherapy
pCR	Pathological complete response
PR	Progesterone receptor
RCB	Residual cancer burden

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10549-019-05252-6) contains supplementary material, which is available to authorized users.

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RD Residual disease
sTILs Stromal tumor-infiltrating lymphocytes
TNBC Triple-negative breast cancer
UHL University Hospitals Leuven

Introduction

Triple-negative breast cancers (TNBC) lack the immune-histochemical (IHC) expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). In Western countries, 10–15% of all primary diagnosed early cases are TNBC [1, 2]. Most TNBCs have a worse outcome than in case any or all receptors (ER, PR, HER2) are expressed, since most TNBCs have a high proliferation, high tumor grade, and lack a therapeutic target such as ER and/or HER2 to block [3]. So far, only (neo)-adjuvant poly-/single-agent chemotherapy (NACT) is available to prevent or decrease relapse prior to or following local therapy with surgery and radiotherapy. In the absence of NACT-induced pathological complete response (pCR) or once TNBC metastasizes, survival is poor. However, a recent clinical trial has shown promising results with a combination of immunotherapy and chemotherapy for advanced TNBC [4].

TNBC is a very heterogeneous disease and between 10 and 50% have been reported to express the androgen receptor (AR) at the protein level [5–8]. This percentage is variable due to, the use of diverse antibodies, detection systems and scoring methods to evaluate AR-expression on paraffin-embedded tissue [5–8]. The precise role of AR in the progression of breast carcinomas and particularly in TNBC, remains elusive. Indeed, patients with AR expressing TNBC have shown a worse [9], a better [6, 8, 10] or no difference [11–13] in outcome compared to TNBC without AR expression. This discordance of results might be due to other important variables that might influence outcome that were previously not taken into account for NACT-induced pCR and outcome in TNBC like age, menopausal status, BMI, tumor load and sTILs [14–19]. In non-metastatic TNBC, the highest pCR rates following NACT have been observed with high levels of stromal tumor-infiltrating lymphocytes (sTILs) [14, 20, 21]. In contrast, the lowest pCR rate has been reported in AR-positive TNBC with AR determined by IHC or molecular profiling [22, 23].

Interestingly, in stage IV TNBC, AR is now being targeted with anti-androgen therapies such as bicalutamide, enzalutamide, or a combination of one of those with a PIK3CA or CDK4/6 inhibitors, since objective responses with anti-androgen monotherapy are rather limited [24–27]. We have also recently described a single case of a postmenopausal woman with an AR-positive triple-negative apocrine carcinoma who experienced 1 year of clinical benefit due to androgen deprivation by chronic adrenal suppression using a low-dose hydrocortisone following several treatment lines [28].

Little is known about the relation between AR-expression and factors already proven to be important for pCR in TNBC. In addition, there is little knowledge about the difference in AR-expression between diagnostic core-needle biopsy, (CNB), and residual disease (RD) on the resection specimen and outcome of TNBC. Therefore, we studied AR-expression on CNB and RD in NACT-treated TNBC patients. Next, we investigated the associations between AR with different cut-offs on CNB and the relation between the clinical and pathological variables including, age, tumor load, menopausal status, sTILs and body mass index (BMI) for pCR or RD using also multiple correspondence analysis (MCA). Finally, we evaluated the potential prognostic value of AR-expression on pre-NACT CNB and post-NACT RD for (time to) metastasis, distant disease-free survival (DDFS) and breast cancer-specific survival (BCSS).

Materials and methods

Patients

We retrospectively selected women treated with NACT for early invasive TNBC from the prospectively collected database of the Multidisciplinary Breast Center at the University Hospitals Leuven (UHL). TNBC was defined ER and PR-negative according to ASCO/CAP guidelines on IHC-stained slides and HER2-negative by IHC (in case of score 0 or 1 +) or in case of the absence of *HER2* amplification by fluorescent in situ hybridization (FISH) for cases with IHC score of 2 + or 3 + [29]. pCR was defined as ypT0/isN0, tumors not achieving pCR are further referred to as RD. These patients were consecutively diagnosed between January 1, 2000 and August 31, 2017. They were either primary operable defined as cT1-3N0-1 or locally advanced (cT4 or cN2-3). Exclusion criteria were men, synchronous metastasis, external biopsy and/or surgery and invasive lobular carcinoma. The type of NACT followed international standards at the time of diagnosis. Information about receptor status and clinical characteristics (i.e. age, menopausal status, BMI etc.) were retrieved from pathology reports and medical files.

Histopathology

AR-expression was determined on a full-face 5 µm formalin-fixed paraffin-embedded (FFPE) tumor tissue. The sections were placed in the automated IHC stainer (BOND MAX (Leica Biosystems, Australia) with the 3,3′-diaminobenzidin tetrahydrochloride (DAB) kit Bond polymer

refine detection system (Leica, United Kingdom). First, pre-treatment process of deparaffinization, rehydration and epitope retrieval were combined at pH 6 at 98 °C for 20 min. Next, the endogenous peroxidases were blocked using peroxidase-blocking solution for 5 min and washed in wash buffer. Subsequently, the primary antibody (1:100), monoclonal mouse anti-human AR (clone AR411, DAKO), was incubated for 30 min at room temperature. Next, the slides were washed with wash buffer and the secondary antibody was incubated for 30 min at room temperature. Thereafter, the slides were washed with wash buffer and incubated with DAB for 10 min at room temperature. At last, the slides were washed with wash buffer and counterstained with hematoxylin (Leica Autostainer XL (ST5010), Germany), dehydrated and mounted (Leica Robotic Coverslipper (CV5030), Germany).

We recorded the proportion of positive tumor cells and the intensity of staining slide of all CNB and RD. Normal breast tissue or apocrine changes present within the same FFPE block served as positive control. We evaluated two common used cut-offs for positive AR-expression ($\geq 1\%$ and $\geq 10\%$ positive tumor cells) and also categorized AR using semi-quantitative expression. The $\geq 1\%$, was the same cut-off as recommended by ASCO/CAP in 2009 for ER and PR [29], was used for the primary endpoint of the study because this was frequently chosen in other studies [9, 13, 30, 31]. As others used the $\geq 10\%$ cut-off [7, 32, 33], our data were also assessed accordingly. The semi-quantitative score for proportion of cells staining for AR we used, was as defined by the Allred method for proportion irrespective of the staining intensity. This stratified AR-positive tumors in AR-high (proportion score 4–5; $\geq 34\%$ positive tumor cells) and AR-low tumors (proportion score 2–3; 1–34% positive tumor cells).

sTILs were scored on the same FFPE block as the AR on CNB and RD, using a hematoxylin and eosin staining according to the recommendations by the international TILs working group [34]. We investigated the threshold of on average $\geq 50\%$ infiltration of sTILs (also known as lymphocyte-predominant breast cancer, LPBC) as a descriptive term for tumors that contain "more lymphocytes than tumor cells" and the on average $\geq 30\%$ infiltration of the stromal area with TILs as a threshold which was recently determined to be an excellent prognostic factor in patients with TNBC [14, 20, 21, 35].

In order to improve the predictive information of our study, we used the residual cancer burden (RCB) scoring method as recommended by Symmans et al. [36] by retrieving all H&E stained slides of the cases lacking pCR. All histopathological assessments were performed by a board-certified pathologist (GF).

Statistical analysis

To evaluate the correlation between AR-expression ($\geq 1\%$, $\geq 10\%$, and $< 1\%$ vs. 1–34% vs. $\geq 34\%$ threshold) and patients demographic, clinical, pathological characteristics, pCR and time to metastasis we used Fisher's exact test for categorical variables or Mann–Whitney *U* test for continuous variables if not mentioned otherwise. To determine if AR-expression on the CNB and on the RD is prognostic for DDFS (time from diagnosis of TNBC until date of distant metastatic disease and death of other cause as competing event) and/or BCSS we used the cumulative incidence function and the comparisons between two groups was performed by the Fine and Gray model. BCSS is the time between metastasis and death of BC (event) or death of other cause (competing event) or end of follow-up (censored, data lock for these patients was July 2018; cases with unknown cause of death were excluded.). Analysis were performed using SAS software (version 9.4 of the SAS System for Windows).

The MCA was performed as a descriptive visual analysis to explore possible relationships among the categories of the eight different variables. Continuous variables were categorized according to defined thresholds and labeled accordingly. The MCA was done using "FactoMineR" package (version 1.34), and visualizations generated with "factoextra" package (version 1.0.5). The scree plot was used to determine the contribution of each dimensions to the variance of the data. The squared cosine (cos2) was used to assess the representation quality of each category to the dimensions. If a variable category is well represented by two dimensions, the sum of the cos2 is close to one. Category clustering was performed with the k-means algorithm from the stat package (version 3.5.2). The optimal number of clusters for each couple of dimensions was retrieved through the elbow method (Supplementary Fig. 1). These analyses were performed using the R software version 3.5.1 (https://www.r-project.org/).

Results

Patient and pathological characteristics according to AR-expression in TNBC

In UHL, 317 patients received NACT for TNBC (with combinations of the following: anthracyclines, cyclophosphamide, taxanes, platinum, 5-fluorouracil or capecitabine). One hundred and forty-eight patients were not selected as the CNB was performed in another hospital. Sufficient remaining tissue for measuring AR was available in 71 of 158 remaining patients (Fig. 1). Importantly, patient demographic, clinical characteristics, pCR rate and 5-years DDFS

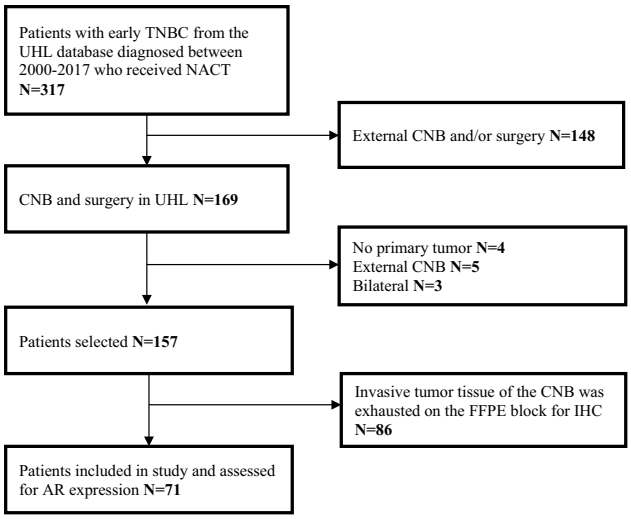

Fig. 1 Flowchart of the patient selection and final inclusion. *TNBC* triple-negative breast cancer, *UHL* University Hospitals Leuven, *NACT* neo-adjuvant chemotherapy, *FFPE* formalin-fixed paraffin embedded, *IHC* immunohistochemistry, *AR* androgen receptor, *CNB* core-needle biopsy,

did not differ between the groups with and without exhausted CNB-tissue for AR-measurement (data not shown).

The 71 included patients had a median age of 51.0 years at diagnosis; 49% were premenopausal, the mean BMI was 25.8 kg/m^2 (median 24.5 kg/m^2), and the median follow-up period was 6.7 years. The AR (cut-off 1%) on CNB was expressed in 23 patients (32%). Of the 28 patients, prospectively tested for germline *BRCA1/2*-mutations, 14% were carriers; one patient had a *BRCA1*-mutation (AR-positive) and three patients *BRCA2*-mutation (AR negative).

Considering the ≥ 1% cut-off, AR-expression did not correlate with age, menopausal status and BMI at diagnosis, nor for tumor grade, size, sTILs (≥ 30% cut-off), nodal involvement, or primary inoperability (Table 1). A higher cut-off for AR (≥ 10%) had similar results for patient and tumor characteristics (data not shown). When categorizing AR expression as follows: < 1% (AR-negative), 1–34% (AR-low) and ≥ 34% (AR-high), we observed a significant correlation with age. Younger patients carried more frequently AR-low tumors as compared to the older ones who had more frequently AR-high tumors ($p = 0.03$). Consequently, we also observed a correlation with menopausal status (AR-low tumors were more often observed in premenopausal and AR-high tumors were more often present in postmenopausal patients, $p = 0.008$) (Supplementary Table 1).

Response to NACT by AR expression

In our series, pCR was chemotherapy-schedule-independent and was achieved in 39% of the patients (Table 1). The probability of pCR was AR independent, 39% if AR-positive and 40% if AR negative (Table 1). Correlation with RCB-score/class is described in Supplementary Table 2). In univariate analysis, only sTILs (≥ 30% threshold) ($p = 0.03$) were related to pCR (Table 2). The alternative cut-offs for AR expression (≥ 10% and < 1%, 1–34%, and ≥ 34%) did also not predict pCR (Supplementary Table 3).

AR expression levels changed between CNB and RD in 9 of the 14 AR-positive patients with RD. In eight patients, we observed a complete switch from AR positive to AR negative on the RD, while in one patient we observed increased AR expression from 1% in the CNB to 30% in the RD. AR-low tumors switched more frequently ($n = 5/6$) to AR negative in RD as compared to AR-high tumors ($n = 3/8$). We did not observe receptor status switch in the RD of tumors that proved to be negative for AR (< 1%), ER, PR and HER2 on CNB.

Relations between variable categories using MCA

To identify the pattern of relationships of the different variables in our dataset, we performed an MCA analysis. In Fig. 2, we present the relationship between categories of the different variables in 2-dimensional plots: the couple dimensions 1 and 2 (Fig. 2a), 1 and 3 (Fig. 2b), and 2 and 3 (Fig. 2c). These three dimensions of the MCA accounted for 47.4% of the variability of our cohort, 19.7% in the first dimension, 14.2% in the second dimension, and 13.5% in the third dimension (Supplementary Fig. 2). The analysis showed an association between postmenopausal patients, age (≥ 60 years) and partly with AR-high tumors (clusters V and XI). On the contrary, premenopausal patients were associated with age (< 50 years) and AR-low tumors (cluster I and VI). These three categories were also associated with pCR, although weaker represented (cluster VI). High sTILs (≥ 30%) were associated with overweight patients (cluster XV). Using another couple of dimensions, high sTILs were associated in a weaker manner with pCR, normal weight (BMI < 25 kg/m^2), smaller tumor size (cT1-2), and negative lymph nodes (cluster II). At the opposite, low sTILs (< 30%) were associated with RD (cluster XIII) as well as with larger tumor size (cT3-4) and in a weaker manner to positive lymph nodes (cluster III). AR-high was weakly represented overall, therefore, association has to be taken with cautions. The MCA described a possible association of AR-high with, low sTILs, older age, obesity, and RD (cluster IX), as well as with node negativity, small tumor size, normal weight, postmenopausal status, and age (50–59 years) (cluster XVII). AR-negative category appeared in clusters III, VIII, and XVI but these clusters were not reported due to poor representativeness in these dimensions.

Table 1 Patient demographic and tumor characteristics according to AR expression (≥ 1%) on the CNB

Variable	Statistic	All	AR-positive (≥ 1%)	AR negative (< 1%)	*p* value
		71	23	48	
All patients	%	100	32.4	67.6	
Age diagnosis	Median	51.0	51.0	51.0	0.907
BMI	Median	24.5	24.4	24.7	0.690
Postmenopausal					1.000
Yes	*n* (%)	36 (50.1)	12 (52.2)	24 (50.0)	
No	*n* (%)	35 (49.3)	11 (47.8)	24 (50.0)	
Grade					0.718
2	*n* (%)	10 (14.1)	4 (17.4)	6 (12.5)	
3	*n* (%)	61 (85.9)	19 (82.6)	42 (87.5)	
cT					0.651
1	*n* (%)	8 (11.3)	4 (17.4)	4 (8.3)	
2	*n* (%)	27 (38.0)	7 (30.4)	20 (41.7)	
3	*n* (%)	13 (18.3)	4 (17.4)	9 (18.8)	
4	*n* (%)	23 (32.4)	8 (34.8)	15 (31.3)	
cN					0.473
0	*n* (%)	23 (32.4)	9 (39.1)	14 (29.2)	
1	*n* (%)	23 (32.4)	9 (39.1)	14 (29.2)	
2	*n* (%)	6 (8.5)	1 (4.4)	5 (10.4)	
3	*n* (%)	19 (26.8)	4 (17.4)	15 (31.3)	
Primary operable					0.447
Yes	*n* (%)	34 (47.9)	13 (56.5)	21 (43.8)	
No	*n* (%)	37 (52.1)	10 (43.5)	27 (56.3)	
sTILs CNB (%)	Mean	21.0	17.0	22.0	0.726
sTILs CNB (%)					1.000
< 30%	*n* (%)	52 (73.2)	17 (73.9)	35 (72.9)	
≥ 30%	*n* (%)	19 (26.8)	6 (26.1)	13 (27.1)	
LPBC%					0.482
Yes	*n* (%)	10 (14.1)	2 (8.7)	8 (16.7)	
No	*n* (%)	61 (85.9)	21 (91.3)	40 (83.3)	
NACT					0.876
Tax + Ant	*n* (%)	42 (59.2)	13 (56.5)	29 (60.4)	
Tax + Ant + Platinum	*n* (%)	19 (26.8)	6 (26.1)	13 (27.1)	
Tax + Cap	*n* (%)	10 (14.1)	4 (17.4)	6 (12.5)	
pCR					1.000
No	*n* (%)	43 (60.6)	14 (60.9)	29 (60.4)	
Yes	*n* (%)	28 (39.4)	9 (39.1)	19 (39.6)	
Distant metastasis					0.774
No	*n* (%)	54 (76.1)	17 (73.9)	37 (77.1)	
Yes	*n* (%)	17 (23.9)	6 (26.1)	11 (22.9)	
Time to metastasis	Median	17.0	20.0	16.0	0.642

AR androgen receptor, *BMI* body mass index, *sTILs* stromal tumor infiltrating lymphocytes, *LPBC* lymphocyte-predominant breast cancer, *NACT* neo-adjuvant chemotherapy, *CNB* core-needle biopsy, *Tax + Ant* taxanes and antracyclines, *Tax + cap* taxanes + capecitabine, *pCR* pathological complete response

Survival according to AR expression

Globally, there was no difference in survival after a median follow-up of 6.7 years for any of the investigated survival endpoints between AR-positive and AR-negative cases, as assessed in the CNB. When considering the ≥ 1% cut-off, 24% of the patients developed metastasis: 26% in the AR-positive and 23% in the AR-negative group (Supplementary Table 4). Overall, the median time-to-metastasis was 17 months, 20 and 16 months, respectively, for the

Table 2 Pathological complete response (pCR) compared to non-pCR in subgroups according to androgen receptor (AR)-status on the core-needle biopsy, (CNB)

Variable	Statistic	All pCR	All no-pCR	*p* value	pCR in		P value	No-pCR in		*p* value
					AR+	AR−		AR+	AR−	
		28	43		9	19		14	29	
All patients	%	39.4	60.6		39.1	39.6		60.9	60.4	
Age at diagnosis	Median	51.0	51.0	0.469	47.0	52.0	0.279	52.0	51.0	0.476
Age at diagnosis				1.000			0.432			1.000
≤ 50 year	*n* (%)	12 (40.0)	18 (60.0)		5 (55.6)	7 (36.8)		6 (42.9)	12 (41.4)	
> 50 year	*n* (%)	16 (39.0)	25 (61.0)		4 (44.4)	12 (63.2)		8 (57.1)	17 (58.6)	
Postmenopausal				0.337			1.000			0.523
Yes	n (%)	12 (33.3)	24 (66.7)		3 (33.3)	9 (47.4)		9 (64.3)	15 (51.7)	
No	n (%)	16 (45.7)	19 (54.3)		6 (67.7)	10 (52.6)		5 (35.7)	14 (48.3)	
BMI	Median	24.1	25.3	0.082	23.3	24.3	0.461	25.0	25.0	0.913
Primary operable				0.812			1.000			0.515
Yes	*n* (%)	14 (41.2)	20 (58.8)		5 (55.6)	9 (47.4)		8 (57.1)	12 (41.4)	
No	*n* (%)	14 (37.8)	23 (62.2)		4 (44.4)	10 (52.6)		6 (42.9)	17 (58.6)	
Grade				0.730			0.234			1.000
2	*n* (%)	3 (30.0)	7 (70.0)		2 (22.2)	1 (5.3)		2 (14.3)	5 (17.2)	
3	*n* (%)	25 (41.0)	36 (59.0)		7 (77.8)	18 (94.7)		12 (85.7)	24 (82.8)	
sTILs CNB (%)	Mean	28.0	16.0	0.109	20.0	31.0	0.337	15.0	16.0	0.763
sTILs CNB				0.027			1.000			1.000
< 30%	*n* (%)	16 (30.8)	36 (69.2)		5 (55.6)	11 (57.9)		12 (85.7)	24 (82.8)	
≥ 30%	*n* (%)	12 (63.2)	7 (36.8)		4 (44.4)	8 (42.1)		2 (14.3)	5 (17.2)	
LPBC				0.177			0.630			1.000
Yes	*n* (%)	6 (60.0)	4 (40.0)		1 (11.1)	5 (26.3)		1 (7.1)	3 (10.3)	
No	*n* (%)	22 (36.1)	39 (63.9)		8 (88.9)	14 (73.7)		13 (92.9)	26 (89.7)	
NACT				0.742			0.617			0.535
Tax + Ant	*n* (%)	18 (42.9)	24 (57.1)		5 (55.6)	13 (68.4)		8 (57.1)	16 (55.2)	
Tax + Ant + Platinum	*n* (%)	6 (31.6)	13 (68.4)		3 (33.3)	3 (15.8)		3 (21.4)	10 (34.5)	
Tax + Cap	*n* (%)	4 (40.0)	6 (60.0)		1 (11.1)	3 (15.8)		3 (21.4)	3 (10.3)	
Metastasis				0.001			1.000			0.739
Yes	*n* (%)	1 (5.9)	16 (94.1)		0 (0)	1 (5.3)		6 (42.9)	10 (34.5)	
No	*n* (%)	27 (50.0)	27 (50.0)		9 (100)	18 (94.7)		8 (57.1)	19 (65.5)	
Time to metastasis	Median	17		–	0	34	–	27	15.0	0.128

BMI Body mass Index, *NPI* Nottingham Prognostic Index, *AR* androgen receptor, *sTILs* stromal tumor infiltrating lymphocytes, *LPBC* lymphocyte-predominant breast cancer, *NACT* neo-adjuvant chemotherapy, *CNB* core-needle biopsy,, *pCR* pathological complete response, for the patients who developed metastatic breast cancer

AR-positive and AR-negative patients ($p = 0.6$). AR was not prognostic for DDFS and BCSS. For the 23 patients with AR-positive (≥ 1%) tumors, the 5-year DDFS was 73.5% (95% CI 52.61–90.54) compared to 73.8% for the 48 AR-negative patients (95% CI 59.61–86.16; $p = 0.6$) (Supplementary: Table 5; Fig. 3). Similarly, the 5-year BCSS in the AR-positive patients was 83.5% (95% CI 26.38–87.86) and 75.4% in the AR-negative patients (95% CI 60.39–88.00; $p = 0.8$) (Supplementary: Table 6; Fig. 4).

Of note, pCR and RCB class were independent prognostic parameters for DDFS and BCSS (Supplementary: Table 7; Fig. 5, and Table 8; Fig. 6, respectively).

In the univariate analysis, metastasis was associated with higher BMI, lymph node positivity, and RD ($p = 0.008$, $p = 0.042$, and $p = 0.02$, respectively; Supplementary Table 3).

As opposed to the AR-status on the CNB, the AR-status on the RD using the different cut-offs was prognostic for DDFS but not significantly for BCSS. AR-positive (≥ 1%) patients with RD following NACT had a lower 5-year DDFS rate as compared to AR-negative patients following NACT (25.0% (95% CI 3.94–87.21) and 61.6% (95% CI 44.26–79.14), respectively; p = 0.01). The three different cut-off for AR expression on RD demonstrated, although

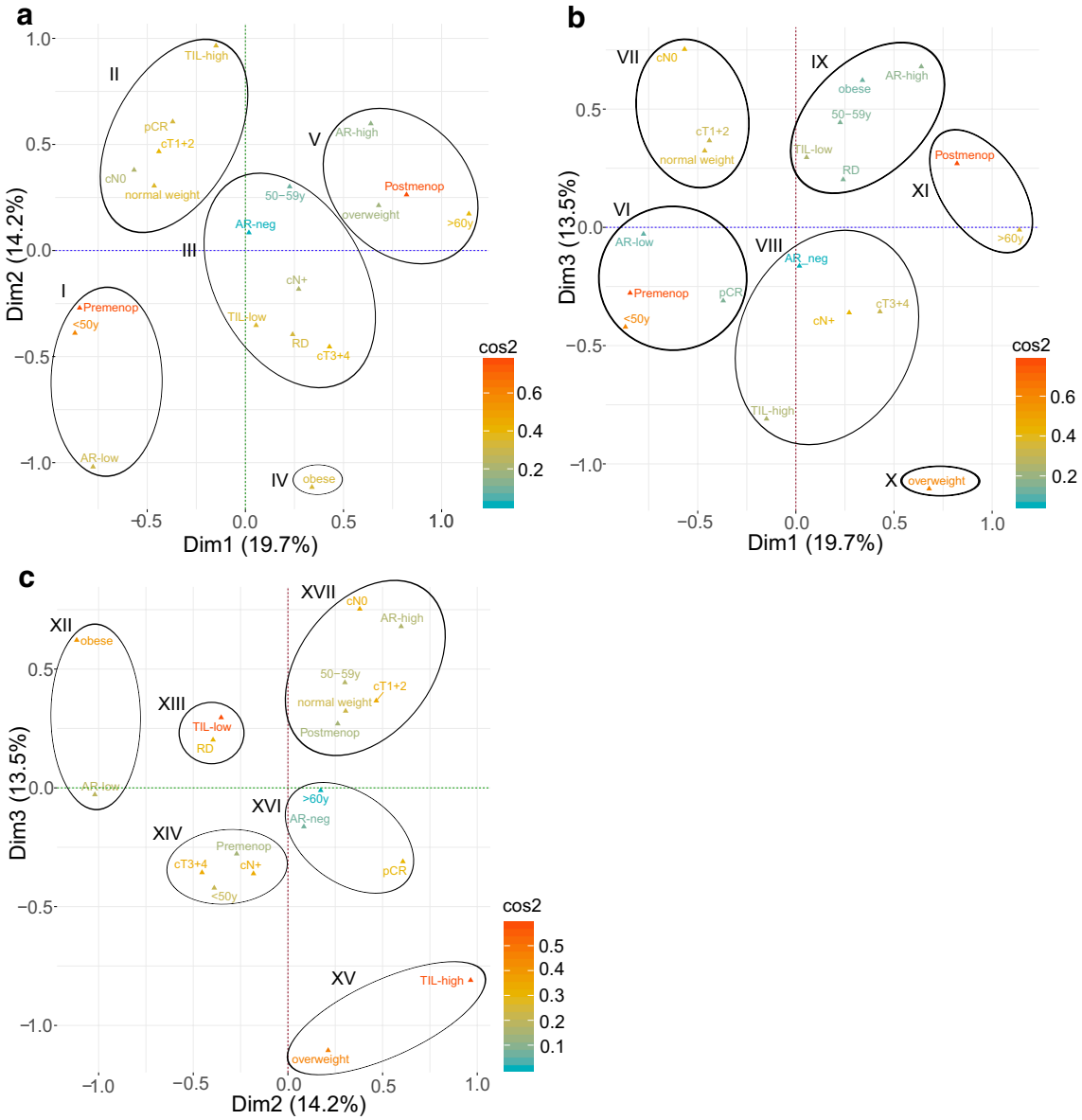

Fig. 2 Multiple correspondence analysis (MCA) showing the clustering (numbered from I to XVII) of different variable categories with a similar relation, of all 71 patients with TNBC. The squared cosine (cos2) is a measurement of the degree of association between variable categories and a particular axis (dimension). **a** Dimension 1 and 2. **b** Dimension 1 and 3. **c** Dimension 2 and 3

with small numbers, that AR-low had a better 5-year DDFS (100%) than AR negative [61.6% (95% CI 44.26–79.14)] and AR-high (0% (95% CI 5.48–94.59)) ($p = 0.006$).

Discussion

In this study, we aimed at better characterizing AR-expressing tumors in a consecutive institutional series of TNBC patients treated with NACT. In our analyses, we considered different cut-offs for AR, 1% and 10% which have been reported frequently in the literature [7, 9, 13, 30–33], and the three categories < 1% (negative), 1–34% (AR-low) and ≥ 34% (AR-high). Considering the 1% and 10% cut-off, AR was expressed in 32% and 27% of the cases, respectively. Considering the three categories, we found 68% AR negative, 15% AR-low, and 17% AR-high. We first assessed the association between AR-expression and the clinical and pathological features. We observed a significant associations of AR expression (three categories cut-off) with age and menopausal status, but not for the other clinical and pathological variables. Younger and premenopausal patients carried more often AR-low tumors, while AR-high tumors were observed more often in older and postmenopausal patients. AR-negative tumors were in the middle regarding patient age, and were observed just as much in pre- as postmenopausal patients.

In our cohort, the pCR rate was 39% in TNBC, a rate which is similar to what has been reported by others [23, 37, 38]. In the univariate analysis, only sTILs (≥ 30% threshold) but not AR-expression in CNB predicted pCR and distant metastasis. This is a confirmation for our sTILs findings to predict pCR [35], and for AR to not predict pCR [39–42]. However, AR-expression in TNBC has been observed to be prognostic for outcome when it was not predictive for pCR following NACT [42]. Differences with our study might be explained by NACT type as we collected cases over a 17 years period.

In the MCA, we visualized possible relationships among the different categorized variables in clusters. Since we observed no difference in the predictive role of AR on pCR we hypothesized that looking further into low and high AR-positive tumors would provide more insight on the role of AR. It was confirmed that AR-high was related to menopause in TNBC patients. Consistently, it also confirmed that AR-low was related to age (< 50 years) and premenopausal status, and in a weaker way also to pCR. The other AR-low tumors that were not represented in the previous cluster were associated with obesity in one cluster in the same quadrant of dimension 2 and 3 with RD and low sTILs. AR-high might be related to poor prognostic markers like low sTILs, obesity and RD in one cluster and to better prognostic markers in another cluster (e.g. negative lymph nodes, small tumors, and a normal BMI). Numerically, in this series of 52 TNBC patients with low sTILs, pCR was lowest if AR-high (12.5%) confirming Masuda's data using molecular profiling [37]; these figures in our series for TNBC with low sTILs were 44.4% and 31.4% respectively if AR-low and AR negative. Postmenopausal patients, which carry more often AR-high tumors, are also more often linked to a higher BMI [43–45]. BMI appeared to affect the predictive role of both AR-low and AR-high tumors, associations which deserve to be further investigated. AR-negative tumors were weakly represented due to the large heterogeneity in this subgroup.

We speculate that AR-low and AR-high tumors are weakly and highly dependent on AR activity, respectively. In this regard, we speculate that the higher pCR rate observed in younger premenopausal normal BMI patients with AR-low tumors might be explained by the chemotherapy-induced ovarian suppression, which completely suppresses the androgen serum levels. However, if these patients have a high BMI, local androgens synthesized by adipose tissue might also be the source of the tumor which is not affected by chemotherapy. In older postmenopausal women with a higher BMI the source of the tumor is not dependent on the serum androgens and more dependent on the local androgen levels (from the adrenal glands and adipose tissue) or use other tumor growth pathways. Therefore, these latter patients benefit less from chemotherapy since their source of tumor growth is still intact. In case they have a normal BMI their local source is smaller and, therefore, might achieve more often pCR.

Interestingly, the MCA demonstrated that low sTILs were always clustered with RD. As opposed to high TILs which were only clustered with pCR when it was clustered with normal weight. Since high sTILs are such a high predictive marker for pCR [14] the relation between BMI and the predictive role of high sTILs would be interesting to explore further in larger cohorts for validation and has to the best of our knowledge, never been described before.

In our series, metastasis developed in 24% after a median follow-up of 6.7 years; AR-expression was not prognostic for DDFS or BCSS in contrast to two meta-analysis in which AR was prognostic only for DDFS in TNBC [46, 47]. However, in most of these large studies, the patients received adjuvant chemotherapy instead of NACT; cohorts were larger and follow-up longer.

A switch in receptor status after NACT is a very well-known phenomenon [48, 49] that has been recently described also in AR-positive TNBC following NACT. However, in contrast to Liu et al. we did not observe a switch in AR-status when AR was negative on pre-NACT CNB [50]. Switch in receptor status may be explained by the different activity of the chemotherapy in different tumor cell populations present in heterogeneous tumors. Interestingly, we observed for the first time that AR-loss in RD significantly improved DDFS as compared to those that remained AR positive. Looking further to these patients, we suggested that this smaller DDFS was caused by the AR-high tumors and not by the AR-low who had the best DDFS on RD. Although numbers were small, it suggests that the low and high percentage of AR-expression is important for prognosis. However, this should be confirmed in a larger population.

To conclude, AR expression on CNB does not predict pCR in TNBC treated with NACT. No specific clinical or pathological correlations were observed in AR-positive TNBC, except for age and menopausal status when tumors were sub-stratified according to percentage of positive tumor cells. Our exploratory MCA suggested new insights that BMI might affect the predictive role of both AR-low and AR-high tumors. AR on CNB was not prognostic for DDFS and BCSS. However, AR-loss on RD could be a potentially favorable prognostic factor for DDFS. Validation of our findings in larger cohorts is warranted.

Acknowledgements We would like to thank Funds of Friends for Cancer Research Leuven for supporting this research. The authors would also like to thank Sarah Cumps, Eef Allegaert and Kathleen Van den Eynde from the Department of Imaging and Pathology, Translational Cell & Tissue Research of the KU Leuven, for their technical support.

Compliance with ethical standards

Conflict of interest Hans Wildiers received travel support from Roche and Pfizer, and his institution received consulting fees and honoraria

from Roche, Astra Zeneca, Amgen, Lilly, Novartis, Abbvie, Vifor Pharma, Pfizer, Celldex therapeutics, Janssen-CILAG, TRM Oncology, PUMA Biotechnology, ORION corporation and an unrestricted research grant from Roche. Christine Desmedt received speakers fee from Roche Diagnostics for her institution. Kevin Punie received travel support from Astra Zeneca, Novartis, Pfize, Pharma Mar and Roche. His institution received research funding from Sanofi, honoraria for consulting or advisory roles from Astra Zeneca, Novartis, Pfizer, Roche and Vifor Pharma and speaker fees from Novartis and Pfizer. Ann Smeets received for her institution a grant from MSD for medication for a window of opportunity trial. Patrick Neven received speaker's fees, consulting fees, and research support from Roche (all provided to his institute). Patrick Neven is on the advisory board of Novartis, AstraZeneca, Lilly, and Pfizer (all consulting fees are provided to his institute). Ignace Vergote is on the advisory board of: Advaxis, Inc., Eisai Inc., MSD Belgium, Roche NV, Genmab A/S, Genmab US, F. Hoffmann-La Roche Ltd, Pharmamar, Millennium Pharmaceuticals, Clovis Oncology Inc., AstraZeneca NV, Tesaro Bio GmbH, Tesaro Inc, Oncoinvent AS, and Immunogen Inc. Ignace Vergote has contracted research (via KU Leuven) of: Oncoinvent AS, and Genmab A/S – Genmab B.V. Ignace Vergote receives corporate sponsored research grants by Amgen and Roche. Ignace Vergote received travel support by: Takeda Oncology, Pharma Mar, Genmab, Roche, Astrazeneca, and Tesaro. All other authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee (UHL, Belgium) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the UHL in Belgium before the study started.

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