Role of androgen receptor expression in early stage ER+/PgR–/HER2– breast cancer

Barbara Tagliaferri*, Erica Quaquarini*, Raffaella Palumbo, Emanuela Balletti, Daniele Presti, Alberto Malovini, Manuela Agozzino, Cristina Maria Teragni, Andrea Terzoni, Antonio Bernardo, Laura Villani and Federico Sottotetti

Abstract

Background: Progesterone receptor (PgR) negative breast cancer (BC) is an aggressive subtype with poor prognosis and reduced response to endocrine treatments. Several studies have suggested that androgen receptor (AR) expression is associated with a favorable tumor biology, longer recurrence free survival (RFS), and overall survival. In the literature no data exist regarding the role of AR expression in early stage estrogen receptor (ER)+/PgR– BCs. The aim of this study was to evaluate the prognostic role of AR expression in this setting.

Patients and methods: This is a monocentric retrospective study in which 208 patients who underwent surgical intervention for ER+/PgR–/Human Epidermal growth factor Receptor 2 (HER2)– BC were included. The primary objective was to analyze the relationship between AR expression and RFS.

Results: At a median follow-up of 77 months, 75 patients (36%) had a disease relapse (all sites included). AR expression was significantly higher in patients who did not relapse compared with those who relapsed with an impact on RFS (hazard ratio [HR] = 0.99, p = 0.025). Patients with AR expression ≥80% had a lower risk of relapse compared with those with AR <80% (HR = 0.53, p = 0.008). In addition, breast tumors with higher AR expression had good biological features (low ki67 and nuclear grade) compared with BCs with lower AR expression, at least partly explaining the different outcome.

Conclusions: The results of this study support the potential prognostic role of AR in patients with ER+/PgR– BCs and may contribute to the identification of subgroups of high-risk patients.

Keywords: androgen receptor, early breast cancer, immunohistochemistry, progesterone receptor negative, relapse-free survival

Correspondence to:
Erica Quaquarini
Medical Oncology Unit, ICS Maugeri-IRCCS SpA SB, via Maugeri 10, Pavia, 27100, Italy

Experimental Medicine School, University of Pavia, Pavia, 27100, Italy
erica.quaquarini@ icsmaugeri.it

Barbara Tagliaferri
Raffaella Palumbo
Emanuela Balletti
Daniele Presti
Cristina Maria Teragni
Andrea Terzoni
Antonio Bernardo
Federico Sottotetti
Medical Oncology Unit, ICS Maugeri-IRCCS SpA SB, Pavia, Italy

Alberto Malovini
Laboratory of Informatics and System Engineering for Clinical Research, ICS Maugeri-IRCCS SpA SB, Pavia, Italy

Manuela Agozzino
Laura Villani
Operative Unit of Anatomic Pathology, ICS Maugeri-IRCCS SpA SB, Pavia, Italy

*These Authors have contributed equally to this work so that they are considered co – first Authors

Introduction

Breast cancer (BC) is classified according to estrogen (ER), progesterone (PgR), and Human Epidermal growth factor Receptor 2 (HER2) expression, each of them playing an important predictive and prognostic role.

PgR gene is estrogen-dependent, and most ER positive (+) tumors are also PgR+. On the other hand, almost all ER negative (−) cancers are PgR−. PgR pathway plays a role in mammary gland growth as well as in the development of BC.[1] The receptor is expressed in two isoforms (PgR-A and PgR-B), whose relationship has a functional and morphogenic role.[2] In clinical practice, the lack of PgR expression is a well-known marker of worse prognosis in luminal cancers; in tumors defined as ER+ and having ki67 >14%, the loss of PgR was identified as an adverse prognostic factor.[3,4] In addition, luminal B cancers, classified as ER+, HER2−, ki67 >14% and PgR <20%, have worse prognosis than tumors with PgR >20%.[5,6]

Several studies demonstrated that ER and PgR expression can vary during cancer natural history and during treatments; indeed, ER expression may be reduced during endocrine therapy, in particular with tamoxifen and, in 50% of cases, its complete loss together with the development of resistance to endocrine treatments has been described.[7,8]

Androgen receptor (AR) is expressed in more than 85% of early stage BCs and in about 75% of metastatic BCs.[9] Recent gene expression profiling studies have identified four different BC subgroups: luminal subtypes A and B, both of which are ER and/or PgR positive; the HER2 subtype; and the basal-like group.[10] Collins *et al.* evaluated the expression of AR among these molecularly-defined categories of invasive BC and found that AR expression was present in 91% of luminal A cancers, 68% of luminal B cancers, 59% of HER2-type cancers, 32% of basal-like cancers, and in 32% of unclassified carcinomas (those that were ER, PgR, HER2, CK 5/6 and epidermal growth factor [EGFR] negative).[11] As concerning tumor histotype, AR has been reported to be expressed in about 71% of ductal, 96% of lobular, 81% of mucinous and 100% of tubular cancers.[12] Apocrine carcinomas are typically characterized by AR expression without ER and PgR expression and most of them fitted into the luminal AR (LAR) molecular subtype of triple negative BC. However, a small subset of LAR cancers lacks an apocrine morphology.[13]

In BCs expressing ER and AR, an AR/ER crosstalk exists and AR competes with ER to block ER cascade.[14] In this setting, several studies have described that, in PgR− BCs, AR could have a pro-tumorigenic role, promoting the transcription of ER-dependent genes.[15] On the other hand, other papers suggested that, in early BC, AR expression represents a positive prognostic factor associated with a longer relapse free survival (RFS), favorable biologic features (lower tumor size, nuclear grade, proliferation index and ki67), longer overall survival (OS) whereas AR hyperexpression (AR/ER ratio ⩾2) appears to be detrimental in patients treated with endocrine therapies.[12,16–18] The results regarding the expression of AR in BC subtypes are heterogeneous.[19–21] A recent analysis of the PREPARE and TECHNO trials was published, trying to investigate the predictive and/or prognostic role of AR expression in high-risk BC patients.[22] In particular, in the phase III PREPARE trial, patients with high-risk primary BC stratified for tumors ⩾3 cm or inflammatory BC were randomly assigned to receive concurrent preoperative epirubicin/paclitaxel every 3 weeks or dose-dense and dose-escalated sequential epirubicin followed by paclitaxel every 2 weeks. All patients received three cycles of cyclophosphamide, methotrexate, and fluorouracil chemotherapy after surgery.[23] The primary objective of the trial was to compare preoperative dose-dense chemotherapy with conventionally scheduled preoperative chemotherapy in terms of pathologic complete response, disease-free survival (DFS) and OS. In the phase III TECHNO trial, patients with HER2-overexpressing BC (⩾2 cm or inflammatory) received four 3-week cycles of epirubicin and cyclophosphamide followed by four 3-week cycles of paclitaxel and trastuzumab before surgery.[24] Trastuzumab was continued after surgery to complete 1 year of treatment. The primary endpoint was pathologic complete response. Overall, high AR mRNA levels were found to be associated with lower pathological complete response rates (odds ratio [OR] = 0.77, 95% confidence interval [CI] = 0.67–0.88, $p = 0.0002$) but also with better prognosis in terms of DFS (hazard ratio [HR] = 0.57, 95% CI = 0.39–0.85, $p = 0.0054$) and OS (HR = 0.43, 95% CI = 0.26−0.71, $p = 0.0011$).[22] In the PREPARE trial, survival differences for patients with high and low AR1 mRNA levels could only be seen in the standard chemotherapy arm but not in the dose-dense treatment arm (OS: HR = 0.41, 95% CI = 0.22−0.74 *versus* HR = 1.05, 95% CI = 0.52−2.13; $p = 0.0459$).

To our knowledge, in the literature no data are reported regarding the role of AR expression in early stage ER+/PgR− BCs. The aim of this study was to evaluate the prognostic role of AR expression in this setting after a long follow-up (median 77 months).

Patients and methods

Study setting and design
We report the results of a monocentric retrospective analysis in which consecutive patients who underwent surgical intervention for ER+/PgR−/HER2− BC at Istituti Clinici Scientifici Maugeri (Pavia, Italy) between January 2005 and December 2014 were included. Patients with a triple negative BC, apocrine histotype and immunohistochemistry (IHC) for HER2 3+ or HER2 2+ and fluorescence *in situ* hybridization

test positive for amplification were excluded. According to the literature, BC was considered ER+ if an ER expression higher than 10% was seen immunohistochemically.[25] Data collection ended the 30th June 2019 for the analysis.

Information including BC history, previous and subsequent treatments in (neo)adjuvant setting, menopausal status, staging at diagnosis, type of surgical intervention, response to treatments, tumor biology including histotype, grade, ki67, vascular invasion and lymphoplasmacytic infiltrate, relapse sites, and date of last follow-up or death were collected for each patient. AR expression was evaluated by IHC in each tumor surgical specimen. The research was conducted according to the principles of the Declaration of Helsinki and was approved by the Local Institutional Ethical Committee (ICS Maugeri IRCCS Pavia Ethic Committee; approval number 2409). All patients involved in this study signed an authorization form for the use of their data.

Study objectives

The primary objective of the study was the evaluation of the relationship between AR expression in tumor specimen and the RFS.

Secondary objectives were: the evaluation of the relationship between AR expression in the tumor specimen and OS; the correlation between AR/ER ratio and RFS; the correlation between AR/ER ratio and OS; the correlation between AR expression and the tumor biology including histotype, grade, ki67, vascular invasion, and lymphoplasmacytic infiltrate; the correlation between AR expression and patient characteristics, in particular stage, age at diagnosis, and menopausal status.

Study assessment and outcomes

As a primary objective, RFS was defined as the time between the diagnosis of BC and the evidence of first disease relapse (all sites included). As a secondary objective, OS was defined as the time between the diagnosis of BC and the date of cancer-specific death or to the last date of follow-up, estimated using the Kaplan−Meier method. Finally, multivariate and univariate analyses were conducted in order to identify factors potentially linked with AR expression and disease outcomes.

Immunohistochemically determination of AR expression

The expression of the AR on BC tissues was evaluated using IHC analysis. Surgical specimens were fixed in formalin and included in paraffin. For each sample, 3 µm sections were obtained. The IHC analysis was performed using VENTANA BenchMark Ultra (Ventana Medical Systems, Tucson, AZ, USA) with ultraView Universal DAB kit (Ventana Medical System).

A primary monoclonal rabbit antibody directed *versus* AR (SP107 Cell Marque, Ventana Medical System) and pre-diluted by the supplier was used. After immunostaining, the slides were counterstained with Harris hematoxylin de-hydrated and mounted for microscopic reading. Nuclear staining was expressed as the percentage of stained cells (0−100%) over the total number of viable cells. Two expert pathologists in BC (LV and MA) assessed the expression of AR together, and disagreement was resolved through consensus.

Statistical analysis

Sample size was calculated according to the primary endpoint. It was estimated that 207 patients were necessary to demonstrate the presence of a statistically significant difference in 5-year relapse probability in patients with high AR expression (presumed to be 15%) than patients with low or absent AR expression (presumed to be 35%), with a statistical power (1 − beta) of 90% assuming a two-sided confidence level (1 − alpha) of 95%. The sample size was calculated using OpenEpi.[26] Continuous variables' distribution was expressed in terms of median and interquartile range (25th−75th percentiles) since most of the variables deviated significantly from the normal distribution, and the categorical variables' distribution was described by absolute and relative frequency (%). AR and ER distributions were discretized into two levels using Receiver Operating Characteristic (ROC) method and their median value. The presence of a statistically significant difference in terms of variable distribution by outcomes' values was assessed by the non-parametric Wilcoxon rank sum test (for numeric explanatory variables) or by Fisher's exact tests for categorical explanatory variables. The quantile regression with stepwise selection was applied to evaluate the impact of variables on non-Gaussian numeric dependent variables. The log-rank test was applied to compare survival profiles between AR groups;

survival curves were defined by the Kaplan–Meier method. Cox regression was applied to estimate the time-dependent risk of the outcomes of interest. The statistical significance threshold was set to $p < 0.05$ for all analyses. All statistical procedures were performed by the *R* statistical software tool (https://www.r-project.org).

Results

We included 237 women with early stage ER+/PgR−/HER2− BC; 29 patients were excluded from the analysis because they were lost at follow-up (12/29) or AR expression evaluation could not be performed (17/29) because cancer tissue was no longer available. For these reasons, a total of 208 patients were analyzed; their baseline characteristics are reported in Table 1. AR expression was present in 184 patients (88.5%); among them, 5% had an AR expression ⩽5% whereas 29% had ⩾90% (Figure 1). The median AR expression value was 80%; about 90% of patients had an AR/ER ratio <2.

At a median follow-up of 77 months (range 18–168 months), 75 patients (36%) had a disease relapse. Among them, 32 patients (43%) had a visceral recurrence, 20 (27%) a bone progression, 18 patients (23%) a loco-regional relapse, and 5 patients (6%) a mediastinal lymph node progression.

We first tested for the presence of statistically significant differences in terms of AR and ER values distribution in relapsed and non-relapsed patients by the Wilcoxon rank sum test and found that the median AR expression was significantly higher in patients who did not relapse compared with those who had relapsed (median AR values = 80% *versus* 70%, respectively, $p = 0.011$) with an impact on RFS (HR = 0.99, $p = 0.025$) (Table 2). Similarly, ER expression was significantly higher in patients who did not relapse compared with the rest of the cohort (median ER values = 80% *versus* 70%, respectively, $p = 0.038$) but this data had no statistically significant impact on the probability of RFS (HR = 0.99, 95% CI = 0.99 − 1, $p = 0.068$).

After, it was tested whether an AR and ER value threshold could be informative in terms of RFS and OS. Both AR and ER were discretized according to their median value (AR = 80%, ER = 80%) and the prognostic role of these cut-off values had been assessed by univariate

analysis. Results showed that patients with AR expression ⩾80% had a lower risk of relapse compared with those with an AR <80% (HR = 0.53, 95% CI = 0.34 − 0.85, $p = 0.008$), while women with an ER expression ⩾80% had no different risk of relapse compared with the rest of the cohort (HR = 0.694, 95% CI = 0.440 − 1.094, $p = 0.116$). Extremely similar thresholds to discriminate patients who relapsed from those who did not relapse had been obtained by applying the ROC method (ROC method: AR = 75% and ER = 75%). Of note, the two thresholds derived from the median method and the ROC method were able to distinguish the same patients: individuals with AR values ⩾75% ($n = 110/208$) also had AR values ⩾80% ($n = 110/208$) while patients with ER values ⩾75% ($n = 113/208$) also had ER values ⩾80% ($n = 113/208$).

Patients with AR expression <80% had a median RFS of 8.3 months whereas the median RFS value was not reached in patients with AR ⩾80%. The Kaplan–Meier curve comparing the probability of RFS in the two cohorts (AR ⩾80% and AR <80%) is shown in Figure 2A ($p = 0.007$). Instead, no association between AR expression and OS was observed, as shown in Figure 2B ($p = 0.40$).

We also evaluated if the AR/ER ratio could have a prognostic role; this variable was not informative for RFS ($p > 0.05$) but had a statistically significant association with OS (HR = 1.21, 95% CI = 1.01 − 1.45, $p = 0.035$). If discretized according to a threshold corresponding to a ratio of 2, no statistically significant difference in terms of AR/ER was observed neither with RFS nor with OS ($p > 0.05$).[27]

We then investigated if the presence of AR (defined as an AR expression ⩾5%) was associated with particular tumor biological characteristics compared with AR negative BCs and we found that patients with an AR expression ⩾5% had more frequently low lymphoplasmacytic infiltrate ($p = 0.021$), ki67 <20% ($p = 0.016$), and lobular histotype ($p = 0.010$) (Table 3). When considering an AR expression ⩾80%, tumors more frequently had a low ki67 (<20%, $p = 0.001$), a low tumor grade ($p = 0.003$), and they were more commonly related to lobular histotype ($p = 0.003$) (Table 3).

When performing multivariate analyses for RFS including all potential confounders (demographic

Table 1. Patients baseline characteristics.

Variable	Whole sample
Median age (range)	65 (33–88)
Menopausal status	
Pre-menopausal	23 (11%)
Post-menopausal	185 (89%)
Stage	
I	102 (49%)
II	73 (35%)
III	33 (16%)
ER	
⩽10%	16 (8%)
10–60%	23 (11%)
⩾60%	169 (81%)
AR	
0	24 (12%)
⩽5%	11 (5%)
5–90%	112 (54%)
⩾90%	61 (29%)
Histotype	
NOS/Ductal	131 (63%)
Lobular	67 (32%)
Other	10 (5%)
Grade	
G1	18 (9%)
G2	130 (62%)
G3	60 (29%)
Ki67	
<20%	148 (71%)
⩾20%	60 (29%)
Vascular invasion	
Absent	83 (40%)
Mild	68 (33%)
Diffuse	13 (6%)
Not available	44 (21%)
Lymphoplasmacytic infiltrate	
Low	149 (72%)
Moderate	13 (6%)

(Continued)

Table 1. (Continued)

Variable	Whole sample
Not available	46 (22%)
Type of surgical intervention	
Quadranctectomy + SLNB	86 (41%)
Quadrantectomy + ALND	58 (28%)
Mastectomy + SLNB	15 (7%)
Mastectomy + ALND	49 (24%)
Radiotherapy	
Yes	141 (68%)
No	67 (32%)
Neoadjuvant therapy	
No	171 (82%)
Yes	37 (18%)
Anthracycline	15 (41%)
Taxane	1 (3%)
Anthracycline + taxane	9 (24%)
Endocrine treatment	11 (30%)
Other (CMF)	1 (2%)
Adjuvant chemotherapy	
No	142 (68%)
Yes	66 (32%)
Anthracycline	24 (36%)
Taxane	10 (15%)
Anthracycline + taxane	23 (35%)
Other	9 (14%)
Adjuvant endocrine therapy	
No	20 (10%)
Yes	188 (90%)
Tamoxifen	19 (10%)
Aromatase inhibitors	150 (80%)
Tamoxifen + LHRH analogue	11 (6%)
Sequential	8 (4%)

ALND, axillary lymph node dissection; AR, androgen receptor; CMF, cyclophosphamide-methotrexate-fluorouracil; ER, estrogen receptor; LHRH, luteinizing hormone-releasing hormone; NOS, not otherwise specified; SLNB, sentinel lymph node biopsy; variable, analyzed variable; whole sample, variables' distribution in the whole sample: median (25th–75th percentiles) or absolute and relative frequency (%).
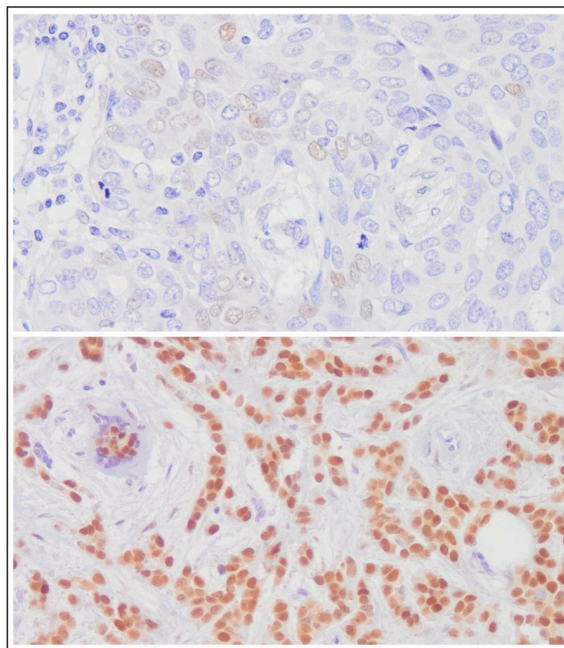
Figure 1. Representative histologic images showing immunohistochemical expression of androgen receptor in breast cancer tissue (upper image: androgen receptor expression ≤5%; lower image: androgen receptor expression ≥90%) (original magnification, ×100).

and biological characteristics and type of treatments), neither AR nor ER expression reached statistical significance.

Moreover, in the whole cohort, the only other characteristics associated with a higher probability of relapse were ki67 ≥20% (HR = 2.3, $p < 0.001$, 95% CI = 1.44–3.66) and presence of low or diffuse vascular invasion (HR = 1.80, 95% CI = 1.0–3.26, $p = 0.051$ and HR = 2.23, 95% CI = 0.89–5.60, $p = 0.086$, respectively) (Table 2). Stepwise quantile regression showed that ER expression correlated significantly with AR distribution (quantile regression coefficient = +0.80, SE = 0.26, $p = 0.002$) independently from the chemotherapy or endocrine treatment performed. The scatterplot in Figure 3 describes the correlation between ER and AR expression (Spearman coefficient = +35).

Multivariate Cox regression models including AR and ER expression as well as demographic factors, tumor characteristics, and type of therapies performed suggested that neither AR nor ER expression were informative with respect to the probability of survival ($p > 0.05$). The only

variables associated with OS were disease relapse (HR = 4.85, 95% CI = 2.39–9.84, $p < 0.001$) and having performed adjuvant chemotherapy (HR = 0.38, 95% CI = 0.15–0.99, $p = 0.048$).

Discussion

To the best of our knowledge, this is the first study evaluating the potential prognostic role of AR in ER+/PgR–/HER2– BC. In the literature, this BC subtype has a worse survival compared with tumors with the same histotype and PgR+.[28–31] PgR expression is linked to ER expression since PgR is an estrogen-regulated gene. For this reason, PgR absence may be due, at least in part, to the presence of a non-functional ER.[7,32] This fact could explain why the subgroup of PgR– BC has a lower response to endocrine therapies.[31] A recent study examined the clinical and biological features of BC women stratified according to ER and PgR tumor expression as double positive (ER+, PgR+), single positive (ER+, PgR–) and double negative (ER–, PgR–).[33] The results confirm the poor prognosis of ER+/PgR–/HER2– BC patients that resulted in being similar to the triple negative subtype.

The increasing interest for the potential predictive and/or prognostic role of AR is well portrayed in the literature. Several retrospective studies described an association between high AR expression and older age at diagnosis, lower tumor grade, lower ki67, and smaller tumor size. In addition, AR seems to be involved in the development of drug resistance in luminal BC treated with aromatase inhibitors (AIs) or tamoxifen.[34] AR and ER can compete for the binding to estrogen response elements (EREs) on specific genes. Therefore, the binding of AR to EREs reduces the estrogen proliferative action, thus inducing antiproliferative effects.[35] Conversely, ER can bind to androgen response elements, obtaining the opposite effect. This mechanism could explain the role of AR in the resistance to standard endocrine treatments. Moreover, a high AR expression can activate the EGFR, promoting an agonist effect of tamoxifen on the ER pathway. Recent meta-analysis demonstrated that AR expression is a positive prognostic factor independent from ER, being associated with a lower risk of disease recurrence in all BC subtypes and with a better OS in ER+ breast tumors.[36–38] In our study, AR resulted in being expressed in about 90% of patients but with variable values. The majority of patients had an AR expression between 5% and 90% (54%), 29%

Table 2. Univariate analysis on relapse probability.

Variable	Value	Relapse			Relapse free survival	
		No	Yes	*p*	HR (95% CI)	*p*
AR (%)		80 (60–90)	70 (12.5–80)	0.011	0.99 (0.99–1)	0.025
	<80%	52 (53.06%)	46 (46.94%)	0.002	Baseline	
	≥80%	81 (73.64%)	29 (26.36%)		0.53 (0.34–0.85)	0.008
ER (%)		80 (70–80)	70 (60–80)	0.038	0.99 (0.98–1)	0.068
	<80%	54 (40.6%)	41 (54.67%)	0.060	Baseline	
	≥80%	79 (59.4%)	34 (45.33%)		0.69 (0.44–1.09)	0.116
AR/ER ratio		1 (0.8–1.12)	0.89 (0.29–1.12)	0.281	1.06 (0.88–1.27)	0.536
	<2	125 (64.77%)	68 (35.23%)	0.410	Baseline	
	≥2	8 (53.33%)	7 (47.67%)		1.47 (0.67–3.20)	0.334
Grade	G1 – G2	97 (65.54%)	51 (34.46%)	0.524	Baseline	
	G3	36 (60%)	24 (40%)		1.24 (0.76–2.01)	0.394
Stage	I	76 (81.72%)	17 (18.28%)	1	Baseline	
	yI	6 (85.71%)	1 (14.29%)		0.89 (0.12–6.77)	0.912
	II	32 (55.17%)	26 (44.83%)	1	Baseline	
	yII	8 (53.33%)	7 (46.67%)		0.79 (0.34–1.83)	0.581
	III	10 (50%)	10 (50%)	0.022	Baseline	
	yIII	1 (7.69%)	12 (92.31%)		1.46 (0.6–3.52)	0.405
Vascular invasion	Absent	64 (77.11%)	19 (22.89%)	0.061	Baseline	
	Mild	42 (61.76%)	26 (38.24%)		1.80 (1–3.26)	0.051
	Diffuse	7 (53.85%)	6 (46.15%)		2.23 (0.89–5.6)	0.086
Histotype	NOS/ductal	87 (66.41%)	44 (33.59%)	0.485	Baseline	
	Lobular	39 (58.21%)	28 (41.79%)		1.13 (0.7–1.81)	0.620
	Other	7 (70.00%)	3 (30.00%)		0.89 (0.28–2.88)	0.850
ki67	<20%	103 (69.59%)	45 (30.41%)	0.011	Baseline	
	≥20%	30 (50%)	30 (50%)		2.3 (1.44–3.66)	<0.001

AR, androgen receptor; baseline, intercept of the regression; CI, 95% confidence interval; ER, estrogen receptor; HR, hazard ratio; NOS, not otherwise specified; *p* = unadjusted *p*-value from Wilcoxon rank sum test, Chi-square test, or Fisher's exact test as appropriate; value, value that the variable assumes; variable, analyzed variable; yI – yII – yIII, patients who performed neoadjuvant treatment.

an AR ≥90%; 17% had an AR expression ≤5%. At a median follow-up of 77 months, 75 patients (36%) experienced a disease relapse. Among them, 42% had a visceral recurrence suggesting a higher aggressiveness of this subgroup.[33] This study also confirmed that in the ER+/PgR– BC subtype an AR expression >80% is a prognostic factor for RFS. In fact, in patients with AR ≥80%

Table 3. Biological and clinical characteristics according to AR expression.

Variable	Value	AR			Median AR		
		⩽5%	>5%	*p*	<80%	⩾80%	*p*
Grade	G1-G2	21 (60%)	127 (73.41%)	0.110	60 (61.22%)	88 (80%)	0.003
	G3	14 (40%)	46 (26.59%)		38 (38.78%)	22 (20%)	
Stage	I	13 (92.86%)	80 (93.02%)	1	37 (92.5%)	56 (93.33%)	1
	yI	1 (7.14%)	6 (6.98%)		3 (7.5%)	4 (6.67%)	
	II	12 (92.31%)	46 (76.67%)	0.279	32 (80%)	26 (78.79%)	0.898
	yII	1 (7.69%)	14 (23.33%)		8 (20%)	7 (21.21%)	
	III	4 (50%)	16 (64%)	0.681	9 (52.94%)	11 (68.75%)	0.481
	yIII	4 (50%)	9 (36%)		8 (47.06%)	5 (31.25%)	
LPI infiltrate	low	29 (82.86%)	163 (94.22%)	0.021	91 (92.86%)	101 (91.82%)	0.779
	high	6 (17.14%)	10 (5.78%)		7 (7.14%)	9 (8.18%)	
Vascular invasion	absent	12 (34.29%)	71 (41.04%)	0.770	41 (41.84%)	42 (38.18%)	0.611
	mild	13 (37.14%)	55 (31.79%)		29 (29.59%)	39 (35.45%)	
	diffuse	2 (5.71%)	11 (6.36%)		5 (5.1%)	8 (7.27%)	
Histotype	NOS/ductal	26 (74.29%)	105 (60.69%)	0.010	65 (66.33%)	66 (60%)	0.003
	lobular	5 (14.29%)	62 (35.84%)		24 (24.49%)	43 (39.09%)	
	other	4 (11.43%)	6 (3.47%)		9 (9.18%)	1 (0.91%)	
ki67	<20%	19 (54.29%)	129 (74.57%)	0.016	59 (60.2%)	89 (80.91%)	0.001
	⩾20%	16 (45.71%)	44 (25.43%)		39 (39.8%)	21 (19.09%)	

AR, androgen receptor; LPI, lymphoplasmacytic infiltrate; NOS, not otherwise specified; value, value that the variable assumes; variable, analyzed variable; yI – yII – yIII, patients who performed neoadjuvant treatment.
Variables' distribution within AR category is described by absolute and relative frequency (%).

BCs, risk recurrence is reduced by 47% (HR = 0.53, 95% CI = 0.34 – 0.85, *p* = 0.008). *Post hoc* statistical power calculations showed that the statistical power to detect the observed differences in terms of probability of relapse in patients with AR <80% (*n* = 46/98, 46.94%) and those with AR ⩾80% (*n* = 29/110, 26.36%) was 84% assuming a bilateral confidence level of 95%. The analysis of independent cohorts of patients will allow validation of these findings. Of note, extremely similar thresholds to discriminate patients who had relapsed from those who did not relapse can be obtained by applying the supervised ROC method (AR = 75%). The median value and the ROC method provided thresholds able to distinguish the same patients: the same individuals with AR values ⩾75% calculated with the ROC method (*n* = 110/208) also had AR values ⩾80% calculated as median value (*n* = 110/208).

Regarding the relationship between AR expression, type of endocrine treatment performed, and RFS, data in the literature are scarce with discordant results. In particular, a recent study described that patients with a high AR/ERα ratio (⩾2.0) or prostate-derived Ets factor (PDEF)/ERα ratio (⩾2.0) receiving adjuvant tamoxifen treatment had a two-fold increased risk of failure and that both the AR/ERα ratio and PDEF/ERα ratio were independently associated with the risk of tamoxifen treatment failure.[27] On the other hand, the results of another trial evidenced that AR
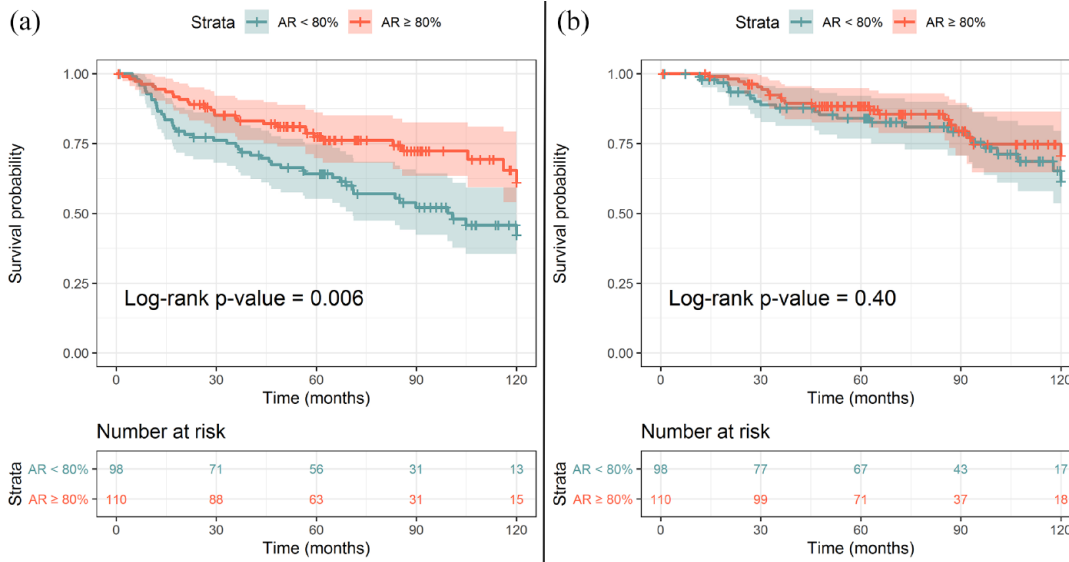
Figure 2. Kaplan–Meier curve showing the relationship between androgen receptor expression and probability of relapse (A) and between androgen receptor expression and probability of survival (B). The numbers in each table describe the number of patients at risk by stratum at different time points. The shaded areas represent the 95% confidence interval of each curve. *p*-values were generated by the Log-rank test. AR, androgen receptor.
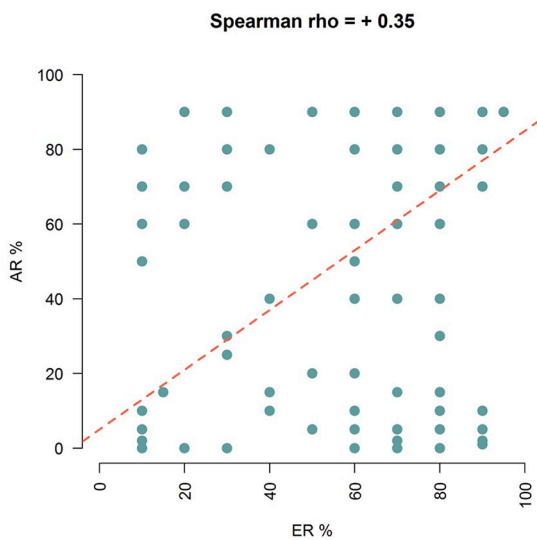

Figure 3. Scatterplot describing the correlation between estrogen receptor (ER) and androgen receptor (AR) expression. The quantile regression line is represented in red.

expression is not an informative biomarker for the selection of adjuvant endocrine therapy in post-menopausal women with ER+ BCs.[39] In this study, no associations between the type of endocrine adjuvant treatment, AR expression, and RFS were evident.

The link between AR expression and RFS may vary according to ER expression.[39] In the work by Castellano and colleagues, AR-ER co-expression was reported to be associated with a better outcome in terms of time to relapse and disease-specific survival.[40] Two subsequent studies described that a high AR/ER ratio is detrimental although the AR/ER co-expression is related to a better prognosis.[16,17] In the first work, Authors established that an AR/ER ratio $\geqslant 2$ identified a subgroup of patients with a four times higher probability of disease recurrence, in particular to lymph nodes, and a shorter DFS.[16] In the other work, a high AR/ER ratio was associated with poor clinic-biological characteristics; in particular, higher tumor size at diagnosis, lymph nodes involvement, and higher tumor grade. In addition, an AR/ER ratio $\geqslant 2$ was related to a worse survival with shorter DFS.[17] In our study, an AR/ER ratio $\geqslant 2$ was not associated with a higher risk of recurrence since statistical analysis did not identify an AR/ER ratio that could split the population in two different groups of patients with different prognosis. Moreover, no correlations between AR expression and OS were identified. In addition, from univariate analysis, patients with a ki67 $\geqslant 20\%$ had a higher probability to develop disease recurrence than patients with a ki67 $<20\%$ (HR = 2.3, 95% CI = 1.44 − 3.66, $p < 0.001$) as well as patients with tumor vascular invasion compared with patient with absent tumor

vascular invasion (HR = 1.80, 95% CI = 1.0 – 3.26, p = 0.051 and HR = 2.23, 95% CI = 0.89 – 5.60, p = 0.086 for low and diffuse vascular invasion, respectively). We also found that, compared with AR < 80%, AR+ (≥80%) BCs had a higher probability to have lobular histotype while having a lower ki67 and tumor grade. On the other hand, 65% of the tumors with ki67 ≥20% (luminal B like) and 63% poorly differentiated (G3) tumors had an AR expression <80%.

The retrospective design of the study limits its significance, but a long follow-up period was considered to strengthen our hypothesis. Nevertheless, these data might help in developing further prospective and mechanistic studies and add more information to the identification of subgroups of high-risk patients for daily decision making in clinical practice.

Conclusion

The results of this retrospective observational trial on early ER+/PgR– BC patients support the potential prognostic role of AR in this peculiar population. Breast tumors with high AR expression usually have good biological features (low ki67 and good nuclear differentiation) whereas BCs with lower AR expression often show adverse biological features, at least partly explaining the better outcome of patients with ER+/PgR– coupled with high AR expression BCs.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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