RESEARCH COMMUNICATION

Factors Associated with HER2 Overexpression in Breast Cancer: Experience in an Asian Developing Country

Gie-Hooi Tan[1], Wan-Yuen Choo[2], Nur Aishah Taib[1], Cheng-Har Yip[1]*

Abstract

Introduction: The HER2 gene is amplified in up to 30% of human breast cancers, leading to overexpression of the HER2 protein on the cell surface. Overexpression of HER2 is associated with a more aggressive cancer and hence a poorer overall survival. Objective: To evaluate the association between clinico-pathological features and HER2 overexpression in breast cancer. Methods: This is a retrospective study conducted in the Department of Surgery, University Malaya Medical Centre. The association between HER2 overexpression, determined by immunohistochemistry, and other clinicopathological factors was evaluated in 996 patients with newly diagnosed breast cancer treated from 2005 to 2007 using univariate and multivariate logistic regression. Results: HER2 overexpression occurred in 30.3% of patients. On bivariate analysis, HER2 overexpression was inversely related to ER expression (p<0.01) and PR expression (p<0.01). This overexpression was associated with a higher tumour grade, lymphovascular positivity and infiltrating ductal carcinoma subtype. On multivariate analysis, HER2 overexpression was significantly associated with higher tumour grade (p=0.018, CI 1.25-11.04), PR negativity (p=0.002, CI 0.30-0.77) and lymphovascular positivity (p=0.042, CI 1.01-2.12). Conclusions: HER2 overexpression was observed in 30.3% of Malaysian female breast cancer patients. This group of patients represents a more aggressive subtype of breast cancer with higher tumour grade, PR negativity and lymphovascular positivity. No significant relationship was established between HER2 overexpression and age, race, lymph node, ER, pathology subtype and stage of disease from this study.

Key Words: Breast cancer - HE2 overexpression - clinicopathological factors

Asian Pacific J Cancer Prev, **10**, 837-840

Introduction

Human epidermal growth factor receptor 2 (HER2) overexpressed breast cancer is one of the subtypes of breast cancer which is gaining major interest with the discovery of targeted therapy as an effective treatment modality. The HER2 gene is located on chromosome 17q. The HER2 gene encodes a 185 kDa transmembrane phosphoglycoprotein with tyrosine kinase activity. HER2 gene amplification is found in 10-35% of invasive breast cancer. Cells transfected with HER2 acquire a more malignant phenotype as they are correlated with poor prognostic tumour characteristics eg. higher histological grade, increased tumour size, lymph node involvement, lymphovascular invasion and lower ER expression. Amplification of the HER2 gene is a significant predictor of both overall survival and time to relapse in patients with lymph-node positive breast cancer. (Slamon et al., 1987)

The aim of this study is to evaluate the association between HER2 overexpression in breast cancer and other clinicopathological parameters such as race, tumour histological type and grade, estrogen and progesterone receptor status, tumour size, patients' age, stage of disease at presentation,lymph node metastases and lymphovascular invasion.

We conducted a retrospective review of 1147 patients with newly diagnosed breast cancer treated in the University Malaya Medical Center from January 2005 to December 2007. The patients' age at onset, self-reported race, stage of disease, histological type, size, tumour grade, lymphovascular invasion and lymph node status were evaluated from pathological and clinical reports. The patients were staged according to the American Joint Commission on Cancer (AJCC) Cancer Staging Manual 6th Ed (Greene et al., 2002). We excluded 151 patients where the ER, PR and HER2 status was not available in the patient records, and the final analysis was conducted on 996 patients.

The ER, PR and HER2 status was determined in each case by immunohistochemical staining. HER2 was scored using a scale from 0 to 3 according to the criteria set by DAKO. Only a score of 3+ was considered as indicating HER2 overexpression. Fluorescence *in situ* hybridization (FISH) was not performed for the tumours in the present study.

*[1]Department of Surgery, University of Malaya Medical Center, [2]Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, *For Correspondence: yipch@um.med.my*

Materials and Methods

Statistical analysis

Descriptive and inferential analyses were performed using SPSS Inc. software (Version 16). Continuous variables were summarized as means and standard deviation. Categorical variables were summarized into frequency and proportion. Univariate logistic regression analysis was used to determine the association between expression of HER2 and each clinicopathological parameters. Multivariate Logistic Regression was used to determine the independent predictors of HER2 overexpression after adjusting for confounders. All variables with $p<0.25$ and biological plausibles were entered for multivariate analysis. The final model included all variables with $p<0.05$ and concluded based on the Principle of Parsimony. Both crude and adjusted odds ratio with 95% confidence interval was reported. The Hosmer-Lemeshow (H-L) approach was used to test the overall goodness-of-fit of the final model. To measure the model's ability to discriminate between women with HER-2 overexpressed versus those who did not, the area under the ROC (Receiver Operating Characteristic) curve was plotted.

Results

The clinicopathological characteristics of the patients are summarized in Table 1. The age ranged from 22 to 88 years (mean age 53.3 years). 125 (12.6%) patients were less than 40 years old. 70.1% of the patients were Chinese, 17.6% were Malays and 12.3% were Indians. The size of the tumour ranged from 0.2cm to 23cm (mean size: 3.92cm). It was between 2 and 5 cm in 45.4% of the cases, less than 2cm in 35.9% and more than 5cm in 18.7%. The histological grading was done according to Bloom-Richardson scoring system. 7.3% were grade 1, 47.9% were grade 2 and 44.7% were grade 3. The majority of the tumours were infiltrating ductal carcinoma (87.3%). 79.5% of patients presented with early breast cancer (stage 1 and 2).

Overall HER2 overexpression was found to be 30.3% in this study. ER positivity was 56.0% and PR positivity 48.2%. In bivariate analysis, ER found to be inversely related to HER2 overexpression; HER2 was overexpressed in only 21.5% of ER positive breast cancer, compared to 41.6% of ER negative breast cancer ($p<0.01$). A similar association between HER2 over-expression and PR was seen, being 18.8% in PR positive cancers compared to 41.1% in PR negative cancers. ($p<0.01$) There was no difference between age of onset in HER2 overexpressed and non-HER2 overexpressed cancers. HER2 overexpression was found to be less common in non-infiltrating ductal carcinomas. There was no association of HER2 overexpression with stage, size and lymph node positivity ($p>0.05$). However there was a significant association with higher tumour grade ($p<0.01$), and lymphovascular invasion ($p=0.03$).

In multivariate analysis, the HER-2 overexpression was significantly associated with higher tumour grade ($p=0.018$, 95% CI 1.25-11.04), PR negativity ($p=0.002$, 95% CI 0.30-0.77) and lymphovascular invasion ($p=0.042$, 95% CI 1.01-2.12) (Table 2).

Discussion

The HER2 oncogene was first described in 1985 as a cell receptor of the tyrosine kinase gene family, with a primary sequence similar to the human epidermal growth factor receptor. (Coussens et al., 1985; Schechter et al.,

Table 1. Clinicopathological Features of HER2 Over-Expressed Breast Cancer

		Total	HER2 overexpressed	Crude OR	95% CI	p value
Age	Less than 40	125	38 (30.4%)	Reference		0.98
	40 and above	871	264 (30.3%)	0.99	0.66-1.50	
Race	Chinese	686	210(30.6%)	Reference		0.74
	Malays	172	48 (27.6%)	0.88	0.61-1.27	
	Indians	120	38 (31.7%)	1.05	0.69-1.60	
Histological type	IDC	870	281 (32.3%)	Reference		<0.01
	Non IDC	126	21 (16.7%)	0.419	0.26-0.68	
Stage	1-2	779	223(28.6%)	Reference		0.29
	3-4	204	68 (33.3%)	1.19	0.86-1.66	
Size	2 cm and less	343	96 (30.0%)	Reference		0.05
	2-5 cm	434	123 28.3%)	1.02	0.74-1.39	
	>5 cm	179	67 (37.4%)	1.54	1.05-2.26	
Grade	1	57	6 (10.5%)	Reference		<0.01
	2	373	92 (24.7%)	2.78	1.16-6.70	
	3	347	129 (37.2%)	5.00	2.09-12.0	
Lymph node	Negative	538	156 (29.0%)	Reference		0.27
involvement	Positive	450	145 (32.2%)	1.164	0.89-1.53	
ER	Negative	438	182 (41.6%)	Reference		<0.01
	Positive	558	120 (21.5%)	0.39	0.29-0.51	
PR	Negative	516	212 (41.1%)	Reference		<0.01
	Positive	480	90 (18.8%)	0.33	0.25-0.44	
Lymphovascular	Negative	433	116 (26.8%)	Reference		0.04
invasion	Positive	374	125 (33.4%)	1.37	1.01-1.86	

Table 2. Multivariate Logistics Regression for Predictors Associated with HER2 Overexpresssion

Variables		Adjusted ORs*	95% confidence interval	p value
Tumour grade[1]	2	2.83	0.97 - 8.25	0.057
	3	3.82	1.29 - 11.3	0.015
PR status[1]		0.47	0.29 - 0.74	0.001
Invasion[1]		1.51	1.05 - 2.16	0.025

*Adjusted for histology subtype and ER status; [1]Referent groups, Grade 1, PR negative, lymphovascular invasion negative Overall goodness-of-fit test shows that the model fits the data (p=0.935). Area under the ROC curve was 0.672 indicating the model to have acceptable discrimination

1985). Slamon et al later demonstrated that amplification of the HER2 was a significant predictor of both overall survival and time to relapse in patients with breast cancer. (Slamon et al., 1987), and this was confirmed by other studies. (Tandon et al., 1989; Borg et al., 1990). The prognostic impact of HER2 overexpression was strongest in node positive patients, whereas its prognostic impact was weak and not statistically significant in the node negative patients. (Menard et al., 2002).

In most studies, there seems to be no significant difference in incidence of HER2 overexpression between different ethnic groups. Unlike triple negative breast cancer (ER negative, PR negative and HER2 negative) which is more common in African-Americans compared to White Americans, there is no difference in HER2 overexpression rate between the two groups. (Stark et al., 2008). Another study had shown no difference in Asians, Hispanics, Blacks or Whites in a socioeconomically homogenous cohort in a New York public hospital. (Marti et al., 2008). Similarly we did not find any differences in HER-2 over-expression among the Malays, Chinese and Indians in this multiethnic group of patients in Malaysia.

Previous studies on HER2 have been carried out in predominantly Caucasian populations with little data in the Asian region. Reported incidences have ranged from 21% (Yau et al., 2008) to 44% (Looi and Cheah, 1998). A recent publication in a tertiary teaching hospital in Malaysia showed a 32% rate of HER2 overexpression by immunohistochemistry (IHC), which is similar to the rate found in the present study. However, the current methods of determining overexpression HER2 in breast cancer, that is, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) have shown considerable problems in accuracy and interlaboratory reproducibility. A study in Hong Kong found that more than 25% of HER2 overexpression identified by IHC assays in this Hong Kong cohort could not be verified by confirmatory FISH assays.Compliance with the latest guidelines for HER2 testing should improve the future accuracy and concordance (Yau et al., 2008). When carefully validated testing is performed, available data do not clearly demonstrate the superiority of either immunohistochemistry (IHC) or in situ hybridization (Wolff et al., 2007)

HER2 overexpression has been associated with tumour type, being very rare in non-infiltrating ductal carcinoma (Ariga et al., 2005). It is also associated with higher grade and hormone receptor negativity (Looi and Cheah, 1998; Ariga et al., 2005; Selvarajan et al., 2006), and not to be associated with the size of tumour and age at presentation (Supanaranond et al., 1997; Ariga et al., 2005) while other studies also showed that HER2 overexpression was associated with large tumours and lymphovascular invasion (Menard et al., 2002). A significant association with higher grade, hormone receptor positivity and lymphovascular invasion was seen in our study. Although there appears to be no association with stage and lymph node positivity (Menard et al., 2002; Yau et al., 2008) the prognostic influence of HER2 overexpression increases arithmatically with increasing number of involved axillary lymph nodes. (Mittra et al., 1995). This present study is similar in that HER2 overexpression was found to be associated with a higher grade, hormone receptor negativity and lymphovascular invasion, but not to size, stage and lymph node positivity, although in multivariate analysis, high grade, PR negativity and lymphovasulcar invasion remained as significant factors.

Although problems with reproducibility can be partly alleviated by the use of validated, standardised 'kits', there may be considerable cost involved in their use. Prior to testing it may therefore be an advantage to be able to predict from basic pathology data whether a cancer is likely to overexpress HER2. Infiltrating lobular and special types of carcinoma and Grade 1 tumours may not need to be routinely tested at presentation as only 1.4% have been shown to overexpress HER2 (Bilous et al., 2003).

Limitations of this study should be discussed. Determination of ER and PR (by IHC) has been carried out since 1996 in the University Malaya Medical Centre, and although the pathology laboratory started HER2 testing in 2000, it initially had problems with standardization, and it was not until 2005 when the test became more reliable. Hence, the period of this study was from 2005-2007, and outcome data are not yet available. The data on the ER, PR and HER2 were obtained from the pathology reports, and reporting was done by different pathologists. FISH is not available in our centre.

In conclusion, HER2 overexpression was seen in 30.3% of breast cancers in our institution, and is associated with poor prognostic factors such as high grade, hormone receptor negativity, and lymphovascular invasion. There was no association with age, lymph node status, size and stage at presentation. On multivariate analysis, high grade, PR negativity and lymphovascular invasion remained significant associations.

References

Ariga R, Zarif A, et al (2005). Correlation of her-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. *Breast J*, **11**, 278-80.

Bilous M, Ades C, et al (2003). Predicting the HER2 status of breast cancer from basic histopathology data: an analysis of 1500 breast cancers as part of the HER2000 International Study. *Breast*, **12**, 92-8.

Borg A, Tandon AK, et al (1990). HER-2/neu amplification predicts poor survival in node-positive breast cancer. *Cancer Res*, **50**, 4332-7.

Coussens L, Yang-Feng TL, et al (1985). Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science*, **230**, 1132-9.

Greene FL, Fleming ID et al (2002). AJCC Cancer Staging Manual 6th Ed. New York. Springer.

Looi LM, Cheah PL (1998). C-erbB-2 oncoprotein amplification in infiltrating ductal carcinoma of breast relates to high histological grade and loss of oestrogen receptor protein. *Malays J Pathol*, **20**, 19-23.

Marti JL, Guth A, et al (2008). Receptor status and ethnicity of indigent patients with breast cancer in New York City. *Arch Surg*, **143**, 1227-30.

Menard S, Balsari A, et al (2002). HER-2-positive breast carcinomas as a particular subset with peculiar clinical behaviors. *Clin Cancer Res*, **8**, 520-5.

Mittra I, Redkar AA, et al (1995). Prognosis of breast cancer: evidence for interaction between c-erbB-2 overexpression and number of involved axillary lymph nodes. *J Surg Oncol*, **60**, 106-11.

Schechter AL, Hung MC, et al (1985). The neu gene: an erbB-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. *Science*, **229**, 976-8.

Selvarajan S, Wong KY, et al (2006). Over-expression of c-erbB-2 correlates with nuclear morphometry and prognosis in breast carcinoma in Asian women. *Pathology*, **38**, 528-33.

Slamon DJ, Clark GM, et al (1987). Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, **235**, 177-82.

Stark A, Kapke A, et al (2008). Advanced stages and poorly differentiated grade are associated with an increased risk of HER2/neu positive breast carcinoma only in White women: findings from a prospective cohort study of African-American and White-American women. *Breast Cancer Res Treat*, **107**, 405-14.

Supanaranond K, Sukarayodhin S, et al (1997). The significance of HER-2/neu/c-erbB-2 gene amplification in benign and malignant breast disease. *Southeast Asian J Trop Med Public Health*, **28**, 631-40.

Tandon AK, Clark GM, et al (1989). HER-2/neu oncogene protein and prognosis in breast cancer. *J Clin Oncol*, **7**, 1120-8.

Wolff AC, Hammond ME, et al (2007). American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*, **25**, 118-45.

Yau TK, Sze H, et al (2008). HER2 overexpression of breast cancers in Hong Kong: prevalence and concordance between immunohistochemistry and in-situ hybridisation assays. *Hong Kong Med J*, **14**, 130-5.