RESEARCH COMMUNICATION

C-erbB-2 Onco-Protein Expression in Breast Cancer: Relationship to Tumour Characterisitcs and Short-Term Survival in Universiti Kebansaan Malaysia Medical Centre

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Abstract

Breast cancer is the commonest cancer affecting females in Malaysia, contributing 31% of all newly diagnosed cases amongst Malaysian women. The present retrospective cohort study evaluated the relationship between c-erbB-2 onco-protein overexpression with various tumour characteristics and survival rate of breast cancer patients treated at the Universiti Kebangsaan Malaysia Medical Centre (UKMMC) between 1996-2000. C-erbB-2 oncoprotein overexpression was determined by immunohistochemistry (IHC) and tumors showing 2+ positivity were verified by Fluorescence In Situ Hybridization (FISH). One hundred and seventy two patients were eligible for the study with a short-term follow-up (median) of 5.1 years. C-erbB-2 oncoprotein overexpression correlated with lymph node positivity, oestrogen receptor (ER) and progesterone receptor (PR) negativity. Univariate analyses showed shorter disease free survival (DFS) and overall survival (OS) in patients with c-erbB-2 oncoprotein overexpression, Malay ethnicity, higher tumour grade, lymph node positivity, ER and PR negativity. In a subgroup of patients with c-erbB-2 oncoprotein overexpression, a shorter OS was observed in those with lymph node positivity, ER and PR negativity. In multivariate prognostic analysis, lymph node status, ER status and tumour grading were the strongest independent prognostic factors for both OS and DFS. However, c-erbB-2 status was not a significantly independent prognostic factor, even in subsets with lymph node positive or negative group. C-erbB-2 oncoprotein overexpression correlated well with lymph node status, ER and PR. Shorter OS and DFS were significantly observed in patients with c-erbB-2 oncoprotein overexpression. Lymph node status, ER status and tumour grading were the only three independent prognostic factors for OS and DFS in this study. Although c-erbB-2 expression is obviously important from a biological standpoint, multivariate analysis showed that it is not an independent prognostic indicator in breast carcinoma in the local population.

Key Words: C-erbB-2 oncoprotein, breast carcinoma, prognostic factor, disease-free survival, overall survival

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Introduction

Breast cancer is one of the most common malignancies in women and the incidence of this malignancy is increasing worldwide. Breast cancer is the leading cause of cancer deaths in Malaysian women. In the year 2003, 3738 (31.0% of all female cancer) new cases were reported. Of these, 47.0% were from women less than 50 years old (Lim and Halimah, 2004). The age-standardized incidence rate (ASR) of breast cancer was 46.2 per 100,000 women, which means than 1 in 20 women in Malaysia will develop breast cancer in their life. In Malaysia, the survival rate of breast cancer patients is so far unknown due to the under-reported mortality data. However, the overall 5-year survival rate of a group of patients diagnosed in another local university was estimated to be 58% (Yip et al., 2006). The estimated 5-year survival rate of 58% is far lower than in developed countries (73%).

The prognostic determinants of breast cancer are related to patient characteristics, tumour morphology and biology. Some of the latest therapeutic options for breast carcinoma, which will improve the prognosis of the disease, is dependent on the patient and tumour characteristics. These medical developments highlight the increasing importance of prognostic and predictive factors in the management of patients with breast cancer. The conventional prognostic factors for breast carcinoma include distant metastases, locally advanced disease, axillary nodal status, histological type, grading, tumour size, hormone receptor status (oestrogen and progesterone receptor) and c-erbB-2 oncoprotein overexpression (Mori

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et al., 2002).

The measurement of oestrogen receptor (ER) in breast cancer tissue is important to discriminate between hormone-dependent and -independent tumours in order to determine whether endocrine treatments (Tamoxifen) should be administered.

Of patients whose primary tumours demonstrate elevated ER, 55-60% respond to hormone therapy. In patients, whose tumours are positive for both ER and Progesterone receptor (PR), as many as 80% respond favourably. Patients with elevated levels of ER and PR also experience a longer disease-free interval and longer survival than those with low levels (Tesch et al., 1993). Tamoxifen is still currently the golden adjuvant therapy in the treatment of hormone-dependent breast cancer.

The c-erbB-2 oncogene (also known as HER-2/neu) encodes for a transmembrane receptor. The c-erbB-2 oncogene, located on chromosome 17q21, has been shown to be amplified in 10–30% of surgically resected breast cancer cases. It has been suggested that c-erbB-2 oncogene is an independent prognostic indicator for node-positive, but not node-negative, breast cancers (Clark and McGuire, 1991). Amplification of the oncogene and the subsequent over-expression of the protein in breast cancer patients are associated with a high risk of metastasis and tumour recurrence. Recent studies shown that the presence of the c-erbB-2 oncogene protein product on the cell membranes of neoplastic cells may indicate a favourable response to immunotherapy. Trastuzumab, a monoclonal IgG1 class humanized anti-c-erbB-2 antibody, has been developed as a novel anticancer drug, targeting amplified and overexpressed c-erbB-2 (Cobleigh et al., 1999). In the phase III pivot trial, adding trastuzumab to chemotherapy was associated with a longer time to disease progression, longer survival rate and 20% reduction in the risk of death (Slamon et al., 2001). Since only around 10-30% of the breast cancers carry the overexpressed c-erbB-2 oncogene to which this treatment is directed, patient selection is very important in determining eligibility for the drug. Hence, accurate and meaningful interpretation of the c-erbB-2 oncogene overexpression is crucial to provide better yield for this expensive treatment. In some studies, c-erbB-2 oncogene overexpression's ability to predict survival was better than tumour size or hormone receptor status, but inferior to nodal status. The c-erbB-2 is not of value in screening for malignancy or for following therapy (Ross et al., 2003). It has also been described in tumours of ovarian, uterine, and gastrointestinal origin.

There is still a lack of local data on the c-erbB-2 onco-protein, ER and PR expression in breast cancer among Malaysian patients for the purpose of referral and initiation of the latest treatment. A local study has shown a high proportion (38.4%) of overexpressed c-erbB-2 in 112 samples of infiltrating ductal carcinoma of the breast (Looi et al., 1997). Ong and Yip (2003) concluded that 59.9% of the breast tumours expressed oestrogen receptor from 337 local patients. This study aimed to determine the the relationship between c-erbB-2 onco-protein overexpression with various tumour characteristics and short term survival rate in a cohort of breast cancer patients treated in UKMMC.

Materials and Methods

This is a retrospective cohort study using sections of formalin-fixed, paraffin-embedded tissue (PET) samples from primary invasive breast carcinomas (ductal and nonductal) retrieved from UKMMC. A total number of 223 patients were operated (mastectomy or lumpectomy) in UKMMC from 1995 to 2000. However, 46 cases were excluded due to loss of tissue blocks or unsuitable tissue for further laboratory analysis. Therefore only 172 patients were eligible for this study.

The tumour specimens were received and reported between 1996-2000. The samples included female, primary tumour, operated (mastectomy or lumpectomy); unilateral breast cancer with no other primary cancers at the time of diagnosis. All the tissue blocks had been previously fixed in 10% formalin and embedded in paraffin. All haematoxylin and eosin (H&E) stained slides were reviewed for confirmation of the diagnosis and reassessment of histological grading. Grading of the primary invasive breast carcinomas is done according to the modified Bloom-Richardson criteria (Elston and Ellis, 1998). For each case, the most appropriate block was selected for further c-erbB-2 oncoprotein, ER and PR testing.

Patient's clinical data and follow up information were traced using hospital, pathology laboratory, and oncology or radiotherapy records. The staging of the disease at the time of diagnosis was done according to the American Joint Committee on Cancer (AJCC) cancer staging manual, 5th edition 1997 (AJCC Cancer Staging Manual, 1997). If patient was lost from follow-up, direct mailing or telephone call were attempted.

Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS) programme version 13.0 (SPSS Inc.,Chicago, IL, USA). Clinical and histological variables were separated into groupings for the purposes of statistical analyses as described in specific tables and associated text.

Univariate analyses were preformed by using the Chi-squared test to evaluate the association of the categorical variables, especially with c-erbB-2 oncogene in this study. The univariate relationships between prognostic indicators and DFS and OS were computed by using Kaplan–Meier survival curves (Kaplan and Meier, 1958). The statistical differences among the survival curves were calculated by using the log-rank test (Mantel, 1966). Multivariable analysis based on the Cox proportional hazards model was used to assess the relative importance of the prognostic factors (Cox, 1972).

Disease free survival (DFS) is defined as the time between diagnosis and the occurrence of local recurrence or distant metastasis or the end of the study. Overall survival (OS) is defined as the time between diagnosis and the time of death or the end of the study. Patients who died of causes unrelated to breast cancer were considered as censored at the time of death. Therefore, 'death' refers to breast cancer-related death for overall survival.

In this study, all deaths were attributed to breast cancer. All the selected cases were followed-up till December 2005.This study was approved by the UKM Research and

Table 1. Results of the Immunohistochemical Interpretation for c-erbB-2 Oncoprotein

Grade	Interpretation	Microscopic Findings
0	Negative	No membranous staining identified
1+	Negative	Faint or blush staining involving a portion of the circumference of the cytoplasmic membrane in at least 10% of the neoplastic cell population.
2+	Positive	Weak but definitive staining of the membrane involving 100% of the cytoplasmic circumference in at least 10% of the neoplastic cell population.
3+	Positive	Strong positive staining of the membrane involving 100% of the cytoplasmic circumference in at least 10% of the neoplastic cell population.

Ethics Committee (FF-043-2005).

Results of the c-erbB-2 (HER-2/neu) Tissue Assay (Paraffin) were interpreted in a semi-quantitative manner (see Figure 1). The results scored as 0 to 3+. A score of 0 and 1+ were considered as negative and a score of 3+ was considered as positive (Table 1). A score of 2+ was considered as equivocal and Fluorescence In Situ Hybridisation (FISH) was performed to confirm the results (see Figure 2).

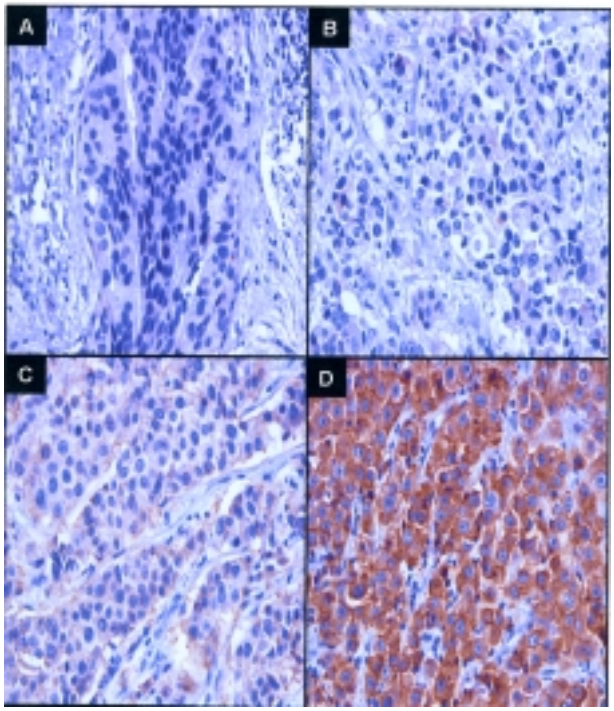

Figure 1. C-erbB-2 Oncoprotein Overexpression by Immunohistochemistry (IHC). A) no staining, score 0; B) weak, 1+; C) moderate, 2+; D, strong, 3+ (X 400)

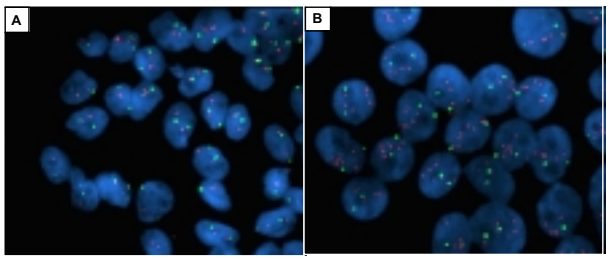

Figure 2. C-erbB-2 Gene Amplification using FISH. A, Green signals are CEP17 (chromosome enumeration probe 17); orange signals are LSI-HER2 (locus specific identifier, HER2). Two each in a single cell nucleusmakes a ratio of 1.00; B, HER-2 gene amplification with a ratio of 3.00 (x100)

Table 2. Patient and Tumour Characteristics

Age at diagnosis, range (years)		50.8 ± 11.5, 25 - 80	
Age categories	<30	3	1.7
	31-40	29	16.9
	41-50	54	31.4
	≥51	86	50.0
Ethnic group	Malay	90	52.3
	Chinese	64	37.2
	Indian	10	5.8
	Others	8	4.7
Histological type			
Infiltrating ductal carcinoma		153	89.0
Lobular carcinoma		6	3.5
Other carcinoma		13	7.5
Tumour grading	I	63	36.6
	II	52	30.2
	III	57	33.2
Tumour largest diameter category, cm			
	0.1-2.0	21	12.2
	2.1-5.0	89	51.7
	>5.0	62	36.1
Lymph node status	Positive	82	47.7
	Negative	90	52.3
Number of positive lymph nodes involved, n = 82 (%)			
	1-3	45	54.9
	4-9	25	30.5
	>10	12	14.6
Stage at diagnosis	I	24	14.0
	II	80	46.5
	III	62	36.0
	IV	6	3.5
Oestrogen receptor status			
	Positive	80	46.5
	Negative	92	53.5
Progesterone receptor status			
	Positive	86	50.0
	Negative	86	50.0
c-erbB-2 IHC status	0	101	58.7
	1+	16	9.3
	2+	13	7.6
	3+	42	24.4
C-erbB-2 amplification for IHC 2+ tumour			
	using FISH	13	100.0
	Positive	4	30.8
	Negative	9	69.2
Final c-erbB-2 status			
	Positive	46	26.7
	Negative	126	73.3

Data are n (%)

Results

Patient, tumour histological and biological marker characteristics

Patient's clinical characteristics and their tumour histological characteristics are shown in Table 2. Exactly 50% of the patients were more than 50 years old. Six patients diagnosed at stage IV were included in this study as they underwent toilet mastectomy or wide excision, which fulfilled the study criteria.

The tumour biological characteristics, which included ER, PR and c-erbB-2 oncogene, are also summarized in Table 2. Based on IHC staining, the c-erbB-2 oncoprotein overexpression was 32.0%. After retesting the respective tumour blocks with FISH, only four (30.8%) showed

Table 3. Clinical Outcome and Treatment Characteristics (n, %)

Local recurrence or distant metastasis			
	Yes	61	35.5
Death	Yes	56	32.6
Tamoxifen use	Yes	120	69.8
	No	52	30.2
Post mastectomy radiotherapy			
	Yes	112	65.1
	No	60	34.9
Chemotherapy	Yes	140	81.4
	No	32	18.6
Neo-adjuvant chemotherapy			
	CMF	94	67.1
	CAF	40	28.6
	Palliative chemo	6	4.3
Type of operation			
	Mastectomy with axillary clearance	121	70.3
	Simple mastectomy	20	11.7
	Toilet mastectomy	3	1.7
	Wide excision	28	16.3

CMF: cyclophosphamide, methotrexate and 5-fluorouracil
CAF: cyclophosphamide, doxorubicin, and 5-fluorouracil

Table 4. Relationships between c-erbB-2 and Classical Variables (χ2 test)

Variables		N (%)	c-erbB-2 status Positive	Negative	P value
Age	≤50	86 (50.0)	22	64	0.863
	≥51	86 (50.0)	24	62	
Ethnic group	Malay	90 (52.3)	26	64	0.605
	Non-Malay	82 (47.7)	20	62	
Histological type					
Infiltrating ductal carc		153 (89.0)	42	111	0.598
Lobular carcinoma		6 (3.5)	2	4	
Other		13 (7.5)	2	11	
Tumour largest diameter (cm)					
	T1, 0.1-2.0	21 (12.2)	3	18	0.178
	T2, 2.1-5.0	89 (51.7)	22	67	
	T3, >5.0	62 (36.1)	21	41	
Tumour grade	I	63 (36.6)	11	52	0.086
	II	52 (30.2)	15	37	
	III	57 (33.2)	20	37	
Lymph node status					
	Positive	82 (48.8)	28	54	0.040
	Negative	90 (51.2)	18	72	
Stage	I	24 (14.0)	3	21	0.039
	II	80 (46.5)	17	63	
	III	62 (36.0)	24	38	
	IV	6 (3.5)	2	4	
Oestrogen receptor status					
	Positive	80 (46.5)	11	69	<0.001
	Negative	92 (53.5)	35	57	
Progesterone receptor status					
	Positive	86 (50.0)	13	73	0.001
	Negative	86 (50.0)	33	53	

amplifications; two of which showed low level amplification (ratio of 3 and 4) and the other two showed high level amplification (ratio of more than 5). The c-erbB-2 status was much lower (26.7%) after retesting the cases that showed IHC 2+ with FISH.

Table 3 summarizes data for clinical outcome and treatment characteristics. The mean follow-up for this cohort was 4.7 years with the standard deviation (SD) of 2.4. The range of follow-up was from 0.1 to 9.26 years and the median was 5.10 years. During this period of follow up, 61 (35.5%) of the cohort group had local recurrence or distant metastasis and 56 patients (32.6%) died from breast cancer. One hundred twenty (69.8%) patients received hormonal therapy and 112 (65.1%) patients received radiotherapy. One hundred twenty one (90.3%) patients underwent mastectomy with axillary clearance. Only 28 (16.3%) patients received conservative surgery (wide excision).

Table 5. OS and DFS Rates at 5 years: Comparison with Clinical, Histological and Biological Variables

Variables	N (%)	OS	P value	DFS	P value
Age categories					
50 or less	86 (50.0)	64	0.129	60	0.320
51 or more	86 (50.0)	80		72	
Ethnic group					
Malay	90 (52.3)	61	0.040	60	0.034
Chinese	64 (37.2)	87		80	
Indian	10 (5.8)	60		60	
Other	8 (4.7)	76		63	
Ethnic group					
Malay	90 (52.3)	60	0.024	60	0.008
Non-Malay	82 (47.7)	81		76	
Histological type					
IDC	153 (89.0)	70	0.107	67	0.231
Lobular	6 (3.5)	50		50	
Other	13 (7.5)	92		92	
Tumour grading					
I	63 (36.6)	89	<0.001	88	<0.001
II	52 (30.2)	70		66	
III	57 (33.2)	51		50	
Tumour largest diameter category (cm)					
T1, 0.1-2.0	21 (12.2)	87	<0.001	80	<0.001
T2, 2.1-5.0	89 (51.7)	80		84	
T3, >5.0	62 (36.1)	46		41	
Lymph node status					
Positive	82 (48.8)	50	<0.001	49	<0.001
Negative	90 (51.2)	91		90	
Stage at diagnosis					
I	24 (14.0)	93	<0.001	95	<0.001
II	80 (46.5)	86		85	
III	62 (36.0)	45		42	
IV	6 (3.5)	0		0	
Oestrogen receptor status					
Positive	80 (46.5)	85	<0.000	80	0.002
Negative	92 (53.5)	60		58	
Progesterone receptor status					
Positive	86 (50.0)	84	<0.001	78	0.001
Negative	86 (50.0)	57		56	
c-erbB-2 status					
Positive	46 (26.7)	53	0.020	52	0.001
Negative	126 (73.3)	76		74	

P value = log-rank test

Relationship between c-erbB-2 and the other variables

The correlations between c-erbB-2 and the classical and biological variables are presented in Table 4. C-erbB-2 status did not correlate with age, ethnic group, histological type, tumour size and tumour grade. PR and ER negative tumours were more likely to be c-erbB-2 positive ($p<0.001$). C-erbB-2 positive status also

Table 6. Univariate Analysis of the Prognostic Factors for DFS

Variables	RR	95% CI	P value
Age categories			
≥51 vs ≤50	1.40	0.83-2.38	0.212
Ethnic group			
Non-Malay vs Malay	1.87	1.08-3.24	0.025
Histological type			
LC vs IDC	4.94	0.68-35.7	0.114
other vs IDC	9.32	0.97-89.7	0.053
Tumour grading			
I vs II	2.59	1.17-5.78	0.020
I vs III	4.71	2.23-9.95	<0.001
Tumour largest diameter category			
T1 vs T2	0.69	0.25-1.91	0.473
T1 vs T3	3.63	1.42-8.25	0.007
Lymph node status			
Negative vs Positive	5.95	3.07-1.53	<0.001
Oestrogen receptor status			
Positive vs Negative	2.80	1.55-5.06	0.001
Progesterone receptor status			
Positive vs Negative	2.80	1.58-4.95	<0.001
c-erbB-2 status			
Negative vs Positive	2.25	1.31-3.83	0.003

RR, relative risk

Table 7. Univariate Analysis of the Prognostic Factors for DFS

Variables	RR	95% CI	P value
Age categories			
≥51 vs ≤50	1.19	0.71-1.97	0.506
Ethnic group			
Non-Malay vs Malay	2.01	1.18-3.40	0.010
Histological type			
LC vs IDC	2.67	0.65-11.0	0.172
other vs IDC	4.48	0.74-26.8	0.101
Tumour grading			
I vs II	2.77	1.30-5.88	0.008
I vs III	4.42	2.16-9.05	<0.001
Tumour largest diameter category			
T1 vs T2	0.90	0.33-2.42	0.828
T1 vs T3	3.9	1.53-9.94	0.004
Lymph node status			
Negative vs Positive	4.53	2.52-8.13	<0.001
Oestrogen receptor status			
Positive vs Negative	2.31	1.34-3.98	0.002
Progesterone receptor status			
Positive vs Negative	2.36	1.39-4.00	0.002
c-erbB-2 status			
Negative vs Positive	??		

RR, relative risk

correlated well with positive lymph node status (p=0.040) and higher tumour staging (p=0.039).

Univariate prognostic analysis

Figure 3 illustrates the 5-year survival rate of OS and DFS by using Kaplan–Meier survival curves with statistical differences calculated using the log-rank test. Shorter OS and DFS were significantly observed in patients with higher tumour grading, larger tumour size , lymph node positive status and higher staging (Table 5). Similar findings were significantly observed among Malay patients. ER positivity, PR positivity and negative c-erbB-2 status were shown to be significantly associated with longer OS and DFS.

Table 6 and Table 7 summarize data from univariate analyses of the prognostic factors by using Cox regression to demonstrate the relative risk for OS and DFS, respectively.

Univariate prognostic analysis for c-erbB-2 status in subgroup defined classical prognostic factors

We also studied the combination of prognostic factors (Table 4) and c-erbB-2 status for their influence on DFS and OS. There was no significant difference in DFS in combinations of c-erbB-2 status in subgroups of various prognostic factors. However, we observed significant shorter OS in c-erbB-2 positive patients with ER negativity (p=0.24), PR negativity (p=0.027) and positive nodal status (p=0.030, Figure 4). There was no significant observation in shorter OS & DFS in Malay ethnicity and c-erbB-2 status positive patients.

Multivariate prognostic analysis

By comparing all the prognostic factors in Table 4, multivariate analysis of the OS and DFS (Table 8 and 9) showed that lymph node status was the strongest independent prognostic factor for both OS and DFS (P>0.0001). Patients with positive lymph nodes were 3.69 times more likely to relapse than those with negative lymph nodes.

ER status was the second most significant independent prognostic factor for OS (p=0.0075). Tumour grading was

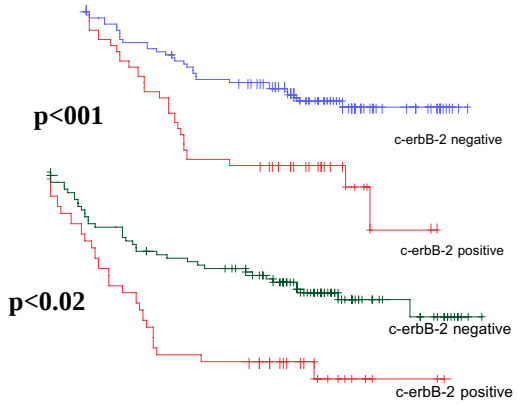

Figure 3. Kaplan-Meyer Curves for A) OS and B) DFS in 172 Patients According to c-erbB-2 Expression

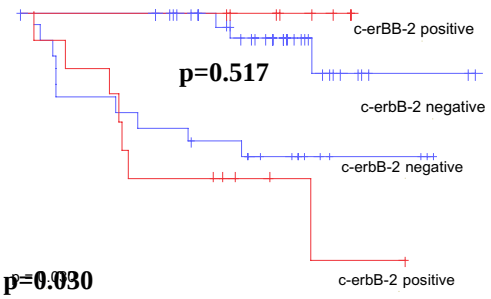

Figure 4. Kaplan-Meyer Curves for OS with A) Lymph Node Negative and B) Positive Cases According to c-erbB-2 Expression

Table 8. Multivariate Analysis of Prognostic Factors for OS

Variables	RR	95% CI	P value
Oestrogen receptor status			
Positive vs Negative	2.37	1.26-4.46	0.0075
Lymph node status			
Negative vs Positive	5.18	2.56-10.5	<0.0001
Tumour largest diameter category			
T1 vs T2	0.27	0.09-0.81	0.0189
T1 vs T3	1.01	0.37-2.77	0.9886
Tumour grade			
I vs II	2.96	1.30-6.72	0.0098
I vs III	3.14	1.45-6.79	0.0036
Progesterone receptor status			
Positive vs Negative	1.23	0.52-2.89	0.6401
c-erbB-2 status			
Negative vs Positive	1.01	0.55-1.86	0.9792

RR, relative risk

Table 9. Multivariate Analysis of Prognostic Factors for DFS

Variables	RR	95% CI	P value
Oestrogen receptor status			
Negative vs Positive	1.95	1.09-3.47	0.0243
Lymph node status			
Negative vs Positive	3.69	2.00-6.81	<0.0001
Tumour largest diameter category			
T1 vs T2	0.46	0.16-1.28	0.1357
T1 vs T3	1.43	0.53-3.84	0.4766
Tumour grading			
I vs II	2.80	1.29-6.06	0.0089
I vs III	2.90	1.39-6.06	0.0046
Progesterone receptor status			
Negative vs Positive	1.14	0.51-2.54	0.7515
c-erbB-2 status			
Negative vs Positive	1.01	0.56-1.82	0.9604

RR, relative risk

noted significantly to be an independent prognostic factor in OS and DFS. However, c-erbB-2 status was not a significantly independent prognostic factor, even in the subsets of lymph node positive or negative group.

Discussion

Among the variables studied, the most striking was the difference among ethnic groups. The ethnic composition of this cohort group was consistent with the Malaysian ethnicity composition; 52.3% Malay and 47.7% non-Malay. There were no significant correlations between Malay patients with age groups, c-erbB-2 oncoprotein overexpression, ER and PR status. However, the findings of Malay patients presenting with larger tumours and at adverse stages were statistically significant. Compared to the other groups, Malay patients had a shorter OS and DFS rate in univariate analysis (P=0.024, P=0.008 respectively). The Malay patients also showed a poorer 5-year survival rate of 61% compared to non-Malay patients at 81%. This is not surprising as most Malay patients presented with advanced stage of breast cancer. The social and cultural perceptions of breast cancer are the most important contributor of the late presentation among Malay patients. Frequently, some of them turned to alternative medicine for treatment. Furthermore, even for those who are diagnosed at an early stage, they will default further treatment or come back later with florid presentations or advanced stages. Approximately 5% of breast cancer patients diagnosed annually defaulted further treatment in Kuala Lumpur Hospital (Hisham and Yip, 2003). In the subgroup univariate analysis of OS, the Malay patients did not show significant difference in their status of c-erbB-2 (Figure5). Similarly, in the multivariate analysis, Malay ethnicity failed to prove as an independent adverse factor. Thus, health education programmes and awareness on excellent early surgical outcome have to target at this particular ethnic group.

Since the first report that c-erbB2 oncogene amplification in breast carcinoma correlates with poor prognosis by Slamon et al (1987), detection of the c-erbB2 oncogene status has become increasingly important. Moreover, the selection of patients for trastuzumab therapy relies on the amplification of this oncogene. In view of trastuzumab's toxicity and expense but with the promising outcome, accurate determination of c-erbB-2 oncogene status is very crucial. Currently, most centers perform Fluorescence In Situ Hybridisation (FISH) only on tumours showing 2+ positivity by immunohistochemistry (IHC) to obtain the most reliable result. Therefore, we incorporated this practice in our study (Bilous et al., 2003).

Based on IHC staining, the c-erbB-2 oncoprotein overexpression was 32% in our study, which is in keeping with both the western (Heintz et al., 1990) as well as the eastern (Tsuda et al., 1990) population figures, with a variety of techniques. However, the figures were much lower (26.7%) after retesting the cases that showed IHC 2+ with FISH. Out of the 13 cases, only 4 (30.8%) cases showed amplification of the oncogene by FISH. This is because c-erbB-2 oncoprotein overexpression can occur without concurrent gene amplification, a phenomenon attributed to chromosome 17 polyploidy (Perez et al., 2002) and to posttranscriptional regulation leading to increased surface receptor expression (Earp et al., 1995). Patients with these apparent IHC-positive, FISH-negative tumours appear to have clinical outcomes similar to patients with no alteration (Pauletti et al., 2000). However, the c-erbB-2 status by neither method showed an independent prognostic factor in multivariate analysis.

We found that c-erbB-2 oncoprotein overexpression correlated with lymph node status (p=0.04) which was in keeping with Berger et al (1988). C-erbB-2 oncogene overexpression indicate a more aggressive breast cancer phenotype which tend to have lymph node metastasis. Nodal status is the most reliable and standard prognostic indicator in breast carcinoma. Lymph node status, along with ER status and tumour grading were the only three independent prognostic factors for OS and DFS in this study. Some study even suggested that patients with four or more involved nodes at initial diagnosis have a worse outcome after relapse than node-negative cases, regardless of the duration of the disease-free interval (Jatoi et al., 1999). Therefore, pathologists should provide the most accurate nodal assessment to provide optimum care for the patients.

Estrogen and progesterone receptor determination are

established procedures in the routine management of patients with breast cancer, primarily as predictive factors for response to hormonal therapy. In this study, we observed strong correlation between c-erbB-2 oncogene overexpression with ER and PR negativity ($p<0.0001$ and $p=0.0001$, respectively). Although several studies have indicated correlation between c-erbB-2 oncoprotein overexpression with steroid receptor negativity, the mechanism whereby c-erbB-2 mediates ER loss remains unclear (Looi and Cheah, 1998). The benign breast epithelium is oestrogen sensitive in its normal physiological state. The loss of oestrogen receptor protein in breast cancer is regarded as a regressive phenomenon occurring during cellular dedifferentiation and malignant transformation. Since c-erbB-2 oncogene features substantial homology with the epidermal growth factor receptor (EGFR) gene, a well-established receptor mediating mitogenesis, we expect a worse prognosis among patients with c-erbB-2 oncoprotein overexpressed tumour and ER negativity. This was well illustrated in the univariate analysis of this study (Figure 4.) The 5 year OS rate for c-erbB-2 overexpressed and ER negative patients was 46% whereas the patients with c-erbB-2 negative status and ER positive was 80%.

Over the past 20 years, numerous studies utilizing both morphology-based or molecular- based techniques have been used to determine the status of breast cancer. Seventy four out of the 81 studies (91%) that featured univariate analysis demonstrated c-erbB-2 oncogene amplification as a significant prognostic factor (Ross et al., 2003).

However, of the 73 studies that featured multivariate analysis reviewed by Ross et al (2003), 52 (71%) showed c-erbB-2 oncogene amplification to be independent of all other prognostic variables.

In our study, a significant correlation between c-erbB-2 oncoprotein overeexpression and disease outcome was noted in the univariate analysis. However, we failed to demonstrate c-erbB-2 oncogene amplification as an independent prognostic variable in the multivariate analysis.

The discrepancy of c-erbB2 oncogene as an independent prognostic factor in the multivariate analysis is possibly due to the issue of immunohistochemical technique performed on archival, fixed, paraffin-embedded tissues. Prolonged storage of the archival paraffin-embedded tissues can cause significant loss of this tumour marker immunostaining intensity. The impact of the fixative has been considered and shown to have a significant effect on c-erbB-2 oncogene (Penault-Llorca et al., 1994). Many laboratory factors such as method of tissue processing, temperature of the paraffin embedding procedure can influence the e-erbB-2 protein antigen loss. The latest studies showed that gene amplification (FISH technique) and immunohistochemistry on frozen sections or enzyme immunoassays on fresh tumour derived the most consistent result (el-Ahmady et al., 2002; Joensuu et al., 2003).

The results on the prognostic value of c-erbB-2 are still somewhat contradictory, with the majority of studies reporting significant prognostic relevance in large series based on immunohistochemistry on frozen sections or enzyme immunoassays on fresh tumour (el-Ahmady et al., 2002; Joensuu et al., 2003); whereas, some studies showed only significance of c-erbB-2 expression as independent of all other prognostic variables in node-negative patients (Elston and Ellis, 1998; Jatoi et al., 1999).

In our study, even in the subset of lymph node negativity, c-erbB-2 failed to prove as a short-term independent prognostic indicator. However, the long-term prognostic relevance of c-erbB-2 in this cohort group is yet to be determined in the future. However, c-erbB-2 oncoprotein overexpression still remains a potential prognostic factor and predictor of response to hormonal therapy and to different anti-tumour drugs.

In conclusion, C-erbB-2 overexpression showed significant correlation with higher tumour grade, lymph node positivity, ER and PR negativity. Shorter OS and DFS were significantly observed in patients with c-erbB-2 overexpression. Lymph node status, ER status and tumour grading were the only three independent prognostic factors for OS and DFS in this study. Although c-erbB-2 expression was obviously important from a biological standpoint, in this study multivariate analysis showed that c-erbB-2 was not recognised as an independent prognostic indicator in breast carcinoma in the local population.

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