

ORIGINAL ARTICLE

Association between tumour characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer

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Aims: To investigate the association between tumour characteristics and HER-2/neu by immunohistochemistry in primary operable breast cancer.

Methods: The association between HER-2/neu and other clinicopathological factors was evaluated in 1362 consecutive patients with primary breast cancer treated between 2000 and July 2003 in one centre. Microscopic tumour size, tumour grade, lymph node status, patient's age, oestrogen receptor (ER), progesterone receptor (PR), and joint ER/PR status were evaluated, using the χ^2 test for univariate analysis and logistic regression for multivariate analysis. The hormone receptors and HER-2/neu were studied immunohistochemically. Using the HER-2/neu DAKO scoring system, scores of 0, 1+, or 2+ were defined as negative and 3+ as positive. Data for DAKO scores 2+/3+ versus 0/1+ are also presented.

Results: Hormone receptor negative breast cancers were more often HER-2/neu positive than hormone receptor positive cancers, both for ER (28.7% v 6.8%) and PR (19.9% v 5.9%). In multivariate analysis, both ER, PR, and tumour grade were independently associated with HER-2/neu. In ER⁺ tumours, HER-2/neu overexpression was significantly lower in PR⁺ than in PR⁻ cases (11.5% v 5.4%). HER-2/neu overexpression (2.7%) was lowest in the large subgroup of ER⁺PR⁺ tumours with low tumour grade (grade 1–2), comprising 46.1% of all patients.

Conclusions: ER, PR, and tumour grade are independent predictors for HER-2/neu overexpression in women with primary operable breast cancer. ER and PR are negatively associated with HER-2/neu, whereas tumour grade is positively associated with HER-2/neu. In women with ER⁺ tumours, PR status also affects the likelihood of HER-2/neu expression.

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The HER-2 gene encodes a 185 kDa transmembrane phosphoglycoprotein with tyrosine kinase activity and is a member of the human epidermal growth factor receptor gene family.¹ Cells transfected with HER-2/neu acquire a more malignant phenotype, with stimulation of cell proliferation, invasion, and metastasis.² This has been confirmed in the clinic: women with HER-2/neu positive breast cancer have a worse prognosis than those with HER-2/neu negative cancers; this is also true for T1N0M0 tumours.^{3–6} Furthermore, HER-2/neu overexpression has been correlated with poor prognostic tumour characteristics such as higher histological grade, S phase fraction, increased tumour size, number of involved lymph nodes, lymphoid infiltration, p53 mutation, absence of bcl-2, absence of lobular histology, and negative or lower oestrogen receptor (ER) expression.^{7–15} As a consequence, several, but not all, studies have confirmed that HER-2/neu overexpressing tumours show a lower response to tamoxifen in metastatic or early breast cancer.^{16–21}

“Women with HER-2/neu positive breast cancer have a worse prognosis than those with HER-2/neu negative cancers”

Several techniques are available for the genetic testing of HER-2/neu amplification. Fluorescence in situ hybridisation (FISH) has become popular over the past few years because it is a reliable method. Semiquantitative measurement using immunohistochemistry (IHC) for the HER-2/neu membrane receptor protein can also accurately predict gene amplification.²² FISH for HER-2/neu has a higher failure rate and

reagent cost than IHC, and it takes longer to carry out and interpret than IHC. Testing for HER-2/neu is currently standard practice because it has a prognostic role and predicts response to the anti-HER-2/neu antibody, trastuzumab, which offers an extra treatment option, in monotherapy and also together with chemotherapy in women with metastatic disease.^{23–25} Trastuzumab is currently under extensive evaluation in major clinical trials and HER-2/neu is being tested as a predictor of response to other treatments in large prospective clinical outcome studies.

It has recently been suggested in a univariate model that there is an inverse relation between the expression of the progesterone receptor (PR) and HER-2/neu in women with ER⁺ breast cancer.^{11–12} However, ER⁺ breast cancers that are PR⁻ are more likely to be of a high grade, resistant to tamoxifen, and more aggressive, independent of treatment.^{26–28} Therefore, it is not clear whether tumour grade and PR remain independent predictors for HER-2/neu in a multivariate model. We examined in a multivariate analysis the relation between HER-2/neu overexpression and other clinicopathological factors in women with operable breast cancer.

MATERIALS AND METHODS

Charts from 1688 consecutive women with breast cancer, treated between January 2000 and July 2003 at Leuven University Hospital, Belgium, were retrospectively evaluated.

Abbreviations: CI, confidence interval; ER, oestrogen receptor; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry; OR, odds ratio; PR, progesterone receptor

Women who had recurrent tumours or received neoadjuvant treatment, in addition to those with missing data on the tumour characteristics, were excluded; 1362 patients remained. The following factors were evaluated: patient's age at diagnosis, tumour size, tumour grade, axillary lymph node status, ER, PR, and HER-2/neu status.

IHC staining for ER, PR, and HER-2/neu was carried out according to the Envision method as a standard procedure for clinical purposes using the NCL-ER-6F11/2, NCL-PgR-312, and CB11 primary monoclonal antibodies, respectively. ER, PR, and HER-2/neu IHC staining was evaluated semiquantitatively. Using the H score for ER and PR, a negative result was defined as a score of ≤ 50 , weakly positive as 51–100, moderately positive as 101–200, and strongly positive as > 200 . The DAKO scoring system for HER-2/neu was defined as negative for scores of 0, 1+, or 2+ and positive for tumours with a score of 3+. In a small subgroup ($n = 41$) of the 149 women with a HER-2/neu DAKO score 3+, FISH data were available for HER-2/neu. Because some HER-2/neu DAKO score 2+ cases will be FISH positive for HER-2/neu, we also compared DAKO score 0 or 1+ with DAKO score 2+ or 3+ cases separately. Tumour grading was performed according to the Ellis and Elston grading system.²⁹

Using univariate and multivariate analyses, we identified the following factors to predict HER-2/neu status: ER status, PR status, tumour size, tumour grade, axillary lymph node status, and patient's age at diagnosis. The χ^2 test was used to examine the categorical variables and the association between HER-2/neu status and other clinicopathological variables in univariate analysis. In multivariate analysis, logistic regression was used to detect the independent factors predicting HER-2/neu overexpression. The frequency of HER-2/neu expression according to joint ER/PR status and the distribution of the hormone receptor status (ER, PR, and joint ER/PR) according to HER-2/neu were also calculated. All statistical tests were two sided and $p < 0.05$ was considered significant. All statistical analyses were performed with SPSS software version 11.0.1 for Windows (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Table 1 summarises the clinicopathological features of all 1362 women with primary operable breast cancer. HER-2/neu was overexpressed as defined by a DAKO score 3+ and a DAKO score 2+ or 3+ in 10.9% and 17.7% of all patients, respectively. Table 2 shows data for DAKO score 3+ versus 0, 1+, or 2+ cases. ER⁺ tumours overexpressed HER-2/neu in 6.8% of cases, and ER⁻ tumours in 28.7% of cases ($p < 0.001$). Similarly, women with PR⁺ tumours overexpressed HER-2/neu in 5.9% of cases, whereas PR⁻ tumours overexpressed HER-2/neu in 19.9% of cases ($p < 0.001$). Tumour grade also predicted HER-2/neu status: 4.6% of all grade 1–2 breast cancers overexpressed HER-2/neu compared with 20.8% of grade 3 lesions ($p < 0.001$). There was no correlation between HER-2/neu status and tumour size, axillary lymph node status, or age at diagnosis (table 2). Multivariate analysis with logistic regression revealed that HER-2/neu overexpression was predicted by ER expression (negative *v* positive; odds ratio (OR), 2.16; 95% confidence interval (CI), 1.34 to 3.51; $p = 0.002$), PR expression (negative *v* positive; OR, 1.74; 95% CI, 1.10 to 2.78; $p = 0.019$), and tumour grade (grade 3 *v* grade 1–2; OR, 3.27; 95% CI, 2.12 to 5.05; $p < 0.001$) (table 3). Tables 4 and 5 show these data for HER-2/neu DAKO scores 2+ or 3+ versus 0 or 1+. The results are similar to those for HER-2/neu DAKO scores 3+ versus 0, 1+, or 2+. However, the predictors for HER-2/neu overexpression in the multivariate model—ER, PR, and tumour grade—have a higher OR when using DAKO score 3+ cases against 0–2+ rather than DAKO scores

Table 1 Clinicopathological features ($n = 1362$)

	N	%
HER-2/neu status*		
Negative (score 0, 1+)	1121	82.3%
Positive (score 2+, 3+)	241	17.7%
HER-2/neu status†		
Negative (score 0, 1+, 2+)	1213	89.1%
Positive (score 3+)	149	10.9%
ER expression‡		
Negative	258	18.9%
Positive	1104	81.1%
PR expression‡		
Negative	488	35.8%
Positive	874	64.2%
Tumour grade		
1–2	828	60.8%
3	534	39.2%
Tumour size		
≤ 20 mm	721	52.9%
> 20 mm	641	47.1%
Lymph node		
Negative	880	64.6%
Positive	482	35.4%
Age		
≤ 50 years	429	31.5%
> 50 years	933	68.5%
Total	1362	100.0%

*HER-2/neu was defined as negative when the DAKO score was 0 or 1+, and positive when 2+ or 3+; †HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+, and positive when 3+; ‡ER and PR were defined as negative when the H score was ≤ 50 , and positive when 51–300.
ER, oestrogen receptor; PR, progesterone receptor.

2+ or 3+ against 0–1+ cases. Tables 6 and 7 show the frequency of HER-2/neu positivity (DAKO score 3+) in the different ER/PR phenotypes (ER⁻PR⁻, ER⁻PR⁺, ER⁺PR⁻, and ER⁺PR⁺) of breast cancer and for low and high tumour grade lesions. Differences in HER-2/neu expression between the ER⁻PR⁻, ER⁺PR⁻, and ER⁺PR⁺ phenotypes were significant ($p < 0.001$). The frequency of HER-2/neu overexpression decreased significantly from ER⁻PR⁻ to ER⁺PR⁻ (28.8% to

Table 2 Factors predicting HER-2/neu overexpression in primary operable breast cancers ($n = 1362$): univariate analysis

	HER-2/neu expression*		Odds ratio†	p Value
	Negative	Positive		
ER status‡				
Negative	184 (71.3%)	74 (28.7%)	5.52	< 0.001
Positive	1029 (93.2%)	75 (6.8%)	1	
PR status‡				
Negative	391 (80.1%)	97 (19.9%)	3.92	< 0.001
Positive	822 (94.1%)	52 (5.9%)	1	
Tumour grade				
1–2	790 (95.4%)	38 (4.6%)	1	< 0.001
3	423 (79.2%)	111 (20.8%)	5.46	
Tumour size				
≤ 20 mm	645 (89.5%)	76 (10.5%)	1	0.617
> 20 mm	568 (88.6%)	73 (11.4%)	1.09	
Lymph node				
Negative	790 (89.8%)	90 (10.2%)	1	0.255
Positive	423 (87.8%)	59 (12.2%)	1.22	
Age				
≤ 50 years	377 (87.9%)	52 (12.1%)	1.19	0.344
> 50 years	836 (89.6%)	97 (10.4%)	1	

Data are number of patients (%).

*HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+ and positive when 3+; †odds ratio adjusted for all variables in table; ‡ER and PR were defined as negative when the H score was ≤ 50 , and positive when 51–300.

ER, oestrogen receptor; PR, progesterone receptor.

Table 3 Factors predicting HER-2/neu overexpression* in primary operable breast cancers (n = 1362): multivariate analysis

	Odds ratio† (95% CI)	p Value
ER status‡ (negative v positive)	2.16 (1.34 to 3.51)	0.002
PR status‡ (negative v positive)	1.74 (1.10 to 2.78)	0.019
Tumour grade (grade 3 v 1–2)	3.27 (2.12 to 5.05)	<0.001
Tumour size (>20 mm v ≤20 mm)	–	0.351
Lymph node (positive v negative)	–	0.646
Age (>50 years v ≤50 years)	–	0.989

*HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+ and positive when 3+; †odds ratio adjusted for all variables in table; ‡ER and PR were defined as negative when the H score was ≤50, and positive when 51–300.
CI, confidence interval; ER, oestrogen receptor; PR, progesterone receptor.

Table 5 Factors predicting HER-2/neu overexpression* in primary operable breast cancers (n = 1362): multivariate analysis

	Odds ratio† (95% CI)	p Value
ER status‡ (negative versus positive)	1.56 (1.03 to 2.35)	0.034
PR status‡ (negative v positive)	1.51 (1.10 to 2.25)	0.014
Tumour grade (grade 3 v 1–2)	2.15 (1.81 to 3.48)	<0.001
Tumour size (>20 v ≤20 mm)	–	0.670
Lymph node (positive v negative)	–	0.178
Age (>50 v ≤50 years)	–	0.736

*HER-2/neu was defined as negative when the DAKO score was 0 or 1+, and positive when 2+ or 3+; †odds ratio adjusted for all variables in table; ‡ER and PR were defined as negative when the H score was ≤50, and positive when 51–300.
CI, confidence interval; ER, oestrogen receptor; PR, progesterone receptor.

11.5%; p < 0.001) and from ER⁺PR⁻ to ER⁺PR⁺ (11.5% to 5.4%; p = 0.001) cases. These data were similar when comparing HER-2/neu DAKO score 0 or 1+ with HER-2/neu DAKO score 2+ or 3+ cases (data not shown). Tables 8 and 9 show the frequency of hormone receptor expression according to HER-2/neu status. HER-2/neu positive tumours are more often hormone receptor negative than HER-2/neu non-overexpressing tumours. Tables 10 and 11 show the predictive value of the clinicopathological factors for HER-2/neu in women with an ER⁺ breast cancer. In multivariate analysis, PR and tumour grade remained independent predictors for HER-2/neu overexpression in this subgroup of patients with breast cancer. In 41 of the 149 women with a HER-2/neu DAKO score 3+, we obtained FISH data for HER-2/neu gene amplification. All 41 cases were FISH positive.

DISCUSSION

We compared HER-2/neu overexpression in hormone receptor positive and hormone receptor negative breast cancers, considering ER, PR, and joint ER/PR expression. In a multivariate model, ER negativity, PR negativity, and high

Table 4 Factors predicting HER-2/neu overexpression in primary operable breast cancers (n = 1362): univariate analysis

	HER-2/neu expression*		Odds ratio†	p Value
	Negative	Positive		
ER status‡				
Negative	168 (65.1%)	90 (34.9%)	3.38	<0.001
Positive	953 (86.3%)	151 (13.7%)	1	
PR status‡				
Negative	355 (72.7%)	133 (27.3%)	2.66	<0.001
Positive	766 (87.6%)	108 (12.4%)	1	
Tumour grade				
1–2	741 (89.5%)	87 (10.5%)	1	<0.001
3	380 (71.2%)	154 (28.8%)	3.45	
Tumour size				
≤20 mm	600 (83.2%)	121 (16.8%)	1	0.349
>20 mm	521 (81.3%)	120 (18.7%)	1.14	
Lymph node				
Negative	738 (83.9%)	142 (16.1%)	1	0.042
Positive	383 (79.5%)	99 (20.5%)	1.34	
Age				
≤50 years	346 (80.7%)	83 (19.3%)	1.18	0.278
>50 years	775 (83.1%)	158 (16.9%)	1	

Data are number of patients (%).
*HER-2/neu was defined as negative when the DAKO score was 0 or 1+, and positive when 2+ or 3+; †odds ratio adjusted for all variables in table; ‡ER and PR were defined as negative when the H score was ≤50, and positive when 51–300.
ER, oestrogen receptor; PR, progesterone receptor.

Table 6 Frequency of HER-2/neu expression by joint ER/PR status

	HER-2/neu expression*				Total
	Negative		Positive		
	N	%	N	%	
ER ⁻ PR ⁻ †	168	71.2	68	28.8	236
ER ⁻ PR ⁺	16	72.7	6	27.3	22
ER ⁺ PR ⁻	223	88.5	29	11.5	252
ER ⁺ PR ⁺	806	94.6	46	5.4	852
Total	1213	89.1	149	10.9	1362

*HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+ and positive when 3+; †ER or PR was defined as negative when the H score was ≤50, and positive when 51–300.
ER, oestrogen receptor; PR, progesterone receptor.

tumour grade were independent predictors of HER-2/neu positivity. In the subgroup of women with an ER⁺ tumour, PR negativity remained an independent predictor for HER-2/neu overexpression. For the joint ER/PR subgroup, the likelihood of a positive HER-2/neu status decreased significantly from ER⁻PR⁻ to ER⁺PR⁻ and from ER⁺PR⁻ to ER⁺PR⁺ cases.

The presence of ER and oestrogen in human breast cancer cell lines results in a reduction of the concentration of the neu protein.³⁰ The inverse association between steroid hormone receptors and HER-2/neu has also been described in clinical studies.^{8 9 11 12} Most studies on this inverse

Table 7 Frequency of HER-2/neu expression for tumour grade 1–2 and grade 3 lesions by joint ER/PR status

	Grade 1–2			Grade 3		
	HER-2/neu*		Total	HER-2/neu*		Total
	N	Positive		N	Positive	
ER ⁻ PR ⁻ †	20 (69.0%)	9 (31.0%)	29	148 (71.5%)	59 (28.5%)	207
ER ⁻ PR ⁺	9 (100.0%)	0 (0.0%)	9	7 (53.8%)	6 (46.2%)	13
ER ⁺ PR ⁻	150 (92.6%)	12 (7.4%)	162	73 (81.1%)	17 (18.9%)	90
ER ⁺ PR ⁺	611 (97.3%)	17 (2.7%)	628	195 (87.1%)	29 (12.9%)	224
Total	790 (95.4%)	38 (4.6%)	828	423 (79.2%)	111 (20.8%)	534

Data are numbers of patients (%).
*HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+ and positive when 3+; †ER or PR was defined as negative when the H score was ≤50, and positive when 51–300.
ER, oestrogen receptor; PR, progesterone receptor.

Table 8 Frequency of ER or PR expression by HER-2/neu status

HER-2/neu*	ER†		PR‡		Total
	Negative	Positive	Negative	Positive	
Negative	184 (15.2%)	1029 (84.8%)	391 (32.2%)	822 (67.8%)	1213
Positive	74 (49.7%)	75 (50.3%)	97 (65.1%)	52 (34.9%)	149
Total	258 (18.9%)	1104 (81.1%)	488 (35.8%)	874 (64.2%)	1362

Data are numbers of patients (%).
 *HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+ and positive when 3+; †ER or PR was defined as negative when the H score was ≤ 50, and positive when 51–300.
 ER, oestrogen receptor; PR, progesterone receptor.

association have focused on ER or PR alone, and only two studies considered the effect of the absence of PR in women with an ER⁺ tumour.^{11 12} Our results confirm the data of Taucher *et al* on the association between steroid hormone and HER-2/neu receptors using IHC.¹² PR⁻ breast cancers, even if they are ER⁺, are more aggressive than the PR⁺ phenotype.^{26–28 31}

PR⁻ tumours, even if they are ER⁺, are more likely to be of high grade than PR⁺ tumours.²⁶ Tumour grade is one of the best predictors for HER-2/neu overexpression, and may therefore interfere with PR as an independent predictor for HER-2/neu. However, our findings showed that this is not the case. We were able to show that PR is an independent predictor for HER-2/neu overexpression in a multivariate model taking other predictors such as tumour grade into account. We agree that the HER-2/neu overexpression rate is low in a large subgroup of patients with breast cancer.¹² In our study, the population of ER⁺ tumours with a low grade (grade 1–2) comprises 58% of the entire population and these tumours are HER-2/neu positive in only 3.7% of cases. In this low grade ER⁺ population, PR is also predictive for HER-2/neu positivity, which was 2.7% and 7.4% in PR⁺ and PR⁻ tumours, respectively (p = 0.005). Therefore, our data on the importance of PR in predicting HER-2/neu overexpression in low and high grade tumour lesions add to the existing data on this topic and show consistency between populations, so that we still believe in the importance of measuring PR in women with an ER⁺ breast cancer, in contrast to recent reports suggesting otherwise.³²

The inverse association between HER-2/neu and hormone receptors leads to lower or absent hormone receptors in women with HER-2/neu positive breast cancers. This is one of the reasons why women who overexpress HER-2/neu may be resistant to tamoxifen.^{16–20} In women with an ER⁺ breast cancer, HER-2/neu overexpression implies a greater likelihood of the tumour being PR⁻. Rhodes *et al* have shown that the ER⁺PR⁻ variant is a menopause related phenotype.³³ Endogenous oestrogen may be too low to upregulate PR and

Table 10 Factors predicting HER-2/neu overexpression in ER⁺ breast cancers (n = 1104): univariate analysis

	HER-2/neu expression*		Odds ratio†	p Value
	Negative	Positive		
PR expression‡				
Negative	223 (88.5%)	29 (11.5%)	2.28	0.001
Positive	806 (94.6%)	46 (5.4%)	1	
Tumour grade				
1–2	761 (96.3%)	29 (03.7%)	1	<0.001
3	268 (85.4%)	46 (14.6%)	4.50	
Tumour size				
≤ 20 mm	563 (93.4%)	40 (6.6%)	1	0.817
> 20 mm	466 (93.0%)	35 (7.0%)	1.06	
Lymph node				
Negative	676 (93.9%)	44 (6.1%)	1	0.217
Positive	353 (91.9%)	31 (8.1%)	1.35	
Age				
≤ 50 years	299 (91.2%)	29 (8.8%)	1.54	0.079
> 50 years	730 (94.1%)	46 (5.9%)	1	

Data are number of patients (%).
 *HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+ and positive when 3+; †odds ratio adjusted for all variables in table; ‡ER and PR were defined as negative when the H score was ≤ 50, and positive when 51–300.
 ER, oestrogen receptor; PR, progesterone receptor.

Table 11 Factors predicting HER-2/neu overexpression* in ER⁺ breast cancers (n = 1104): multivariate analysis

	Odds ratio† (95% CI)	p Value
PR expression‡ (negative v positive)	2.01 (1.22–3.32)	0.006
Tumour grade (grade 3 v 1–2)	4.27 (2.62 to 6.97)	<0.001
Tumour size (>20 v ≤ 20 mm)	–	0.300
Lymph node (positive v negative)	–	0.692
Age (≤ 50 v > 50 years)	–	0.088

*HER-2/neu was defined as negative when the DAKO score was 0, 1+, or 2+ and positive when 3+; †odds ratio adjusted for all variables in table; ‡ER and PR were defined as negative when the H score was ≤ 50, and positive when 51–300.
 CI, confidence interval; ER, oestrogen receptor; PR, progesterone receptor.

repress HER-2/neu by oestrogen binding to ER, so that the inverse association might be restricted to postmenopausal women. Recently, it has been shown that the ER⁺PR⁻ phenotype is predictive of tamoxifen resistance in postmenopausal women. ER⁺PR⁻ tumours have a higher response to aromatase inhibitors and no further response beyond two years of tamoxifen use compared with the ER⁺PR⁺ phenotype.^{34 35} However, these randomised controlled studies did not associate HER-2/neu positivity with the ER⁺PR⁻ phenotype, but our findings and those of others suggest this as one of the underlying mechanisms for

Table 9 Frequency of joint ER/PR expression by HER-2/neu status

HER-2/neu*	Joint ER/PR†				Total
	ER ⁻ PR ⁻	ER ⁺ PR ⁻	ER ⁻ PR ⁺	ER ⁺ PR ⁺	
Negative	168 (13.8%)	16 (1.3%)	223 (18.4%)	806 (66.4%)	1213
Positive	68 (45.6%)	6 (4.0%)	29 (19.5%)	46 (30.9%)	149
Total	236 (17.3%)	22 (1.6%)	252 (18.5%)	852 (62.6%)	1362

Data are number of patients (%).
 *HER-2/neu was defined as negative when the DAKO score was 0, 1+, and 2+ and positive when 3+; †ER or PR was defined as negative when the H score was ≤ 50, and positive when 51–300.
 ER, oestrogen receptor; PR, progesterone receptor.

Take home messages

- Using multivariate analysis, we found that the oestrogen receptor (ER), progesterone receptor (PR), and tumour grade were independent predictors of HER-2/neu overexpression in women with operable breast cancer
- ER and PR were inversely related to HER-2/neu overexpression, whereas tumour grade was positively associated with HER-2/neu overexpression
- In women with ER⁺ tumours, the expression of PR affects the likelihood of HER-2/neu overexpression, and it may be that women with ER⁺ PR⁻ tumours should be targeted with more aggressive treatment than those with ER⁺ PR⁺ tumours

tamoxifen resistance in such patients. This is another reason to measure PR in women with an ER⁺ tumour.³² The mechanism behind PR negativity in premenopausal women with an ER⁺ breast cancer and large amounts of circulating oestrogen is not known, but may be different from that in postmenopausal women. Whether HER-2/neu overexpression also implies a greater likelihood for PR negativity in premenopausal women with an ER⁺ breast cancer is the subject of our own ongoing research, but one study indicated that HER-2/neu overexpression is not a predictor for resistance to antioestrogen treatment in premenopausal women with early breast cancer.³⁶

“We were able to show that PR is an independent predictor for HER-2/neu overexpression in a multivariate model taking other predictors such as tumour grade into account”

Our IHC definition of HER-2/neu positivity has been shown to be comparable to FISH testing for HER-2/neu,²² and we found 100% agreement between IHC 3+ and FISH gene amplification for HER-2/neu in a small subgroup of women with a FISH result available. A small number of patients with a lower than 3+ score for HER-2/neu may also test positive using FISH.³⁷ Most studies on HER-2/neu expression examined HER-2/neu by enzyme linked immunosorbent assay or considered DAKO score 2+ cases in the group of HER-2/neu positive tumours when using IHC. This may explain why our frequency data for HER-2/neu overexpression are lower than in other reports. When we considered DAKO score 2+ or 3+ versus 0 or 1+ as HER-2/neu positive and negative, respectively, the inverse association between HER-2/neu and hormone receptors remained significant, but the odds ratios of the different variables predicting HER-2/neu positivity were slightly lower. Furthermore, our data are also reliable in that we used a large number of patients from one centre where the scoring analysis was validated by one pathologist (Dr M Drijckoning).

In conclusion, we examined whether ER, PR, tumour grade, and other clinicopathological factors in all women with breast cancer and in women with an ER⁺ breast cancer were associated with HER-2/neu positivity. We found such a predictive role for ER and PR in a multivariate model also including tumour grade. We suggest that the inverse relation between loss of ER and HER-2/neu overexpression should be extended to loss of PR in ER⁺ breast cancers. Whether women with an ER⁺PR⁻ breast cancer need more aggressive treatment or a combination of hormone treatment and

anti-HER-2/neu antibodies in contrast to women with an ER⁺PR⁺ breast cancer will become clear from the results of major breast cancer treatment trials taking all these different variables into consideration.

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