The Prognostic Value of Androgen Receptors in Breast Cancer Subtypes

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Abstract

Purpose Androgen receptor (AR) expression is frequent in breast cancer and has been associated with good prognosis in several studies.

The present study investigates AR-expression in relation to molecular subtypes, clinicopathological features and prognosis in 1297 primary tumours and 336 paired axillary lymph node metastases (LNM) from two cohorts of Norwegian patients.

Methods Immunohistochemistry for AR was performed on tumours previously reclassified into molecular subtypes using immunohistochemistry and *in situ* hybridisation. Associations between AR-expression and clinical features were studied using chi-square tests. Cumulative incidence of breast cancer death and Cox regression analyses were used to assess prognosis.

Results AR-positivity was found in 78.0% of all cases, 84.9% of luminal and 45.1% of non-luminal tumours. The highest proportion of AR-positivity was found in Luminal B tumours, and the lowest in the Basal phenotype. Discordance in AR-status between primary tumours and lymph node metastases was observed in 21.4% of cases. A switch from AR^- primary tumour to AR^+ lymph node metastasis was seen in 60/72 discrepant cases. AR-expression in primary tumours was an independent and favourable prognostic marker (HR 0.70, 95% CI 0.55-0.90), particularly in the Luminal A subtype, and in grade 3 tumours.

Conclusions AR is an independent predictor of good prognosis in BC, particularly in grade 3 and Luminal A tumours. Discordant AR-expression between primary tumour and LNM was observed in 21.4% of cases and most often there was a switch from AR^- primary tumour to AR^+ axillary LNM.

Keywords Breast cancer; Androgen receptor; Molecular subtypes; Lymph node metastasis; Prognosis

Introduction

The androgen receptor (AR) is frequently expressed in breast cancer (BC) [1-3] and has been associated with favourable prognosis in several studies [4], especially oestrogen receptor (ER) positive tumours [5, 6]. In BCs negative for ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), also called triple negative BC (TNBC), some report better prognosis in AR^+ patients [7-10], while others do not [5, 6, 11]. Current guidelines include assessment of ER, PR, HER2 and the proliferation marker Ki67 [12]. Due to its high prevalence, and because it is the only sex steroid receptor expressed in some BCs, AR may provide additional prognostic information [5, 6, 13] and could be a target for therapy [1, 6, 8].

Gene expression analyses have shown that BCs can be separated into molecular subtypes with differing biology and prognosis [14, 15]. Immunohistochemistry (IHC) and *in situ* hybridisation (ISH) can be used as surrogates for gene expression analyses to reclassify BCs into molecular subtypes [16-19]. While discrepancies between the intrinsic subtypes and surrogate subtypes have been demonstrated [20], subtyping using IHC and ISH has been shown to correlate well with prognosis [16, 21]. AR-expression has been shown to be most common in luminal tumours (ER^+ and/or PR^+) and rare in the Basal phenotype (BP), defined as basal marker positive TNBC [2, 13, 22, 23]. However, few studies have investigated AR-expression across molecular subtypes of BC [2, 4, 13, 22, 23], and even fewer of AR-expression in lymph node metastases (LNM) [3, 24, 25].

The aims of this study were to investigate AR-expression in relation to clinicopathological features, molecular subtypes and BC prognosis in primary BC tumours and corresponding axillary LNM in two cohorts of Norwegian BC patients with long-term follow-up.

Materials and methods

Study population

The study population comprised 1297 cases of primary, invasive BCs from two prospective cohorts of women in Nord-Trøndelag County, Norway (Figure 1).

Cohort 1 comprised women invited to participate in a survey for early BC detection in 1956-1959 [26, 27]. Of the 1379 BCs diagnosed 1961-2008, 909 were reclassified into molecular subtypes [17]. Cohort 2 comprised women who participated in the Nord-Trøndelag Health Study (1995-1997) [28]. From participation until 2009, 728 developed BC. Some were included in Cohort 1. In total, 514 BCs were reclassified into molecular subtypes [29]. According to treatment guidelines at time of diagnosis, most patients in Cohort 1 were treated with surgery alone, whereas patients in Cohort 2 had access to systemic adjuvant therapy. Individual treatment data was unavailable.

Cases were identified through data linkage with the Cancer Registry of Norway. Pathology reports and tissue blocks were retrieved from the Department of Pathology, St Olav's Hospital, Norway. Date and cause of death were obtained from the Norwegian Cause of Death Registry. Follow-up was from date of diagnosis until death from BC, death from other causes or end of follow-up (December 31st 2010 (cohort 1) and December 31st 2013 (cohort 2)), whichever came first. Only cases in tissue microarrays (TMA) (n=1340) were included in the present study. Forty-three cases were excluded due to lack of tumour tissue or poor IHC quality, leaving 1297

cases for analysis. Axillary LNM diagnosed at or within six months of primary diagnosis, were available for 336 patients.

Specimen characteristics

As previously described [17, 29], tumours were first classified into histopathological type [30] and grade [31]. TMAs comprised three 1mm cores from the periphery of the primary tumours and LNM. Tumours were reclassified into molecular subtypes (Figure 2) using IHC and ISH as surrogates for gene expression analysis [17, 29].

Androgen receptor immunohistochemistry

In the present study, a monoclonal anti-human androgen receptor antibody (M3562, clone AR441, (DAKO)) was used. The antibody is registered and validated in the Human Protein Atlas [32-34] and has been used in several previous BC studies [3, 4, 35, 36]. Tissue sections (4µm) from TMAs and 57 of the original tissue blocks, were retrieved from storage (-20°C), heated at 60°C for 1 hour, deparaffinized and rehydrated. Heat Induced Epitope Retrieval was done in a Pre-Treatment Link with EnVision FLEX Target Retrieval Solution K8004 (DAKO, DK-2600 Glostrup, Denmark), at 97°C for 20 minutes, then cooled to 65°C. IHC was done at room temperature using Dako Autostainer Plus (DAKO). After enzyme blocking with Dako REAL Peroxidase Blocking Solution S2023 (DAKO) (10 minutes), the antibody with Dako REAL Antibody Diluent S2022 (1:50), was applied and incubated (40 minutes), followed by HRP Rabbit/Mouse EnVision Polymer (30 minutes). Sections were rinsed between steps with Dako Wash Buffer (DAKO) S3006 1:10, incubated for 10 minutes in DAB+ Chromogen (Dako REAL EnVision Detection System K5007), and contrast-stained with hematoxylin. Figure 3 shows haematoxylin-erythrosin-saffron (HES) and IHC staining for AR.

Negative and positive controls were included in all IHC runs. For negative controls, the primary antibody was omitted in a TMA section from the study series. Isotype control staining was performed on one TMA section by incubating with Mouse IgG1, kappa Isotype Control (Clone MOPC-31, antbodies-online, GmbH Germany) 6,08mg/L, using the same protocol as for AR-IHC. The isotope-stained section was analysed using a bright-field microscope, and some cases showed sporadic, faint brown nuclear staining in tumour cells. In the AR-stained section, the corresponding cases were either strongly AR-positive in >75% of the tumour cells, or AR-negative.

Scoring and reporting

Primary tumour TMA slides were scanned at 20x magnification using Ariol^{TM} SL-50 3.3 Scan system and analysis station (Genetix), and observers assessed AR-status on a computer screen (Ariol review station). LNM TMAs were scanned using the Olympus Virtual Slide System (VS120-S5) and the scanning program VS-ASW (OlyVIA 2.9 software for image visualisation, build 13753, Olympus, Germany, free download available). Based on researcher preference, LNM sections were evaluated on a computer screen or a bright-field microscope. AR-staining was scored by two researchers independently, one of whom was a pathologist. Both were blinded for clinical information and 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). In cases of disagreement, consensus was reached after discussion. The cut-off level for AR-positivity was set at ≥10% positive stained tumour cell nuclei.

The study is in accordance with the REMARK recommendations for tumor marker studies [37].

Statistical analyses

Chi-squared tests were used for associations between AR-expression and clinicopathological features. Differences in prognosis between AR^+ and AR^- cases were estimated by calculating the cumulative incidence of death from BC, treating death from other causes as a competing event. Gray's test was used to compare the equality of cumulative incidence curves. Multiple Cox regression analysis was used to estimate risk of death from BC (relative rate of BC death) according to AR-expression, censoring at death from other causes. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated, adjusting, when applicable, for categorical variables: year of diagnosis (before 1995, 1995 or later), age at diagnosis (<50, 50-59, 60-69, 70-79, ≥80 years), stage (I-IV), histopathological grade (1-3) and molecular subtype. Survival analyses were done for all cases combined, and separately according to ER-status, grade and molecular subtype. Likelihood ratio tests were used to compare associations between subgroups. Statistical analyses were performed using STATA 14.2 (Stata Corp., College Station, TX, USA).

Results

Tables 1 and 2 show characteristics of the study population and tumours, according to AR-expression. The median age at diagnosis was 68 years (range 33-96). In all, 1011 (79 %) cases were AR^+.

AR-expression in primary tumours and clinicopathological features

AR-expression in primary tumours was inversely associated with age at diagnosis, where 87.2% diagnosed before the age of 50 were AR^+, compared to 70.9% diagnosed at 80 years or older.

AR was associated with tumour diameter and stage. The proportion of AR^+ tumours decreased with increasing tumour size, from 83.5% AR^+ in tumours <20mm in diameter to 43.8% AR^+ when >50mm. AR-positivity was highest in stage I (82.1%) and lowest in stage III (71.6%).

The highest percentage of AR^+ tumours was observed in lobular carcinomas (86.3%, compared to 80.3% in carcinomas of no special type (NST) and 58.7% in all other types combined). The highest proportion of AR^+ cases was seen in histopathological grade 2 (84.0%) tumours, the lowest in grade 3 (68.7%).

AR was expressed in 84.9% of ER^+ compared to 45.1% of ER^- tumours. Among TNBCs, 31.1% were AR^+. AR-expression was inversely associated with basal marker expression, but not associated with Ki67 or HER2.

In molecular subtypes, the highest proportion of AR^+ tumours was found in the Luminal B($HER2^-$) (88.1%) and Luminal B($HER2^+$) (87.1%) subtypes, and the lowest in the BP (24.3%).

AR-expression in primary tumour and prognosis

Figure 4 shows the cumulative incidence of BC death, based on AR-expression (<1%, 1-9% and ≥10%). Patients with AR-expression ≥10% had a better prognosis than those with AR <1% and 1-9% (Gray's test $p<0.001$). No difference in survival was observed between patients with <1% and 1-9% AR^+ tumours (Gray's test $p=0.902$).

Table 3 shows the cumulative incidence and relative rate of death from BC according to AR-status. Cox analyses at 1% cut-off are shown in Supplementary Table 1. Cumulative incidence of death for AR^- cases was 27.0% (95% CI 22.3%-32.6%) five years after diagnosis, and 40.6% (95% CI 34.8%-46.9%) after 15 years. The corresponding risks for AR^+ cases were 16.1% (95% CI 13.9%-18.5%) and 27.7% (95% CI 24.9%-30.8%),

respectively. AR^+ cases had a better prognosis compared to AR^- in both unadjusted and adjusted analyses (adjusted HR 0.70, 95% CI 0.55-0.90). AR-expression in LNM was not associated with prognosis (data not shown).

Stratified for histopathological grade, AR-expression was associated with favourable prognosis in grades 2 and 3 tumours in unadjusted Cox analyses. AR was an independent prognostic factor only in grade 3 (HR 0.61, 95% CI 0.42-0.89, Table 3). However, in the likelihood ratio test, there was no evidence that associations between AR and risk of death from BC differed between grades (likelihood ratio p-value 0.47).

AR-expression was associated with a better prognosis in ER^+ cases in unadjusted analyses (Figure 5), and this finding persisted after adjustments (HR 0.67, 95% CI 0.50-0. 91). In ER^- cases, neither the unadjusted (HR 0.91, 95% CI 0.61-1.37), nor the adjusted analyses (HR 0.79, 95% CI 0.52-1.21) supported an association between AR and prognosis (Table 3). Despite this, there was no clear evidence that the associations between AR and risk of death from BC differed between women with ER^+ and ER^- tumours (p-value from likelihood ratio test 0.61).

In molecular subtypes, there was a general trend towards better survival for AR^+ cases in the unadjusted analyses in all subtypes except BP (Table 4 and Figure 5). However, this was clearly observed only for Luminal A (HR 0.49, 95% CI 0.33-0.72) and Luminal B($HER2^-$) (HR 0.52, 95% CI 0.31-0.87). For both, estimates were somewhat attenuated after adjustments, but a clear prognostic value persisted for Luminal A (HR 0.61, 95% CI 0.41-0.91). There was an indication that the association between AR and risk of BC death might differ according to BC subtype (likelihood ratio p-value 0.06).

AR-expression in paired primary tumours and axillary lymph node metastases

Of the 336 cases with LNM, 293 (87.2%) were AR^+ (Table 2). AR-status differed between the primary tumour and its corresponding LNM in 72 cases (21.4%). Of the 91 cases with AR^- primary tumours, 60 cases (65.9%) showed AR-expression ≥10% in the LNM. In cases with AR^+ primary tumours, 4 cases had <1% and 8 cases 1-9% AR-expression in the LNM. Table 5 shows AR-expression in LNM and discordant AR-status between primary tumour and LNM, according to molecular subtype.

AR in full-face sections

AR was assessed in full-face sections in a small subset of 57 cases. A total of 45 cases were classified in the same category as the TMA. Using the full-face sections as a gold standard, this resulted in a sensitivity of 72.2% (10 discrepant cases) and a specificity of 90.5% (2 discrepant cases). Of the ten cases scoring <10% in the TMA and ≥10% in the full-face section, seven scored 1-9% in TMA and three scored <1%. Two cases that scored ≥10% in the TMA, scored 1-9% in the whole section.

Discussion

In this study of 1297 BC patients, AR was expressed in 78% of primary tumours at 10% cut-off. The highest proportion of AR-expression was seen in Luminal B subtypes, and the lowest in BP. Patients with AR^+ tumours had a better prognosis than those with AR^-. This was most evident in Luminal A and Luminal B($HER2^-$) subtypes, and in grade 3 tumours. However, although precision was low, a trend towards better prognosis in AR^+ cases was seen in all subtypes except BP. Discrepancy in AR-expression between primary tumour and

LNM occurred in 21.4% of cases, and in the majority of discrepant cases, there was a switch from AR^- primary tumour to AR^+ LNM.

To the best of our knowledge, ours is one of the largest studies of the prognostic value of AR-expression across molecular subtypes of BC. It is also the largest study of AR-expression in BC LNM. We assessed AR-expression in 1297 primary BCs and 336 LNM from two well-described, prospective cohorts. Molecular subtyping had been carried out at the same laboratory, using the same antibodies and subtyping algorithm in all tumours [17, 29]. All IHC markers were assessed by two researchers independently. Reliable information on BC incidence and follow-up data were available from high-quality national registries [38].

The study was performed on TMAs, which is a cost-effective means of analysing large numbers of samples under similar laboratory conditions. Punches were taken from the tumour periphery. However, the method could be vulnerable to intratumour heterogeneity. AR was assessed in whole sections in a subset of 57 cases. Using the 10% cut-off, 45 cases were classified in the same category as the TMA resulting in a sensitivity of 72.2% and a specificity of 90.5%. However, among 12 cases with discrepant results at the 10% cut-off, nine scored 1-9%.

Tumour tissue from 1961-2009 was used, and preanalytical conditions may have varied. This may have influenced our results. However, it has been shown that antigenicity is, for the most part, preserved in paraffin blocks over decades [39]. Although one study has demonstrated that loss of antigenicity during slide storage is minimal and that modern antigen retrieval probably compensates for the effect of storage [40], others have shown that staining intensity is weakened in tissue sections stored over time at room temperature or at $0^{\circ}C$, though associations between IHC-findings and clinicopathological parameters appear to be conserved [41]. Storing TMA sections at $-20^{\circ}C$ may have resulted in weaker staining. Staining intensity was not quantified in this study.

AR-expression and clinicopathological features

In accordance with others, we found higher AR-expression in lobular carcinomas than carcinomas of NST and other types, [2, 3, 23, 42]. Histopathological grade 2 tumours comprise a heterogenous group of patients with varying prognoses [17], and there is a need for further subclassification into prognostic groups. The present study found the highest percentage of AR-positivity in grade 2 and the lowest in grade 3, which is in accordance with one study [3]. However, others have shown highest AR-expression in grade 1 and lowest in grade 3 [11, 22, 23, 42].

Few studies have assessed AR-expression in molecular subtypes of BC [2, 13, 22, 23], and results vary. In one, AR was most frequently expressed in Luminal B [22], which complies with our findings. Others found AR to be most frequent in Luminal A [2, 13, 23]. Similar to our findings, three found the lowest frequency of AR^+ in BP, followed by the five negative phenotype (5NP) and HER2 type [2, 22, 23]. Another found AR-positivity to be more frequent in 5NP than the HER2 type [13].

TNBCs have aggressive clinicopathological features and poor prognosis [43], and are a heterogeneous group of tumours, both histologically and molecularly [44]. These patients qualify for neither endocrine therapy nor targeted anti-HER2 therapy. Hence, they are offered only adjuvant chemotherapy [12]. AR-expression has been reported in 8% to 53% [3, 5-11, 22, 23, 45-48] of TNBCs. In the present study, 33.1% of TNBCs were AR^+ at

10% cut-off, in agreement with previous studies using the same cut-off level [35, 46]. AR is recognized as a driver of TNBC and AR^+ TNBC patients may benefit from targeted antiandrogen therapy [49], which has been implemented in the treatment of prostate cancer [50]. Phase 2 studies of antiandrogens in metastatic or advanced ER^- BC show promising results [51-53]. Clinical trials are planned or under way, such as enzalutamide in AR^+, early-stage TNBC (NCT 02750358), and antiandrogens in combination with drugs that target CDK4/6 or the PI3K/AKT/mTOR pathway (NCT02457910, NCT02605486, NCT03090165).

Current guidelines classify 5NP and BP as TNBC [12]. However, their AR-expression differs considerably. 5NP is among the subtypes with poorest prognosis [16], showing the second poorest prognosis after HER2 type in our first cohort [17]. Twenty-one of 43 (48.8%) 5NP tumours were AR^+, suggesting that a large proportion of patients with 5NP tumours could be candidates for antiandrogen treatment.

AR-expression is low in BP, and inversely associated with basal markers [2, 13, 22, 45]. Our findings support this, with the lowest prevalence of AR^+ in BP tumours (25.3%). Some suggest that absence of basal markers predicts response to antiandrogen therapy in TNBCs [52]. In our study, 17 BP cases expressed AR in 1- 9% of tumour cells. In a study of AR in TNBC, 13.8% of AR^+ cases were between 1 and 9%. However, this study did not subdivide TNBCs into BP and 5NP [11].

Few studies describe AR-expression in BC metastases, with cases varying from 16 to 134 [3, 24, 25, 54-56]. To the best of our knowledge, ours is the largest study of AR-expression in BC LNM. AR-status was discordant in 21.4% of LNM compared to primary tumours. Grogg et al demonstrated a discordance in 4.3% of 117 cases using the same clone as ours [3]. However, they used a 1% cut-off and this may account for the difference beween their results and ours. In our study, a switch from AR^- primary tumour to AR^+ LNM accounted for the greatest proportion of discrepancies. Of the 91 cases with AR^- primary tumours, 65.9% had AR^+ LNM. Although the numbers are low, 12 of 14 TNBC cases with discordant AR-status showed a switch from AR^- primary tumour to AR^+ LNM. Should antiandrogen treatment become a therapeutic option in BC, then AR-status in LNM could be of importance in the presence of an AR^- primary tumour, particularly in TNBC where treatment options are limited.

AR-expression and prognosis

Several studies have shown that AR-expression is associated with good prognosis in BC. Of three meta-analyses of AR in BC [4, 57, 58], the most recent (10,004 patients from 22 studies) found that patients with AR^+ tumours had better disease-free and overall survival in both uni- and multivariate analyses [4]. This is in accordance with our study, where AR was an independent prognostic factor in all patients combined.

In our study, AR-expression was not associated with survival in grade 1 tumours, but was an independent predictor of good prognosis in grade 3. Another study including 403 cases did not demonstrate an association between AR and prognosis when stratified for grade [59].

AR was clearly associated with prognosis only in Luminal A and Luminal B($HER2^-$). However, there was an indication that the association between AR and risk of BC death might differ across BC subtypes. Others have reported AR to be associated with prognosis in all subtypes except the HER2 type [13], or Luminal B($HER2^+$) only [22].

We found no association between AR-expression and prognosis in TNBC (data not shown), in accordance with Park et al [6]. While AR-positivity in TNBC has been associated with better prognosis by some [7-10, 45], others report an association with poor prognosis [5, 11]. The meta-analysis including the most TNBC studies, found that AR-positivity was associated with improved disease-free survival, but not overall survival [60]. The most recent meta-analysis shows that AR-positivity predicts better overall survival [4].Varying definitions of TNBC, cut-off levels, cohort characteristics and laboratory procedures could explain some of these differences.

There is no consensus regarding cut-off levels for AR-positivity, and cut-off varies from >0% to ≥75% [2, 4, 6-8, 10, 11, 13, 45, 46, 61]. This must be taken into account when comparing studies. In our study, the 10% cut-off showed independent prognostic value, whereas 1% did not. Furthermore, patients with tumours expressing AR in 1-9%, had a similar prognosis to those with <1%. Based on this, we support the 10% cut-off for AR-positivity as a prognostic factor.

Conclusions

In the present study, AR-expression in ≥10% of tumour cells was an independent predictor of good prognosis in BC, particularly in grade 3, and Luminal A tumours. AR-expression was highest in the Luminal B subtypes, and higher in 5NP than BP. Discordant AR-expression between primary tumour and LNM was observed in 21.4% of cases and most often there was a switch from AR^- primary tumour to AR^+ axillary LNM. Our results indicate that it is important to examine AR-status in LNM in addition to the primary tumor.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval for this study, including dispensation from the requirement of patient consent, was granted by the Regional Committee for Medical and Health Sciences Research Ethics (REK, Midt-Norge, ref. nr: 836/2009).

Data availability The datasets generated and/or analysed in the current study are not publicly available but are available from the corresponding author on reasonable request.

Conflicts of interest The authors declare that they have no conflict of interest.

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Table 1 Descriptive characteristics and chi-square tests for the 1297 breast cancer patients according to androgen receptor status

	Total	**<10%**	**≥10%**	**Chi^2**
Number of cases (%)	1297	286 (22.1)	1011 (78.0)	
Year of diagnosis				
Before 1995	620	148 (23.9)	472 (76.1)	
1995 or later	677	138 (20.4)	539 (79.6)	
Median age at diagnosis, years (IQR)	68 (58-76)	71 (62-79)	67 (57-75)	
Median follow-up time, years (IQR)	7.6 (4.0-12.5)	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)	2.7 (1.5-6.4)	4.2 (2.0-7.7)	
Age at diagnosis, years (%)				0.001
<50	141	18 (12.8)	123 (87.2)	
50-59	218	41 (18.8)	177 (81.2)	
60-69	340	68 (20.0)	272 (80.0)	
70-79	361	90 (24.9)	271 (75.1)	
≥80	237	69 (29.1)	168 (70.9)	
Tumour diameter (mm), n (%)				<0.001
≤20	681	112 (16.5)	569 (83.6)	
>20 ≤50	247	73 (29.6)	174 (70.5)	
>50	16	9 (56.3)	7 (43.8)	
Uncertain, but ≥20	141	40 (28.4)	101 (71.6)	
Unknown*	212	52	160	
Lymph node status, n (%)				0.051
Negative	620	128 (20.7)	492 (79.4)	
Positive	443	114 (25.7)	329 (74.3)	
Unknown*	234	44	190	
Stage, n (%)				
I	652	117 (17.9)	535 (82.1)	0.003
II	522	137 (26.3)	385 (73.8)	
III	67	19 (28.4)	48 (71.6)	
IV	50	13 (26.0)	37 (74.0)	
Missing*	6	0	6	

*Chi^2 test does not include cases with missing values.

Table 2 Descriptive characteristics and chi-square tests for the 1297 breast cancer tumours according to androgen receptor status

	Total	**<10%**	**≥10%**	**Chi²**
Number of cases (%)	1297	286 (22.1)	1011 (78.0)	
Type, n (%)				<0.001
No special type	953	188 (19.7)	765 (80.3)	
Lobular	160	22 (13.8)	138 (86.3)	
Other types	184	76 (41.3)	108 (58.7)	
Grade, n (%)				<0.001
1	186	41 (22.0)	145 (78.0)	
2	674	108 (16.0)	566 (84.0)	
3	437	137 (31.4)	300 (68.7)	
ER, n (%)				<0.001
Negative	224	123 (54.9)	101 (45.1)	
Positive	1071	162 (15.1)	909 (84.9)	
Missing*	2	1	1	
PR, n (%)				<0.001
Negative	487	192 (39.4)	295 (60.6)	
Positive	809	93 (11.5)	716 (88.5)	
Missing*	1	1	0	
HER2, n (%)				0.355
Negative	1119	242 (21.6)	877 (78.4)	
Positive	178	44 (24.7)	134 (75.3)	
Ki67, n (%)				0.094
<15 %	732	149 (20.4)	583 (79.6)	
≥15 %	565	137 (24.3)	428 (75.8)	
CK5, n (%)				<0.001
Negative	983	193 (19.6)	790 (80.4)	
Positive	313	92 (29.4)	221 (70.6)	
Missing*	1	1	0	
EGFR, n (%)				<0.001
Negative	1221	245 (20.1)	976 (79.9)	
Positive	75	41 (54.7)	34 (45.3)	
Missing*	1	0	1	
Subtype, n (%)				<0.001
Luminal A	644	114 (17.7)	530 (82.3)	
Luminal B(HER2-)	345	41 (11.9)	304 (88.1)	
Luminal B(HER2+)	101	13 (12.9)	88 (87.1)	
HER2 type	77	31 (40.3)	46 (59.7)	
5NP	43	22 (51.2)	21 (48.8)	
BP	87	65 (74.7)	22 (25.3)	
AR in lymph nodes				<0.001
<1%	22	18 (81.8)	4 (18.2)	
1-9%	21	13 (61.9)	8 (38.1)	
≥10%	293	60 (20.5)	233 (79.5)	
Missing*	961	195	766	

*Chi² test does not include cases with missing values

Table 3 Breast cancer prognosis according to androgen receptor (AR) in all cases, and stratified for grade and ER status

		Cumulative incidence of death					Cox regression analyses			
			Until 5 years after diagnosis		Until 15 years after diagnosis		Entire follow-up period			
		Cases (n)	BC deaths (n)	Cum. risk of death (%), (95 % CI)	BC deaths (n)	Cum. risk of death (%), (95 % CI)	BC deaths (n)	HR (95% CI) Unadjusted	HR (95% CI) Adjusted*	HR (95% CI) Adjusted†
All cases	**All cases**									
	AR- (<10%)	286	77	27.0 (22.3-32.6)	108	40.6 (34.8-46.9)	111	1	1	1
	AR+ (≥10%)	1011	161	16.1 (13.9-18.5)	255	27.7 (24.9-30.8)	272	0.56 (0.45-0.70)	0.63 (0.51-0.79)	0.70 (0.55-0.90)
	p-value							<0.001	<0.001	0.005
Grade	**Grade 1**									
	AR- (<10%)	41	4	9.9 (3.9-24.4)	8	21.4 (11.3-38.6)	9	1	1	1
	AR+ (≥10%)	145	9	6.3 (3.3-11.7)	18	15.1 (9.6-23.2)	21	0.48 (0.22-1.05)	0.63 (0.28-1.41)	0.56 (0.24-1.34)
	p-value							0.066	0.264	0.193
	Grade 2									
	AR- (<10%)	108	22	20.5 (14.0-29.4)	39	40.5 (31.2-51.3)	39	1	1	1
	AR+ (≥10%)	566	77	13.8 (11.2-16.9)	135	26.5 (22.8-30.6)	145	0.58 (0.41-0.83)	0.74 (0.52-1.06)	0.79 (0.54-1.15)
	p-value							0.003	0.104	0.223
	Grade 3									
	AR- (<10%)	137	51	37.3 (29.8-46.0)	61	46.6 (38.3-55.7)	63	1	1	1
	AR+ (≥10%)	300	75	25.1 (20.6-30.4)	102	36.0 (30.6-42.0)	106	0.65 (0.48-0.89)	0.65 (0.48-0.89)	0.61 (0.42-0.89)
	p-value							0.007	0.007	0.011
ER status	**ER+ (≥1%)**									
	AR- (<10%)	162	36	22.4 (16.7-29.6)	55	37.2 (29.8-45.7)	58	1	1	1
	AR+ (≥10%)	909	124	13.8 (11.7-16.2)	213	26.2 (23.2-29.4)	229	0.56 (0.42-0.75)	0.71 (0.53-0.95)	0.67 (0.50-0.91)
	p-value							<0.001	0.022	0.009
	ER- (<1%)									
	AR- (<10%)	123	41	33.5 (25.9-42.6)	52	44.5 (35.9-54.2)	52	1	1	1
	AR+ (≥10%)	101	37	36.6 (28.1-46.8)	42	41.9 (32.9-52.2)	43	0.91 (0.61-1.37)	0.85 (0.56-1.28)	0.79 (0.52-1.21)
	p-value							0.655	0.430	0.286

*Adjusted for age and year of diagnosis. †Adjusted for age, stage, grade, subtype in all cases. Adjusted for age, stage, subtype when stratified for grade. Adjusted for age, stage and grade when stratified for ER status.

Table 4 Breast cancer prognosis according to androgen receptor (AR), stratified for subtypes

	Cumulative incidence of death					Cox regression analyses			
		Until 5 years after diagnosis		Until 15 years after diagnosis		Entire follow-up period			
	Cases (n)	BC deaths (n)	Cum. risk of death (%), (95 % CI)	BC deaths (n)	Cum. risk of death (%), (95 % CI)	BC deaths (n)	HR (95% CI) Unadjusted	HR (95% CI) Adjusted*	HR (95% CI) Adjusted†
Luminal A									
AR- (<10%)	114	20	17.7 (11.8-26.1)	35	34.4 (25.9-44.8)	36	1	1	1
AR+ (≥10%)	530	52	10.0 (7.7-12.9)	97	21.1 (17.6-25.3)	105	0.49 (0.33-0.72)	0.63 (0.43-0.94)	0.61 (0.41-0.91)
p-value							<0.001	0.022	0.016
Luminal B(HER2-)									
AR- (<10%)	41	14	34.2 (21.8-50.7)	16	40.4 (26.9-57.5)	17	1	1	1
AR+ (≥10%)	304	49	16.2 (12.5-20.9)	87	31.2 (26.0-37.1)	93	0.52 (0.31-0.87)	0.66 (0.39-1.13)	0.64 (0.38-1.11)
p-value							0.013	0.130	0.111
Luminal B(HER2+)									
AR- (<10%)	13	3	23.1 (8.1-55.8)	6	51.2 (26.6-81.0)	7	1	1	1
AR+ (≥10%)	88	24	27.5 (19.4-38.2	32	38.4 (28.8-50.0)	34	0.76 (0.34-1.72)	0.91 (0.39-2.10)	0.82 (0.32-2.08)
p-value							0.515	0.819	0.670
HER2 type									
AR- (<10%)	31	14	45.2 (29.7-64.0)	17	56.0 (39.4-74.0)	17	1	1	1
AR+ (≥10%)	46	20	43.5 (30.6-58.9)	21	45.7 (32.7-61.1)	22	0.72 (0.38-1.36)	0.70 (0.36-1.37)	0.62 (0.30-1.27)
p-value							0.310	0.300	0.195
5NP									
AR- (<10%)	22	8	36.4 (20.1-59.7)	11	50.8 (32.0-72.7)	11	1	1	1
AR+ (≥10%)	21	8	38.1 (21.2-61.9)	9	43.3 (25.3-66.8)	9	0.88 (0.36-2.12)	0.53 (0.17-1.64)	0.39 (0.10-1.51)
p-value							0.769	0.270	0.173
BP									
AR- (<10%)	65	18	27.7 (18.4-40.3)	23	38.1 (26.9-52.0)	23	1	1	1
AR+ (≥10%)	22	8	36.4 (20.1-59.7)	9	41.3 (24.0-64.4)	9	1.22 (0.57-2.64)	0.97 (0.43-2.20)	1.30 (0.52-3.24)
p-value							0.610	0.944	0.572

*Adjusted for age and year of diagnosis. †Adjusted for age, stage and grade.

Table 5. AR-expression in lymph node metastases, and discordant AR-status between primary tumour and lymph node metastasis (LNM), according to molecular subtype of the primary tumour (PT)

	Total LNM	**<10%**	**≥10%**	**Discordant PT and LNM**	**AR^- PT and AR^+ LNM**	**AR^+ PT and AR^- LNM**
Number of cases (%)	336	43 (12.8)	293 (87.2)	72	60	12
Subtype, n (%)						
Luminal A	143	10 (7.0)	133 (93.0)	30 (21.0)	28	2
Luminal B(HER2-)	96	9 (9.4)	87 (90.6)	16 (16.7%)	11	5
Luminal B(HER2+)	28	5 (17.9)	23 (82.1)	4 (14.2%)	1	3
HER2 type	32	6 (18.8)	26 (81.3)	8 (25.0%)	8	0
TNBC	37	13 (35.1)	24 (64.9)	14 (37.8%)	12	2
5NP	13	3 (23.1)	10 (76.9)	4 (30.8%)	4	0
BP	24	10 (41.7)	14 (58.3)	10 (41.7%)	8	2

PT: Primary tumour. LNM: Lymph node metastasis.

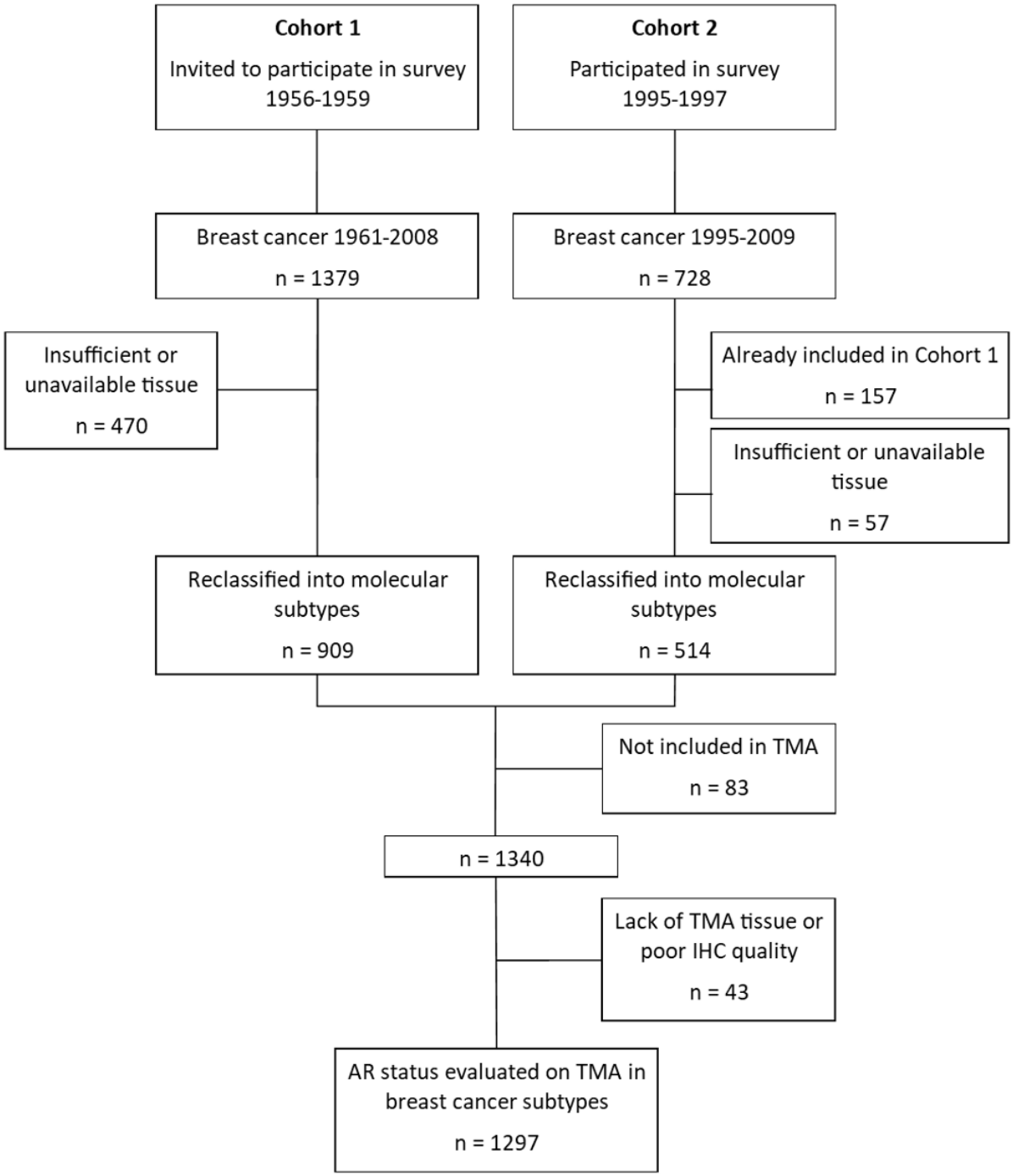

Fig. 1 Flow-chart of the study population, showing inclusion and exclusion of cases in the study

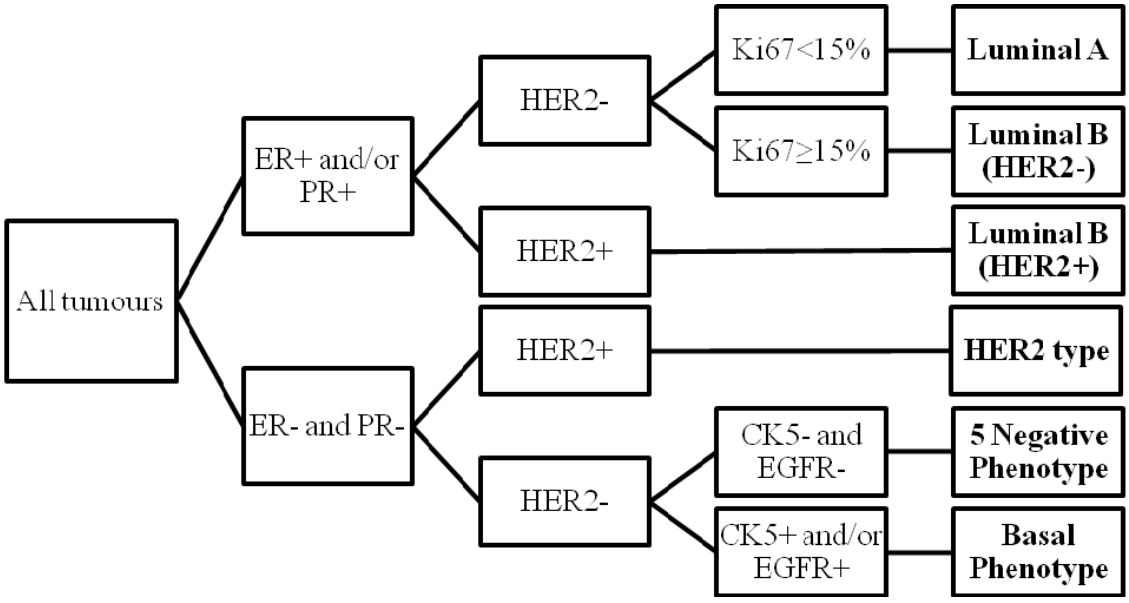

Fig. 2 Algorithm for molecular subtyping. Luminal A (n=644), Luminal B(HER2-) (n=345), Luminal B(HER2+) (n=101), HER2 type (n=77), 5 Negative Phenotype (n=43), Basal Phenotype (n=87). Oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Cytokeratin 5 (CK5), epidermal growth factor receptor (EGFR). Adapted from Breast Cancer Res Treat, 2013, Vol 140, Engstrøm et al, *Molecular subtypes, histopathological grade and survival in a historic cohort of breast cancer patients* [17] Figure 2, page 466. Open access, permission for reprint not required.

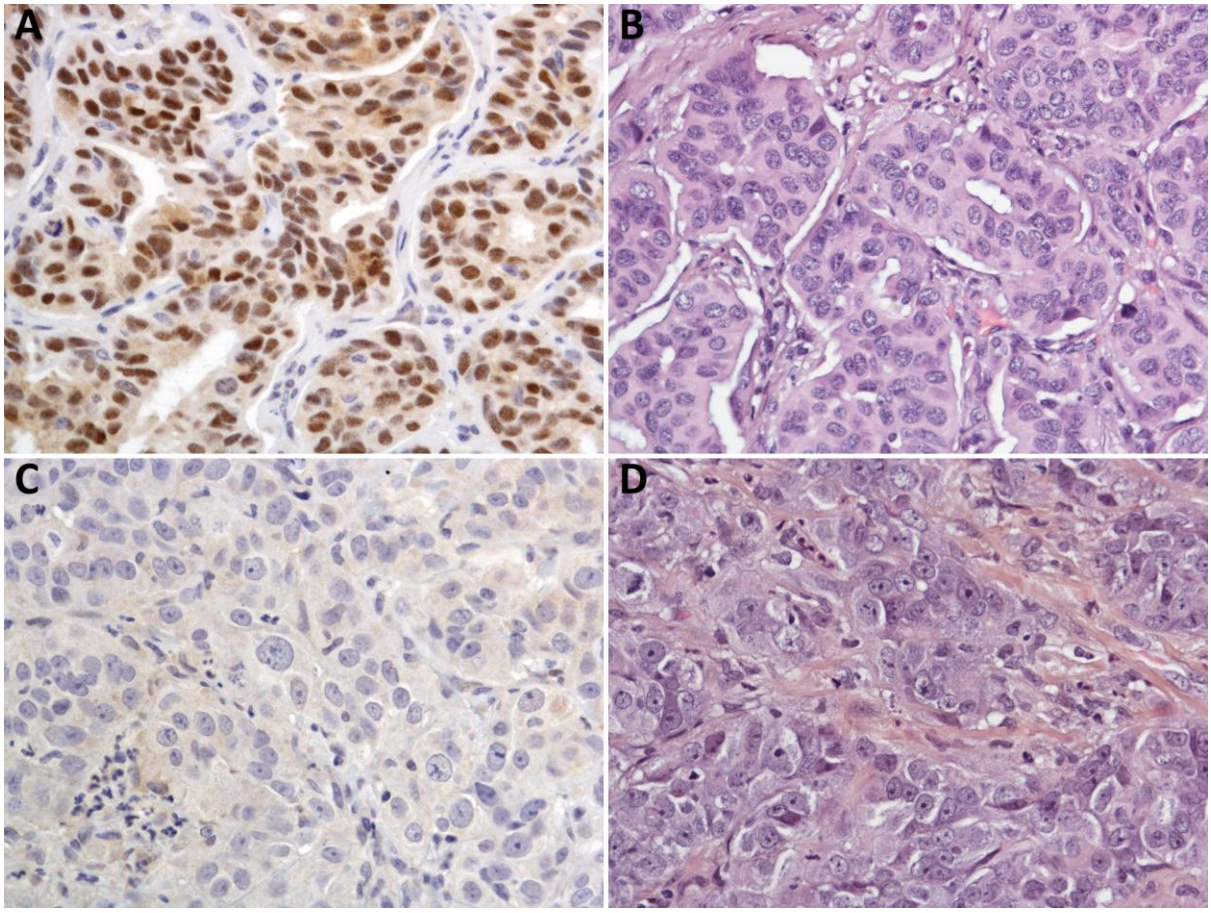

Fig. 3 Immunohistochemical staining for androgen receptor (AR) and haematoxylin-erythrosin and saffron (HES) in two cases. Case number 4046: AR-positive nuclear staining in ≥10% (A), HES (B) (400x). Case number 4023: AR-negative (C) and HES (D) (400x)

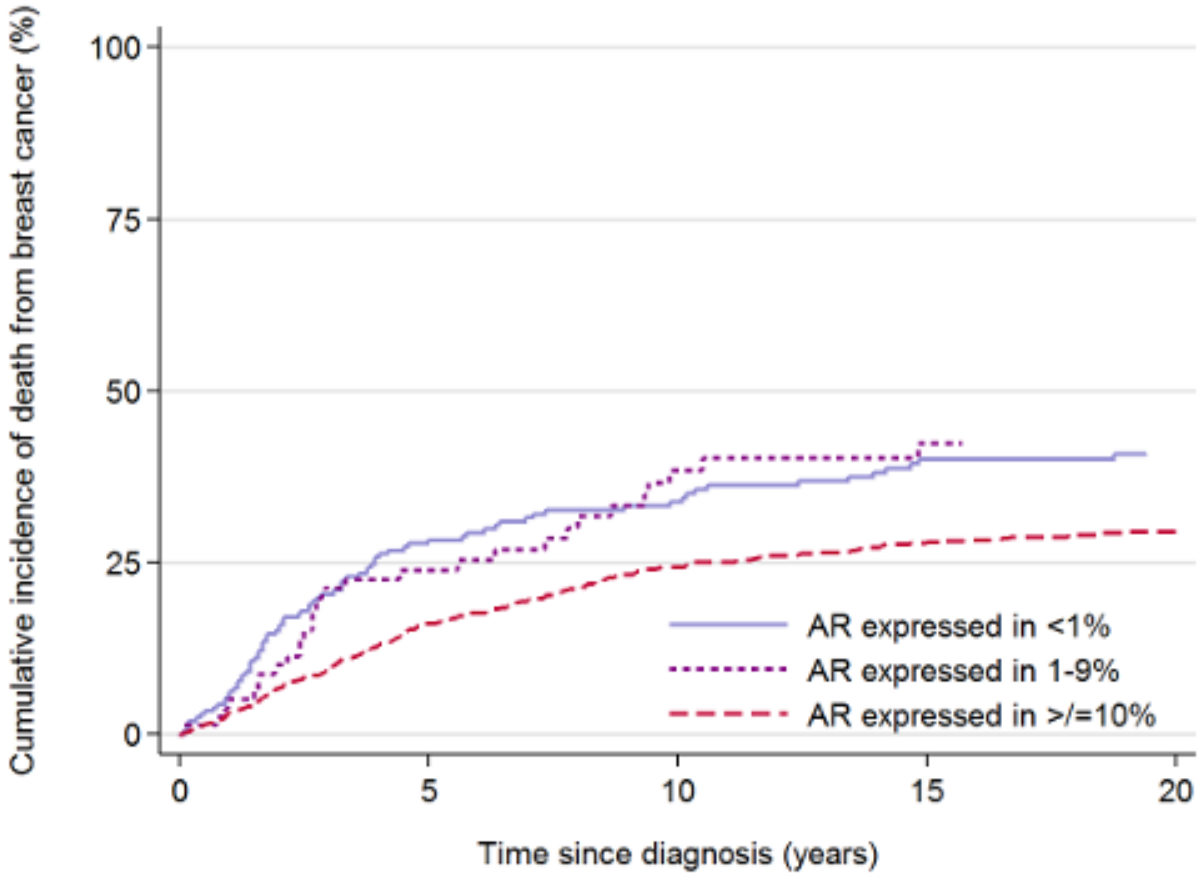

Fig. 4 Cumulative incidence of breast cancer death according to androgen receptor (AR) positivity in all patients. Cut-off at 10% for AR positivity. Gray's test: p<0.001

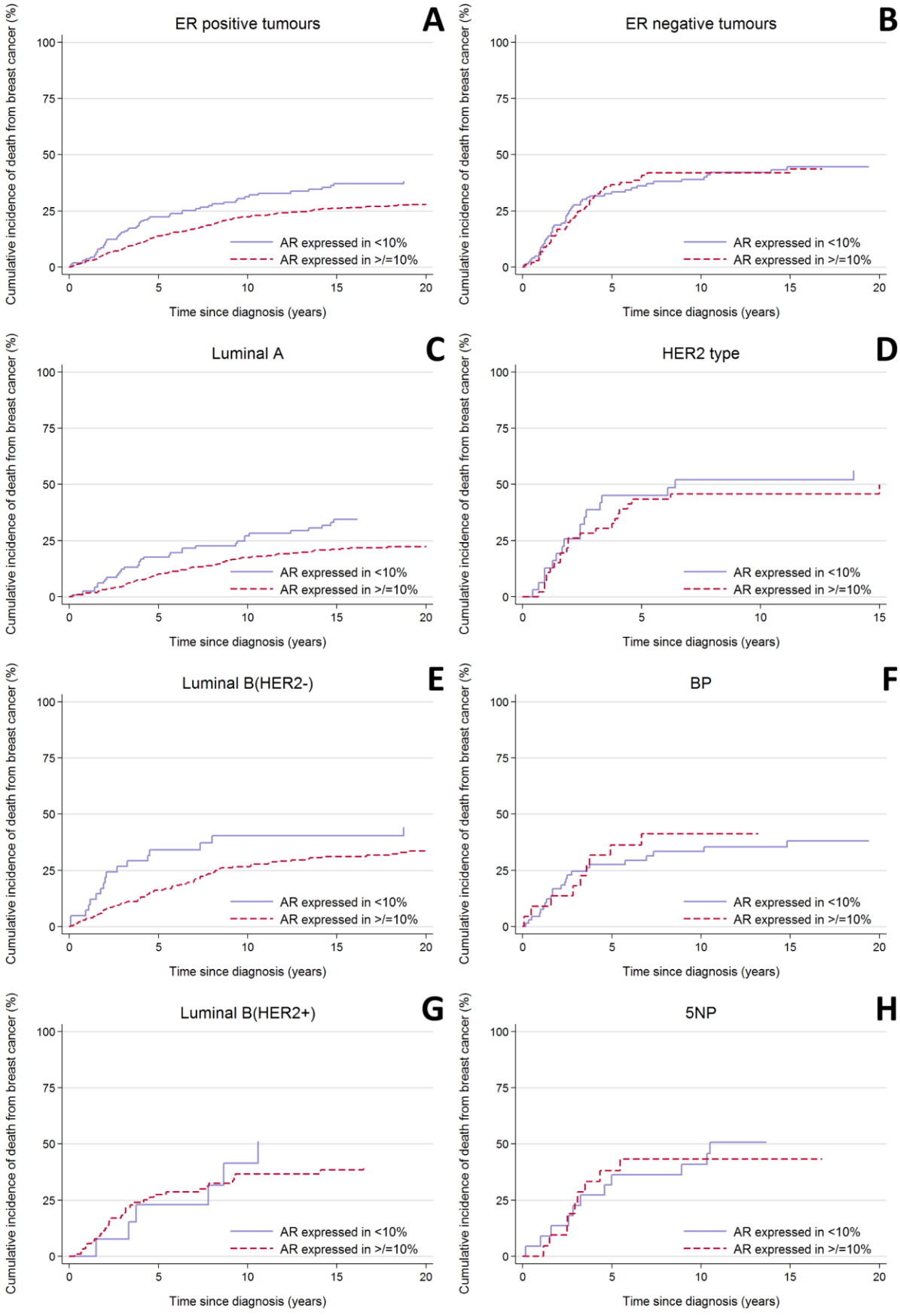
A
ER positive tumours
B
ER negative tumours
C
Luminal A
D
HER2 type
E
Luminal B(HER2-)
F
BP
G
Luminal B(HER2+)
H
5NP
Cumulative incidence of death from breast cancer (%)
Time since diagnosis (years)
AR expressed in <10%
AR expressed in >/=10%

Fig. 5 Cumulative incidence of breast cancer death according to androgen- and oestrogen receptor, and molecular subtype. Cut-off at 10% for androgen receptor positivity, and at 1% for oestrogen receptor (ER) positivity. a: ER positive tumours, Gray's test: p=0.005. b: ER negative tumours, Gray's test: p=0.94. c: Luminal A, Gray's test: p=0.009. d: HER2 type Gray's test: p=0.543. e: Luminal B HER2-, Gray's test: p=0.070. f: Basal phenotype Gray's test: p=0.751. g: Luminal B HER2+, Gray's test: p=0.491. h: 5 negative phenotype Gray's test: p=0.821