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Original Article

Association between hormone receptors and HER-2/neu is age-related

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Abstract: Purpose: To investigate the association between hormone receptors and HER-2/neu in different age groups of women with breast cancers. Methods: A total of 1036 women with breast cancers were recruited. All the patients were divided into nine groups. The expression of hormone receptors and HER-2/neu was studied by IHC, while FISH test was used to determine HER-2/neu status in cases scored IHC 2+. The association between hormone receptors and HER-2/neu in different age groups was evaluated using the χ^2 test. Multivariate analysis was used to find out the independent factors predicting HER-2/neu amplification. Significant findings: The expression of ER and PR was inversely correlated with HER-2/neu status in women aged >40 years. By multivariate analysis, as far as the overall groups were concerned, PR, lymph node status and tumor grade were independently associated with HER-2/neu; Considering the younger age group (≤40), the only predictor for HER-2/neu was the tumor grade; Considering the older age group (>40), tumor grade, PR status, tumor size and lymph node status were associated with HER-2/neu overexpression. Conclusions: Our data suggest that the association between ER, PR and HER-2/neu is age-related. The negative relationship is only applied for women aged >40 years.

Keywords: ER, PR, HER-2/neu, breast cancer

Introduction

Breast cancer is the most commonly diagnosed carcinoma and the second leading cause of cancer deaths among women in China [1]. In recent years, both the incidence and mortality of breast cancers are steadily rising [2-4]. Breast cancers are biologically heterogeneous. The subtype classifications most often used in clinical settings are based on the commonly measured tumor markers ER, PR and HER-2/neu, which offer imperfect but practical surrogates for genomic profiling [5, 6]. It is increasingly recognized that breast cancer subtypes vary in occurrence (especially by race/ethnicity) [5-9], in their detection by screening mammography [6, 10, 11], and in their risk association with other factors [6, 12-16]. Prognosis and management also depend on breast cancer subtypes.

Despite accumulating evidence that breast cancer subtypes should be considered separately, it is still a routine to present statistics that consider the disease as a single entity [6]. This single estimate does not convey age-related variation in breast cancer risks. One clinically important example is that hormone receptors do not predict the HER-2/neu status in all age groups of women [17]. Moreover, different races can also contribute to the variations of breast cancer subtypes. It is reported that the characteristics of breast cancer in Asia is very different from that in non-Asian countries, which is characterized by the early tumor onset, showing a relatively younger median age at diagnosis [18]. In this study, we aimed to investigate the relationship between different age groups and the status of hormone receptors (ER and PR) and HER-2/neu in our local population.

Patients and methods

Patients

From January 2010 to October 2014 all the primary breast cancer diagnosed at the

Table 1. Association between ER and HER-2/neu in women by age range of 5 years (n=1036)

Age groups (years)	ER	HER-2/neu status-number (%)		Total	χ^2	*P*
		Negative	Positive			
≤35	-	9 (69.2)	4 (30.8)	13	0	1.0
	+	23 (74.2)	8 (25.8)	31		
36-40	-	18 (72.0)	7 (28.0)	25	0.003	0.960
	+	37 (72.5)	14 (27.5)	51		
41-45	-	22 (62.9)	13 (37.1)	35	6.674	0.010
	+	91 (83.5)	18 (16.5)	109		
46-50	-	29 (46.8)	33 (53.2)	62	26.672	0.000
	+	110 (82.7)	23 (17.3)	133		
51-55	-	25 (43.1)	33 (56.9)	58	22.187	0.000
	+	69 (81.2)	16 (18.8)	85		
56-60	-	28 (41.8)	39 (58.2)	67	31.672	0.000
	+	91 (82.7)	19 (17.3)	110		
61-65	-	23 (53.5)	20 (46.5)	43	13.645	0.000
	+	69 (84.1)	13 (15.9)	82		
66-70	-	12 (63.2)	7 (36.8)	19	3.866	0.049
	+	33 (89.2)	4 (10.8)	37		
>70	-	11 (47.8)	12 (52.2)	23	16.873	0.000
	+	48 (90.6)	5 (9.4)	53		

Table 2. Association between PR and HER-2/neu in women by age range of 5 years (n=1036)

Age groups (years)	PR	HER-2/neu status-number (%)		Total	χ^2	*P*
		Negative	Positive			
≤35	-	10 (71.4)	4 (28.6)	14	0.000	1.000
	+	22 (73.3)	8 (26.7)	30		
36-40	-	15 (65.2)	8 (34.8)	23	0.843	0.358
	+	40 (75.5)	13 (24.5)	53		
41-45	-	24 (66.7)	12 (33.3)	36	3.960	0.047
	+	89 (82.4)	19 (17.6)	108		
46-50	-	39 (52.0)	36 (48.0)	75	22.135	0.000
	+	100 (83.3)	20 (16.7)	120		
51-55	-	30 (44.1)	38 (55.9)	68	26.897	0.000
	+	64 (85.3)	11 (14.7)	75		
56-60	-	36 (44.4)	45 (55.6)	81	35.200	0.000
	+	83 (86.5)	13 (13.5)	96		
61-65	-	29 (52.7)	26 (47.3)	55	22.022	0.000
	+	63 (90.0)	7 (10.0)	70		
66-70	-	15 (65.2)	8 (34.8)	23	4.157	0.041
	+	30 (90.9)	3 (9.1)	33		
>70	-	19 (57.6)	14 (42.4)	33	13.510	0.000
	+	40 (93.0)	3 (7.0)	43		

Department of Pathology, the First Affiliated Hospital of Zhejiang University, Hangzhou, China, were included in our research work. Women who had recurrent tumors, as well as those with missing data on the tumor characteristics were excluded. The remaining 1036 cases (922 from mastectomies, 114 from needle core biopsies: 14 in women aged ≤40; 100 in women aged >40) were considered in the analyses. This research was approved by the ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. The ethics committee waived the need for consent for the data were analyzed anonymously.

Methods

The expression of ER, PR and HER-2/neu status was examined using 10% neutral formalin-fixed paraffin-embedded tissues. Automated IHC of HER2/neu (4B5, Ventana Medical Systems, Tucson, AZ, USA), ER (SP1, 1:200, DAKO, Denmark), PR (PgR636, 1:200, DAKO, Denmark) was performed on 4 μm thick tissue sections using an automated slide stainer, the Ventana Benchmark XT (Ventana Medical Systems, Tucson, AZ, USA). HER-2/neu immunoreactivity was evaluated in a semi-quantitative way. IHC3+ (positive): circumferential membrane staining that is complete, intense and within >10% of tumor cells. IHC2+ (equivocal): circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of tumor cells

Table 3. Association between clinical-pathological features and different age groups (n=1036)

Variable	Age-number (%)		Total	χ^2	*P*
	≤40	>40			
ER expression					
Negative	38 (11.0)	307 (89.0)	345	0.163	0.686
Positive	82 (11.9)	609 (88.1)	691		
PR expression					
Negative	37 (9.1)	371 (90.9)	408	4.155	0.042
Positive	83 (13.2)	545 (86.8)	628		
HER-2/neu status					
Negative	87 (11.6)	661 (88.4)	748	0.006	0.938
Positive	33 (11.5)	255 (88.5)	288		
Tumor size					
≤2 cm	61 (11.6)	466 (88.4)	527	0.007	0.931
>2 cm	45 (11.4)	350 (88.6)	395		
(Excluding: breast core biopsies n=114)					
Lymph node					
Negative	66 (12.3)	469 (87.7)	535	0.883	0.347
Positive	40 (10.3)	347 (89.7)	387		
(Excluding: breast core biopsies n=114)					
Tumor grade					
I	9 (8.1)	102 (91.9)	111	2.404	0.301
II	38 (10.7)	317 (89.3)	355		
III	59 (12.9)	397 (87.1)	456		
(Excluding: breast core biopsies n=114)					

or complete and circumferential membrane staining that is intense and within ≤10% of tumor cells. IHC 1+ (negative): incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells. IHC 0 (negative): no staining is observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of tumor cells. The ER and PR results were interpreted as positive when more than 1% of tumor cells showed positive nuclear staining.

FISH test was used to determine the HER-2/neu status in cases scored IHC 2+. 4 μm sections were cut for FISH analysis. The sections were baked overnight at 60°C, deparaffinized in two 10-min changes of xylene, transferred through two 3-min changes of 100% ethanol, one 3-min change of 85% ethanol, one 3-min change of 70% ethanol and immersed for 25 min in distilled water at 90°C. The slides were then incubated for 10 min in protease solution at 37°C. After that, the slides were briefly washed in sodium saline citrate (SSC, pH 7.2) at room temperature, dehydrated through 70%, 85%, 100% ethanol and acetone. After drying in the open-air, 10 μl of probe (Jinpujia, Beijing, China) was applied onto each slide, cover slip was placed and sealed with rubber cement, and then the slides were transferred to the hybridization oven (S500-24, Abbott molecular, USA). The procedure was as follows: denature at 83°C for 5 min, and hybridization overnight at 42°C. After that, the slides were washed in 46°C preheated post-hybridization buffer (2XSSC/0.1% sodium dodecyl sulfate) for 5 min and rinsed in 70% ethanol. After air-drying, the slides were counterstained with 15 μl DAPI and cover slip applied.

Thirty randomly selected invasive tumor nuclei in each of two separate, distinct microscopic areas were evaluated. Positive for HER-2/neu is defined as HER-2/CEP 17 ratio ≥2.0 or HER-2/CEP17ratio <2.0 with an average HER-2 copy number ≥6.0. Equivocal for HER-2 is defined as HER-2/CEP17 ratio <2.0 with an average HER-2 copy number ≥4.0 and <6.0 signals/cell. Negative for HER-2 is defined as HER-2/CEP17 ratio <2.0 with an average HER-2 copy number <4.0 signals/cell.

Statistical analyses

The correlation analysis of nominal variables was performed using the χ^2 test (SPSS 13.0). Multivariate analysis with logistic regression was used to find out the independent factors predicting HER-2/neu amplification. $P<0.05$ was considered as significant. If there is no significance between the variable and HER2/neu status from the univariate analysis, the variable will not be taken into account in the multivariate analysis

Results

The age of the patients was from 25 to 93-year-old, with an average age of 53.3-year-old, 35.2% of who were between 40 and 50-year-old. According to H. J. Huang's methodology [17], we divided the patients into nine age groups with a range of 5 years starting at age ≤35 years and ending at age >70 years.

Table 4. Univariate analysis of association between clinical-pathological features and HER2/neu status in all the patients included in our study (n=1036)

Variable	HER-2/neu status-number (%)		Total	OR	*P*
	Negative	Positive			
Age (years)					
≤40	87 (72.5)	33 (27.5)	120	1.017	0.938
>40	661 (72.2)	255 (27.8)	916		
ER expression					
Negative	177 (51.3)	168 (48.7)	345	4.516	0.000
Positive	571 (82.6)	120 (17.4)	691		
PR expression					
Negative	217 (53.2)	191 (46.8)	408	4.818	0.000
Positive	531 (84.6)	97 (15.4)	628		
Tumor size					
≤2 cm	412 (78.2)	115 (21.8)	527	0.595	0.000
>2 cm	259 (65.6)	136 (34.4)	395		
(Excluding: breast core biopsies n=114)					
Lymph node					
Negative	413 (77.2)	122 (22.8)	535	0.591	0.000
Positive	258 (66.7)	129 (33.3)	387		
(Excluding: breast core biopsies n=114)					
Tumor grade					
I- II	421 (90.3)	45 (9.7)	466	0.130	0.000
III	250 (54.8)	206 (45.2)	456		
(Excluding: breast core biopsies n=114)					

OR: odds ratio.

Table 5. Multivariate analysis of association between clinical-pathological features and HER-2/neu status in all the patients included in our study (n=1036)

Variable	OR (95% CI)	*P*
PR status (negative vs positive)	2.892 (2.063-4.054)	0.000
Lymph node (negative vs positive)	0.575 (0.414-0.799)	0.001
Tumor grade (II + I vs III)	0.182 (0.125-0.265)	0.000

OR: odds ratio; 95% CI: 95% confidence interval.

The results for the association between HER-2/neu status and ER expression were shown in **Table 1**. There was a negative correlation between ER and HER-2/neu in women aged >40 years old. However, no relationship was observed in women aged ≤40 years old. Similarly, PR inversely correlated with HER-2/neu in women aged >40 years old, but this was not the case in other age groups (**Table 2**). From these results, we divided the entire patients into two age groups (≤40, >40). The clinical-pathological features in different age groups were presented in **Table 3**. Compared with women aged >40, those ≤40 were more likely to be PR-positive.

Table 4 showed the association between clinical-pathological features and HER-2/neu status in overall groups from univariate analysis. HER-2/neu positive tumors were more often ER and PR negative, larger tumor size, lymph node positive and higher tumor grade. In the multivariate model, the only valuable predictors for HER-2/neu amplification were PR,lymph node status and tumor grade (**Table 5**).

Table 6 summarized the association between clinical-pathological features and HER-2/neu status in women aged ≤40. From univariate analysis and multivariate analysis, only the tumor grade independently predicted the HER-2/neu overexpression (**Table 7**).

The results from univariate analysis and multivariate analysis for the association between clinical-pathological features and HER-2/neu status in women aged >40 were presented in **Tables 8** and **9**. ER and PR negatively associated with HER-2/neu amplification, while lymph node status,tumor size and tumor grade positively correlated with HER-2/neu overexpression (**Table 8**). Multivariate analysis revealed that HER-2/neu amplification was independently predicted by PR status, tumor grade, tumor size and lymph node status (**Table 9**).

Discussion

Breast cancer in Asia is characterized by a lower incidence than in Western population. Age is the major factor on breast cancer incidence. The most affected women in Asia countries are between 40 and 50 years old, whereas the peak age in the Western countries is between 60 and 70 years [18]. In this study, the patients were in the age group of 25 to 93-year-old, with an average age of 53.3-year-old, 35.2% of who were between 40 and 50-year-old. Several factors might account for the dis-

Table 6. Univariate analysis of association between clinical-pathological features and HER2/neu status in women aged ≤40 (n=120)

Variable	HER-2/neu status-number (%)		Total	OR	*P*
	Negative	Positive			
ER expression					
Negative	27 (71.1)	11 (28.9)	38	1.111	0.809
Positive	60 (73.2)	22 (26.8)	82		
PR expression					
Negative	25 (67.6)	12 (32.4)	37	1.417	0.420
Positive	62 (74.7)	21 (25.3)	83		
Tumor size					
≤2 cm	43 (70.5)	18 (29.5)	61	1.117	0.792
>2 cm	32 (71.1)	13 (28.9)	45		
(Excluding: breast core biopsies n=14)					
Lymph node					
Negative	50 (75.8)	16 (24.2)	66	0.533	0.148
Positive	25 (62.5)	15 (37.5)	40		
(Excluding: breast core biopsies n=14)					
Tumor grade					
I-II	39 (83.0)	8 (17.0)	47	0.321	0.016
III	36 (61.0)	23 (39.0)	59		
(Excluding: breast core biopsies n=14)					

OR: odds ratio.

Table 7. Multivariate analysis of association between clinical-pathological features and HER2/neu status in women aged ≤40 (n=120)

Variable	OR (95% CI)	*P*
Tumor grade (II + I vs III)	0.321(0.128-0.808)	0.000

OR: odds ratio; 95% CI: 95% confidence interval.

parities in epidemiology of breast cancer between different races, including differences in biological characteristics of the tumor [19-21], differences in treatment received [19, 22, 23], lack of access to care [19, 24] or inadequate follow-up after abnormal screening mammography or treatment [19, 25, 26], and overall differences in income and insurance coverage [19, 27]. Further studies regarding diagnosis, screening activities, lifestyle and genetic susceptibility are needed in order to clarify the reasons for these dissimilarities.

The positive percentage of HER-2/neu amplification in our study was 27.8%, which was consistent with the commonly accepted rate of 20% to 30% [28], but lower than some neighboring countries such as India [29]. The correlation between hormone receptors and HER-2/neu has been reported in many published literatures. It has been well-defined that there is an inverse relationship between the expression of ER, PR and HER-2/neu amplification in both preclinical and clinical studies [30-34]. This inverse relationship has been linked with the fact that estrogens and its receptor are required to suppress HER-2/neu [35]. ER and HER-2/neu signaling are inversely related through a transcriptional repression of HER-2/neu by estradiol binging to ER [35]. But this reverse relationship cannot explain some clinical trials. One example is that premenopausal hormone responsive breast cancers remain sensitive to anti-estrogens, whereas a lower response to tamoxifen has been observed in postmenopausal women with HER-2/neu overexpression [36]. Therefore, it is reasonable to suppose that the inverse association may differ by age. Huang et al noted that ER, PR and tumor grade were associated with HER-2/neu only in women aged >45 years [17]. Sharif MA et al also determined that PR only showed association with HER-2/neu in the pen-menopausal and postmenopausal women [37]. In our study, we concluded that there was an inverse association between the expression of ER, PR and HER-2/neu amplification only in women aged >40 years. Considering the entire patients, PR was a valuable independent predictor for HER-2/neu amplification from the multivariate analysis. Multivariate analysis also revealed that PR was negatively associated with HER-2/neu amplification in women aged >40 years, but not in women aged ≤40. Moreover, multivariate analysis showed that only the tumor grade independently predicted the HER-2/neu overexpression in women aged ≤40. It was concordant with the fact that the prognostic effect of PR was confined to postmenopausal women because a high tumor grade overrules any other prognostic tumor characteristic in premenopausal women [17, 38].

Table 8. Univariate analysis of association between clinical-pathological features and HER2/neu status in women aged >40 (n=916)

Variable	HER-2/neu status-number (%)		Total	OR	*P*
	Negative	Positive			
ER expression					
Negative	150 (48.9)	157 (51.1)	307	5.458	0.000
Positive	511 (83.9)	98 (16.1)	609		
PR expression					
Negative	192 (51.8)	179 (48.2)	371	5.753	0.000
Positive	469 (86.1)	76 (13.9)	545		
Tumor size					
≤2 cm	369 (79.2)	97 (20.8)	466	0.565	0.000
>2 cm	227 (64.9)	123 (35.1)	350		
(Excluding: breast core biopsies n=100)					
Lymph node					
Negative	363 (77.4)	106 (22.6)	469	0.597	0.001
Positive	233 (67.1)	114 (32.9)	347		
(Excluding: breast core biopsies n=100)					
Tumor grade					
I-II	382 (91.2)	37 (8.8)	419	0.113	0.000
III	214 (53.9)	183 (46.1)	397		
(Excluding: breast core biopsies n=100)					

OR: odds ratio.

Table 9. Multivariate analysis of association between clinical-pathological features and HER2/neu status in women aged >40 (n=916)

Variable	OR (95% CI)	*P*
Tumor grade (II + I vs III)	0.175 (0.116-0.264)	0.000
PR status (negative vs positive)	3.438 (2.377-4.971)	0.000
Tumor size	0.669 (0.464-0.964)	0.031
Lymph node (negative vs positive)	0.660 (0.457-0.952)	0.026

OR: odds ratio; 95% CI: 95% confidence interval.

Controversy on the correlation between lymph node status and HER-2/neu amplification still surrounds. Susanne et al described the lack of a correlation between lymph node involvement and HER-2/neu status (*P*=0.382) in patients with primary breast carcinoma, which indicated that HER-2/neu positivity was equally distributed in lymph node-negative and lymph node-positive patients [31]. Huang et al observed that HER-2/neu overexpression was not associated with a positive lymph node status [17]. However, there are still a number of literatures holding different opinion. Vaidyanathan et al concluded that lymph node status was positively associated with ErbB-2 status [29]. Similarly, Tiwari et al also found that amplification and overexpression of HER-2/neu was significantly correlated with the status of the axillary lymph nodes (*P*=0.02) [39]. Regarding our research, patients with positive lymph node status were more likely to be HER-2/neu amplification from univariate analysis. Lymph node status was also a valuable independent predictor in women aged >40. Moreover, differences in HER-2/neu status between tumor grades were significant. Tumors with higher tumor grade were more often positive for HER-2/neu amplification. The underlying reason for the disparity on the relationship between lymph node status and HER-2/neu is that the number of cases included in these studies was so limited that it was difficult to make confident statistical statements [40]. As a result, a much larger scale of patients should be enrolled to resolve the dilemma.

The shortcoming of these researches is that they did not use FISH to measure HER-2/neu status in cases scored IHC 2+. Nowadays, FISH method has been regarded as a golden standard for its sensitivity and specificity. Though a high concordance rate in breast carcinomas with IHC score 3+ or 1+/0 between IHC and FISH has been well established, discrepancies regarding the equivocal cases (IHC 2+) still remain. We previously demonstrated that 65.5% of IHC 2+ patients were negative for HER-2/neu amplification, 29.0% were positive [33]. It may lead to bias to consider all the breast cancer cases with IHC score 2+ as negativity. This may be another reason for the differences of the conclusions between ours and other literatures.

Taken together, our data suggest that the association between hormone receptors and HER-2/neu is age-related. The negative relationship between ER, PR and HER-2/neu is just applied

for women aged >40 years. When we use HER-2/neu as a predictive factor and make clinical decision, this relationship should be taken into consideration. Above all, the accurate evaluation of hormone receptors and HER-2/neu is utmost important.

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Disclosure of conflict of interest

None.

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