Breast cancer in Brunei Darussalam -Incidence and the role of evaluation of molecular markers

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ABSTRACT

Introduction: Molecular markers determined by immunohistochemistry are routinely used for predicting and prognosticating cancers including breast cancer. Molecular markers for breast cancer such as oestrogen and progesterone receptors, Her2, p53, pS2, Bcl2, EGFR, Ki67, CD34 and Cathepsin D have been used in Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital. This study assessed the ethnic variations in breast cancer incidence as well as the relationships between expression of these markers with tumour stage and grade and patient survival in Brunei Darussalam . **Materials and Methods**: Records of breast cancer patients between 2001 and 2009 were retrieved and abstracted from the Cancer Registry maintained by the Department of Pathology at the RIPAS Hospital. These were reviewed and analysed using appropriate statistical methods. **Results**: Overall, the mean age at diagnosis was 49.2 years. The incidence rate of breast cancer among Chinese (56.4 per 100,000 per year) was significantly higher than Malays (27.8) and the other ethnic groups (12.3). The expression of oestrogen and progesterone receptors and pS2 was significantly greater in the more differentiated tumours while that of Ki67 and p53 in tumours and CD34 in blood vessels within the tumour was significantly greater in the less differentiated tumours. Positivity for oestrogen receptor was significantly associated with the absence of metastases in regional lymph nodes. Expression of oestrogen and progesterone receptors in tumour cells was significantly associated with enhanced patient survival, while the detection of CD34 in blood vessels within tumours was associated with poorer survival. Survival trends seen for other markers were not statistically significant. **Conclusions**: The different incidence of breast cancer among the different ethnic groups merits more detailed investigation of the responsible genetic, social and environmental factors. Oestrogen and progesterone receptors and CD34 are confirmed as useful markers for prognosis among our population with breast cancer. Expression of p53, Ki67 and pS2 may also be useful. These markers will be helpful in determining treatment options and for patient education.

Keywords: Breast cancer, cancer incidence, ethnic variations, molecular markers, predictive value, prognostic value

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INTRODUCTION

Breast cancer is a major cause of morbidity and mortality among women. In 2007, breast

cancer was the fifth leading cause of cancer mortality both in Brunei Darussalam and globally. [1, 2] It is therefore of major concern in regard to women's health and has implications for their self-esteem and social interactions.

The prognosis for an individual diagnosed with breast cancer can be estimated using systems such as the Nottingham Prognostic Index which takes into account the tumour grade and stage. [3] In recent years, various methods have been developed to determine the expression of individual genes or proteins in tissue samples. Immunohistochemical (IHC) staining is a widely used option to profile different tumours based on the expression of proteins as it can be used in resource-limited laboratories. The prognostic and predictive values of hormone receptors and the human epidermal growth factor receptor-2 (Her2, also termed HER2/neu or c-erb B2) are well established. [4] Other markers such as cyclins D1 and E, the cyclin-dependent kinase inhibitors $p21^{WAF1}$ and p27, and cell-proliferation associated nuclear antigen Ki67 have also been reported to have independent prognostic and predictive value based on meta-analysis of published data. [5] Another recent meta-analysis demonstrated that expression of the anti-apoptotic protein Bcl2 may have prognostic potential for breast cancer. [6] Mutation of p53, a tumour suppressor protein, is common in breast cancer and a meta-analysis concluded that the IHC detection of p53 is associated with poorer prognosis. [7] Cathepsin D, a lysosomal protease, and pS2, an oestrogen regulated protein, have also been investigated as prognostic markers in breast cancer. [8] Expression of CD34, a marker of angiogenesis, has been associated with poor prognosis in breast cancer. [9]

Our earlier study looking at data from breast cancer patients in a two year period (2004 to 2005) failed to show any significant relationship between some of these markers and survival or tumour grade and stage. [10] The lack of significant relationships in that study was interpreted as being possibly due to an insufficient sample size. The present study examined the relationship between the expression of the oestrogen receptor (OR), progesterone receptor (PR), Her2, p53, Ki67, pS2, Bcl2, CD34, the epidermal growth factor receptor (EGFR) and cathepsin D in breast cancer and disease progression in a much larger number of patients over a nine year period from 2001 to 2009 in Brunei Darussalam. We also investigated the possible variation in breast cancer incidence among different ethnic communities in the country.

MATERIALS AND METHODS

All cases of breast cancer diagnosis registered in the Cancer Registry maintained by the Department of Pathology at RIPAS hospital from 1st January 2001 to 31st December 2009 were identified. A total of 481 reported cases of breast cancer were identified but only 200 patients had complete records of all the parameters needed for the different analyses. A significant proportion of patients lacked aspects of data, e.g. tumour grade, nodal status or expression of specific markers by IHC , and these are reflected in the numbers available for the various analyses. Four patients in the registry were later found not to have breast cancer. Data from six male patients recorded during this time were not included in this study. Data was taken from histology reports generated in the State Pathology Laboratory

and from the individual patient's medical record. Information regarding deaths was taken from the Birth, Death and Child adoption Registration Section, Ministry of Home Affairs. Abstracted data from female patients with confirmed breast cancer was anonymised and then entered into a Microsoft Excel spreadsheet.

For the calculation of the incidence rates, the population estimate for 2011 was used (Division of Economic Planning, Ministry of Finance); approximately 400,000 composing of about 66% Malays, 11% Chinese, and 22% "Others" made up of foreigners of different nationalities and indigenous people of Borneo. [12]

Patients suspected to have breast cancer typically underwent fine needle aspiration cytology to diagnose breast cancer. Upon diagnosis of breast cancer, patients would or would not have required surgical intervention. Patients who did not require surgery were unlikely to have been comprehensively tested for molecular markers as no excisions were made and consequently, no sample sent for IHC staining. IHC staining was carried out on samples of excised primary tumour with the following primary antibodies: OR (Product code M7047), PR (M3569), Her2 (A0485), p53 (M7001), pS2 (M7184), EGFR (M3563), CD34 (M7165), Ki67 (M7240), Cathepsin D (A0561) and Bcl2 (M0887) using conditions recommended by the manufacturer (Dako, Denmark). The OR, PR, p53, pS2 and Ki67 are nuclear proteins, Her2 and EGFR are plasma membrane proteins, and Bcl2 and Cathepsin D are present in the cytoplasm of tumour cells. CD34 is localised to the membrane of blood vessels within the tumour.

Histological grading of the tumours was assessed according to the standard Bloom and Richardson system. [11] A positive IHC result was defined as staining of at least 25% of the tumour cells visually examined and categorized as 1+ (25-50% stained cells), 2+ (50-75% stained cells) and 3+ (>75% stained cells) as previously described. [10]

Statistical analysis was carried out using SPSS version 16 (IBM, New York, USA). Predictive value of tumour markers was analysed using univariate analysis. Survival analyses were performed using Mantel-Cox log rank analysis in association with Kaplan-Meier actual survival curves for all patients from 1st January 2001 to 31st December 2007. Incidence analysis was only carried out for patients up to 2008.

This study was approved by the Medical and Health Research Ethics Committee, Ministry of Health, Brunei Darussalam.

RESULTS

The ethnic distribution among the breast cancer patients reflected the general population distribution in the country. The majority of breast cancer patients were Malay, followed by Chinese and others (Table 1). Among the different tumour types, ductal tumours were the majority (83.9%) as shown in Table 1.

The mean age at diagnosis was 49.2 ± 11.6 years with a peak of incidence occurring in the 45-49 year age group (Figure 1). The incidence of breast cancer was significantly greater ($p<0.05$) in Chinese compared to Malays and others (Table 2).

Among all the tumour markers analysed, only the presence of OR in tumour cells was significantly associated with nodal negativity (p=0.020) but the expression of Ki67 and Cathepsin D were associated with a non-significant trend towards nodal metastases (Table 3).

Stronger statistical associations were however observed between tumour grade and the expression of specific molecular markers. The expression of OR, PR and pS2 were significantly associated with greater differentiation of tumours, at diagnosis (Table 4). In contrast, the expression of Ki67, p53 and CD34 were associated with poorer differentiation of tumours at diagnosis . There was a tendency for Bcl2 expression to be associated with more differentiated tumours at diagnosis but the result was not statistically significant (p=0.090).

Using Log Rank analysis in association with Kaplan Meier Survival Analysis (KMSA), statistically significant differences in survival could (Figures 2a-c) be seen favouring patients with OR positivity (p=0.049), PR positivity (p=0.012) and CD34 negativity (p= 0.034) (Table 5). Trends on Kaplan Meier

Table 1: Breast cancer characteristics in Brunei Darussalam (2001-2009).

Variables	n	Cases (%)
Race	461	
Malay		323 (70.1)
Chinese		93 (20.2)
Others		45 (9.8)
Tumour type	461	
Ductal		387 (83.9)
Lobular		24 (5.2)
Other		50 (10.8)
Tumour grade	328	
1		46 (14.0)
2		86 (26.2)
3		196 (59.8)
Node status	292	
Positive		167 (57.2)
Negative		125 (42.8)
Tumour (T) stage	331	
1		107 (31.8)
2		146 (43.3)
3		36 (10.7)
4		48 (14.2)

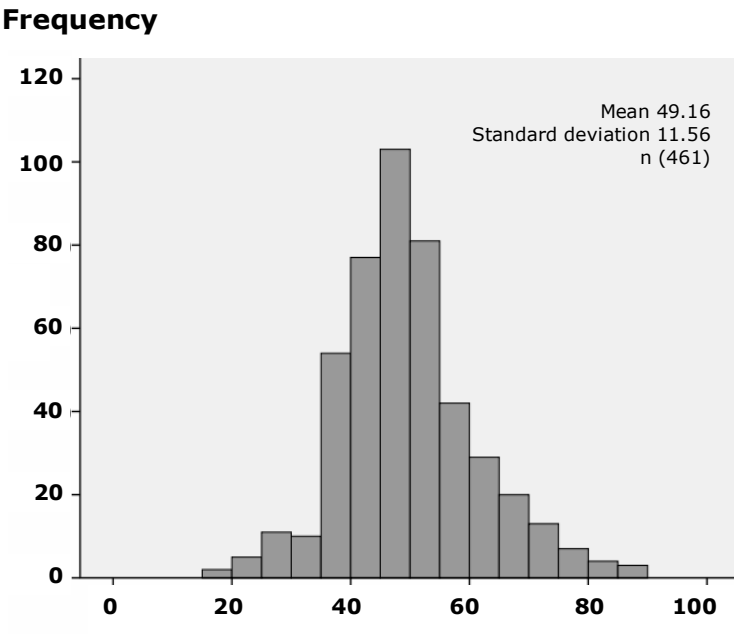

Fig. 1: The age distribution of breast cancer in Brunei Darussalam in the period 2001-2007. The numbers of new cancer cases detected in five-year age blocks of the patient population are presented in the bar chart .

Table 2: Breast cancer incidence among the various ethnic groups in Brunei Darussalam.

Race	Incidence (cases/100,000 women/year)	95% CI
Malays	27.8	19.5 to 40.5
Chinese	56.4	43.2 to 72.7
Others	12.3	6.9 to 20.9
Overall	26.6	19.5 to 40.5

CI: Confidence interval

survival analyses could also be seen favoring patients with pS2 positivity (Table 5; p= 0.052). Patients without nodal involvement also showed significantly increased survival compared to patients with nodal infiltration (5-yr survival rate 90% vs. 60% respectively; p<0.001).

DISCUSSION

Our study showed that there is an ethnic variation in the incidence of breast cancer in Brunei Darussalam similar to other cancers. The higher incidence among our Bruneian Chinese compared to the Malays and other Bruneians is similar to the patterns seen in other countries with a similar ethnic mix in Southeast Asia. In Singapore, the Chinese have a significantly higher incidence (54.9 per 100,000) compared to the Malays (44.8 per 100,000). [13] In Peninsular Malaysia, the Chinese also have higher incidence (59.7 per 100,000) compared to the Malays (33.9 per 100,000). [14] Overall, the incidence among Bruneian Chinese is similar to both Singapore and Peninsular Malaysia. However, the incidence among our Malay population is much lower than their Singaporean counterpart [10] and close to the rates reported in Peninsular Malaysia. [14] However the incidence of breast cancer in Brunei Darussalam, Malaysia and Singapore is about half of what has been reported in the United States of America. [15]

Table 3: Relationships between molecular markers and nodal metastasis.

Markers	n	Node status		*p* value
		Negative (%)#	Positive (%)#	
OR	275	60.9	46.3	0.020 *
PR	276	55.7	53.4	0.806
Her2	271	64.3	69.8	0.359
p53	248	42.5	35.9	0.356
pS2	237	49.5	45.6	0.599
Bcl2	245	33.0	35.2	0.786
EGFR	247	14.4	21.7	0.186
Ki67	229	18.8	30.1	0.064
CD34	202	43.0	44.8	0.886
Cathepsin D	247	73.8	84.0	0.055

the columns show the percentage of node positive and node negative cases where each marker was detected as positive at any level by IHC

Table 4: Relationship between molecular markers and tumour grades.

Markers	n	Tumour grades			*p* value
		I (%*)	II (%*)	III (%*)	
OR	296	82.9	61.0	46.4	<0.001
PR	297	62.9	70.7	44.4	<0.001
Her2	294	57.1	67.1	71.8	0.219
p53	269	18.2	31.0	44.2	0.008
pS2	254	53.3	58.9	40.4	0.026
Bcl2	269	45.5	41.3	29.8	0.090
EGFR	268	9.7	13.7	22.0	0.133
Ki67	250	12.1	10.3	33.6	<0.001
CD34	220	16.1	53.9	43.8	0.003
Cathepsin D	269	80.0	78.4	85.5	0.367

%* = Percentage of cases for which marker is positive i.e. ≥1+

Similarly, there are also variations in the incidence rates in the United States with much higher incidence reported for the Caucasian population compared to either Afro-Americans or Native American Indians. [15]

The differences between Chinese and Malay populations in Brunei, Singapore and Malaysia are consistent with a well-established role for genetic and culturally related behavioural factors in the development of breast cancer. [16] Despite this, different incidence of breast cancer among our Chinese and Malay populations merits more detailed investigation of the responsible genetic, social and environmental factors that might be specific to Brunei Darussalam and the region. Consistent with the first study with smaller numbers of patients, [10] the present study also showed that the mean age of diagnosis and the age distribution of breast cancer is comparable to that seen in Malaysia [14] and Singapore [13], but different from the United States where the median age of diagnosis is 61 years. [15]

In our previous study which was limited to a two year period (2004-2005), no statistically significant relationships between any of the molecular markers studied and tumour staging or grading could be established. [10] The present results however show that positivity for OR was significantly associated with a lower rate of nodal metastasis and lower tumour grades, i.e. more differentiated tumours, indicating a better prognosis for the patient. Significant reduction in PR expression was also found in higher grade or more poorly differentiated tumours, indicating an addition-

Table 5: Summary of Kaplan-Meier survival analysis of the significance of molecular Markers.

Markers	n	deaths	Log Rank (Mantel-Cox) Probability value
OR	177	45	0.049
PR	177	44	0.012
Her2	174	44	0.966
p53	153	39	0.212
pS2	154	40	0.052
Bcl2	151	38	0.349
EGFR	153	39	0.387
Ki67	144	34	0.622
CD34	124	31	0.034
Cathepsin D	40	40	0.231

n is the number of cases with relevant data

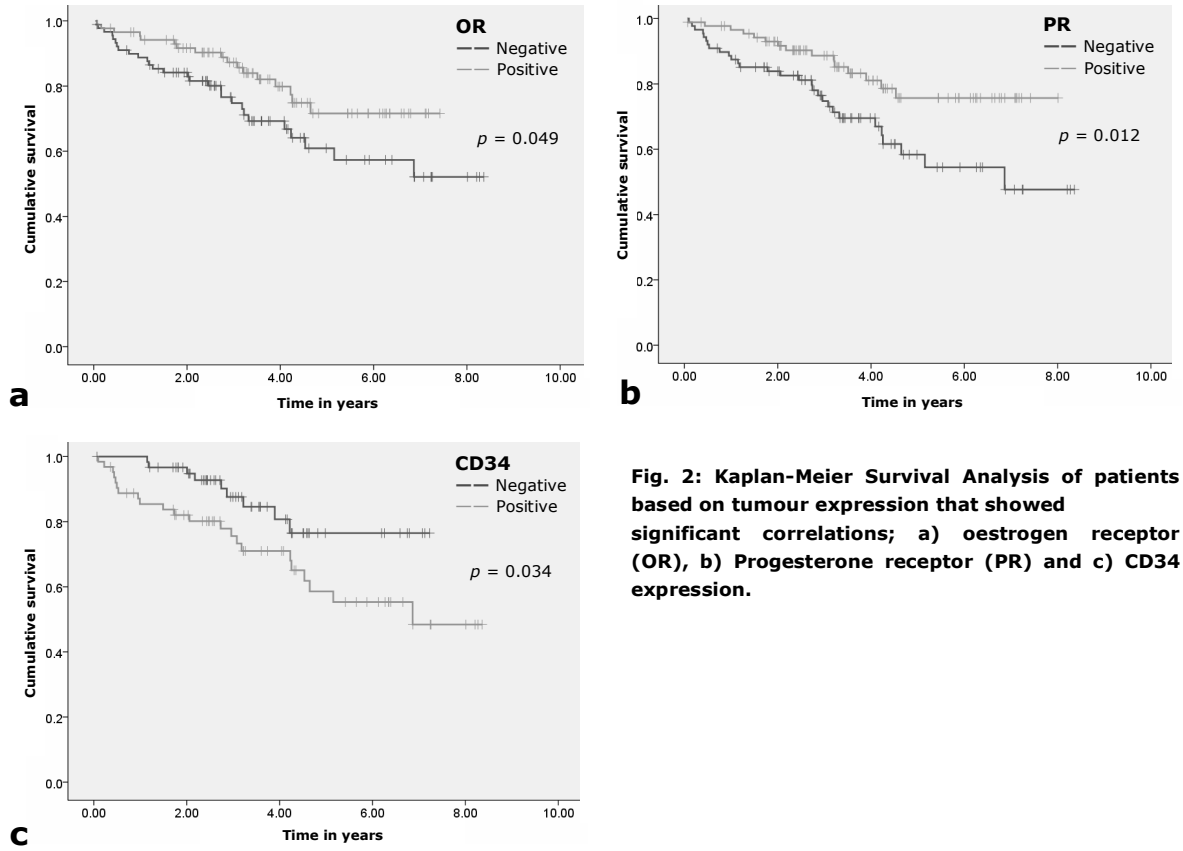

Fig. 2: Kaplan-Meier Survival Analysis of patients based on tumour expression that showed significant correlations; a) oestrogen receptor (OR), b) Progesterone receptor (PR) and c) CD34 expression.

al prognostic value for this marker. A similar relationship was found between pS2 and tumour grade, and this is expected as the expression of pS2 is induced by oestrogen acting through the OR. There is evidence to suggest that pS2 has prognostic and predictive value independent of OR. [17] All patients who are found to have OR +ve breast cancers are offered therapy with the oestrogen receptor antagonist (Tamoxifen), in Brunei Darussalam. OR +ve breast cancers are more responsive to therapy with Tamoxifen resulting in significantly better survival. Current guidelines in the United States emphasise the importance of determining status of OR and PR in breast cancer and their value in hormone therapy. [8, 18] The present findings also support the use of OR and PR as determined by IHC for the management and treatment of breast cancer in our local setting. The additional use of pS2 as a marker in breast cancer is also indicated but further studies are needed to establish its role as a marker independent from the two hormone receptors OR and PR.

Her2 belongs to the epidermal growth factor receptor family with tyrosine kinase activity, and is amplified in 18-20% of breast cancers. [8, 19] Current guidelines recommend its determination in breast cancers particularly because of the use of anti-Her2 antibody Trastuzumab (Herceptin; Genentech, San Francisco, CA) in treatment. [19] However Her2 expression has been associated with an overall poor prognosis. [8, 19] On the other hand, Trastuzumab and specific small molecule tyrosine kinases inhibitors that target Her2 have

been shown to have significant benefits in the treatment of breast cancer. [19] Patients who are Her2 positive at the 3+ level by IHC are routinely offered treatment with Trastuzumab in Brunei Darussalam. Our study showed that there is no overall statistically significant survival in patients who express Her2 at ≥1+ compared to those who are Her2 negative. This data is consistent with the known better prognosis for Her2 negative tumours [8, 19] and the protection afforded by treatment with Trastuzumab. A few patients who were Her2 positive at the 3+ level opted not to receive Trastuzumab, but their numbers were too few to determine statistical differences in survival in comparison with those Her2 3+ patients that received Trastuzumab.

There was significantly greater expression of Ki67 in higher grade tumours. This is to be expected as Ki67 is a marker of cellular proliferation and therefore its expression levels would be higher in less differentiated tumour cells. There is an indication in the present study that expression of Ki67 may be associated with a tendency towards nodal involvement. Current guidelines in the USA suggest that Ki67 is a useful marker for prognosis and also for prediction of response to Letrazole therapy. [21] The Kaplan Meier survival analyses did not, unlike in the case of OR, PR and CD34, show a significant statistical relationship between Ki67 expression and overall survival in Bruneian patients. However a meta-analysis on the much larger number of 12,155 patients showed that Ki67 positivity produces worse survival and poorer prognosis in patients with early breast cancer. [20]

Bcl2 is a member of a protein family that regulates apoptosis and is an oncogene in many lymphomas. [22, 23] Wild type Bcl-2 itself antagonises apoptosis.[23] However, paradoxically, Bcl2 overexpression has been associated with better prognostic features in breast cancer (lower grade of tumour, OR positivity and better survival). [24] The observations in the present study that Bcl2 tends to be expressed in more differentiated tumours is therefore consistent with this finding. However, no statistically significant relationship was observed between nodal metastasis or overall survival and Bcl2 expression.

There was a tendency, although not statistically significant, for EGFR expression to be associated with nodal metastasis and a higher grade of tumour in the present study. No firm conclusion can therefore be drawn about its use in prognosis although it has been suggested that it may be indirectly useful as a predictive factor in relation to hormone therapy. [17]

The IHC technique used at RIPAS Hospital will detect accumulated wild type p53 as well most types of mutant p53 other than those with large deletions that have lost the epitope recognised by the antibody used in IHC. [7] Statistically significant effects of p53 detection on survival or nodal metastasis were not observed in the present study. However the results showed that p53 was more highly expressed in the poorly differentiated tumours. Current clinical guidelines suggest that there is insufficient evidence to recommend the use of p53 detected by IHC for management of patients. [8]

There was a tendency for the detect–able expression of Cathepsin D to be associated with nodal metastasis but not with grade

of tumour or overall survival of patients, in Brunei Darussalam. This may indicate a role for the protease in promoting the metastatic process and further investigation may improve understanding of breast tumour biology. However there is no clear indication from the present study that IHC determination of Cathepsin D is of value in patient management, a finding that is consistent with the conclusions of the American Society of Clinical Oncologists expert group. [8]

An important finding from this study was that CD34, a molecule present in vascular endothelial cells and therefore a marker for angiogenesis, was significantly associated with higher grade of tumour and poorer overall survival but not significantly with nodal metastasis. It was previously associated only with a poorer prognosis in breast cancer. [9] Sustained angiogenesis is one of the hallmarks of malignant solid tumours and therefore our observation is consistent with current paradigm. [25] Therefore determination of CD34 is of prognostic value in breast cancer and may have implications for management and treatment that need to be explored further.

A limitation of our study was that even with data on patients over a nine-year period there were trends in association between some markers and tumour characteristics and patient survival that suggest that analysis of data from a larger sample population may show even more statistically significant relationships. A larger data set from Brunei Darussalam will require a much longer period of time to collect. Another limitation was that complete data were not available for all the patients diagnosed with breast cancer in the nine-year period. This was compounded by the fact that not all patients had the same molecular markers tested. Therefore a meta-analysis of available data from Brunei Darussalam and other countries in the region can be valuable.

In conclusion, this study shows the value of determining specific molecular markers by IHC in the prognosis, treatment and management of breast cancer. It further highlights the importance of establishing a complete registry of data from breast cancer patients for these purposes in Brunei Darussalam. Similar considerations are also likely to be relevant in other cancers.

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