Combined Androgen and Estrogen Receptor Status in Breast Cancer: Treatment

Prediction and Prognosis in a Population-based Prospective Cohort

Karin Elebro¹ ², Signe Borgquist¹ ³, Maria Simonsson¹, Andrea Markkula¹, Karin Jirström¹, Christian Ingvar⁴, Carsten Rose⁵, Helena Jernström¹

¹Division of Oncology and Pathology, Department of Clinical Sciences, Lund, Lund University ²Department of Plastic and Reconstructive Surgery, Skåne University Hospital ³Department of Oncology, Skåne University Hospital ⁴Division of Surgery, Department of Clinical Sciences, Lund, Lund University ⁵CREATE Health and Department of Immunotechnology, Lund University, Sweden

Financial support

This work was supported by grants from The Swedish Cancer Society (CAN 2011/497), the Medical Research Council (K2012-54X-22027-01-3), the Medical Faculty at Lund University, the Mrs. Berta Kamprad Foundation, the Gunnar Nilsson Foundation the South Swedish Health Care Region (Region Skåne ALF), Konung Gustaf V:s Jubileumsfond (PI H Jernström), the Swedish Breast Cancer Group (BRO), the Lund Hospital Fund, the RATHER consortium (http://www.ratherproject.com/), and the Seventh Framework programme.

Corresponding author:

Associate Professor Helena Jernström, PhD

Division of Oncology and Pathology, Department of Clinical Sciences, Lund, Lund University Cancer Center/Kamprad, Barngatan 2B, SE-22185 Lund, Sweden

Phone: +46 46 17 76 19, fax: +46 46 14 73 27

E-mail: helena.jernstrom@med.lu.se
Contributing authors’ work telephone numbers and institutional postal addresses:

Karin Elebro (+46-40-336039), Signe Borgquist (+46-46-17 75 71), Maria Simonsson (+46-46-17 75 71), Andrea Markkula (+46-46-17 75 71), and Karin Jirström (+46-46-2220829):

Same address as the corresponding author.

Christian Ingvar (+46-46-171438):
Division of Surgery, Department of Clinical Sciences, Lund, Lund University and Skane University Hospital, SE-221 85 Lund, Sweden

Carsten Rose (+46-46-70257509):
CREATE Health and Department of Immunotechnology, Lund University, Medicon Village, Building 406, Scheelevägen 2, SE-223 81 Lund, Sweden

All authors’ email addresses:
karin.elebro@med.lu.se, signe.borgquist@med.lu.se, maria.simonsson@med.lu.se,
andrea.markkula@med.lu.se, karin.jirstrom@med.lu.se, christian.ingvar@med.lu.se,
carsten.rose@immun.lth.se, helena.jernstrom@med.lu.se

Competing interests
The authors declare that they have no competing interests.
Running title: Androgen and Estrogen Receptor Status in Breast Cancer

Word count: 3770

Total number of figures and table: 6

Key words:
breast cancer; androgen receptor; estrogen receptor; prognosis; treatment-prediction;
population-based cohort

List of abbreviations
AI aromatase inhibitors
AR androgen receptor
BMI body mass index
CI confidence interval
CYP17 cytochrome P450 c17
ER estrogen receptor alpha
HER2 human epidermal growth factor receptor 2
HR hazard ratio
OR odds ratio
pN pathological axillary lymph node involvement
pT pathological tumor size
PR progesterone receptor
TAM tamoxifen
TMA tissue microarray
Translational relevance

In this prospective, population-based study, a differential role of AR in ER-positive versus ER-negative tumors is demonstrated. Further, patients with tumors of discordant receptor status (ER+AR- or ER-AR+) had worse prognosis in multivariable models compared to patients with tumors of concordant receptor status (ER+AR+ or ER-AR-), suggesting a need for new treatment options for these patients. ER+AR- indicated early treatment failure with aromatase inhibitors among chemonaïve patients aged 50 or older, a finding of interest to investigate in future randomized trials.

Thus, we suggest caution when considering treatment strategies targeting AR, because either inhibitory or stimulatory action might be beneficial depending on the patient’s breast cancer ER status. Several clinical trials with either anti-androgens or selective androgen receptor modulators are ongoing. More studies on AR stratified by ER status are needed.
Abstract

Purpose: To evaluate whether tumor androgen receptor (AR) expression was prognostic and/or predictive for endocrine treatment alone or in combination with estrogen receptor (ER). The AR has been hypothesized to have differential prognostic roles in breast cancer depending on tumor ER-status, and to influence endocrine treatment response.

Experimental Design: A population-based prospective cohort of 1026 patients diagnosed with primary invasive breast cancer in Lund, Sweden between 2002 and 2012 was followed until June 2014. Associations between immunohistochemical AR expression in tumor tissue microarrays, patient and tumor characteristics, and AR genotypes were analyzed. Disease-free survival (DFS) by AR status, and combined ER/AR status was assessed in various treatment groups.

Results: AR expression was assessable in 913 tumors. AR+ tumors (85.0%) were associated with higher age ($P=0.036$) and favorable tumor characteristics. The AR+ status was a prognostic marker for DFS (LogRank $P=0.025$). There was an interaction between AR and ER expression with respect to prognosis (adjusted $P_{\text{interaction}}\leq0.024$). Tumors with discordant hormone receptor expressions (ER+AR- or ER-AR+) demonstrated worse prognosis compared to concordant tumor expressions (ER+AR+ or ER-AR-) in multivariable models (adjusted hazard ratios (95% confidence intervals); $\geq1.99$ (1.28-3.10), $P\leq0.002$). ER+AR- indicated early treatment failure with aromatase inhibitors (AI) among chemonaïve patients aged 50 or older.

Conclusions: Prediction of breast cancer prognosis and treatment response was improved by combining AR and ER status. AR negativity predicted early treatment failure with AI but not tamoxifen, a finding that warrants confirmation in a randomized setting. Patients may benefit from anti-androgens or selective androgen receptor modulators.
Introduction

The heterogeneity of breast cancer calls for a deeper understanding of prognostic and predictive markers to improve breast cancer survival. The critical gaps in breast cancer research have recently been portrayed (1), and the androgen receptor (AR) was highlighted as an interesting prognostic and treatment-predictive marker because of its interplay with the estrogen receptor-alpha (ER). Based on preclinical findings, Vera-Badillo et al. hypothesized AR to be a good prognostic marker in ER+ tumors, but a poor prognostic marker in ER- tumors (2). However, this hypothesis was not confirmed in their meta-analysis of clinical studies. Moreover, high AR expression has been proposed as a positive predictive marker for endocrine treatment response; however, endocrine treatment type was not considered (3).

Conversely, preclinical data suggest that AR overexpression causes tamoxifen (TAM) and/or aromatase inhibitor (AI) resistance (4, 5). Ongoing clinical trials with anti-androgens (6, 7) further highlight the need for additional studies of AR alone and in combination with ER in breast cancer (8).

In a previous study, we reported a treatment predictive value of AR genotyping for adjuvant TAM, but not for AIs (9). In the present study, we hypothesized that the prognostic value of tumor AR expression may depend on ER status and have predictive value for endocrine treatment response. Our primary aim was to analyze AR expression in breast tumors from patients included in the prospective population-based BC Blood Study and to relate AR expression to patient and tumor characteristics. We also aimed to examine if germline AR genotypes were associated with AR tumor expression. Finally, we aimed to elucidate whether AR tumor expression was prognostic and/or predictive for endocrine treatment either alone or in combination with ER.
Methods

Patients

The BC Blood Study is an ongoing epidemiological cohort study at the Skåne University Hospital in Lund, Sweden, exploring factors, which may be associated with prognosis and treatment response in primary breast cancer. Starting in October 2002, patients with primary breast cancers were invited to participate at the preoperative visit. Patients with a previous cancer during the last ten years were excluded.

The current study included patients enrolled between October 2002 and June 2012. After a written informed consent was signed, a questionnaire regarding lifestyle, reproductive factors, and medications was completed (10). Blood samples were taken and body measurements, including breast volume, were recorded by a research nurse. Breast volume was defined as the total bilateral breast volume among patients without previous breast surgeries and was measured with plastic cups (11, 12). Specific treatment data were collected from patient charts and follow-up questionnaires to enable assessment of adherence to the prescribed therapy (13). Follow-up included additional questionnaires and collection of clinical data after 3 to 6 months, 7 to 9 months, and after 1, 2, 3, 5, 7, 9 and 11 years from inclusion. Information on breast cancer events and date of death was obtained from patient charts, the regional tumor registry, and the population registry. Genotyping of the six haplotypes tagging single nucleotide polymorphisms in the AR (rs1337080, rs17302090, rs6152, rs7061037, rs5031002, and rs5964607) was done as previously described (9).

Between October 2002 and June 2012, a total of 1116 patients were included. Patients who had received preoperative treatment ($n=51$) and patients with only ductal carcinoma in-situ ($n=39$) were excluded. The total study cohort therefore consisted of 1026 patients (Figure 1).
The study was approved by the Lund University Ethics Committee (Dnr LU75-02, LU37-08, LU658-09, LU58-12, and LU379-12).

**Histopathological analyses**

Tumor tissue microarrays (TMAs) were constructed by sampling cores from representative non-necrotic tumor regions of formalin-fixed paraffin-embedded tissue blocks. Duplicate cores (1.0 mm) from the primary tumors were mounted into recipient blocks. For immunohistochemical analysis, 4-μm sections were automatically pre-treated using the PT Link system and stained for the monoclonal antibody of AR (clone AR441, dilution 1:200; Thermo Scientific, Fremont, California, US) in an Autostainer Plus (Dako, Glostrup, Denmark).

A senior breast pathologist (AE) was consulted to ensure assessability and invasiveness of the TMA sections. Scoring was then performed twice independently (KE), and blinded for clinical data. In cases of discrepancies (2%), a third scoring was done to reach consensus (KE+SB). Scoring included nuclear staining fractions (0%, 1%-10%, 11%-50%, 51%-75%, 76%-100%) and intensity score (negative, weak, moderate, strong). Tumors were considered AR+ if more than 10% of the nuclei were stained, independent of intensity. In an alternative analysis, a cut-off of >75 % of stained nuclei was used to define AR75 positive tumors (AR75+). If the duplicate cores were heterogeneous, the fraction of positively stained nuclei was estimated across both sampled cores.

Tumor characteristics collected from patient charts and pathology reports included invasive tumor size, histological grade, axillary lymph node involvement, ER, and progesterone.
receptor (PR) status (positive if greater than 10% nuclei were stained according to standard
clinical practice in Sweden) (14, 15). Human epidermal growth factor receptor 2
(HER2) assessment was routinely analyzed as of November 2005 in patients younger than 70
years. HER2 status determined by Fluorescent In Situ Hybridization (16) was thus included in
subgroup analyses of patients included in the study between November 2005 and June 2012
(n=738, of which 50 patients (6.8%) had missing HER2 status), Figure 1.

Statistical analyses
All analyses were performed using SPSS Statistics 19 (IBM, Chicago, Illinois, US). The
anthropometric variables weight (kgs), height (m), body mass index (BMI) (kgs/m²) and
waist-to-hip ratio were used as continuous variables. Breast volume was analyzed as a
continuous variable and as a dichotomous variable (≥850 mL; yes/no) as per previous reports
from this cohort (11, 12). Reproductive factors such as age at menarche and age at first full-
term pregnancy were used as continuous variables (years) whereas parity (yes/no), current
smoker prior to surgery (yes/no), and abstainer (yes/no) were used as dichotomous variables.
Information on treatment by last follow-up prior to any event was entered as dichotomous
variables defined as patients who had received postoperative chemotherapy (yes/no),
radiotherapy (yes/no) and endocrine therapy (yes/no), respectively. The endocrine therapy
group was stratified according to TAM treatment (yes/no) and AI treatment (yes/no).
Trastuzumab treatment (yes/no) was entered as a co-variate in subgroup analyses of patients
included as of November 2005. Tumor characteristics were categorized as invasive
pathological tumor size (pT; 1–4 or 2+), pathological axillary lymph node involvement (pN;
yes/no) or number of involved lymph nodes (0, 1–3, 4+), and histological grade (I–III or I–II
vs. III).
Associations between patient and tumor characteristics and AR status were assessed. Since some variables were not normally distributed, the Mann-Whitney U-test was used. Categorized variables were analyzed using Chi-square tests and logistic regression; odds ratios (ORs) with 95% confidence intervals (CIs) are presented.

Disease-free survival (DFS) in relation to AR status was assessed by the Kaplan-Meier method and the LogRank test. Crude and adjusted Cox proportional hazards regression models provided hazard ratios (HRs) with 95% CIs. Adjustments were performed using four models. Model 1: age (continuous) and tumor characteristics (pT 2+ yes/no, pN yes/no, histological grade I–II vs. III, ER status [+/-], and AR status [+/-]). Model 2: age, tumor characteristics, BMI (≥25 kg/m² yes/no), and smoking (yes/no). Model 3: age, tumor characteristics, and treatment (chemotherapy yes/no, radiotherapy yes/no, TAM yes/no, AI yes/no). Model 4: model 3 variables with the addition of trastuzumab treatment yes/no and restricted to patients included as of November 2005. An interaction term between ER and AR was calculated and used in the Cox regression analysis, and adjustments using models 1, 2 and 3 were performed. Thereafter, combined ER and AR status (ER+AR+, ER-AR-, ER+AR- or ER-AR+) were used in the remaining multivariable analyses, using ER+AR+ as the reference group.

Survival was calculated from inclusion to a first breast cancer event, death from non-breast cancer related cause or last follow-up by 30th of June 2014, whichever came first. Patients with distant metastases detected on the postoperative metastases screen at 0.3 years of inclusion (n=8) or earlier were excluded from the survival analyses. Finally, patients with tumors without available AR status (n=113) were excluded, resulting in 905 patients in the
survival analyses (Figure 1). Breast cancer events were defined as local or regional
recurrences, contralateral cancer, or distant metastases.

Prior power calculations assuming 900 patients with an accrual interval of 10 years and
additional follow-up time of 0.5 years and a frequency of 15% AR- tumors showed that the
study was able to detect true HRs between 0.731 and 1.403 with 80% power and alpha of 5%
(Power and Sample size calculation program, PS, version 3.0, developed by Dupont and
Plummer; http://biostat.mc.vanderbilt.edu/wiki/Main/).

P-values < 0.05 were considered significant. All P-values were two-tailed. Since this was an
exploratory study, nominal P-values are presented without adjustments for multiple testing.
The report follows the REMARK criteria (17).
Results

AR in relation to patient characteristics

Eighty-nine percent of the patients had tumors for which AR status were available (n=913). The majority of tumors were AR+ (n=776, 85.0%; Figure 1). Higher age at diagnosis was significantly associated with AR+ (Table 1). This association remained significant among patients younger than 50 years (P=0.008), indicating that the association was driven by the younger patients (Supplementary Table 1). Smaller breast volumes (<850mL) were more common among patients with AR+ tumors (Table 1). This association was more apparent among patients aged less than 50 years, where an association was seen both for the continuous (P=0.03) and the dichotomized variables (OR=0.45; 95% CI 0.20-1.03; Supplementary Table 1). No other anthropometric measure was associated with AR status. Reproductive factors, exogenous hormone use, and smoking and alcohol habits were not associated with AR status. Tumor AR expression was not significantly associated with the germline AR diplotypes (data not shown).

AR in relation to tumor characteristics

Tumors <21 mm were more likely to be AR+ compared to larger tumors or tumors with skin or muscle involvement independent of size. No association between axillary lymph node involvement and AR status was observed. Lower histological grade was significantly associated with AR+ tumors. Tumor AR was highly co-expressed with ER and PR status. HER2 amplified tumors were evenly distributed between AR+ and AR- tumors. However, AR+ was significantly positively associated with HER2 amplification in the ER-PR- tumors (Table 2).
AR and disease-free survival

Patients were followed for up to 11 years (median follow-up for patients still at risk 5.0 years), and 107 events were observed. In general, patients with AR- tumors had significantly worse prognoses compared to patients with AR+ tumors (Figure 2A, Table 3). However, stratification by ER status revealed AR was a positive prognostic marker in patients with ER+ tumors, but conferred a worse prognosis in patients with ER- tumors (Figures 2B-2D, Table 3). A significant interaction between AR and ER expressions was seen ($P_{\text{interaction}}=0.010$ univariable and adjusted; $P_{\text{interaction}}=0.019$ (model 1), $P_{\text{interaction}}=0.024$ (model 2), $P_{\text{interaction}}=0.014$ (model 3) adjusted for age and tumor characteristics, BMI and smoking, or treatment.

Multivariable analyses for combined ER and AR status showed worse prognosis for the ER-AR+ tumors compared to all other combinations (Table 3). The ER-AR+ tumors showed significantly worse prognosis compared to ER+AR+ tumors. This association remained after adjusting for age and tumor characteristics (model 1), when BMI and smoking were added to the model (model 2), and when adjustments for treatment were added to the model (model 3). When breast size was added to model 2, results remained essentially the same (data not shown). In model 3, which incorporated adjuvant treatment data, double negative (ER-AR-) tumors demonstrated a reduced risk compared to the other models. Since tumors with discordant ER and AR status (ER+AR- or ER-AR+) showed worse prognosis compared to concordant ER and AR status (ER+AR+ or ER-AR-), a variable for discordant ER and AR status was constructed. Tumors of discordant ER and AR status demonstrated significantly worse prognosis compared to concordant ER and AR status in all multivariable models, including model 4, which was based on the subgroup of patients with data on trastuzumab treatment (Table 3).
AR as a treatment-predictive marker

Tumor AR status did not provide a treatment predictive value for adjuvant chemotherapy alone or in combination with endocrine treatment. This was analyzed among all patients who ever received chemotherapy, and in patients with ER- tumors who received chemotherapy only, as well as in patients with ER+ tumors who received chemotherapy followed by endocrine treatment (all LogRank $P$s $\geq 0.17$). In order to explore whether AR status had a treatment predictive value for endocrine treatment, patients with ER+ tumors who had not received chemotherapy were included in further analyses. Further, patients aged less than 50 years were excluded because AIs are rarely prescribed to premenopausal patients.

Tumor AR status did not provide a prognostic value among patients who never received endocrine treatments (Figure 3A). However, AR- was predictive of early failure of sequential treatments with TAM/AI or AI/TAM (Figure 3B). The association was weaker among patients who had ever received TAM, including those sequentially treated with AIs (Figure 3C), whereas the predictive value of AR remained strong in the subgroup of patients who had ever received AIs, including those sequentially treated with TAM (Figure 3D). To further differentiate between TAM and AIs, patients with endocrine monotherapy were analyzed separately. Still, AR- suggested early failure of AI treatment (Figure 3F) whereas no predictive value was found for patients who received TAM alone (Figure 3E).

High AR expression (>75%) as an alternative cut-off for AR positivity

Since AR overexpression may impact on endocrine treatment response, analyses were repeated with an alternative cut-off of >75 % stained nuclei for AR (AR$_{75^+}$). This resulted in a near equal distribution of AR$_{75^+}$ tumors (n=463, 50.7%) and AR$_{75^-}$ negative (AR$_{75^-}$) tumors.
(n=450, 49.3%). However, the associations between tumor AR status and age, invasive tumor size and breast size were lost. The associations between AR status and grade or ER/PR co-expression were weakened with this cut-off. The association between AR status and HER2 amplification in the ER-PR- subgroup was lost. In contrast, a new association between AR75 negativity (AR75-) and HER2 amplification emerged among all tumors unselected for ER status. The prognostic role of AR75+ in the entire cohort and in the ER+ subgroup was lost, as well as the interaction between AR75+ and ER status (data not shown). In terms of treatment response, there was still no association between AR75 status and DFS in the group of TAM-treated patients 50 years and older, who had not received chemotherapy. Similarly, the early events seen among the AI treated patients with AR75- tumors remained with the new cut-off and became stronger. Among the patients who received AI only, there were six events among 33 patients with AR75- tumors, and only one event among the 53 patients with AR75+ tumors (LogRank \( P=0.023 \), adjusted HR (model 1) 7.18 (0.84-61.53), \( P=0.072 \)).
Discussion

In this study, the prognostic value of AR expression was significantly different depending on the ER status of the tumor. Concomitant AR and ER expression was associated with superior prognosis compared to all other AR/ER combinations. In contrast, AR expression among ER- tumors presented a worse prognosis than ER-AR- tumors. Similar findings have been reported by others (18, 19).

No association was seen between tumor AR expression and the AR germline diplotypes that we previously reported were associated with response to TAM but not AI treatment (9). In contrast, lack of tumor AR expression was predictive of early failure of AI treatment among postmenopausal patients who never received chemotherapy. To our knowledge, this has not been reported previously.

The prognostic role of tumor AR expression in ER+ breast cancer has consistently been reported to be associated with favorable clinical outcomes (18-24). Results from clinical studies on ER- breast cancer have, however, been inconsistent, demonstrating positive or negative as well as no associations of AR with clinical outcomes (2, 18-20, 25-27). Many factors may have contributed to the reported inconsistent results; small sample sizes, heterogeneity of study populations, selection of included studies in the meta-analyses and lack of standardized methods and cut-offs for AR assessment but also for ER assessment, and the biological heterogeneity among ER- tumors.

The differential role of AR depending on ER status has been suggested to be related to the competitive interaction between AR and ER (20). In the presence of ER, AR interacts with estrogen response elements on ER, blocking downstream estrogen target genes thus
inhibiting ER-stimulated tumor growth (28, 29). In the absence of ER, AR instead interacts
with androgen receptor elements and functions as an oncogene promoting tumor growth
(28). Recently, three reviews highlighted this complexity and called for awareness when
implementing AR targeting agents in clinic practice; certain settings might require anti-
androgens, whereas in other settings androgen agonists, such as selective androgen
receptor modulators (30), might be warranted (8, 26, 31).

In this study, a significant interaction between AR and ER expression with respect to DFS
was found, indicating the importance of stratifying according to ER status in analyses of
the prognostic value of AR. In accordance with this finding, results from the Nurses’
Health Study also reported that AR positivity confers a negative prognosis for patients with
ER- breast cancer and demonstrated a formal interaction analysis, which to our knowledge
has not been performed by other groups. Further, it was shown that discordant ER and AR
expression conferred a worse prognosis than concordant ER and AR expression (19),
which corresponds to the findings from our multivariable analyses. Herein, the increased
risk for early events among the patients with double negative tumors (ER-AR-) was no
longer evident in the multivariable model adjusted for treatment in all patients, indicating
that chemotherapy had the intended effect for this group of patients, and that chemotherapy
may be a confounder in this setting. In the model where trastuzumab treatment was added
to the multivariable model, ER-AR- tumor status was associated with worse prognosis than
ER+AR+ tumor status but better prognosis than discordant tumors. Since this model only
included a subgroup of patients it is not fully comparable to the other models. However,
patients with ER-AR+ tumors had the worst outcome compared to all other groups,
irrespective of the adjustment model used. The anti-androgen enzalutamide,
(ClinicalTrials.gov identifier: NCT01889238) (6, 7, 32) or indirect modulators such as the
CYP17 inhibitor abiraterone acetate (ClinicalTrials.gov identifier: NCT00755885) could be novel treatment options in the group of ER-AR+ patients. Recently, abiraterone acetate with or without concomitant exemestane was reported not to improve outcome in metastatic breast cancer patients. However, all patients had ER+ tumors and were unselected for AR status (33).

Previous studies on endocrine treatment have, to the best of our knowledge, not reported the predictive value of AR stratified by type of endocrine treatment. However, this would be of value because TAM and AI inhibit breast cancer growth by different mechanisms (28). The AIs block androgen conversion to estrogens resulting in very low circulating estradiol levels (34). They might also confer higher androgen levels during treatment (35). Studies have indicated that increased androgens and AR expression following AI treatment may contribute to reduced tumor cell proliferation. This was explained by the growth inhibitory effect of androgens via the AR being revealed in the low estrogen environment (36, 37). Recently, Patani et al compared the transcriptional response to the AI anastrazole with the transcriptional response to the selected ER down-regulator, fulvestrant. Compared to anastrazole mediated estrogen reduction, fulvestrant treatment induced a stronger and more differential transcriptional response, potentially attributable to arrest of estrogen independent ERalpha activity. The study suggested the involvement of AR associated genes, and it would therefore be of future interest to evaluate AR status in relation to fulvestrant treatment response (38).

Hickey et al. has suggested there may be scope for revisiting the combination TAM and androgen treatment in the subgroup of patients not reaching optimal blockage by TAM alone (28). The preclinical findings indicating AR overexpression as a novel mechanism of
endocrine resistance (4, 5), could not be confirmed in our study. When analyses were repeated using the high cut-off AR75, the finding of early events among patients with AR75-tumors remained. Thus, our results indicate that patients with ER+AR- do not benefit from AIs and that alternative treatment strategies should be considered for this group of patients. Since the associations between AR75 status and conventional tumor characteristics and prognosis were less pronounced, and the interaction between AR and ER status disappeared, the original cut-off (>10%) for AR+ was assumed to be more biologically relevant and of higher clinical utility to guide clinicians in terms of prognosis and treatment selection. Other groups have previously used the same antibody with a cut-off at 10% or lower (19, 39-42). The AR/ER ratio has been reported to predict endocrine resistance (43). Unfortunately, our data do not allow assessment of the AR/ER ratio. We also did not find associations between AR and chemo/endocrine-therapy, as previously shown by others (3, 21).

A strength of the current study is that it was a large prospective population-based study. Participation and follow-up rates were high, and included and non-included patients were similar with respect to age and tumor characteristics (9, 44). However, the follow-up was relatively short, and the majority of patients had ER+ tumors, which tend to metastasize late (45). Since the study was population-based, no randomization of treatment was performed. Thus, analyses of the potential treatment predictive value of AR status were restricted to comparisons within treatment groups. Also, patients with more aggressive disease tended to be treated with AIs rather than TAM only. For example, patients aged 50 and older with ER+ tumors who received AI only were over 30-fold more likely to have node positive disease than those treated with TAM only. Since AR and ER are often co-expressed, the expected number of AR- tumors in the analyses of endocrine treatment
response was low and the subgroup analyses should therefore be interpreted with caution.

No predictive value was seen within the chemotherapy group; however chemotherapy regimens differed somewhat during follow-up. Ki67 analysis was routinely introduced as of March 2009 (9), and thus were not incorporated in the present study, though it would be of interest in future studies. The distribution of patient and tumor characteristics for patients with available AR status was comparable to those without available AR status, suggesting that TMAs were representative.

In conclusion, the prognostic value of AR expression was significantly different depending on the ER status of the tumor. Patients with discordant ER and AR tumor expression had significantly worse prognosis compared to patients with concordant ER and AR tumor expression. Depending on the combined ER/AR status, patients may benefit from new treatment options, such as anti-androgens or selective androgen receptor modulators. Finally, AR negativity was found to be predictive of early failure of AI but not TAM treatment, a finding that warrants confirmation in a randomized setting.
Acknowledgements

We wish to thank our research nurses Anette Ahlin Gullers, Monika Meszaros, Maj-Britt Hedenblad, Karin Henriksson, Anette Möller, Helén Thell, Jessica Åkesson, and Linda Ågren. We also wish to thank Erika Bågeman, Maria Henningson, and Maria Hjertberg for data entry, Björn Nodin and Elise Nilsson for TMA construction, Kristina Lövgren for staining, and Catarina Blennow for sectioning, as well as breast pathologist Anna Ehinger (AE) for help with histopathological assessments.

References


### Table 1. Patient characteristics by AR status.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>All Missing total</th>
<th>Patients with available tumor AR status</th>
<th>Missing AR status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 1026</td>
<td>AR negative n = 137</td>
<td>Missing AR status n = 113</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AR positive n = 776</td>
<td>Median (IQR) or %</td>
</tr>
<tr>
<td>Age at diagnosis, yrs</td>
<td>61.1 (52.1-68.1)</td>
<td>59.4 (49.0-68.0)</td>
<td>0.036 60.0 (47.8-68.1)</td>
</tr>
<tr>
<td>Weight, kgs</td>
<td>69.0 (62.0-78.0)</td>
<td>70.0 (60.9-78.6)</td>
<td>0.99 67.8 (61.8-76.0)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.65 (1.62-1.70)</td>
<td>1.65 (1.61-1.70)</td>
<td>0.39 1.66 (1.62-1.69)</td>
</tr>
<tr>
<td>BMI, kgs/m²</td>
<td>25.1 (22.5-28.3)</td>
<td>25.1 (22.5-28.9)</td>
<td>0.97 24.8 (22.3-27.7)</td>
</tr>
<tr>
<td>Waist-Hip Ratio, m/m</td>
<td>0.86 (0.81-0.90)</td>
<td>0.86 (0.81-0.91)</td>
<td>0.96 0.85 (0.80-0.90)</td>
</tr>
<tr>
<td>Total breast volume, mL</td>
<td>1000 (650-1500)</td>
<td>1050 (700-1650)</td>
<td>0.12 1000 (625-1300)</td>
</tr>
<tr>
<td>≥850mL, %</td>
<td>57.3 (160)</td>
<td>65.2 (160)</td>
<td>57.0</td>
</tr>
<tr>
<td>Age at menarche, yrs</td>
<td>13 (12-14)</td>
<td>13 (12-14)</td>
<td>0.24 13 (13-14)</td>
</tr>
<tr>
<td>Parous, %</td>
<td>87.9 (1)</td>
<td>83.9 (1)</td>
<td>1.47 (0.89-2.45)</td>
</tr>
<tr>
<td>Age at first full term pregnancy, yrs</td>
<td>25 (22-28)</td>
<td>25 (21-29)</td>
<td>0.29 25 (22-28)</td>
</tr>
<tr>
<td>Ever use of oral contraceptives, %</td>
<td>70.8 (1)</td>
<td>75.2 (1)</td>
<td>0.75 (0.49-1.14)</td>
</tr>
<tr>
<td>Ever use of HRT, %</td>
<td>43.9 (3)</td>
<td>37.5 (45.9)</td>
<td>1.41 (0.97-2.05)</td>
</tr>
<tr>
<td>Current smoker prior to surgery, %</td>
<td>20.5 (2)</td>
<td>24.8 (19.5)</td>
<td>0.73 (0.48-1.12)</td>
</tr>
<tr>
<td>Abstainer, %</td>
<td>10.5 (7)</td>
<td>11.8 (10.4)</td>
<td>0.87 (0.49-1.54)</td>
</tr>
</tbody>
</table>

*Bold letters indicate statistically significant results.

Abbreviations: AR = androgen receptor; BMI = body mass index; CI = confidence interval; HRT = hormone replacement therapy; IQR = interquartile range; OR = odds ratio.

p25
**Table 2. Tumor characteristics by AR status.**

<table>
<thead>
<tr>
<th>Tumor characteristics</th>
<th>All Missing</th>
<th>AR status</th>
<th>Patients with available tumor AR status</th>
<th>Missing AR status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 1026 n (%)</td>
<td>AR negative</td>
<td>AR positive P-value or OR (95% CI)</td>
<td>AR positive n (%)</td>
</tr>
<tr>
<td><strong>N伞Missing Patients with available tumor AR status</strong></td>
<td></td>
<td>n = 137 n (%)</td>
<td>n = 776 n (%)</td>
<td>n = 113 n (%)</td>
</tr>
<tr>
<td><strong>Tumor characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Invasive tumor size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (≤20 mm)</td>
<td>740 (72.1)</td>
<td>89 (65.0)</td>
<td>564 (72.7)</td>
<td>Ref. 87</td>
</tr>
<tr>
<td>2 (21-50 mm)</td>
<td>269 (26.2)</td>
<td>46 (33.6)</td>
<td>199 (25.6)</td>
<td>24</td>
</tr>
<tr>
<td>3 (&gt;51 mm)</td>
<td>15 (1.5)</td>
<td>2 (1.5)</td>
<td>11 (1.4)</td>
<td>0.70 (0.47-1.02) 2</td>
</tr>
<tr>
<td>4 (skin or muscular involvement independent of size)</td>
<td>2 (0.2)</td>
<td>0</td>
<td>2 (0.3)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Axillary lymph node involvement</strong></td>
<td>2</td>
<td>0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>627 (61.2)</td>
<td>86 (63.2)</td>
<td>466 (60.1)</td>
<td>Ref. 75</td>
</tr>
<tr>
<td>1-3</td>
<td>306 (29.9)</td>
<td>35 (25.7)</td>
<td>239 (30.8)</td>
<td>1.14 (0.78-1.66) 32</td>
</tr>
<tr>
<td>≥4</td>
<td>91 (8.9)</td>
<td>15 (11.0)</td>
<td>70 (9.0)</td>
<td>6</td>
</tr>
<tr>
<td><strong>Histological grade</strong></td>
<td>1</td>
<td>&lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>252 (24.6)</td>
<td>16 (11.7)</td>
<td>202 (26.0)</td>
<td>34</td>
</tr>
<tr>
<td>II</td>
<td>511 (49.9)</td>
<td>43 (31.4)</td>
<td>416 (53.6)</td>
<td>Ref. 52</td>
</tr>
<tr>
<td>III</td>
<td>262 (25.6)</td>
<td>78 (56.9)</td>
<td>158 (20.4)</td>
<td>0.19 (0.13-0.28) 26</td>
</tr>
<tr>
<td><strong>Hormone receptor status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>896 (87.5)</td>
<td>73 (53.7)</td>
<td>726 (93.6)</td>
<td>12.53 (8.05-19.50) 97</td>
</tr>
<tr>
<td>PR+</td>
<td>726 (70.9)</td>
<td>55 (40.4)</td>
<td>594 (76.5)</td>
<td>4.81 (3.29-7.03) 77</td>
</tr>
<tr>
<td><strong>Combined ER and PR status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-PR-</td>
<td>122 (11.9)</td>
<td>61 (44.9)</td>
<td>46 (5.9)</td>
<td>Ref. 15</td>
</tr>
<tr>
<td>ER-PR+</td>
<td>6 (0.6)</td>
<td>2 (1.5)</td>
<td>4 (0.5)</td>
<td>2.65 (0.47-15.11) 0</td>
</tr>
<tr>
<td>ER-PR-</td>
<td>176 (17.2)</td>
<td>20 (14.7)</td>
<td>136 (17.5)</td>
<td>9.02 (4.92-16.52) 20</td>
</tr>
<tr>
<td>ER-PR+</td>
<td>720 (70.3)</td>
<td>53 (39.0)</td>
<td>590 (76.0)</td>
<td>14.76 (9.18-23.73) 77</td>
</tr>
<tr>
<td><strong>HER2 amplification, November 2005 - onwards</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86 (12.5)</td>
<td>11 (14.1)</td>
<td>57 (10.6)</td>
<td>0.73 (0.36-1.45) 18</td>
</tr>
<tr>
<td>ER-PR- subgroup&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28 (32.9)</td>
<td>0</td>
<td>7 (15.6)</td>
<td>5.43 (1.88-15.72) 5</td>
</tr>
<tr>
<td><strong>Treatment by last follow-up</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ever chemotherapy</td>
<td>259 (25.2)</td>
<td>0</td>
<td>57 (41.6)</td>
<td>169 (21.8)</td>
</tr>
<tr>
<td>ever endocrine therapy</td>
<td>694 (77.5)</td>
<td>0</td>
<td>61 (83.6)</td>
<td>567 (78.1)</td>
</tr>
<tr>
<td>ever tamoxifen</td>
<td>528 (58.9)</td>
<td>0</td>
<td>55 (75.3)</td>
<td>420 (57.9)</td>
</tr>
<tr>
<td>ever aromatase inhibitor</td>
<td>345 (38.5)</td>
<td>0</td>
<td>22 (30.1)</td>
<td>289 (39.8)</td>
</tr>
<tr>
<td>ever radiotherapy&lt;sup&gt;e&lt;/sup&gt;</td>
<td>641 (62.5)</td>
<td>0</td>
<td>89 (65.0)</td>
<td>489 (63.0)</td>
</tr>
<tr>
<td>ever trastuzumab&lt;sup&gt;f&lt;/sup&gt;</td>
<td>November 2005 and onwards</td>
<td>66 (8.9)</td>
<td>0</td>
<td>7 (8.2)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Chi Square 3 df
<sup>b</sup>Chi Square 2df
<sup>c</sup>HER2 status routinely analyzed in patients <70 years with invasive tumors as of November 2005. In total 738 patients were included in the study from November 2005 to June 2012, among which 688 were tested for HER2 status and 50 had missing HER2 status.
<sup>d</sup>Among the 738 patients included as of November 2005, totally 85 patients had ER-PR- tumors all of which were tested for HER2 status.
<sup>e</sup>Patients may have received more than one type of treatment.
<sup>f</sup>Data on trastuzumab treatment was available for all patients as of November 2005. However, 50 patients (6.8%) had missing HER2 status.

Bold letters indicate statistically significant results.

Abbreviations: AR = androgen receptor; CI = confidence interval; df = degree of freedom; ER = estrogen receptor; HER2 = human epidermal growth factor-2; OR = odds ratio; PR = progesterone receptor.
Table 3. Disease-free survival by AR, ER and combinations of ER and AR status.

<table>
<thead>
<tr>
<th>Tumor status</th>
<th>Total</th>
<th>Events</th>
<th>Missing</th>
<th>Crude HR</th>
<th>Adjusted HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>All</td>
<td>905</td>
<td>655</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR-</td>
<td>133</td>
<td>107</td>
<td>0</td>
<td>1.67</td>
<td>0.026</td>
</tr>
<tr>
<td>ER-</td>
<td>109</td>
<td>107</td>
<td>1</td>
<td>2.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER+AR+</td>
<td>723</td>
<td>67</td>
<td></td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>ER-AR-</td>
<td>60</td>
<td>9</td>
<td>1</td>
<td>1.98</td>
<td>0.054</td>
</tr>
<tr>
<td>ER+AR-</td>
<td>72</td>
<td>16</td>
<td></td>
<td>1.91</td>
<td>0.021</td>
</tr>
<tr>
<td>ER+AR+</td>
<td>49</td>
<td>15</td>
<td></td>
<td>3.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER+AR- or ER-AR+</td>
<td>121</td>
<td>107</td>
<td>1</td>
<td>2.38</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
</tbody>
</table>

^aAdjusted for age (continuous), invasive tumor size (<21 mm versus ≥21 mm or skin or muscular involvement independent of size), axillary lymph node involvement (yes/no) and tumor grade III (yes/no). Adjusted for ER status (+/-) in AR only analysis, and for AR status (+/-) in ER only analysis. Missing data for 3 patients.

^bAdjusted for Body mass index ≥25.0 kg/m2 (yes/no) and preoperative current smoking (yes/no). Missing data for 31 patients.

^cAdjusted for treatment; tamoxifen, aromatase inhibitors, chemotherapy, and radiation therapy. Missing data for 3 patients.

^dPatients included as of November 2005. In total 655 patients and 46 events. Missing data for 1 patient.

^eAdjusted for trastuzumab treatment.

Bold letters indicate statistically significant results.

Abbreviations: AR = androgen receptor; CI = confidence interval; ER = Estrogen receptor; HR = hazard ratio.
Figure legends

Figure 1. Flow chart of the study population included in various analyses.

Figure 2. The prognostic role of AR alone and in combination with ER. Kaplan-Meier estimates of disease-free survival for A) all patients (n = 905) by AR status, B) ER negative patients by AR status, C) ER positive patients by AR status, D) combinations of AR and ER status, and E) discordant versus concordant AR and ER status (ER+AR- and ER-AR+ vs. ER+AR+ and ER-AR-). Because this is an ongoing cohort, the number of patients decreased with each follow-up. Bold letters indicate statistically significant results.

Figure 3. The predictive role of AR by type of endocrine treatment. Kaplan-Meier estimates of disease-free survival by AR status for patients aged at least 50 years with ER+ tumors who did not receive chemotherapy, regardless of radiotherapy (n = 573). A) Patients who did not receive endocrine therapy (n = 135). B-F) Subgroups of endocrine treatments by TAM and/or AIs (n = 438). Patients may appear in more than one of these graphs. Because this is an ongoing cohort, the number of patients decreased with each follow-up. Bold letters indicate statistically significant results.

Additional material provided:
Figure 1
Figure 2
Figure 3
Supplementary table 1
Patients with primary breast cancer
Oct 2002-June 2012
n = 1116

Preoperative treatment
n = 51

In situ carcinoma
n = 39

Invasive breast cancer
N = 1026

Missing AR score
n = 113

Available AR score
n = 913

AR positive
n = 776 (85.0%)

AR negative
n = 137 (15.0%)

Distant metastasis ≤ 0.3 years from baseline
n = 8

Included in survival analyses
n = 905

AR positive
n = 772 (85.3%)

AR negative
n = 133 (14.7%)

Routine HER2 assessment initiated as of November 2005
HER2 +
n = 86
HER2 -
n = 602
HER2 missing
n = 50

Routine HER2 assessment initiated as of November 2005
HER2 +
n = 66
HER2 -
n = 543
HER2 missing
n = 46

By systemic treatments of total n=905

Ever chemotherapy:
ER+

ever endocrine therapy:
→ with chemotherapy:
→ without chemotherapy

<50 years (n=166)
≥50 years (n=739)

Systemic treatments by age

Ever chemotherapy:
ER+

ever endocrine therapy:
→ with chemotherapy:
→ without chemotherapy

n_{ER+all}=795 (1 missing)
628
143
485
n_{ER+50yr}=139
110
63
47
n_{ER+>50yr}=656 (1 missing)
138
80
438
Figure 2.

A) All patients by AR status (n = 905)

B) Strata ER- by AR status (n= 109)

C) Strata ER+ by AR status (n= 795)

D) All patients by combined ER and AR status

E) All patients by discordant ER/AR vs concordant ER/AR
Figure 3.

A) No endocrine treatment (n = 135)

![Graph showing disease-free survival with LogRank P = 0.31]

B) Sequential TAM/Al or Al/TAM (n = 126)

![Graph showing disease-free survival with LogRank P = 0.019]

C) TAM or sequential TAM/Al or Al/TAM (n = 352)

![Graph showing disease-free survival with LogRank P = 0.21]

D) Al or sequential TAM/Al or Al/TAM (n = 212)

![Graph showing disease-free survival with LogRank P = 0.008]

E) TAM but no Al (n = 226)

![Graph showing disease-free survival with LogRank P = 1.00]

F) Al but no TAM (n = 86)

![Graph showing disease-free survival with LogRank P = 0.061]
Combined Androgen and Estrogen Receptor Status in Breast Cancer: Treatment Prediction and Prognosis in a Population-based Prospective Cohort

Karin Elebro, Signe Borgquist, Maria Simonsson, et al.

Clin Cancer Res Published OnlineFirst April 22, 2015.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-2564

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2015/04/23/1078-0432.CCR-14-2564.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/early/2015/04/22/1078-0432.CCR-14-2564. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.