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The role of the AR/ER ratio in ER-positive breast cancer patients

Nelson Rangel^{1,2}, Milena Rondon-Lagos³, Laura Annaratone¹, Simona Osella-Abate¹, Jasna Metovic¹, Maria Piera Mano⁴, Luca Bertero¹, Paola Cassoni¹, Anna Sapino^{1,5} and Isabella Castellano¹

¹Department of Medical Sciences, University of Turin, Turin, Italy

²Natural and Mathematical Sciences Faculty, Universidad del Rosario, Bogotá, Colombia

³School of Biological Sciences, Universidad Pedagógica y Tecnológica de Colombia, Tunja, Colombia

⁴Department of Surgical Sciences, University of Turin, Turin, Italy

⁵Pathology Unit, Fondazione del Piemonte per l'Oncologia (FPO) Candiolo Cancer Institute (IRCCS), Candiolo, Italy

Correspondence should be addressed to I Castellano: isabella.castellano@unito.it

Abstract

The significance of androgen receptor (AR) in breast cancer (BC) management is not fully defined, and it is still ambiguous how the level of AR expression influences oestrogen receptor-positive (ER+) tumours. The aim of the present study was to analyse the prognostic impact of AR/ER ratio, evaluated by immunohistochemistry (IHC), correlating this value with clinical, pathological and molecular characteristics. We retrospectively selected a cohort of 402 ER+BC patients. On each tumour, IHC analyses for AR, ER, PgR, HER2 and Ki67 were performed and AR+ cases were used to calculate the AR/ER value. A cut-off of ≥ 2 was selected using receiver-operating characteristic (ROC) curve analyses. RNA from 19 cases with AR/ER ≥ 2 was extracted and used for Prosigna-PAM50 assays. Tumours with AR/ER ≥ 2 (6%) showed more frequent metastatic lymph nodes, larger size, higher histological grade and lower PgR levels than cases with AR/ER < 2 . Multivariate analysis confirmed that patients with AR/ER ≥ 2 had worse disease-free interval (DFI) and disease-specific survival (DSS) (hazard ratios (HR)=4.96 for DFI and HR=8.69 for DSS, both $P \leq 0.004$). According to the Prosigna-PAM50 assay, 63% (12/19) of these cases resulted in intermediate or high risk of recurrence categories. Additionally, although all samples were positive for ER assessed by IHC, the molecular test assigned 47.4% (9/19) of BCs to intrinsic non-luminal subtypes. In conclusion, the AR/ER ratio ≥ 2 identifies a subgroup of patients with aggressive biological features and may represent an additional independent marker of worse BC prognosis. Moreover, the Prosigna-PAM50 results indicate that a significant number of cases with AR/ER ≥ 2 could be non-luminal tumours.

Key Words

- breast cancer
- androgen receptor
- oestrogen receptor
- subtypes
- prosigna

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Introduction

Oestrogen receptor (ER) and progesterone receptor (PgR) are expressed in most breast cancers (BCs) (~75%) and both have wide prognostic and predictive utility (Early Breast Cancer Trialists' Collaborative Group 2011). In contrast, the clinical and biological significance of androgen receptor (AR) expression in BC is not fully

defined. AR positivity has been detected in up to 61% of primary and metastatic BC lesions (Park *et al.* 2010, Hu *et al.* 2011, Yu *et al.* 2011) and approximately 75% of ER-positive (ER+) BCs are also AR positive (AR+). Several studies have shown that AR expression in luminal tumours (ER+) is associated with lower tumour

grade, smaller tumour size, lower proliferative index (Ki67 level) and more importantly, AR expression in ER+ tumours is an independent prognostic factor of a good outcome (Castellano *et al.* 2010, Niemeier *et al.* 2010, Aleskandarany *et al.* 2016, Bozovic-Spasojevic *et al.* 2016). On the other hand, up to 31% of ER-negative (ER-) BCs are reported to be AR+ (Niemeier *et al.* 2010, Park *et al.* 2010), but the prognostic impact of AR expression in this subset of BCs is not clear (Luo *et al.* 2010, Hu *et al.* 2011, Park *et al.* 2011, Pistelli *et al.* 2014, Vera-Badillo *et al.* 2014, Hilborn *et al.* 2016, Jiang *et al.* 2016, Asano *et al.* 2017).

While the interaction between the signalling pathways of ER and AR (Peters *et al.* 2009) is well known, it is still ambiguous how the level of AR expression influences ER+ tumours. *In vitro* studies have shown that AR signalling inhibits oestrogen-induced proliferation of ER+ MCF7 BC cells (Ando *et al.* 2002, Greeve *et al.* 2004, Macedo *et al.* 2006, Cops *et al.* 2008). This inhibitory effect seems to be mediated by several mechanisms, but the most important is the ability of AR to compete with ER for binding of oestrogen response elements (EREs), preventing ER-dependent gene transcription (Need *et al.* 2012). In line with this observation, some studies have reported that increasing AR expression results in a greater androgen-dependent inhibition of ER function (Buchanan *et al.* 2005, Peters *et al.* 2009). However, other studies performed on ER+ MCF7 BC cells described an increase in proliferation when the AR signalling pathway is stimulated (Birrell *et al.* 1995, Lin *et al.* 2009). Moreover, Cochrane and coworkers (Cochrane *et al.* 2014) recently reported that high AR levels and low ER levels (higher AR/ER ratio) could be associated with a worse prognosis and tamoxifen (TAM) resistance.

Considering these data, the aim of the present study was to analyse the prognostic impact of AR expression with respect to ER (AR/ER ratio) in a large case series of ER+/HER2-negative (HER2-) BC patients. We evaluated if the AR/ER ratio may identify a subset of tumours with different clinical and pathological characteristics. In addition, in the subgroup of BCs with high AR/ER ratio values, we performed Prosigna-PAM50 assays to assess the molecular subtypes of these BCs.

Patients and methods

Study design and population

We collected a cohort of 402 ER+/HER2- primary invasive BC patients with available follow-up (Fig. 1), who underwent surgery from January 1998 to December 2012

at the Breast Unit of the Città della Salute e della Scienza of Torino, University Hospital of Torino in Turin, Italy. In the diagnostic setting, the cut-off value considered for ER and PgR positivity was $\geq 1\%$, as suggested by the St Gallen and ASCO/CAP Guideline Recommendations (Hammond *et al.* 2010, Coates *et al.* 2015), and the same cut-off was adopted for AR positivity (Castellano *et al.* 2010). For all cases, the following clinico-pathological data were obtained from the clinical charts and pathological reports: age, type of surgery (conservative surgery vs radical mastectomy), tumour size (<15 mm vs ≥ 15 mm), histological type, tumour grade and nodal involvement. Ethical approval for this study was obtained from the Committee for human Biospecimen Utilization (Department of Medical Sciences – ChBU). The project provided an informed consent, obtained from the patients at the time of surgery due to the retrospective approach of the study, which did not impact on their treatment. The procedure for collecting the consent was approved by the Committee for human Biospecimen Utilization (Department of Medical Sciences – ChBU). All the cases were anonymously recorded, and data were accessed anonymously.

Figure 1

Study flowchart. ER – estrogen receptor; HER2 – human epidermal growth factor receptor 2; AR – androgen receptor. *Three additional cases (without follow-up) with a ratio of AR/ER ≥ 2 were included for the Prosigna-PAM50 assay.

Immunohistochemistry (IHC)

For each case, representative blocks were selected and multicore tissue microarrays (TMAs) were prepared, as previously described (Sapino *et al.* 2006). IHC was performed using an automated slide processing platform (Ventana BenchMark AutoStainer, Ventana Medical Systems, Tucson, AZ, USA) with the following primary antibodies: prediluted anti-ER rabbit monoclonal antibody (SP1, Ventana Medical Systems); prediluted anti-PgR rabbit monoclonal antibody (1E2, Ventana Medical Systems); anti-AR mouse monoclonal antibody (AR441, diluted 1:50, Dako) and anti-Ki67 mouse monoclonal antibody (MIB1, diluted 1:50, Dako). Measurement of HER2 expression was performed by an anti-HER2 polyclonal antibody (A0485, diluted 1:800, Dako). IHC equivocal cases (score 2+) were assessed for HER2 status by fluorescence *in situ* hybridization (FISH) (Marchio *et al.* 2009). Positive and negative controls (omission of the primary antibody and IgG-matched serum) were included for each immunohistochemical run. All cases were confirmed as ER+ and HER2–.

For statistical analyses and according to the St Gallen Consensus Recommendations (Coates *et al.* 2015), we adopted a cut-off of 20% for dichotomizing tumours as having low and high levels of PgR and Ki67. In addition, this cut-off agrees with the median Ki67 value of our laboratory, previously established to differentiate tumours with a higher proliferative index (Coates *et al.* 2015, Bustreo *et al.* 2016).

AR/ER ratio calculation

AR and ER nuclear staining percentages were compared. Post estimation ROC curve after logistic regression was used to establish the optimal AR/ER ratio cut-off value, which allowed us to subdivide the patients into those with good and worse prognosis as described below.

Statistical analyses

Pearson's chi-square test and Student's *t*-test were preliminarily performed to compare categorical and continuous variables, respectively, and to evaluate the potential differences in the variable distribution among the groups. The disease-free interval (DFI) was calculated from the date of surgical excision of the primary tumour to the date of the first relapse or last check-up. Disease-specific survival (DSS) was calculated from the surgical excision date of the primary tumour to the date of BC death or last check-up. Survival distribution curves were plotted using the Kaplan–Meier method and the statistical comparisons were performed using the log-rank test. Cox regression analyses were carried out on the DFI and DSS to calculate the crude and adjusted HR and 95% CIs for the different study group. The cases lost to follow-up and cases with non-breast cancer-related deaths were censored at the last follow-up. Models were created to evaluate the prognostic role of different variables. The proportional hazard assumption was assessed with the Schoenfeld residuals. This did not give reasons to suspect a violation of this assumption. The nature of the variables

Figure 2

Immunohistochemical staining. Representative IHC for the androgen receptor (A and C) and estrogen receptor (B and D) in two BC cases with high AR levels with respect to ER ($AR/ER \geq 2$). Although ER is expressed at the IHC level in these cases, the molecular test classified them as non-luminal subtypes.

Table 1 Clinical and pathological characteristics of ER+/AR+ BC patients.

Clinical-pathological features	Total (%)	AR/ER<2 (%)	AR/ER≥2 (%)	P value (Fisher test)
Number of patients	284 (100)	268 (94.3)	16 (5.7)	–
Median age (interval)	62 (31–88)	62 (31–87)	65 (47–88)	0.309*
ER% median (interval)	90 (2–100)	95 (30–100)	18 (2–45)	<0.001*
AR% median (interval)	50 (5–99)	40 (5–99)	80 (25–99)	0.01*
Grade				
1	104 (36.7)	103 (38.4)	1 (6.3)	<0.001
2	128 (45)	122 (45.5)	6 (37.5)	
3	52 (18.3)	43 (16.1)	9 (56.2)	
Tumour size				
<15 mm	149 (52.5)	146 (54.5)	3 (18.7)	0.004
≥15 mm	135 (47.5)	122 (45.5)	13 (81.3)	
Metastatic lymph nodes				
0	191 (67.2)	183 (68.3)	8 (50)	<0.001
1–3	62 (21.8)	61 (22.8)	1 (6.3)	
4–9	22 (7.8)	18 (6.8)	4 (25)	
>9	9 (3.2)	6 (2.1)	3 (18.7)	
Ki67				
<20%	168 (59.2)	162 (60.4)	6 (37.5)	0.075
≥20%	116 (40.8)	106 (39.6)	10 (62.5)	
PgR				
<20%	42 (14.8)	33 (12.3)	9 (56.3)	0.001
≥20%	242 (85.2)	235 (87.7)	7 (43.7)	
Relapse				
No	233 (82)	225 (84)	8 (50)	0.001
Local	5 (1.8)	5 (1.9)	0	
Distant	46 (16.2)	38 (14.1)	8 (50)	
Surgery				
Quadrantectomy	188 (66.2)	181 (67.5)	7 (43.7)	0.036
Mastectomy	96 (33.8)	87 (32.5)	9 (56.3)	
Therapy†				
HT	198 (69.7)	190 (70.9)	8 (50)	0.073
CT	85 (29.4)	77 (28.7)	8 (50)	

Patients were grouped according to AR/ER ratio cut-off ≥2.

*P value from Student's t-test; †1 patient refused therapy.

CT, patients who received hormonal therapy plus chemotherapy; HT, patients who received hormonal therapy.

Table 2 Univariate analysis in the group of ER+/AR+ BC patients.

Clinical-pathological features	DFI		DSS	
	HR (95% CI)	P	HR (95% CI)	P
Age	0.99 (0.96–1.02)	0.694	0.97 0.93–1.01	0.264
Grade	3.02 (1.96–4.66)	<0.001	5.26 (2.37–11.7)	<0.001
Tumor size ≥15 mm	6.97 (3.22–15.06)	<0.001	11.9 (2.74–52)	<0.001
Metastatic lymph nodes				
0	1			
1–3	2.92 (1.37–6.23)	<0.005	2.85 (0.71–11.4)	0.138
4–9	5.58 (2.41–12.9)	<0.001	12.2 (3.44–43.3)	<0.001
>9	15.5 (6.25–38.6)	<0.001	23.17 (5.74–93.3)	<0.001
Ki67≥20%	7.66 (3.55–16.52)	<0.001	12.25 (2.81–53.3)	<0.001
PgR≥20%	0.65 (0.34–1.25)	0.201	0.77 (0.27–2.16)	0.061
ER% expression	0.98 (0.97–0.99)	0.027	0.98 (0.96–0.99)	0.016
AR% expression	1.00 (0.99–1.01)	0.541	0.99 (0.98–1.02)	0.994
AR/ER≥2	7.55 (3.31–17.2)	<0.001	10.84 (3.52–33.3)	<0.001
HT vs CT	3.77 (2.02–7.03)	<0.001	3.85 (1.42–10.42)	0.008

CT, patients who received hormonal therapy plus chemotherapy; DFI, disease-free interval; DSS, disease-specific survival; HR, hazard ratio; HT, patients who received hormonal therapy.

(continue/categorical) included in the models was evaluated considering literature reports and the results of the log-likelihood ratio test. For model selection, the Akaike information criterion (AIC) test was used. All statistical tests were two-sided. P -values <0.05 were considered significant. Statistical analyses were performed using STATA/SE12.0 Statistical Software (STATA, College Station, TX, USA).

Prosigna multigene prognostic assay

Sixteen ER+/HER2– BC cases with an AR/ER ratio ≥ 2 with long follow-up and 3 additional cases collected during the routine diagnostic assessment of ER and AR were selected for Prosigna-PAM50 analysis (NanoString Technologies, Seattle, WA, USA). Briefly, tissue obtained after macrodissection of formalin-fixed paraffin-embedded (FFPE) tumours were processed with a Roche FFPE RNA Isolation Kit (Roche). The isolated RNA was hybridized to 58 gene-specific probe pairs, plus 6 positive and 8 negative controls (Capture and Reporter Probes – Prosigna CodeSet, NanoString Technologies), overnight at 65°C in a single hybridization reaction. The removal of excess probes, followed by binding of the probe–target complexes on the surface of a specific nCounter cartridge, was performed on the nCounter Prep Station (NanoString Technologies). Finally, the nCounter cartridge with immobilized probe/target complexes was read in the nCounter Digital Analyzer (NanoString Technologies). The conversion of gene expression measurements into intrinsic molecular subtypes, risk of recurrence (ROR) scores and risk categories used a fully prespecified algorithm has been previously described (Parker *et al.* 2009, Dowsett *et al.* 2013).

Results

Patients and tumour characteristics

Clinical and pathological features of the 402 ER+/HER2– tumours according to the AR status are shown in [Supplementary Table 1](#) (see section on [supplementary data](#) given at the end of this article). The median time of follow-up was 8 years. The majority of cases, 70.6% (284/402), were AR+. The distribution plots of IHC ER and AR nuclear staining percentages are presented in [Supplementary Fig. 1](#). According to our previous reports (Castellano *et al.* 2010, 2013), we confirmed that AR expression ($\geq 1\%$ nuclear staining) was significantly correlated with a longer DSS ($P=0.0008$; [Supplementary Fig. 2](#)) of ER+ BC patients.

AR/ER ratio and correlation with histological and immunohistochemical features

The median AR/ER ratio was 0.51. Two was the optimal AR/ER ratio that differentiated the cohort by prognosis (AR/ER ≥ 2 : AUC=0.74; $P=0.002$) ([Supplementary Fig. 3](#)). The characteristics of the 284 ER+/HER2–/AR+ BC cases stratified by an AR/ER ratio cut-off are reported in [Table 1](#). Of the 284 AR+/ER+ cases, 268 (94%) had an AR/ER ratio <2 and 16 (6%) an AR/ER ratio ≥ 2 ([Fig. 1](#) and [Fig. 2](#)). In the descriptive analysis, patients with a higher AR/ER ratio carried larger tumours with a higher histological grade and lower PgR levels, and they frequently had more metastatic lymph nodes and had a higher number of relapse events ($P\leq 0.004$) ([Table 1](#)).

Figure 3

Survival curves for AR/ER <2 vs AR/ER ≥ 2 . (A) Disease-free interval (DFI). (B) Disease-specific survival (DSS). *Log-rank test for equality of survivor functions.

Table 3 Multivariate analysis in the group of ER+/AR+ BC patients.

Clinical-pathological features	DFI*		DSS*	
	HR (95% CI)	P	HR (95% CI)	P
Age	1.01 (0.98–1.04)	0.474	0.98 (0.94–1.03)	0.602
Tumor size ≥ 15 mm	4.16 (1.88–9.18)	<0.001	8.87 (1.71–46)	0.009
Metastatic lymph nodes				
0	1			
1–3	1.41 (0.58–3.40)	0.441	1.28 (0.23–7.1)	0.778
4–9	1.59 (0.63–3.99)	0.321	3.45 (0.81–14.7)	0.095
>9	4.42 (1.66–11.79)	0.003	5.71 (1.17–27.7)	0.031
Ki67 $\geq 20\%$	3.98 (1.78–8.86)	<0.001	5.26 (1.12–24.6)	0.035
AR/ER ≥ 2	4.96 (1.95–12.68)	<0.001	8.69 (2.02–37.44)	0.004
HT vs CT	1.64 (0.72–7.03)	0.234	1.02 (0.25–4.18)	0.974

*Test of proportional-hazards assumption global test DFI $P=0.3188$, DSS $P=0.3871$.

CT, patients who received hormonal therapy plus chemotherapy; DFI, disease-free interval; DSS, disease-specific survival; HR, hazard ratio; HT, patients who received hormonal therapy.

AR/ER ratio and impact on prognosis

As shown in Table 2, univariate analysis confirmed that an AR/ER ratio ≥ 2 was one of the most significant markers of poor survival (HR=7.55 for DFI, and HR=10.84 for DSS, both $P<0.001$), together with tumour grade, tumour size ≥ 15 mm, nodal involvement ≥ 4 and high Ki67 index. Moreover, the Kaplan–Meier curves and the log-rank test showed significant differences in the survival times between the two groups (DFI and DSS $P<0.001$) (Fig. 3A and B). In the analysis, we also included ER and AR expression as continuous variables to compare the weight on the prognosis of different levels of the receptor expression with the AR/ER ratio. While the percentage of AR expression did not show any impact on prognosis, the levels of ER were correlated with prognosis although at a lower significance compared to the AR/ER ratio (Table 2). Multivariate analysis confirmed an independent effect on the prognosis of the AR/ER ratio. According to this model, patients with an AR/ER ≥ 2 were five times more likely to relapse (HR=4.96, $P<0.001$ for DFI) and eight times more likely to die of BC (HR=8.69, $P=0.004$ for DSS) compared with patients with a ratio <2 . Tumour size ≥ 15 mm, lymph nodes >9 and a high Ki67 index had an unfavourable effect on DFI and DSS (Table 3). The proportionality assumption was satisfied both for the DFI ($P=0.1227$) and DSS ($P=0.3517$).

To exclude the possibility that prognostic information from the AR/ER ratio was only a consequence of the low ER levels, we additionally tested a cut-off point for ER nuclear staining at 10%. As expected, patients with lower ER levels ($<10\%$) were associated with worse DFI and DSS (Supplementary Fig. 4). However, according to the AIC

Table 4 Characteristics of BC cases evaluated with Prosigna – PAM50 assay.

Clinical and molecular features	AR/ER ≥ 2 n (%)
Grade	
1	1 (5.3)
2	8 (42.1)
3	10 (52.6)
Tumor size	
<15 mm	5 (26.3)
≥ 15 mm	14 (73.7)
Metastatic lymph nodes	
0	11 (57.9)
1–3	1 (5.3)
4–9	4 (21)
>9	3 (15.8)
Ki67	
<20%	8 (42.1)
$\geq 20\%$	11 (57.9)
PgR	
<20	12 (63.2)
≥ 20	7 (36.8)
IHC-based subtype	
Luminal A	3 (15.8)
Luminal B	16 (84.2)
Prosigna-PAM50 molecular subtype	
Luminal A	9 (47.4)
Luminal B	1 (5.3)
HER2-Enriched	2 (10.5)
Basal-like	7 (36.8)
Prosigna-PAM50 risk category	
Low	7 (36.8)
Intermediate	4 (21.1)
High	8 (42.1)
Prosigna-PAM50 risk category – PDR†	
Low	7 (4.57)*
Intermediate	4 (10.25)*
High	8 (34.87)*

†Probability of distant recurrence; *Mean percentage of PDR at 10 years for each risk category.

Figure 4

IHC-based vs intrinsic molecular subtypes.

(A) Following guidelines recommendations, all BC with an AR/ER ratio ≥ 2 (19 cases) were classified as luminal by IHC. However, the Prosigna-PAM50 assay changed the classification of 17 cases (89.5%), almost half of them to the non-luminal subtypes. (B) Correlation of the ER percentage (ER%) nuclear staining (IHC) and intrinsic molecular subtypes in BC with an AR/ER ratio ≥ 2 .

test, which was used for model selection, the AR/ER ratio model received the lowest AIC score (DFI AIC=378.8, DSS AIC=139.7), indicating that this model is more effective at providing prognostic information than the model with an ER cut-off at 10% (DFI AIC=384.6, DSS AIC=145.9). Furthermore, although patients with lower ER levels were more likely to have AR/ER ≥ 2 , 56.2% of tumours (9/16 cases) with a high AR/ER ratio had a high ER level ($\geq 10\%$).

AR/ER ≥ 2 and association with intrinsic molecular subtypes

A Prosigna-PAM50 assay was performed on the 19 cases with a ratio AR/ER ≥ 2 to evaluate their ROR and molecular subtype. Twelve out of the 19 cases (63.2%) resulted in intermediate or high-risk categories (high probability of distant recurrence at 10 years) (Table 4).

We then compared the IHC-based subtypes (Hammond *et al.* 2010, Coates *et al.* 2015) with the intrinsic molecular subtype obtained by the Prosigna-PAM50 assay and the percentage of ER expression. Three cases were classified as IHC luminal A (15.8%) and 16 cases as luminal B (84.2%). The concordance between the IHC subtypes and intrinsic molecular subtypes was very low ($k=0.0583$) since only 2 cases (10.5%) maintained the same subtype (luminal A) using the Prosigna-PAM50 assay. Molecular tests classified 47.4% of samples as luminal A, 5.3% as luminal B, 10.5% as HER2-enriched and 36.8% as a basal-like

subtype (Fig. 4A and Table 4). Thus, gene expression analysis showed that 47.4% of BCs with an AR/ER ratio ≥ 2 were assigned to non-luminal subtypes (Fig. 2, Fig. 4A and Table 4). The correlation with the percentage of ER expression showed that 6 of the cases that switched from luminal to non-luminal had an ER $<10\%$, although two cases classified as luminal A by Prosigna-PAM50 assay had an ER $<10\%$ (2% and 5%, respectively) (Fig. 4B).

Discussion

In our study, we demonstrated that within ER+ BCs, the AR/ER ratio may represent an additional independent prognostic marker. Specifically, we showed that BCs with an AR/ER ratio ≥ 2 had a worse DFF and DSS. This particular subset of tumours is rare within ER+ BCs and, from the molecular point of view, they do not always fit with the luminal subtype.

The prognostic role of AR in ER+ BC has been extensively studied. Several authors have reported that AR expression in luminal cancers is associated with a better outcome compared to AR negative BCs (Park *et al.* 2011, Castellano *et al.* 2013, Kim *et al.* 2015, Bozovic-Spasojevic *et al.* 2016). However, some reports suggest that AR could be related to BC progression (Grogg *et al.* 2015), as it is detected in a significantly higher percentage of ductal carcinomas 'in situ' (DCIS) that are adjacent to invasive carcinomas than in pure DCIS (Yu *et al.* 2011).

Moreover, although the expression of ER and PgR decrease during BC progression (from DCIS to invasive and from G1 to G3), AR expression is highly conserved during BC progression, as it is detected in a high percentage of metastatic tumours (Cimino-Mathews *et al.* 2012, Grogg *et al.* 2015). In addition, Gonzalez and coworkers (Gonzalez *et al.* 2008) found that AR+ tumours are frequently positive for matrix metalloproteinases (MMPs), which have been involved in breast tumour dissemination. Finally, a recent study indicated that AR expression can induce the epithelial-to-mesenchymal transition in ER+ BC cells, conferring them with metastatic potential (Feng *et al.* 2016).

Panet-Raymond and coworkers (Panet-Raymond *et al.* 2000) reported that co-expression of both ER and AR reduces the trans-activation function of AR, and Takagi and coworkers (Takagi *et al.* 2010) suggested that AR signalling is suppressed in BC by high ER signalling activity.

All these results indicate that the interaction between the ER and AR levels may influence the AR activity. In line with this hypothesis, we found that BCs with a high AR/ER ratio are associated with aggressive biological features and worse prognosis.

To the best of our knowledge, only Cochrane and coworkers reported an association between AR/ER ratio and outcome (Cochrane *et al.* 2014). They showed that AR/ER ratio ≥ 2 is a good predictor of DFI and DSS, in a cohort of ER+ BC patients. AR/ER optimal ratio (AR/ER ≥ 2) was further defined and confirmed to predict DFI and DSS in our cohort (ER+/HER2- BC patients) by univariate Cox (HR) analysis. However, they reported a higher percentage of cases with an AR/ER ratio ≥ 2 than in our series (11.4% vs 6% respectively), which is probably related to differences in case selection since we excluded tumours with HER2 positivity.

The molecular analysis with Prosigna-PAM50 confirmed that most cases with AR/ER ≥ 2 had a high-to-intermediate ROR. In addition, Prosigna-PAM50 assay assigned 47.4% of our IHC luminal cases to the non-luminal intrinsic molecular subtypes. These results could suggest that tumours with a high AR/ER ratio could be resistant to hormone therapy. In fact, *in vitro* studies have demonstrated that hormone therapy-resistant tumours express higher levels of AR and lower ER levels than hormone therapy-sensitive tumours (De Amicis *et al.* 2010, Fujii *et al.* 2014, Rechoum *et al.* 2014, Ciupek *et al.* 2015). To confirm these experimental data, Cochrane and coworkers demonstrated that AR/ER ≥ 2 was associated with an increased risk of tamoxifen therapy failure in

BC patients (Cochrane *et al.* 2014). Taken together, these data may suggest that BCs with an AR/ER ≥ 2 could represent tumours that are changing or evolving from ER dependence (luminal subtype) to AR dependence, with the progressive loss of ER expression (non-luminal subtype).

Our study has some limitations due to its retrospective design. We included in the analysis patients with different treatment (hormone therapy and chemotherapy), and we do not have validation setting of patients to confirm our data. To address these limitations and validate our data, future studies need to include larger cohort of patients, who possibly underwent the same therapeutic approach.

In conclusion, our results suggest that tumours with AR/ER ≥ 2 should be carefully evaluated and reinforce the idea of targeting AR for BC treatment.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-17-0417>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Study conception and design: N R, M R L, L A, A S and I C.; Acquisition of data: N R, J M, M P M, L B, P C, S O A, I C.; Analysis and interpretation of data: N R, L A, S O A, M P M, L B, P C, A S; Drafting of manuscript: N R, M R L, J M and I C.; Critical revision: N R, M R L, L A, S O A, J M, M P M, L B, P C, A S and I C.; Final approval of the version to be submitted: N R, M R L, L A, S O A, J M, M P M, L B, P C, A S and I C.

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