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EPIDEMIOLOGY

Age interacts with the expression of steroid and HER-2 receptors in operable invasive breast cancer

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Abstract
Background: The negative association between the oestrogen receptor (ER) and the human epidermal growth factor receptor 2 (HER-2) in breast cancer travels in both directions. ER^+ tumours are less likely $HER\text{-}2^+$ and $HER\text{-}2^+$ tumours are less likely ER^+.
Methods: We studied the age-related immunohistochemical (IHC) expression of ER, progesterone receptor (PR) and HER-2 in 2,227 tumours using age as a continuous variable. Steroid receptors were considered positive for any nuclear staining of invasive cancer cells and for HER-2, either for strong expression by IHC (score 3^+) or gene amplification by fluorescence in situ hybridisation (FISH). Based on nonparametric regression, the age-related association between steroid receptors and HER-2 was presented as likelihood curves.
Results: The association between ER or PR and HER-2 is age-related. The age-related expression of ER and PR is HER-2 dependent. In $HER\text{-}2^-$ cases, the odds ratio (OR) for being ER^+ was 2.594 (95% CI = 1.874–3.591) up to age 50 and age-independent thereafter; for PR-expression the OR was 2.687 (95% CI = 1.780–4.057) up to age 45 and 0.847 (95% CI = 0.761–0.942) thereafter. In $HER\text{-}2^+$ cases, the OR was 0.806 (95% CI = 0.656–0.991) to be ER^+ and 0.722 (95% CI = 0.589–0.886) to be PR^+. The age-related OR for breast cancers to be $HER\text{-}2^+$ is steroid receptor dependent. Taking together, $ER^+PR^+HER\text{-}2^+$ breast cancers appear on average 5.4 years earlier than breast cancers of any other ER/PR/HER-2 phenotype (95% CI = 3.3–7.5; $P < 0.0001$).
Conclusion: There is a qualitative interaction between age and expression of steroid and HER-2 receptors. Our findings suggest a strong age-related selective growth advantage for breast tumour cells belonging to the $ER^+PR^+HER\text{-}2^+$ subgroup.

Keywords Breast cancer · Human epidermal growth factor receptor 2 · Hormone receptors · Interaction

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Introduction

Both steroid receptors and human epidermal growth factor receptor 2 (HER-2) are major biomarkers of breast cancer. Their expression correlates with patient age at diagnosis (1,2). With ageing, ER is more and HER-2 is less likely expressed. There is functional signalling between both pathways of growth also referred to as cross talk (3). $HER\text{-}2^+$ tumours are less likely ER^+ and ER^+ tumours are less likely $HER\text{-}2^+$ (4–9). We and others confirmed in multivariate analyses that loss of PR correlated with HER-2 overexpression in women with an ER^+ breast cancer (7,8). In women with an ER^+ breast cancer, HER-2 predicts response to anti-oestrogens (10–13) but an age difference suggests a qualitative age interaction of functional signalling between steroid and growth factor receptors. Love et al. observed that premenopausal hormone responsive breast cancers remain sensitive to endocrine therapy if HER-2 is also overexpressed (14). In postmenopausal women however, retrospective analyses of clinical studies indicate that ER^+ breast cancers are not entirely endocrine sensitive to tamoxifen or aromatase inhibitors if HER-2 is over-expressed (15–17). In particular, results from central review of ER, PR and HER-2 in the trans-Breast International Group (transBIG 1–98) and *trans*-Anastrazole or Tamoxifen alone or in Combination (*Trans*-ATAC) studies indicate that in the adjuvant setting $HER\text{-}2^+$ and hormone receptor-positive breast cancer tends to be less sensitive to tamoxifen and both aromatase inhibitors than $HER\text{-}2^-$ hormone receptor-positive disease.

We previously suggested an age-related difference for the inverse association between steroid and HER-2 receptors (9) but this has been questioned on the base of low numbers (18). In this larger series, we studied the age-related association between steroid receptors and HER-2 in primary operated breast cancers using age as a continuous variable. We also calculated the mean age at diagnosis and the age-related prevalence of breast cancers by the combined ER/PR/HER-2 status.

Patients and methods

Patients

Charts from 2,227 consecutive women receiving primary surgery for invasive breast cancer, treated between Jan 2000 and Dec 2005 at Leuven University Hospital, were retrospectively evaluated. All had the steroid receptor and HER-2 status of the tumour available.

Measurement of ER, PR and HER-2

Expression was demonstrated by IHC according to the Envision method using the following primary monoclonal antibodies NLC-ER-6F11 for ER, NCL-PgR-312 for PR and CB11 for HER-2 (Novocastra Laboratories, Newcastle-on-Tyne, UK). Since 2005, highly sensitive rabbit monoclonal antibodies were used for the assessment of ER and PR expression (SP1 and SP2, respectively, Labvision Corporation, Fremont, CA, USA). IHC staining was performed according to standard procedures for clinical purposes as previously described (7). For ER and PR, any nuclear staining of invasive tumour cells was considered as positive. For HER-2, either strong expression by IHC (score 3^+) or HER-2 gene amplification by FISH was considered $HER\text{-}2^+$. The following 6 subgroups were distinguished: $ER^-PR^-HER\text{-}2^-$, $ER^-PR^-HER\text{-}2^+$, $ER^+PR^-HER\text{-}2^-$, $ER^+PR^-HER\text{-}2^+$, $ER^+PR^+HER\text{-}2^-$, $ER^+PR^+HER\text{-}2^+$. The last group is further called triple positive (TP).

Statistical analysis

The *t*-test was used to analyse age differences between the 6 subgroups since age appeared to be normally distributed in each subgroup.

The age-related association between steroid receptors and HER-2 was presented as likelihood curves based on nonparametric regression first for being steroid receptor positive by HER-2 and second for being $HER\text{-}2^+$ by the combined steroid receptor status. The main analysis tool consisted of nonparametric regression using the generalized additive models (GAM) theory (19). Whereas logistic regression links the logit transformation of the probability P (e.g. probability of an ER^+ tumour) to a linear combination of predictor variables, GAM links the logit of that probability to an arbitrary smooth function f: "$\log(P/1-P) = \alpha + \beta X$" versus "$\log(P/1-P) = \alpha + f(X)$" where β is a constant. This method makes it possible to model nonlinear relationships between a predictor and the outcome. The effect of age was modelled by a smooth function (using a cubic smoothing spline) rather than by one number such as the odds ratio (OR) in logistic regression. This allows one to detect nonlinear relationships. After the nonparametric regression, the obtained regression lines were approximated using OR resulting from traditional logistic regression. If the nonlinearity in the logit assumption (20) was violated, adjustments to the logistic regression model were made to capture the

nonlinearity as good as possible. ORs were always computed for each decade increase in age. All analyses were performed in SAS v9.1 (SAS Institute, Cary, USA).

Results

We first presented the age-related expression of ER and PR for all cases and by their HER-2 status. Breast cancers were ER^+ in 87.3% and PR^+ in 77.0% of cases. Considered by their HER-2 status, $HER\text{-}2^-$ breast cancers were ER^+ in 90.2% and PR^+ in 80.1% of cases. $HER\text{-}2^+$ breast cancers were ER^+ in 65.6% and PR^+ in 53.5% of cases. From Table 1, it was clear that the proportion of breast cancers being ER^+ (panel A) or PR^+ (panel B) increased with age up to 45–50 years of age where after, a stable proportion being ER^+ and a decreasing proportion being PR^+. Non-parametric regression showed that the relationship between age and both steroid receptors depend on the HER-2 status (Table 1, panel C, D). To approximate these relationships using logistic regression, we allowed a different effect of age for patients younger versus older than 50 and 45 years of age respectively for ER and PR when the tumor was $HER\text{-}2^-$. For being ER^+ (panel C), the OR in $HER\text{-}2^-$

Table 1 Age-related prevalence of breast cancers being respectively ER+ or PR+ for all (panel A, B) or by HER-2 status (panel C, D)

Age	All				$HER\text{-}2^-$			$HER\text{-}2^+$		
	N	%ER^+	%PR^+	%$Her2^+$	*N*	%ER^+	%PR^+	*N*	%ER^+	%PR^+
≤35	56	66.1	57.1	19.6	45	64.4	53.3	11	72.7	72.7
36–45	338	84.6	79.3	15.7	285	85.6	80.7	53	79.3	71.7
46–55	651	89.3	80.3	12.3	571	92.5	84.1	80	66.3	53.8
56–65	584	87.8	75.3	11.0	520	91.5	79.0	64	57.8	45.3
≥65	598	88.5	75.8	8.0	550	91.1	78.9	48	58.3	39.6
All	2,227	87.4	77.1	11.5	1,971	90.2	80.1	256	65.6	53.5

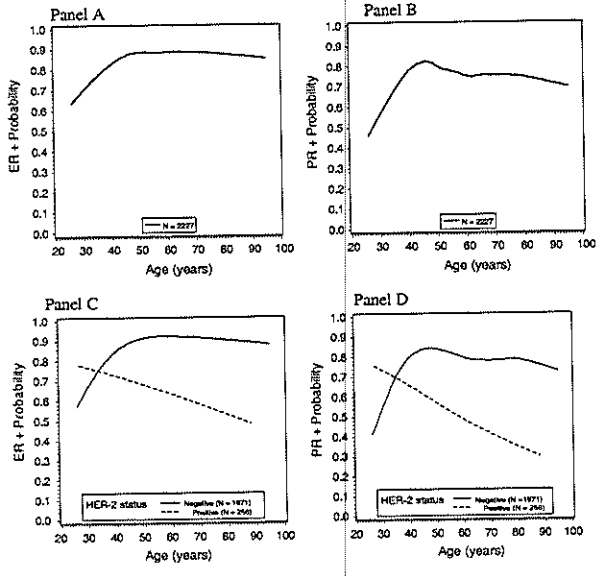

Table 2 Age related prevalence for a HER-2+ status in all patients (panel A) and by the joint hormone receptor status (panel B)

Age	All		ER^-PR^-		ER^+PR^-		ER^+PR^+	
	N	% $HER\text{-}2^+$	*N*	% $HER\text{-}2^+$	*N*	% $HER\text{-}2^+$	*N*	% $HER\text{-}2^+$
≤35	56	19.6	19	15.8	5	0.0	32	25.0
36–45	338	15.7	52	21.2	18	22.2	268	14.2
46–55	651	12.3	70	38.6	58	17.2	523	8.2
56–65	584	11.0	71	38.0	73	11.0	440	6.6
≥65	598	8.0	69	29.0	76	11.8	453	4.2
All	2,227	11.5	281	31.3	230	13.5	1,716	8.0

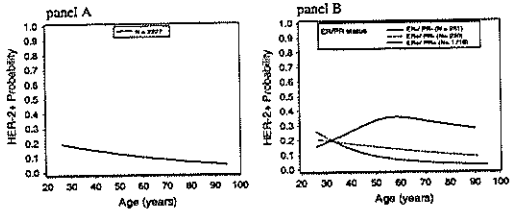

Table 3 (a) A age-related prevalence by ERPRHER-2 status expressed (numbers). (b) Age-related prevalence by ERPRHER-2 status expressed as bar-diagram

Age	*N*	ER^-PR^- $HER\text{-}2^-$ (%)	ER^-PR^- $HER\text{-}2^+$ (%)	ER^+PR^- $HER\text{-}2^-$ (%)	ER^+PR^- $HER\text{-}2^+$ (%)	ER^+PR^+ $HER\text{-}2^-$ (%)	ER^+PR^+ $HER\text{-}2^+$ (%)
≤35	56	28.6	5.4	8.9	0.0	42.8	14.3
36–45	338	12.1	3.3	4.1	1.2	68.1	11.2
46–55	651	6.6	4.1	7.4	1.5	73.8	6.6
56–65	584	7.5	4.6	11.1	1.4	70.4	5.0
≥65	598	8.2	3.3	11.2	1.5	72.6	3.2
All	2,227	8.7	4.0	8.9	1.4	70.8	6.2

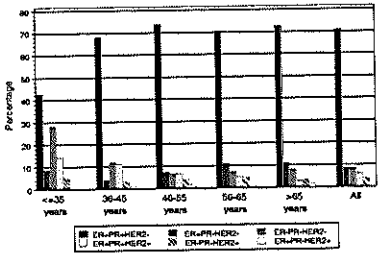

Table 4 (a) Mean and median age per category of breast cancer stratified by ER, PR and HER-2 status. (b) Differences in mean age between TP and cases with a different ER, PR and HER-2 status

Group	*N*	%	Mean (SD)	Median (IQR)	Range
ER^- PR^- $HER2^-$	193	8.7	55.2 (14.32)	55 (23)	26–90
ER^- PR^- $HER2^+$	88	4.0	56.9 (12.33)	57 (15)	32–88
ER^+ PR^- $HER2^-$	199	8.9	60.8 (11.70)	62 (16)	27–91
ER^+ PR^- $HER2^+$	31	1.4	58.6 (12.52)	57 (17)	38–87
ER^+ PR^+ $HER2^-$	1,579	70.8	57.7 (12.08)	57 (18)	26–95
ER^+ PR^+ $HER2^+$ (TP)	137	6.2	52.4 (12.44)	51 (18)	27–86
Overall	2,227	100	57.4 (12.41)	57 (18)	26–95
Comparison	Difference in mean age (years)	95% CI	*P*-value (*t*-test)		
TP versus Others (overall)	5.4	3.3–7.5	<0.0001		
TP versus $ER^-PR^-HER2^-$	2.8	−0.2–5.8	0.0591		
TP versus $ER^-PR^-HER2^+$	4.6	1.2–7.9	0.0075		
TP versus $ER^+PR^-HER2^-$	8.4	5.8–11.1	<0.0001		
TP versus $ER^+PR^-HER2^+$	6.3	1.4–11.2	0.0124		
TP versus $ER^+PR^+HER2^-$	5.4	3.2–7.5	<0.0001		

cases was 2.594 (95% CI = 1.874 – 3.591) up to 50 years and 0.888 (95% CI = 0.748 – 1.053) after 50 years. The OR in $HER\text{-}2^+$ cases was 0.806 (95% CI = 0.656 – 0.991) irrespective of the patient's age. For being PR^+ (panel D), the OR in $HER\text{-}2^-$ cases was 2.687 (95% CI = 1.780 – 4.057) up to age 45 and 0.847 (95% CI = 0.761 – 0.942) after age 45. The OR in $HER\text{-}2^+$ cases was 0.722 (95% CI = 0.589 – 0.886) irrespective of the patient's age.

We secondly presented the age-related expression of HER-2 by the combined ERPR-status. Breast cancers were $HER\text{-}2^+$ in 11.6% of cases. ER^-PR^-, ER^+PR^-, ER^+PR^+ breast cancers were $HER\text{-}2^+$ in respectively 31.3%, 13.5% and 8.0% of cases. From panel A of Table 2, it was clear that the proportion $HER\text{-}2^+$ cancers decreased steadily with age. However, this decline in $HER\text{-}2^+$ disease was restricted to the ER^+PR^+ subset (panel B). The nonparametric regression lines suggested an increase in $HER\text{-}2^+$ cancers for ER^-PR^- lesions until approximately 55 years of age where after it mildly decreased. However, a steady decrease was suggested when the cancer was ER^+PR^- but much more pronounced for ER^+PR^+ cases. A logistic regression model to approximate these relationships was fitted. For patients with an ER^-PR^- tumor, the OR for being $HER\text{-}2^+$ was 1.752 (95% CI = 1.120 – 2.739) up to age 55 and 0.764 (95% CI = 0.532 – 1.097) thereafter. The OR was 0.855 (95% CI = 0.620 – 1.179) for ER^+PR^- and 0.679 (95% CI = 0.581 – 0.794) for ER^+PR^+ tumors, irrespective of age.

Table 3 showed the proportional distribution for any of the 6 ER/PR/HER-2 breast cancer subtypes for all ages and per age-category. In our series, $ER^+PR^+HER\text{-}2^-$ breast cancers constituted the most prevalent phenotype of operable breast cancers (70.8%) whereas 6.2% belong to the $ER^+PR^+HER\text{-}2^+$ or TP-subgroup. With increasing age, breast cancers are more likely $ER^+PR^+HER\text{-}2^-$ and $ER^+PR^-HER\text{-}2^-$ and less likely $ER^-PR^-HER\text{-}2^-$ and $ER^+PR^+HER\text{-}2^+$. The decline in $HER\text{-}2^+$ disease with age is restricted to the ER^+PR^+ subset. Hence, other data enriched in $ER^-PR^-HER2^+$ or $ER^+PR^-HER2^+$ show a constant probability with age.

Table 4 showed the mean and median age per ER-PRHER-2 breast cancer subgroup. Women with an $ER^+PR^+HER\text{-}2^-$ breast cancer presented with a mean age of 57.7 years at diagnosis. Women with an $ER^+PR^+HER\text{-}2^+$ were diagnosed at an age younger than women with any other ER/PR/HER-2 breast cancer phenotype (52.4 years; $P < 0.0001$). Differences in mean age between TP and other ER/PR/HER-2 subgroups were presented in Table 4.

Discussion

Our findings in a large series of primary operable invasive breast cancers clearly suggested a qualitative interaction between age and expression of steroid and HER-2 receptors. The interaction between age and both steroid receptors is well established (1). With increasing age, breast cancers are more likely ER^+ because ER^-PR^- tumours appear earlier in life as they grow faster; they are more likely undifferentiated or aneuploid with a higher S-phase fraction (21, 22). At a certain age, the proportion of breast cancers being ER^+ or ER^-PR^- show a constant probability with age. However, changes continue to appear over time in the proportion of ER^+ breast cancers being PR^+. The PR is induced by an intact oestrogen-ER interaction and the

observed qualitative age-interaction for PR is therefore consistent with higher oestrogen levels before and lower oestrogen levels after menopause (23, 24).

We showed for the first time that the established age-related steroid receptor expression of invasive breast cancers is limited to $HER\text{-}2^-$ cases. The age-related steroid receptor expression of $HER\text{-}2^+$ cases differs from $HER\text{-}2^-$ cases. $HER\text{-}2^+$ breast cancers show an age-related decline in the proportion of tumors being ER^+ or PR^+. We also showed that the age-related decline in $HER\text{-}2^+$ disease is restricted to the ER^+PR^+ subset. Ageing has not only been associated with an increase in ER^+ tumours but also with an increase in their ER content (25). Although it can not be concluded from our study that this increase in ER-content differs on the base of HER-2 amplification our findings of an age-related observation of signalling between steroid and growth factor receptors at least for ER and HER-2 do fit with a lower likelihood to be HER^+ among older ER^+ tumours (with higher ER content) relative to younger ER^+ tumours (with lower ER content). Authors reporting on the HER-2 status of breast cancer did not retain age as a predictor for HER-2 expression, unless breast cancer patients under age 35 were considered (2, 26–28). In our series, mean and median age did also not differ by HER-2 status [53 and 54.4 years for $HER\text{-}2^+$ and 57 and 57.9 years for $HER\text{-}2^-$ cases]. However, combining HER-2 with steroid receptor expression clearly separated HER-2 tumours in an early (ER^+) and later onset type of breast cancer (ER^-). Although the TP breast cancers appeared at an earlier age than the ER^- tumours an even larger series than ours might be required to show significance.

Our findings for the age distribution of breast cancer by their ER/PR/HER-2 status are clinically relevant as breast cancers are not longer classified on the base of ER or PR alone. The combined steroid and HER-2 receptor status of breast cancer has a better prognostic and predictive value than a classification based on either the steroid or HER-2 receptor status alone (29, 30). Also, the intrinsic tumour biology of breast cancer is an additional prognostic factor to characteristics of patients such as age or tumour stage.

Our findings have never been reported before probably because the proportion of breast cancers being $ER^+HER\text{-}2^+$ is low, representing only 6.2% of all breast cancers in our series. Therefore, large series such as ours are required to find such differences. However, HER-2 breast cancers and also those expressing ER are a clinically important category of breast cancers as HER-2 is also prognostic in this group (31).

Although our findings are hypothesis generating and awaiting to be confirmed, the reason why the small group of $ER^+PR^+HER\text{-}2^+$ lesions appear early in life is as yet unclear. However, our findings incorporate some of the interesting biology of $HER\text{-}2^+$ breast cancers and may shed light on the carcinogenic mechanisms for early-onset of $ER^+HER\text{-}2^+$ breast cancers as opposed to $ER^+HER\text{-}2^-$ cases. We suggest that high oestrogen levels in young women drive ER to predominantly regulate classic ER-regulated genes such as PR expression independent of HER-2 whereas in postmenopausal women, low oestrogen levels may allow an increasing effect of growth factor signalling on ER action resulting in a different set of ER-regulated genes being expressed with a lower likelihood for PR-expression. Otherwise said, at young age, $HER\text{-}2^+$ breast cancers are more likely ER^+ through the presence of elevated estrogen levels whereas with ageing and decreasing estrogen levels, the growth of $HER\text{-}2^+$ breast cancers is less dependent of estrogens. This is also reflected in the younger age-of-onset of PR^+ compared with PR^- lesions which is mainly due to the $PR^+HER\text{-}2^+$ subgroup. The biology behind this suggests that premenopausal steroids select ER^+ breast cancers to appear earlier if they are $PR^+HER\text{-}2^+$ than if they are $PR^-HER\text{-}2^-$, $PR^-HER\text{-}2^+$ or $PR^+HER\text{-}2^-$. The absence of a negative association between PR and HER-2 in young women as opposed to elder with an ER^+ breast cancer is in line with these findings (9). These findings may explain the greater sensitivity of $ER^+HER\text{-}2^+$ tumours to anti-estrogens in young as compared to elder women (12, 14–17). It is well known that reproductive hormonal risk factors are different by the ER expression of breast cancer but the drive or risk factors behind the earlier appearance of $ER^+HER\text{-}2^+$ cases compared with other tumour types remains to be explored.

It is unlikely that patients in our series differ from patients in other series. A mean age of 57.5 years in our series compares with other large series. The proportion of women with a $HER\text{-}2^+$ breast cancer in our series (11.9%) is lower than reported by others but compares with data from the Karolinska group in Stockholm only showing HER-2 over-expression in 10% of operable breast cancer cases (personal communication St. Gallen 2007).

In conclusion, our believe that HER-2 expression is estrogen driven in young women with an ER^+ breast cancers is reflected by a mean younger age of diagnosis (52.4 years) of $ER^+PR^+HER\text{-}2^+$ breast cancers, on average 5.4 years younger than lesions with another ER/PR/HER-2 receptor status (95% CI = 3.3–7.5; $P < 0.0001$). The mean age of $ER^+PR^+HER\text{-}2^+$ breast cancers was lower when compared to all other subgroups with the smallest difference occurring with the $ER^-PR^-HER\text{-}2^-$ subgroup (2.8 years younger; 95% CI = −0.2–5.8; $P = 0.0591$). A qualitative age interaction between ER, PR and HER-2 in breast cancers needs confirmation by others as age-related differences between these subgroups may reflect differences in tumour biology, risk factors and sensitivity to sex steroids and anti-estrogens.

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