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Report

Association between HER-2/neu and the progesterone receptor in oestrogen-dependent breast cancer is age-related

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Summary

In oestrogen receptor-positive (ER^+) breast cancer, HER-2/neu and the progesterone receptor (PR) are inversely associated. This explains a lower response to anti-oestrogens if ER^+ breast cancers are HER-2/neu positive. One randomized study however, showed that premenopausal women with an ER^+ breast cancer respond to anti-oestrogens independent of HER-2/neu. We therefore hypothesized an age-related association between HER-2/neu and PR in ER^+ breast cancers. Receptors for ER, PR and HER-2/neu were analysed by immunohistochemistry (IHC). A uni- and multivariate analyses was carried out to assess this relationship in 1104 women with an ER^+ breast cancer. We observed an inverse association between HER-2/neu and PR only after age 45. There were 173 women of age ≤45 and 931 of age >45 years. In multivariate analysis, only tumour grade ($p=0.005$) but not PR status was associated with HER-2/neu in women age ≤45 years. However, in age >45 years group, both PR status ($p=0.001$) and tumour grade ($p = 0.001$) were independently associated with HER-2/neu. In ER^+ breast cancers from women age >45, PR was positive in 76.9% if HER-2/neu negative but in 53.4% if HER-2/neu positive ($p<0.001$) and the median quantitative PR levels are 150 and 75 respectively in HER-2/neu negative and HER-2/neu positive tumours ($p=0.002$). This age effect of HER-2/neu on the PR status was not seen in women age ≤45 years and the median quantitative PR levels are 200 and 220 respectively in HER-2/neu negative and HER-2/neu positive tumours ($p = 0.518$). The study confirms an age-related inverse relationship between HER-2/neu and PR only in women age >45 years but not in women age ≤45 years.

Introduction

In breast cancer, receptors for progesterone and HER-2/neu are in part regulated by oestrogens binding to a functional ER; oestrogens up-regulate PR [1] but down-regulate transcription of HER-2/neu [2]. PR and HER-2/neu are inversely associated in ER^+ breast cancers [2–6]. An absent PR predicts a positive HER-2/neu status and a positive HER-2/neu status predicts an absent PR in women with an ER^+ breast cancer [5, 6]. An absent PR and a positive HER-2/neu status are also considered as predictive factors for a lower response to tamoxifen in women with ER^+ breast cancers [6–13]. This has clearly been shown in postmenopausal women both for PR and HER-2/neu [12, 13]. One recent report however, showed that HER-2/neu overexpression does not predict for a lower response to anti-oestrogens in premenopausal women with an ER^+ breast cancer [14]. There are no data available that explain this observation but this finding maybe reflected by an absent inverse relationship between PR and HER-2/neu in premenopausal women with an ER^+ breast cancer. We assessed the relationship between PR and HER-2/neu in women with an ER^+ breast cancer comparing the age groups ≤45 and >45 years.

Methods

Patient

Charts from 1688 consecutive women with breast cancer, treated between Jan 2000 and July 2003 at Leuven University Hospital were retrospectively evaluated. Women who had recurrent tumour or received neo-adjuvant therapy, as well as those where the steroid receptor or HER-2/neu status of the tumour was not available were excluded, thus 1362 patients receiving primary surgery remained. Another 258 women with an ER-negative tumour were also excluded resulting in the study population of 1104 patients with an operable ER^+ breast tumour. 931 women were in the age group >45 years and 173 were in age group ≤ 45 years.

Measurement of ER, PR, and HER-2/neu

ER, PR and HER-2/neu IHC stains according to the Envision method were performed using the following primary monoclonal antibodies NCL-ER-6F11/2, NCL-PgR-312 and CB11, respectively. ER, PR and HER-2/neu IHC stains were semi-quantitatively evaluated. ER or PR were on a H-score defined negative (−) when staining showed 50 or less, weakly positive (+) between 51 and 100, moderately positive (++) between 101 and 200 and strongly positive (+++) between 201 and 300. The DAKO-scoring system for HER-2/neu was defined negative when score 0 or 1+ or 2+, positive when score 3+ was given. Tumour grading was performed according to the Ellis and Elston grading system [15]. The following factors were included for evaluation: patient's age at diagnosis, tumour size, tumour grade, axillary lymph node status, PR status and HER-2/neu status. To justify which age is the best cut off for an inverse association between HER-2/neu and PR, we used 7 age plots with a range of 5 years starting at age ≤ 45 years and ending at age >70 years. As such we had equally distributed age plots.

Statistical analysis

In univariate analysis, Chi-square test was used to examine the association between HER-2/neu and other categorical variables. In multivariate analysis, logistic regression was used to find out the independent factors predicting HER-2/neu expression. Associations between HER-2/neu (division by DAKO-score 0, 1+, 2+, 3+) and PR status (division by −, +, ++, +++) were analysed using the Spearman's rho correlation. The Spearman rho coefficient (r) indicates agreement between two variables. A value of r approximately equal to 1 indicates good agreement, a value near 0 indicates poor agreement, and negative values indicate inverse correlations. Quantitative PR levels (between 0 and 300) were compared using the Mann–Whitney U test. All statistical tests were two-sided and $p < 0.05$ was considered significant. All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) software version 11.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

Results

In Table 1 we presented the data for the association between HER-2/neu and PR by an age range of 5 years

Table 1. Association between HER-2/neu and PR in women with an ER^+ breast cancer by age range of 5 years from age ≤ 45 years onwards

Age group (years)	PR	HER-2/neu*		Total	*p*-value (Fisher's Exact Test)
		Negative	Positive		
≤45	(−)	21 (91.3%)	2 (8.7%)	23	1.000
	(+)	135 (90.0%)	15 (10.0%)	150	
46–50	(−)	15 (78.9%)	4 (21.1%)	19	0.043
	(+)	128 (94.1%)	8 (5.9%)	136	
51–55	(−)	32 (84.2%)	6 (15.8%)	38	0.218
	(+)	110 (91.7%)	10 (8.3%)	120	
56–60	(−)	31 (83.8%)	6 (16.2%)	37	0.007
	(+)	110 (97.3%)	3 (2.7%)	113	
61–65	(−)	40 (93.0%)	3 (7.0%)	43	0.407
	(+)	104 (96.3%)	4 (3.7%)	108	
66–70	(−)	38 (88.4%)	5 (11.6%)	43	0.109
	(+)	90 (96.8%)	3 (3.2%)	93	
>70	(−)	46 (93.9%)	3 (6.1%)	49	0.346
	(+)	129 (97.7%)	3 (2.3%)	132	

*HER-2/neu was defined negative when DAKO-score 0 or 1+ or 2+, and positive when score 3+.

starting from age ≤45 years onwards. The negative association between HER-2/neu and PR is only seens in women age >45 years. In those age ≤45 years, there is no effect of PR on HER-2/neu overexpression. The tumour characteristics for women with ER^+ tumours according to age group (≤45 versus >45 years) are shown in Table 2. ER^+ breast cancers in women age >45 years were more likely PR-negative, low tumour grade, smaller tumour size and axillary lymph node negative than those in women age ≤45 years. There was no significant difference in HER-2/neu expression between women age ≤45 and >45 years.

The association between the different tumour characteristics and HER-2/neu in univariate analysis is shown in Table 3. The results indicated a statically significant correlation between HER-2/neu and the following variables: PR status, tumour grade and lymph node status in 1104 women with ER^+ breast cancer. Women with an ER^+PR^+ tumour were less likely HER-2/neu positive than women with an ER^+PR^- lesion (5.4% versus 11.5%; $p < 0.001$). Tumour grade also predicted the HER-2/neu status in women with an ER^+ breast cancer; 3.7% of grade 1–2 against 14.6% of tumour grade 3 lesions ($p < 0.001$). After stratification by age 45, the following observations were made. In women age ≤45 years, only tumour grade ($p = 0.002$) but not PR status ($p = 0.845$) was significantly associated with HER-2/neu overexpression; PR status ($p < 0.001$) and tumour grade ($p < 0.001$) were independently associated with HER-2/neu in patients age >45 years (Table 3).

Table 4 shows the association between HER-2/neu and other tumour factors in multivariate models. In all patients with ER^+ tumours, HER-2/neu overexpression was associated with the PR status (negative versus positive; odd ratio (OR) 2.01, 95% confidence interval (CI) 1.22–3.32; $p = 0.006$) and tumour grade (grade 3 versus grade 1–2; ORg 4.27, 95% CIg 2.62–6.97; $p < 0.001$). In women age ≤45 years, only tumour grade (OR 6.36, 95% CI 1.76–23.04; $p = 0.005$) but not PR status ($p = 0.474$) was significantly associated with HER-2/neu overexpression; PR status (OR 2.58, 95% CI 1.49–4.47; $p = 0.001$) and tumour grade (OR 3.77, 95% CI 2.18–6.52; $p = 0.001$) were independently associated with HER-2/neu in patients age >45 years.

The analysis of the association between categorical values of PR (divided by −, +, ++, +++) and HER-2/neu (divided by 0, 1+, 2+, 3+) confirmed a negative association between PR and HER-2/neu in the overall population of women with an ER^+ breast cancer ($r = -0.113$, $p < 0.001$) (Table 5). Again, the inverse correlation between PR and HER-2/neu was only seen

Table 2. Clinicopathological features of ER-positive tumours by age (n = 1104)

Variable	Age ≤45 years		Age >45 years		*p*-value	Total	
	No	%	No	%		No	%
No of patients	173	15.7	931	84.3		1104	100.0
PR expression[a]							
(−)	23	13.3	229	24.6	<0.001	252	22.8
(+)	22	12.7	134	14.4		156	14.1
(++)	41	23.7	249	26.7		290	26.3
(+++)	87	50.3	319	34.3		406	36.8
Tumour grade							
grade 1	15	8.7	189	20.3	<0.001	204	18.5
grade 2	78	45.1	508	54.6		586	53.1
grade 3	80	46.2	234	25.1		314	28.4
Tumour size							
≤20 mm	76	43.9	527	56.6	0.002	603	54.6
>20 mm	97	56.1	404	43.4		501	45.4
Axillary lymph node							
negative	94	54.3	626	67.2	0.001	720	65.2
positive	79	45.7	305	32.8		384	34.8
HER-2/neu status[b]							
score 0	127	73.4	745	80.0	0.154	872	79.0
score 1+	13	7.5	68	7.3		81	7.3
score 2+	16	9.2	60	6.4		76	6.9
score 3+	17	9.8	58	6.2		75	6.8

[a]PR was on a H-score defined negative (−) when staining showed 50 or less, weakly positive (+) between 51 and 100, moderately positive (++) between 101 and 200 and strongly positive (+++) between 201 and 300.
[b]HER-2/neu was defined negative when DAKO-score 0 or 1+ or 2+ and positive when DAKO-score 3+.

Table 3. Univariate analysis of tumour factors predicting HER-2/neu[a] expression in women with an oestrogen-dependent breast cancer by age ($n = 1104$)

	Age ≤45 years				Age >45 years				Total			
	HER-2/neu expression				HER-2/neu expression				HER-2/neu expression			
	Negative	Positive	OR	*p*-value	Negative	Positive	OR	*p*-value	Negative	Positive	OR	*p*-value
PR status[b]												
negative	21 (91.3%)	2 (8.7%)	0.86	0.845	202 (88.2%)	27 (11.8%)	2.89	<0.001	223 (88.5%)	29 (11.5%)	2.28	0.001
positive	135 (90.0%)	15 (10.0%)	1		671 (95.6%)	31 (4.4%)	1		806 (94.6%)	46 (5.4%)	1	
Tumour grade												
grade 1–2	90 (96.8%)	3 (3.2%)	1	0.002	671 (96.3%)	26 (3.7%)	1	<0.001	761 (96.3%)	29 (3.7%)	1	<0.001
grade 3	66 (82.5%)	14 (17.5%)	6.36		202 (86.3%)	32 (13.7%)	4.09		268 (85.4%)	46 (14.6%)	4.50	
Tumour size												
≤20 mm	69 (90.8%)	7 (9.2%)	1	0.810	494 (93.7%)	33 (6.3%)	1.01	0.963	563 (93.4%)	40 (6.6%)	1	0.817
>20 mm	87 (89.7%)	10 (10.3%)	1.13		379 (93.8%)	25 (6.2%)	1		466 (93.0%)	35 (7.0%)	1.06	
Lymph node												
negative	84 (89.4%)	10 (10.6%)	1.22	0.696	592 (94.6%)	34 (5.4%)	1	0.149	676 (93.9%)	44 (6.1%)	1	0.217
positive	72 (91.1%)	7 (8.9%)	1		281 (92.1%)	24 (7.9%)	1.49		353 (91.9%)	31 (8.1%)	1.35	

Data are number of patients (%), OR, odds ratio.
[a]HER-2/neu was defined negative when DAKO-score 0 or 1+ or 2+, and positive when score 3+.
[b]PR was defined negative when H-score ≤50, and positive when H-score 51–300.

Table 4. Multivariate analysis of tumour factors predicting HER-2/neu[a] expression in women with an oestrogen-dependent breast cancer by age ($n = 1104$)

Variable	Age ≤45 years		Age >45 years		Total	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
PR status[b] (negative vs positive)	–	0.474	2.58 (1.49–4.47)	0.001	2.01 (1.22–3.32)	0.006
Tumour grade (grade 3 vs grade 1–2)	6.36 (1.76–23.04)	0.005	3.77 (2.18–6.52)	<0.001	4.27 (2.62–6.97)	<0.001
Tumour size (>20 mm vs ≤20 mm)	–	0.947	–	0.113	–	0.300
Lymph node (positive vs negative)	–	0.372	–	0.354	–	0.692

OR, odds ratio, CI, confidence interval.
[a] HER-2/neu was defined negative when DAKO-score 0 or 1+ or 2+, and positive when score 3+.
[b] PR was defined negative when H-score ≤50, and positive when H-score 51–300.

Table 5. Associations between HER-2/neu expression and progesterone receptor by age

	Spearman correlation*	
	rho coefficient (*r*)	*p*-value
Age ≤45 years	−0.024	0.757
Age >45 years	−0.143	<0.001
Total	−0.113	<0.001

*Correlation between HER-2/neu (division: DAKO-score 0, 1+, 2+, 3+) and PR (division: –, +, ++, +++).

in women age >45 years ($r = -0.143$, $p < 0.001$) but disappeared in women age ≤45 years ($r = -0.024$, $p = 0.757$).

In Figure 1, the frequency for PR-positivity by HER-2/neu status is shown for the overall and for each age group. In women age >45 years, the frequency of PR-positivity in HER-2/neu negative tumours was significantly higher than in HER-2/neu positive tumours (76.9% versus 53.4%, $p < 0.001$). In women age ≤45 years, the frequency of PR-positivity was no significant difference between HER-2/neu negative tumours and positive tumours (86.5% versus 88.2%, $p = 0.845$).

Similar results were seen using semi-quantitative values of PR in Figure 2. The median level of PR in HER-2/neu negative ER^+ breast cancers was higher than HER-2/neu positive ER^+ breast cancers in women age >45 years (150 versus 75, $p = 0.002$). The median level of PR was similar between HER-2/neu negative and positive tumours in women age ≤45 years (200 versus 220, $p = 0.518$).

Discussion

This study analyses the association between HER-2/neu and PR in women with an ER^+ breast cancer. The inverse association is most obvious in women age >45 years. In this age group of women with an ER^+ breast cancer, an absent PR is an independent predictor

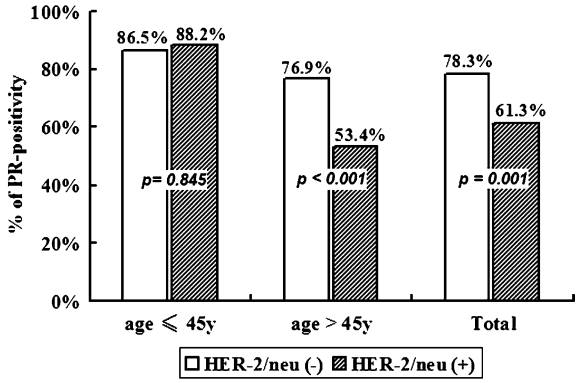

Figure 1. Frequency of PR-positivity in women with an oestrogen-dependent breast cancer by age and HER-2/neu expression. PR was defined negative when H-score ≤50, and positive when H-score 51–300. HER-2/neu was defined negative when DAKO-score 0 or 1+ or 2+, and positive when score 3+.

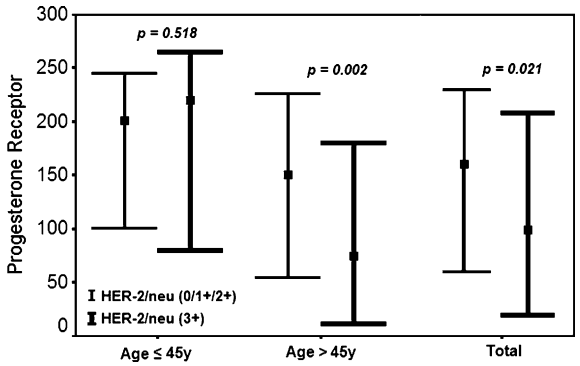

Figure 2. Quantitative progesterone receptor levels in women with an ER-positive by age and HER-2/neu status. The quantitative value of PR using the H-score system is from 0 to 300. The lower and upper lines indicate the lower and upper quartiles, and the central solid boxes indicate the medians. In women age ≤45 years, comparison of the median of PR level between HER-2/neu negative (DAKO-score 0–2+) and positive (DAKO-score 3+) are 200 [interquartile range (IQR) = 101–245] versus 220 [IQR = 80–265] and $p = 0.518$. In women age >45 years, comparison of the median of PR level between HER-2/neu negative and positive are 150 [IQR = 55–226] versus 75 [IQR = 12–180] and $p = 0.002$. In all patients, comparison of the median of PR level between HER-2/neu negative and positive are 160 [IQR = 60–230] versus 99 [IQR = 20–208] and $p = 0.021$.

of a positive HER-2/neu status and is predicted by a positive HER-2/neu status. In younger women (age ≤45 years), HER-2/neu and the PR status are not associated in women with an ER^+ breast cancer.

Compared with the ER^+PR^+ phenotype, ER^+PR^- breast cancers are more common after menopause, more likely high grade and large size [16–19]. We and others were able to show that compared with the ER^+PR^+ phenotype, the ER^+PR^- phenotype is more likely HER-2/neu positive, and this independent of tumour size, grade and lymph node status [5, 6]. According to Konecny et al. [5] the chance to be HER-2neu positive in two different cohorts of women with an ER^+ breast cancer increases by 10.2 to 14.0% if PR is absent. This consistency between populations proves our findings that absence of PR in ER-positive breast cancer predicts HER-2/neu overexpression.

Although a PR-negative status predicts the HER-2/neu status in ER-positive tumours, this is most obvious in women beyond age 45. This age-related difference has never been reported by others [4–6]. Because over two thirds of all women with breast cancer appear beyond age 50, an age or menopause effect may not become apparent when only considering the overall population.

One shortcoming of our study is that we did not use fluorescence in situ hybridisation (FISH) to measure HER-2/neu. In stead, we used IHC staining to analyse ER, PR and HER-2/neu which is easier, safer and less expensive than ligand binding assays with equivalent ability to predict response to anti-HER-2/neu therapy and probably better in predicting response to adjuvant endocrine therapy [20]. Although our conclusion regarding the age-related association between HER-2/neu and PR can only be made for IHC HER-2/neu 3+ breast cancer cases, it is unlikely that a FISH test of the DAKO 2+ cases will considerably change our findings as only 20–30% of these show HER-2/neu gene amplification. Also, the frequency data for HER-2/neu overexpression in our dataset fit with data in the literature using FISH [5, 21]. Furthermore, differences between median PR levels by HER-2/neu status comparing women age ≤ or >45 were larger and significant for the last one in the group of women with a DAKO 3+ score for HER-2/neu overexpression. Another strength of this study is that all the semi-quantitative readings of ER, PR and HER-2/neu were done by one pathologist (Prof Dr Drijkoningen). Successful quality control and quality assurance programs were guaranteed in our laboratory according to requested guidelines.

An inverse relationship between HER-2/neu and PR-expression both for its presence and for its quantitative values only in women beyond age 45 years might explain the potential of HER-2/neu as a predictor for a lower response to tamoxifen in this age group [13]. Our findings may also explain why HER-2/neu overexpressors with an ER-positive tumour remain sensitive to hormone therapy in a recently published population of premenopausal women with ER-positive breast cancer [14]. Unfortunately, data for PR-expression by HER-2/neu status were not provided in their manuscript so that we cannot test our findings for consistency. We do also realise that other factors than PR play a role in response to hormonal therapy as recently shown for cyclin E or its low molecular weight forms [22–24].

A possible hypothesis for an absent inverse relationship between HER-2/neu and PR-expression in younger women are higher levels of circulating oestrogens that continue to stimulate the ER-pathway to express PR and cancer growth despite an active HER-2/neu pathway. Lowering circulating oestrogens by ovarian ablation will treat such patients. Breast cancers appearing after age 45–50 are more likely to appear with lower circulating oestrogen when the ER-pathway is less activated, PR less likely expressed and HER-2/neu more

likely positive. After menopause however, cancer growth remains activated even with low levels of circulating oestrogens from aromatisation of circulating androgens. Tamoxifen is less effective if PR is absent because the HER-2/neu pathway may have taken over and in the long term tamoxifen may work as an oestrogen agonist [8]. Lowering circulating oestrogens through aromatase inhibitors remains an active way of antagonizing cancer growth in women with a HER-2/neu positive breast cancer [13]. Recently, Dowsett et al. [25] presented data that postmenopausal women with an ER-positive breast cancer are more likely to benefit from an oral aormatase inhibitor than from tamoxifen in case PR was not expressed. Unfortunately, data to correlate these finding with a higher rate of HER-2/neu expression were not available.

In conclusion, we did find that ER^+PR^- breast cancers are more likely HER-2/neu positive than ER^+PR^+ tumours and that this observation is limited to women beyond age 45 years. Women age ≤45 years with an ER^+ breast cancer overexpressing HER-2/neu are likely to remain PR-positive which may explain why HER-2/neu overexpression does not predict for a lower activity to anti-oestrogens in these women in contrast to women beyond age 45 years. Future trials will assess whether women with an ER^+PR^- breast cancer need more aggressive therapy or a combination of hormone therapy and HER-2/neu antibodies contrary to women with ER^+PR^+ breast cancer.

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