Maria Ryssdal Kraby

Androgen Receptor Expression in Breast Cancer Subtypes

Student thesis in Medicine

Trondheim, June 2016

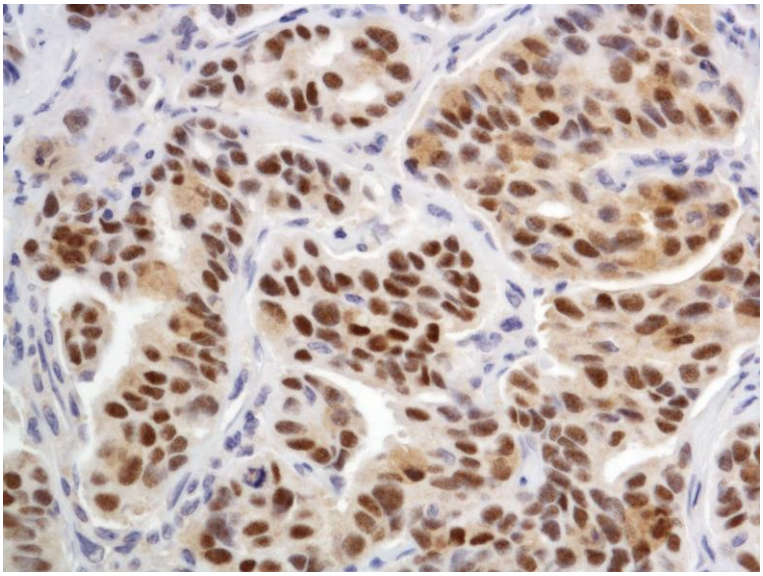

Photo: Case number 4046 x400, Maria Ryssdal Kraby, NTNU

Supervisor:	Professor Anna M. Bofin, Department of Laboratory Medicine, Children's and Women's Health, NTNU
Co-supervisor:	Doctoral research fellow Marit Valla MD, Department of Public Health and General Practice, NTNU

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breast cancer SUBTYPES

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Abstract

Background

The androgen receptor (AR) is frequently expressed in breast cancer, and associated with good prognosis in ER-positive breast cancers. Due to its high prevalence, AR is also a possible target for therapeutic management in breast cancer. More studies are needed to assess AR expression across molecular subtypes of breast cancer.

Aims

The present study aimed to investigate associations between AR expression and breast cancer survival in two well-described cohorts of Norwegian breast cancer patients, and to study AR expression in relation to molecular subtypes and clinicopathological features of breast cancer.

Methods

Immunohistochemistry for AR was carried out on 1340 breast cancers previously reclassified into molecular subtypes. Chi-square tests were used to investigate associations between AR expression and clinicopathological features, while cumulative incidence of breast cancer death and Cox regression analyses were used for survival analyses.

Results

A total of 78.0 % of cases were AR-positive at 10 % cut-off. 45.1 % of oestrogen receptor (ER) negative and 84.9 % of ER-positive tumours were AR positive. AR expression was associated with age, tumour size, stage, histological type, histopathological grade, ER, progesterone receptor, basal biomarkers and molecular subtype. The highest proportion of AR positivity was found in Luminal B tumours, and the lowest in Basal Phenotype tumours. At 10 % cut-off, AR was an independent prognostic marker in breast cancer (HR 0.69 (95 % CI 0.54-0.88)). Stratified for ER status, the prognostic value of AR was limited to ER-positive tumours (HR 0.67 (95 % CI 0.49-0. 91) versus 0.65 (95 % CI 0.41-1.03) in ER-negative tumours). Within the molecular subtypes, AR only showed prognostic value in the HER2-negative Luminal subtypes. Considering grade, AR expression was associated with improved survival in Grade 2 and Grade 3.

Conclusions

In the present study, AR expression in more than 10 % of tumour cells was an independent prognostic factor in breast cancer. Assessment of AR expression in breast cancer could provide additional prognostic information in ER-positive breast cancers. AR is the only steroid receptor expressed in a proportion of triple-negative breast cancers, and its value as a therapeutic target in these tumours should be further studied.

Introduction

The androgen receptor (AR) is a nuclear steroid hormone receptor frequently expressed in both primary and metastatic breast cancer (1-4). AR expression has been associated with favourable clinicopathological features (5-8) and better survival in breast cancer patients (3, 5, 6). Several studies have found that the favourable prognosis associated with AR expression is restricted to women with oestrogen receptor (ER) positive tumours (5, 6, 9). In triple negative (TN) tumours (tumours that are negative for ER, progesterone receptor (PR) and Human Epidermal Growth factor receptor 2 (HER2)), AR positivity has been associated with better prognosis in a number of studies, (10, 11) while others have found no prognostic impact (5), and one paper found AR expression to be associated with poor prognosis (6).

Today, treatment guidelines for classification of breast carcinomas only include the assessment of four biomarkers; ER, PR, HER2 and the proliferation marker, Ki67 (12). Determination of AR status in breast cancer could provide additional prognostic information (3, 5, 6). Due to its high prevalence and the fact that it is the only sex steroid receptor expressed in some breast cancers (1, 2, 5, 6), it has been suggested that AR may serve as a potential target for therapeutic management in breast cancer patients (1, 5, 11).

Gene expression analyses have shown that breast carcinomas can be separated into molecular subtypes that differ in their biology and prognosis (13, 14). Using immunohistochemistry (IHC) and in situ hybridization (ISH) as surrogates for gene expression analyses, it is possible to reclassify archival tumour tissue into molecular subtypes (15, 16).

Only a few studies have considered AR expression across breast cancer subtypes, and the results of these studies vary. In general, the highest prevalence of AR expression is found among Luminal tumours (positive for ER or PR) and the lowest in the Basal phenotype (BP) (TN and positive for basal markers) (3, 7, 8). While one study found AR to be associated with improved survival in all subtypes except the HER2+ type (ER/PR negative and HER2 positive)(3), another study found AR to be associated with prognosis only in the Luminal B(HER2+) subtype (8). A study of TN tumours found AR expression to be associated with improved survival in the 5-negative phenotype (5NP) (TN and negative for basal markers), but not in the BP(10).

The aims of this study were to investigate associations between AR expression and breast cancer survival in two well-described cohorts of Norwegian breast cancer patients, and to study AR expression in relation to clinicopathological features and molecular subtypes of breast cancer.

Materials and methods

Study population

The study population comprised a total of 1340 cases of primary breast cancer from two cohorts of women in Nord-Trøndelag County, Norway. Information on cancer diagnosis was provided by the Cancer Registry of Norway. Pathology reports and formalin-fixed, paraffin embedded (FFPE) tissue from primary tumours were retrieved from the Department of Pathology and Medical Genetics, St Olav's Hospital, Trondheim University Hospital.

Cohort 1 comprised women who were invited to participate in a survey for early breast cancer diagnosis from 1956 to 1959 (17, 18). During follow-up from 1961 to 2008, 1379 women developed breast cancer. Of these, 909 tumours were reclassified into molecular subtypes (16).

Cohort 2 comprised women who participated in the HUNT2 study in 1995-1997 (19). From date of participation until December 31st 2009, 728 women developed breast cancer. A proportion of these women were already included in Cohort 1. Of the remaining, 514 tumours were reclassified into molecular subtypes (Valla et al, manuscript submitted).

Information on date and cause of death was obtained from the Cancer Registry of Norway after linkage with the Causes of Death Registry. Women from both cohorts were followed from date of diagnosis until death from breast cancer, death from other causes or the end of the follow-up period, whichever came first. End of follow-up was December 31st 2010 for Cohort 1 and December 31st 2013 for Cohort 2. In the present study, data from the two cohorts were merged. Of the 1423 subtyped cases, 1340 were stained for AR. Forty-three cases were subsequently excluded due to lack of tumour tissue or poor IHC quality, resulting in a total of 1297 cases in the study.

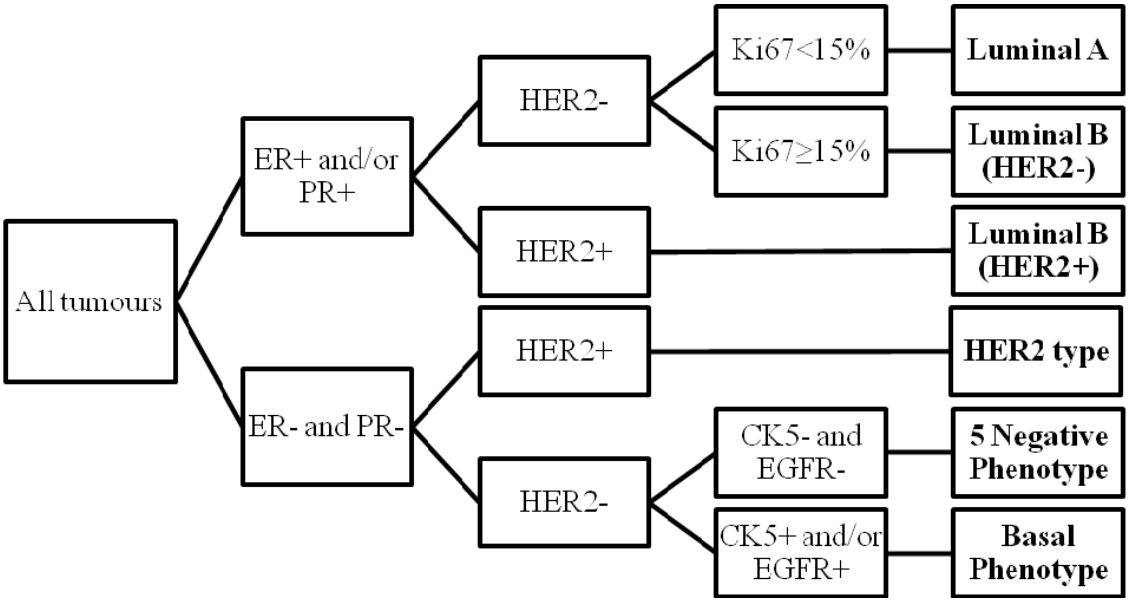

Figure 1. Classification algorithm for molecular subtyping (Engstrøm et al (16)).Cytokeratin 5 (CK5), oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2).

Specimen characteristics

All tumours had previously been classified by two pathologists into histopathological type (20) and Grade (21). Tissue microarrays (TMA) were constructed using Tissue Arrayer MiniCore® with TMA Designer2 software (Alphelys). Three tissue cores of 1mm in diameter were extracted from the periphery of each tumour and inserted into TMA recipient blocks. IHC for ER, PR, HER2, Ki67, cytokeratin 5 (CK5),and epidermal growth factor receptor 1 (EGFR) was done as previously described (16). HES- and IHC-stained slides from the TMA blocks were scanned at 5x and 20x magnification, using the tissue scanner Ariol™ SL-50 3.3 Scan system and analysis station (Genetix). HER2 gene amplification status was assessed using ISH. Tumours were reclassified into six molecular subtypes (Luminal A, Luminal B(HER2+), Luminal B(HER2-), HER2 type, BP and 5NP) as outlined in Figure 1 (16).

Androgen receptor immunohistochemistry

For the present study, 4µm FFPE tissue sections from TMAs were retrieved from storage (-20^{o}C) and put into a heating cabinet at 60^{o}C for 1 hour. The sections were deparaffinised using TissueClear and rehydrated in ethanol and water. For pre-treatment, Heat Induced Epitope Retrieval (HIER) was done in a Pre-Treatment Link (DAKO). Sections were immersed in EnVision FLEX Target Retrieval Solution High pH (DAKO), and diluted 1:50 with dH_20. Next, slides were heated at 97^{o}C for 20 minutes, and then cooled to 65^{o}C. IHC was carried out at room temperature, using Dako Autostainer Plus (DAKO Denmark A/S, Produksjonsvej 42 DK-2600 Glostrup, Denmark). First, enzyme blocking was done with Dako REAL Peroxidase Blocking Solution S2023 (DAKO) for 10 minutes. Next, Monoclonal mouse anti-

human androgen receptor (DAKO), was diluted 1:50 with Dako REAL Antibody Diluent S2022 and incubated for 40 minutes. HRP Rabbit/Mouse EnVision Polymer was incubated for 30 minutes, before sections were rinsed twice. Between each step of the immunostaining process, sections were rinsed with Dako Wash Buffer (DAKO) S3006 1:10. For visualisation, sections were incubated for 10 minutes in DAB+ Chromogen (from Dako REAL EnVision DetectionSystem K5007). Sections were then rinsed twice in dH2O, removed from the autostainer and contrast-stained with Haematoxylin for 30 seconds. Sections were dehydrated and embedded in TissueClear before coverslipping. Figure 2 shows IHC staining for AR in two cases.

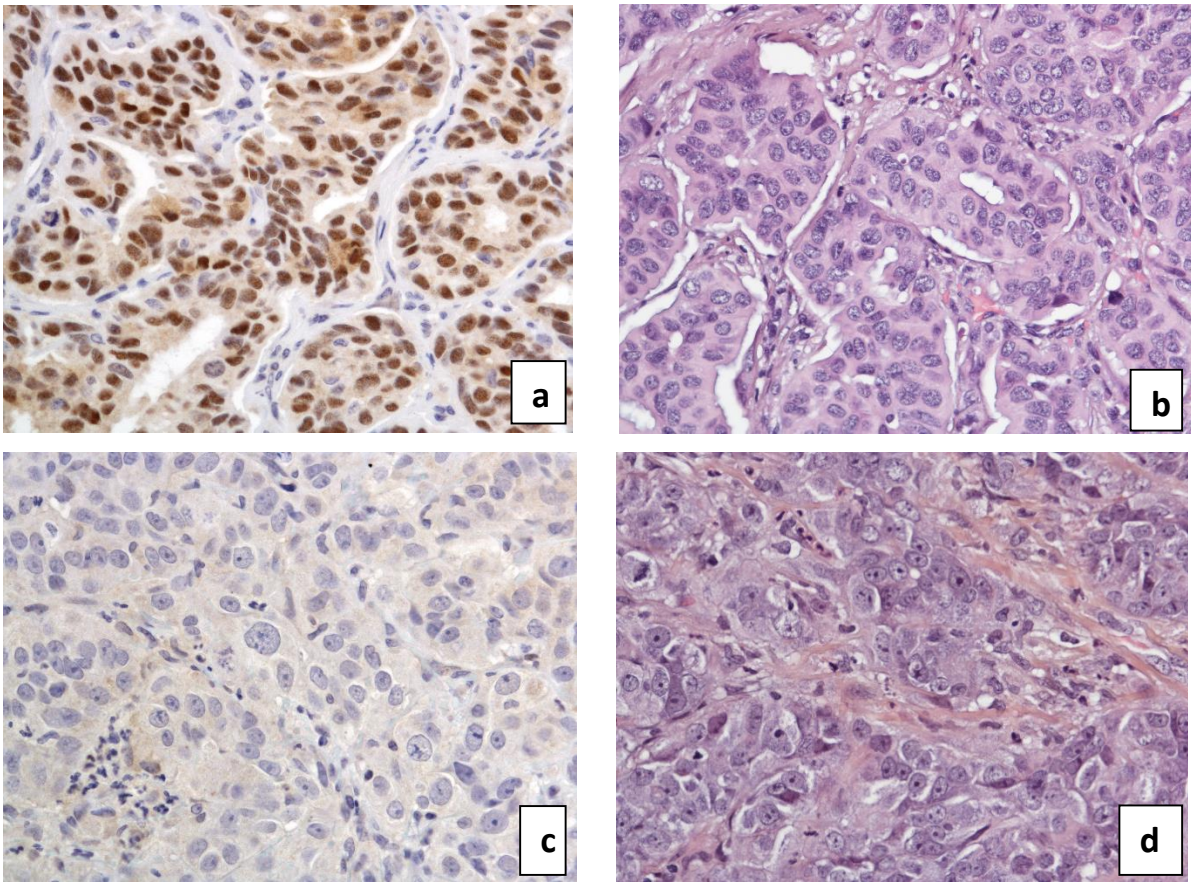

Figure 2. Immunohistochemical staining for androgen receptor (AR) and HES in two cases.
a and b: Case number 4046. AR-positive ≥10 % (a), HES (b)(400x). c and d: Case number 4023. AR-negative (c) and HES (d) (400x).

Scoring and reporting

The AR-stained TMA slides were scanned and then scored by two researchers independently, one of whom was a pathologist. Both observers were blinded for clinical information and staining results for other biomarkers. Nuclear staining was scored from 0 to 2, irrespective of staining intensity (0 = no staining; 1 = 1-9 % positively stained nuclei; 2 = ≥10 % positively stained nuclei). When disagreement between observers occurred, cases were discussed and consensus was reached.

Statistical analyses

Chi-square tests were used to investigate the association between AR expression and clinicopathological features. Breast cancer prognosis in patients with positive and negative staining for AR was compared by calculating the cumulative incidence of death from breast cancer, treating death from other causes as a competing event. Cumulative incidence of death from breast cancer can be interpreted as the risk of dying from breast cancer before dying from other causes (22). Gray's test was used to compare the equality of the cumulative incidence curves. Multiple Cox regression analyses were performed to estimate risk of death from breast cancer according to expression of AR, with censoring at death from other causes. Hazard ratios (HR) with 95 % confidence intervals (CIs) were calculated with adjustment for age at diagnosis, stage, grade and subtype (if applicable). Age at diagnosis was adjusted for as a categorical variable: <45, 5-year intervals from 45 to 80, and 80 years of age or older. Survival analyses were performed for all breast cancer cases combined, and separately for ER status, grade and each molecular subtype. All statistical analyses were performed using STATA 13.1 (Stata Corp., College Station, TX, USA).

Ethics

The study was granted approval including dispensation from the general requirement of patient consent by the Regional Committee for Medical and Health Sciences Research Ethics (REK, Midt-Norge, ref. nr: 836/2009).

Results

Characteristics of the study population are shown in Table 1. The median age at diagnosis was 68 years (range 33-96). Of all cases, 14.3 % were Grade 1, 52.0 % were Grade 2, and 33.7 % were Grade 3. Luminal A tumours comprised 49.7 % of all tumours, while 7.8 % were Luminal B(HER2+), 26.6 % were Luminal B(HER2-), 5.9 % were HER2, 3.3 % were 5NP and 6.7 % were BP. A total of 1091 (84.1 %) cases were AR-positive at 1 % cut-off. When the cut-off was raised to 10 %, 80 cases changed category, resulting in 1011 (78.0 %) AR-positive cases. During follow-up, 29.5 % of patients died from breast cancer. Of the AR-negative cases, 39 % died from breast cancer, irrespective of cut-off. Among AR-positive cases, 27.7 % (1 % as cut-off) and 26.9 % (10 % as cut-off) died from breast cancer during follow-up.

Histopathological grade

AR expression was significantly associated with tumour grade, with the greatest proportion of AR positive cases in Grade 2 and the lowest in Grade 3 tumours, regardless of cut-off level. When the cut-off was set at 10 %, 78 % of Grade 1 tumours, 84 % of Grade 2 and 68.7 % of Grade 3 were AR-positive.

Biomarkers

For both cut-off values, AR expression was significantly associated with expression of ER, PR and basal biomarkers (CK5 and/or EGFR), but not with Ki67 or HER2 status. AR was expressed in a higher proportion of ER-positive tumours compared to ER-negative tumours (45.1 % of ER-negative tumours and 84.9 % of ER-positive tumours at 10 % cut-off). AR expression was inversely associated with basal marker expression. The proportion of AR positivity was lower in TN tumours, compared to non-TN tumours. Using 10 % as cut-off, 33.1 % of TN tumours were AR-positive, and 48.5 % were AR-positive at 1 % cut-off.

Molecular subtype

Molecular subtypes were significantly associated with AR expression. For both cut-offs, the highest proportion of AR positivity was found in the Luminal B(HER2+) and Luminal B(HER2-) subtypes, and the lowest in the BP. At 10 % cut-off, 82.3 % of Luminal A tumours, 87.1 % of Luminal B(HER2+), 88.1 % of Luminal B(HER2-), 59.7 % of the HER2 subtype, 48.8 % of the 5NP and 25.3 % of the BP expressed AR.

Table 1 Descriptive characteristics and chi-square tests for the 1297 breast cancer cases.

	Total	<1 %	≥1 %	Chi^2	<10 %	≥10 %	Chi^2
Number of cases (%)	1297	206(15.9)	1091(84.1)		286(22.1)	1011(78.0)	
Median age at diagnosis, years (IQR)							
	68(58-76)	72(64-80)	67(57-76)		71(62-79)	67(57-75)	
Median follow-up time, years (IQR)							
	7.6(4.0-12.5)	5.4(2.3-10.1)	8(4.4-12.9)		5.6(2.6-10.2)	8.1(4.4-13.0)	
Median time to breast cancer death, years (IQR)							
	3.8(1.8-7.5)	2.8(1.4-6.1)	4.1(2.0-7.8)		2.8(1.5-6.4)	4.3(2.0-7.7)	
Death from breast cancer, n (%)							
No	914	125(13.7)	789(86.3)		175(19.2)	739(80.9)	
Yes	383	81(21.2)	302(78.9)		111(29.0)	272(71.0)	
Age at diagnosis, years (%)				<0.001			0.001
<50	141	9 (6.4)	132(93.6)		18 (12.8)	123(87.2)	
50-59	218	26 (11.9)	192(88.1)		41 (18.8)	177(81.2)	
60-69	340	51 (15.0)	289(85.0)		68 (20.0)	272 (80.0)	
70-79	361	68(18.8)	293(81.2)		90 (24.9)	271 (75.1)	
≥80	237	52 (21.9)	185(78.1)		69 (29.1)	168 (70.9)	
Tumour diameter (mm), n (%)				0.001			<0.001
≤20	684	84 (12.3)	600(87.7)		113(16.5)	571(83.5)	
>20 ≤50	249	48 (19.3)	201(80.7)		75 (30.1)	174(69.9)	
>50	20	6 (30.0)	14 (70.0)		9 (45.0)	11 (55.0)	
Uncertain, but ≥20	141	32 (22.7)	109(77.3)		40 (28.4)	101(71.6)	
Unknown	203	36	167		49	154	
Lymph node status, n (%)				0.081			0.051
Negative	620	90 (14.5)	530(85.5)		128(20.7)	492(79.4)	
Positive	443	82 (18.5)	361(81.5)		114(25.7)	329(74.3)	
Unknown	234	34	200		44	190	
Stage, n (%)							
I	652	83 (12.7)	569(87.3)	0.009	117(17.9)	535(82.1)	0.003
II	522	97 (18.6)	425(81.4)		137(26.3)	385(73.8)	
III	67	16 (23.9)	51 (76.1)		19 (28.4)	48 (71.6)	
IV	50	10 (20.0)	40 (80.0)		13 (26.0)	37 (74.0)	
Missing	6	0	6		0	6	
Type, n (%)				<0.001			<0.001
Ductal	953	136(14.3)	817(85.7)		188(19.7)	765(80.3)	
Lobular	160	14 (8.8)	146(91.3)		22(13.8)	138(86.3)	
Other types	184	56(30.4)	128(69.6)		76(41.3)	108(58.7)	
Grade, n (%)				<0.001			<0.001
1	186	31 (16.7)	155(83.3)		41 (22.0)	145(78.0)	
2	674	72 (10.7)	602(89.3)		108(16.0)	566(84.0)	
3	437	103(23.6)	334(76.4)		137(31.4)	300(68.7)	
ER, n (%)				<0.001			<0.001
Negative	224	95 (42.4)	129(57.6)		123(54.9)	101(45.1)	
Positive	1071	110(10.3)	961(89.7)		162(15.1)	909(84.9)	
Missing	2	1	1		1	1	
PR, n (%)				<0.001			<0.001
Negative	487	144(29.6)	343(70.4)		192(39.4)	295(60.6)	
Positive	809	62(7.7)	747(92.3)		93(11.5)	716(88.5)	
Missing	1	0	1		1	0	
HER2, n (%)				0.41			0.355
Negative	1119	174(15.6)	945(84.5)		242(21.6)	877(78.4)	
Positive	178	32 (18.0)	146(82.0)		44 (24.7)	134(75.3)	
Ki67, n (%)				0.156			0.094

<15 %	732	107(14.6)	625(85.4)		149(20.4)	583(79.6)	
≥15 %	565	99 (17.5)	466(82.5)		137(24.3)	428(75.8)	
CK5, n (%)				<0.001			<0.001
Negative	983	135(13.7)	848(86.3)		193(19.6)	790(80.4)	
Positive	313	70 (22.4)	243(77.6)		92 (29.4)	221(70.6)	
Missing	1	1	0		1	0	
EGFR, n (%)				<0.001			<0.001
Negative	1221	175(14.3)	1046(85.7)		245(20.1)	976(79.9)	
Positive	75	31 (41.3)	44 (58.7)		41 (54.7)	34 (45.3)	
Missing	1	0	1		0	1	
Subtype, n (%)				< 0.001			< 0.001
Luminal A	644	78 (12.1)	566 (87.9)		114 (17.7)	530 (82.3)	
Luminal B (HER2+)	101	8 (7.9)	93 (92.1)		13 (12.9)	88 (87.1)	
Luminal B (HER2-)	345	29 (8.4)	316 (91.6)		41 (11.9)	304(88.1)	
HER2+	77	24 (31.2)	53 (68.8)		31 (40.3)	46 (59.7)	
5NP	43	19 (44.2)	24 (55.8)		22 (51.2)	21 (48.8)	
BP	87	48 (55.2)	39 (44.8)		65 (74.7)	22 (25.3)	

When cut-off was raised from 1 % to 10 %, 17 BP tumours, which represent 19.5 % of all BP tumours, were changed category from AR-positive to AR-negative. Although BP tumours only represent 6.7 % of all cases, they accounted for 20 % of the tumours that changed category when the cut-off level was raised.

Histopathological type

Regardless of cut-off level, AR expression was significantly associated with histopathological type, with the highest proportion of AR positivity in lobular breast cancers, compared to ductal cancers and other types. At 10 % cut-off, 80.3 % of ductal carcinomas, 86.3 % of lobular and 58.7 % of other types were AR-positive.

Age, size and stage

AR expression was inversely associated with age at diagnosis, regardless of cut-off level. At 10 % cut-off, 87.2 % of those who were diagnosed before the age of 50 years were AR-positive, compared to 70.9 % of those diagnosed at 80 years of age or older.

AR expression was significantly associated with tumour diameter and stage, but only weakly with lymph node status. The frequency of AR positivity decreased with increasing tumour size. At 10 % cut-off, 83.5 % of tumours < 20 mm were AR-positive, compared to 69.9% of tumours > 20mm ≤50mm, and 55 % of tumours > 50mm. The same trend was seen at 1 % cut-off. For stage, AR positivity was most frequent in stage I (82.1 % AR-positive), and lowest in stage III (71.6 % AR-positive) at 10 % cut-off. However, 74 % of stage IV tumours were AR-positive.

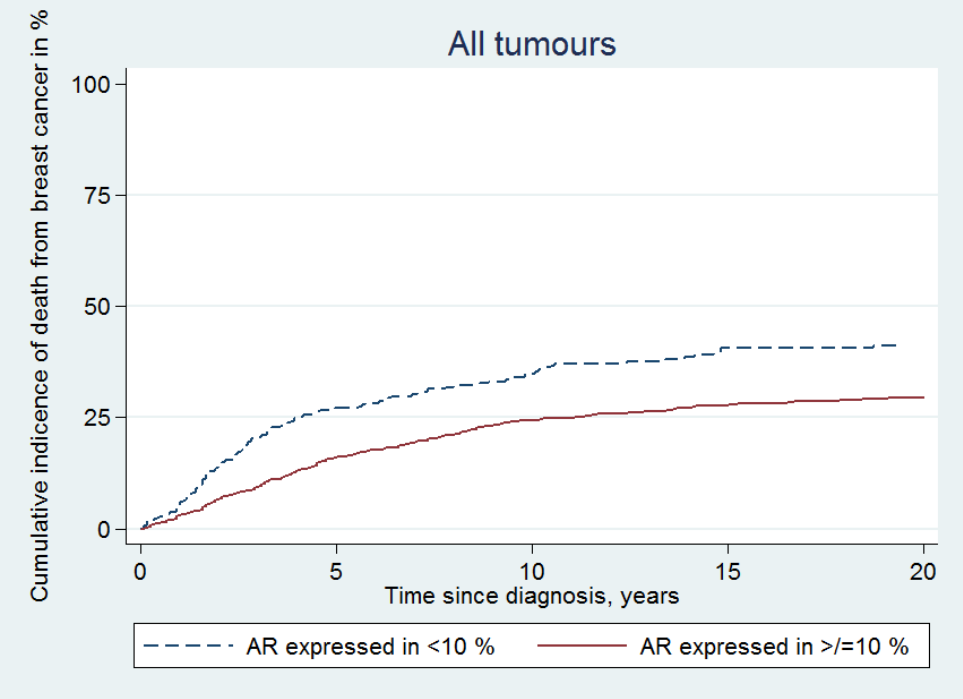

Figure 3. Cumulative incidence curve for breast cancer death according to AR positivity in all patients. Cut-off at 10 %. Gray's test: p<0.001.

Table 2 Cumulative risk of death. Cut-off at 10 %.

		5 years after diagnosis		15 years after diagnosis	
	Total (n)	Breast cancer deaths (n)	Cumulative risk of death (%), (95 % CI)	Breast cancer deaths (n)	Cumulative risk of death (%), (95 % CI)
All cases					
AR-negative	286	77	27.0 (22.3-32.6)	108	40.6 (34.8-46.9)
AR-positive	1011	161	16.1 (13.9-18.5)	225	27.7 (24.9-30.8)
ER-positive					
AR-negative	162	36	22.4 (16.7-29.6)	55	37.2 (29.8-45.7)
AR-positive	909	124	13.8 (11.7-16.2)	213	26.2 (23.2-29.4)
ER-negative					
AR-negative	123	41	33.5 (25.9-42.6)	52	44.5 (35.9-54.2)
AR-positive	101	37	36.6 (28.1-46.8)	42	41.9 (32.9-52.2)

Table 3 Risk of death from breast cancer according to AR status in all tumours. Cut-off at 10 %.

	Unadjusted HR (95 % CI)	p-value	Adjusted[1] HR (95 % CI)	p-value
All cases				
AR-negative	1		1	
AR-positive	0.56 (0.45 to 0.70)	<0.001	0.69 (0.54-0.88)	0.003

[1]Adjusted for age, stage, subtype, grade

Survival

Figure 3 shows cumulative incidence for breast cancer death in all patients. AR-positive tumours were associated with a better prognosis compared to AR-negative in unadjusted analyses at both cut-off levels. Using the 10 % cut-off, cumulative risk of death for AR-negative cases was 27.0 % (95 % CI 22.3-32.6 %) five years after diagnosis, and 40.6 % (95 % CI 34.8-46.9 %) 15 years after diagnosis (Table 2). The corresponding results for cumulative risk of death for AR-positive cases were 16.1 % (95 % CI 13.9-18.5 %) and 27.7 % (95 % CI 24.9-30.8 %), respectively. After adjustment for age, grade, molecular subtype and stage, AR expression was significantly associated with improved survival at the 10 % cut-off, with HR of 0.69 (95 % CI 0.54-0.88) compared to AR-negative cases (Table 3). The same trend was apparent at 1 % cut-off, but did not reach statistical significance.

When stratified for ER status, AR expression was significantly associated with better survival in ER-positive tumours in unadjusted analyses for both cut-off levels. At 10 % cut-off, the cumulative risk of death 15 years after diagnosis was 26.2 % (95 % CI 23.2-29.4 %) for AR-positive cases and 37.2 % (95 % CI 29.8-45.7 %) for AR-negative cases. Figure 5 shows cumulative incidence for breast cancer death in ER-positive and ER-negative patients. When adjusted for age, stage, subtype and grade, HR for risk of death was 0.67 (95 % CI 0.49-0. 91) for AR-positive cases compared to AR-negative (Table 4). Although a similar trend was seen at 1 % cut-off, it did not reach statistical significance. There was no association between AR expression and survival in ER-negative tumours in unadjusted analyses. However, a trend towards improved survival in AR-positive cases was observed in the adjusted analyses (HR 0.65 (95 % CI 0.41-1.03)).

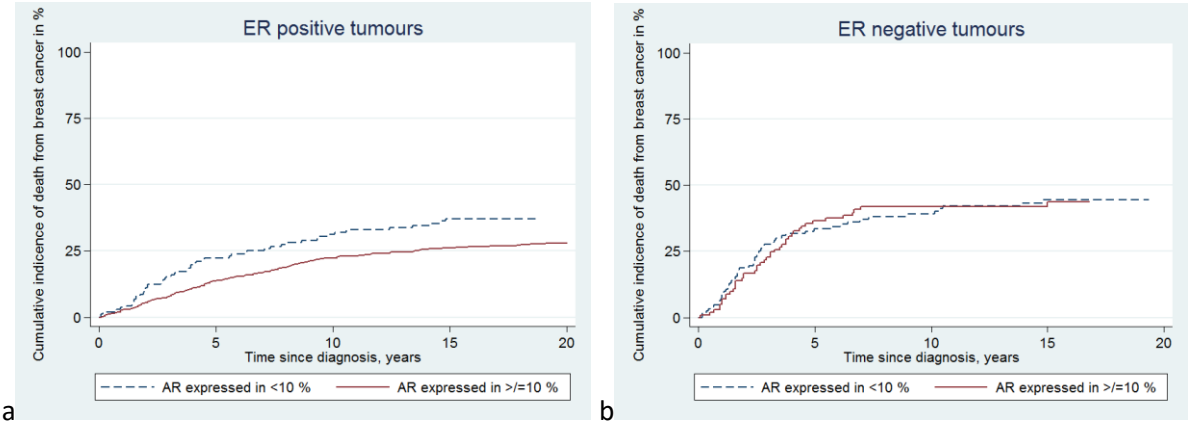

Figure 4. Cumulative incidence curve for breast cancer death according to AR positivity (cut-off at 10 %) and ER positivity (cut-off at 1 %). a: ER-positive tumours, Gray's test: p=0.005 b: ER-negative tumours, Gray's test: p=0.94.

Table 4 Risk of death from breast cancer according to AR status by ER status. Cut-off at 10 %.

	Unadjusted HR (95 % CI)	p-value	Adjusted[1] HR (95 % CI)	p-value
ER-positive				
AR-negative	1		1	
AR-positive	0.56 (0.42-0.75)	<0.001	0.67 (0.49-0.91)	0.01
ER-negative				
AR-negative	1		1	
AR-positive	0.91 (0.61 to 1.37)	0.655	0.65 (0.41-1.03)	0.068

[1]Adjusted for age, stage, subtype, grade

Table 5 Risk of death from breast cancer according to AR status by molecular subtype. Cut-off at 10 %.

	Unadjusted HR (95 % CI)	p-value	Adjusted[1] HR (95 % CI)	p-value
Luminal A				
AR-negative	1		1	
AR-positive	0.49 (0.33 to 0.72)	<0.001	0.62 (0.41-0.92)	0.018
Luminal B(HER2+)				
AR-negative	1		1	
AR-positive	0.76 (0.34 to 1.72)	0.515	0.73 (0.26-2.01)	0.539
Luminal B(HER2-)				
AR-negative	1		1	
AR-positive	0.52 (0.31 to 0.87)	0.013	0.64 (0.37-1.11)	0.113
HER2				
AR-negative	1		1	
AR-positive	0.72 (0.38 to 1.36)	0.310	0.53 (0.25-1.12)	0.094
5NP				
AR-negative	1		1	
AR-positive	0.88 (0.36 to 2.12)	0.769	0.45 (0.11-1.78)	0.257
BP				
AR-negative	1		1	
AR-positive	1.22 (0.57 to 2.64)	0.610	1.15 (0.44-3.03)	0.772

[1]Adjusted for age, stage, grade

Table 6 Risk of death from breast cancer according to AR status by grade. 10 % cut-off.

	Unadjusted HR (95 % CI)	p-value	Adjusted[1] HR(95 % CI)	p-value
Grade 1				
AR-negative	1		1	
AR-positive	0.48 (0.22 to 1.05)	0.066	0.60 (0.25-1.46)	0.259
Grade 2				
AR-negative	1		1	
AR-positive	0.58 (0.41 to 0.83)	0.003	0.80 (0.55-1.16)	0.239
Grade 3				
AR-negative	1		1	
AR-positive	0.65 (0.48 to 0.89)	0.007	0.57 (0.39-0.84)	0.004

[1]Adjusted for age, stage, molecular subtype

When investigating survival in each molecular subtype separately, AR expression was associated with favourable prognosis in both HER2-negative Luminal subtypes in unadjusted analyses (Table 5). For Luminal B(HER2-), this was only significant at 10 % cut-off. In the Luminal A subtype, AR positivity at 10 % cut-off was an independent prognostic factor for breast cancer death (HR 0.62 (95 % CI 0.41-0.92)). A similar trend was observed at 1 % cut-off. AR expression was not associated with survival in Luminal B(HER2+), HER2 type, 5NP or BP. Analyses of the TN group as a whole did not reveal any association between AR expression and prognosis.

When stratified for Grade, AR was associated with improved survival in Grades 2 and 3 in unadjusted analyses at 10 % cut-off. After adjusting for age, stage and subtype, AR was an independent prognostic factor in Grade 3 tumours only (Table 6).

Discussion

In this study, AR expression was an independent prognostic marker in breast cancer. This finding was restricted to ER-positive tumours, in accordance with previous studies (3, 5, 6, 9). Furthermore, these results support the hypothesis that AR has different roles in breast cancer (8), such as an inhibitory effect in ER-positive tumours (9) and a growth stimulating effect in ER-negative tumours (23).

In agreement with others (5-7), the proportion of AR positive cases was comparable to that of ER. However, a considerable proportion of AR-positive cases were found among non-luminal tumours.

AR and subtypes

In the present study, AR expression was highest in Luminal B tumours. Only a few studies have assessed AR expression in molecular subtypes of breast cancer (3, 7, 8). In one study, AR expression was found to be most frequent in Luminal B (8), while two others found it to be most frequently expressed in Luminal A tumours (3, 7). In accordance with the present study, two previous studies found the lowest frequency of AR positivity to be in BP, followed by 5NP and then HER2(7, 8). In a third study, AR positivity was more frequent in 5NP than HER2 tumours, however there were only 25 5NP cases (3). Yu et al found AR to be associated with prognosis in the Luminal B(HER2+) subtype (3), and Tsang et al found that AR was associated with prognosis in all subtypes except the HER2 subtype (8). In the present study, AR was only associated with prognosis in Luminal A and Luminal B(HER2-) tumours.

AR and TN tumours

Some studies have found that AR positivity is associated with better prognosis in TN breast cancer (10, 11, 24). In the present study, we found no association between AR expression and prognosis in TN tumours, supporting the findings of Park et al (5). In one study, AR positivity was associated with poor prognosis (6). These differences could be explained by varying cut-off levels, differing cohort characteristics and laboratory procedures.

TN breast cancers have poor prognosis and are associated with aggressive clinicopathological features (25). These patients do not qualify for endocrine therapy or targeted treatment with trastuzumab. Hence, most TN tumours are only treated with adjuvant chemotherapy (12), and new treatment strategies are needed to improve prognosis for this patient group. AR expression has previously been reported in 12 % to 37 % of TN tumours (5, 6, 8, 10, 11, 24, 26). In the present study, 33.1 % of TN tumours were AR-positive at 10 % cut-off, compared to 48.5 % at 1 % cut-off. AR-positive TN tumours might benefit from antiandrogen receptor therapy. Antiandrogen treatment is already developed for the treatment of prostate cancer (27). Furthermore, a phase II clinical trial with an AR antagonist carried out in a series of patients (n=26) with AR-positive (cut-off 10 %), ER-

negative metastatic breast cancer, showed that anti-AR treatment was well tolerated, and stable disease for >6 months was achieved in 19 % of patients (26).

The 5NP has been shown to be a distinct subtype with different biology and prognosis, compared to other subtypes (15). It is among the breast cancer subtypes with the poorest prognosis (15), and had the second poorest prognosis in our first cohort, after the HER2 type (16). Yet, little is known about the characteristics of the 5NP, and the only adjuvant treatment strategy today is chemotherapy (12). Twenty-one of 43 (48.8 %) 5NP tumours were AR-positive in this study. This implies that a large proportion of patients with 5NP tumours might benefit from anti-AR treatment. Further studies to determine how AR is expressed in 5NP tumours, and whether these tumours could benefit from anti-AR treatment, are needed.

Previous reports state that AR expression is low in BP tumours, and inversely associated with basal markers (3, 7, 8). The findings in the present study support this, where the lowest prevalence of AR positivity was seen in BP tumours. Some have suggested that absence of basal-like IHC markers can predict for response to anti-AR therapy in TN breast cancers (26). Interestingly, 20 % of the BP tumours changed category from AR-positive to AR-negative when the cut-off level was raised from 1 % to 10 %. Based on this, one may speculate whether these "low AR-positive" BP tumours would benefit from anti-AR therapy or not.

AR and Grade

AR expression was not associated with survival in Grade 1 tumours. The strongest association between AR and prognosis was found in Grade 3 tumours. However, the highest proportion of AR positivity was seen in Grade 2 tumours, and the lowest in Grade 3. Other studies have found that AR expression is highest in Grade 1 tumuors and lowest in Grade 3 (2, 8). Grade 2 tumours comprise a heterogenous group with varying prognoses (16). It is possible that AR may be particularly interesting as a potential target for treatment in this group of patients.

AR and type

In the present study, AR expression was higher in lobular carcinomas compared to ductal carcinomas and other types. Similarly, Moinfar et al found a higher proportion of AR positivity in lobular carcinomas compared to ductal carcinomas, although their findings were based on a very low sample size of lobular carcinomas (n=14) (2). Collins et al also report a higher proportion of AR positivity in lobular carcinomas compared to ductal (96.3 % and 71.0 %, respectively), but did not disclose the sample size for lobular carcinomas (7). Compared to invasive ductal carcinomas, lobular carcinomas have a larger proportion of Grade 2 tumours. However, our research group has previously shown that the prognosis for Grade 2 lobular carcinomas is significantly poorer than Grade 2 ductal

carcinomas, and is more similar to that of IDC Grade 3 (28). Still, histopathological type is rarely taken into account when determining treatment strategy (29). The role of AR in lobular carcinomas, and whether patients with lobular carcinoma could benefit from anti-AR treatment, should be further investigated.

Cut-off

Today, there is no consensus with regard to cut-off for AR positivity, and AR positivity is defined differently by different authors (3, 5, 6, 10, 11). This must be taken into account when comparing the results of the various studies. In the present study, the 10 % cut-off showed independent prognostic value, whereas the 1 % cut-off did not.

Weaknesses and strengths

To the best of our knowledge, the present study is among the largest studies of AR expression across molecular subtypes of breast cancer. We have assessed AR expression in 1297 breast cancer tumours. These tumours were previously reclassified into molecular subtypes by IHC and ISH. Molecular subtyping and laboratory work for the present study were carried out at the same laboratory, using the same antibodies, and the same algorithm for subtyping in all tumours. All IHC markers were assessed by two researchers independently. Reliable information on breast cancer incidence and follow-up data were available from national registries.

In this study, we have used archival tumour tissue from 1961-2009. During this time period, preanalytical conditions may have varied. However, we have not identified any particular time periods with poorer IHC or ISH results compared to others. Dowset et al have shown that antigenicity is for the most part preserved in FFPE over decades (30).

Conclusions

In the present study, AR expression in more than 10 % of tumour cells was an independent prognostic factor in breast cancer. Assessment of AR expression in breast cancer could provide additional prognostic information in ER-positive breast cancers.

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