

Breast cancer in Brunei Darussalam – Differential community distribution and an analysis of common molecular tumour markers

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Abstract

The distribution of breast cancer among women of Chinese and Malay origin of different ages presenting at RIPAS hospital, Brunei Darussalam and its relative incidence in the Chinese and Malay populations of the country were determined for the years 2004 and 2005. While the incidence rate for Chinese in Brunei is comparable to that seen in Singapore, the incidence rate for Malays in Brunei is significantly lower than in Singapore. The differences are consistent with genetic or culture associated behavioral factors or both playing a role in the development of breast cancer.

The findings also indicate that breast cancer arises more in a slightly younger age group in the South East Asian populations, including Brunei Darussalam when compared to the USA. The difference may be related to genetic, cultural, behavioural, and environmental factors

The presence of the molecular markers, estrogen and progesterone receptors (ER and PR), p53 protein, HER-2/Neu receptor, Bcl-2, p53 and Ki67 in ductal carcinomas *in situ* and invasive ductal carcinomas was investigated by immunohistochemistry. While no relationship was observed between tumour stage and the expression of the different molecular markers, the results suggest that the expression of both ER and PR, although not statistically significant, tend to be inversely related to grade of the tumour, consistent with observations made elsewhere. Only 3 of 35 tumours were positive for the Ki67 marker of cell division and all these were of tumour grade 3. The findings did not demonstrate a relationship between p53, Bcl-2 or Her 2 and tumour grade. However the detection of Her 2 in the tumours is important for considering Herceptin therapy.

These findings help establish a baseline for more detailed investigations on factors influencing the incidence of breast cancer, and the use of molecular markers in clinical oncology, in Brunei Darussalam.

1. Introduction

Breast cancer is the most common malignancy among women in many countries [1]. The majority of breast cancers are adenocarcinomas. They are classified into *in situ* carcinomas which are neoplasms that are restricted within the ducts or lobules by the basement membrane, and invasive carcinomas that are able penetrate the basement membrane and invade surrounding lymphatic and blood

vasculature and are also able to metastasize to distant regions [2]. *In situ* carcinomas are classified into intraductal carcinomas (accounting for about 5% of all breast cancers) and lobular carcinoma (accounting for about 6% of breast cancers). Invasive carcinomas are classified into several types, of which invasive ductal carcinoma is the most common, accounting for about 70% of all breast cancers [3]. We examined the distribution of breast cancer among women of Chinese and Malay origin of different ages presenting at RIPAS hospital, Brunei Darussalam and estimated its relative incidence in the Chinese and Malay populations of the country.

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Molecular markers characteristic of breast cancer cells, that can be detected by commercially available antibodies using immunohistochemical techniques, have been found

to be useful in prognosis and for devising suitable treatments. Prognostic factors assess the patient's risk of relapse based on intrinsic biological characteristics of the tumour and the disease stage at diagnosis. They help to identify the need for adjuvant therapy [4]. Predictive factors are helpful in assessing the responsiveness of the tumour to particular treatment [5]. Several molecular markers exhibit both prognostic and predictive values; e.g. estrogen and the HER2/neu (c-erb-2) receptors. The predictive and prognostic values of different molecular markers in breast cancer have been extensively reviewed elsewhere [4-9]. In our study we attempt to relate the presence or absence of the estrogen receptor (ER), the progesterone receptor (PR), pS2, HER2/neu (Her2), p53, Bcl2 and Ki67 to the stage and grade of invasive ductal carcinomas and in situ induct breast carcinomas seen in the years 2004-5 at RIPAS hospital, Brunei Darussalam.

The estrogen receptor (ER) and the progesterone receptor (PR) are steroid hormone-activated transcription factors. Estrogens mediate their effects through two specific intracellular ER receptor subtypes, ER α and ER β . Both receptors are expressed from different genes and have similar but not identical ligand binding characteristics [6]. In clinical practice only ER α is currently measured [5]. In terms of its clinical use in breast cancer, overexpression of ER α has been shown to have valuable prognostic and predictive properties [6]. Patients with ER positive tumour have shown to have prolonged disease survival rates compared to those with ER negative tumours regardless of the involvement of nodes [7]. They can also be treated with selective ER modulators such as Tamoxifen. The PR is produced when the estrogen-ER pathway is active. It has similar prognostic value as the ER. The pS2 protein is an estrogen regulated secretory protein that is expressed predominantly in ER-positive tumours [9]. The exact function of the protein is uncertain though it seems to be involved in growth regulation, and act as indicator for an intact cellular estrogen-processing mechanism [7, 9].

Her2-neu (Her2) proto-oncogene, located on chromosome 17q21-q22 [8], codes for a transmembrane tyrosine kinase growth factor receptor. It is a one of a family of epidermal growth factor receptors. This gene has been found to be amplified in 15 – 20% of invasive human carcinomas. Her2 overexpression has been associated with poor prog-

nosis of in both primary operable and advanced breast cancer patients [8]. However, Her2 is the target for treatment of breast cancer with a humanised monoclonal antibody, Herceptin.

p53 is a tumour suppressor gene found on the chromosome 17p13 that acts as a negative regulator of cell proliferation. It does so by binding to DNA and inducing the transcription of *p21* protein which goes on to block the entry of the cell into the S phase of the cell cycle [10]. Mutant p53 is the most common genetic deficit in human cancers, including breast cancer [9]. There is a strong relationship between an abnormal p53 phenotype and poor clinical outcome.

Bcl-2 is an anti-apoptotic protein that suppresses the function of Bax, an apoptosis-inducing protein which forms mitochondrial pores. Opening of such pores releases mitochondrial cytochrome c which then activates caspases to induce apoptosis [11]. Surprisingly, the loss of Bcl 2 expression correlates with the loss of endocrine sensitivity, unfavourable tumour biology and poor prognosis [3, 7]. The reason for this is not known at present.

The Ki67 marker is a monoclonal antibody reactive nuclear antigen, detected in dividing cells [7]. High levels of Ki67 are associated with poor histologic differentiation and with metastasis to the lymph nodes, and are capable of predicting 4 year survivability irrespective of the ER and nodal status.

2. Methods and Materials

2.1 Patient details

All breast cancer patients in Brunei are referred to the RIPAS hospital, Bandar Seri Begawan for treatment. Details of breast cancer patients for the years 2004 and 2005 were obtained from the Cancer Registry of the RIPAS Hospital. Female patients with complete records in 2004 and 2005 that were used in this study numbered 45 and 41 respectively. The one male patient seen during this period was excluded from the study. The records were in the form of case summaries originating from the Oncology Department, RIPAS hospital. Histopathological reports were obtained from the State Pathology Laboratory, RIPAS

hospital. Incomplete or missing patient records due to deferred follow-ups, etc, were excluded from the analysis. For determination of breast cancer incidence and age on diagnosis, the entire set of patients from 2004-5, totalling 86, were used.

2.2 Statistics

The incidence of breast cancer among females in the population or ethnic group, and 95% confidence limits, were calculated as follows using the exact binomial test [12]:

$$\frac{\text{No. of patients with breast cancer}}{\text{Total mid year female population (country or ethnic group) / 100 000 persons}}$$

The significance of differing proportions among tumour molecular markers in the varying grades or stages of tumours was determined by the Fisher's exact test.

2.3 Classification of tumours

From the macroscopic and microscopic results, the size of the tumour (T), the number nodes exhibiting metastasis (N) and the pathological grading were obtained. With these and the metastasis status (M) of patients obtained from the case summaries, the TNM staging of the tumour was determined according to established procedures [13]. The grading of the tumour was based on the microscopic examination of the tumour to assess the level of differentiation of the tumour. G1 indicated a well differentiated tumour cells, G2 indicated moderate or poorly differentiated tumour cells and G3 indicated undifferentiated tumour cells [1].

2.4 Immunohistochemical staining data for markers and their relationship to stage and grade of invasive ductal and intraductal carcinomas

Immunohistochemical staining was performed routinely by the Pathology Department of RIPAS hospital, on the biopsy specimens, using standard procedures. These showed levels of ER, PR, Her 2, Ki-67, p53, Bcl-2 and pS2 proteins. A typical slide of a medullary carcinoma showing the brown immuno-peroxidase reaction for Her 2 is shown

in Figure 1. The specificity of the reaction is shown by the absence of staining in adjacent non-tumour cells that serve as an internal negative control.

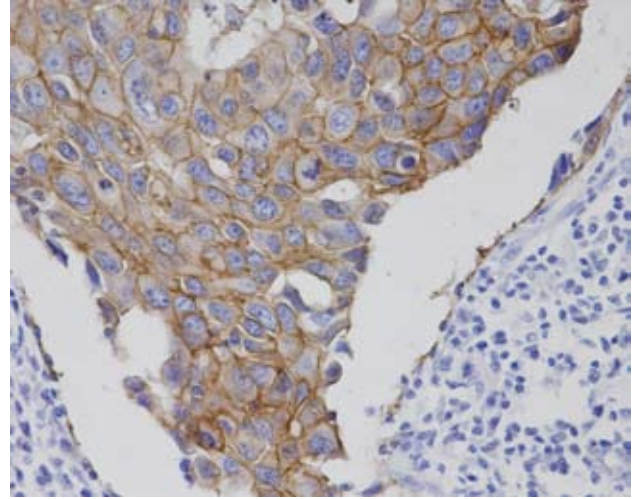


Figure 1. Immuno-peroxidase staining for Her2 in medullary carcinoma of the breast

The units for expression levels of the molecular markers were presented in the form: +1, +2 and +3. The interpretation of these units were based on the microscopic examination of the pathologist as follows:

Units	Proportion of cells that were seen to be positive
-	<25%
1+	25-50%
2+	50-75%
3+	>75%

Table1. Interpretation of immunohistochemistry units

The expression levels of each of the molecular markers were compared with the staging and grading of each of the tumors. In this study, only invasive ductal carcinomas and intraduct carcinomas were analyzed due to the relatively small number of other types of breast carcinomas.

3. Results

3.1 Incidence of breast cancer in the population and among ethnic groups

The incidence rates of breast cancer in the country for the female population of Brunei Darussalam for the period 2004-2005 are shown in Table 2. The results show that the incidence of breast cancer is higher in the Chinese female population than in the Malay female population in Brunei Darussalam. There were seven cases of breast cancer among patients who could not be classified as ethnic Chinese or Malay.

Ethnicity	n ^a	Incidence ^b	95% CI
Malay	51	22.0	16.4, 28.9
Chinese	28	74.5	49.5, 107.6
Total	86	24.9	19.9, 30.8

^a number of breast cancer patients

^b number of cases per 100,000 population per year in the period 2004-2005

CI – confidence interval

Table 2 Incidence of Breast Cancer (BC) for 2004-2005

3.2 Age at diagnosis of the breast cancer

The age at which cancer was first diagnosed in all of the female breast cancer patients is shown in Figure 1. There were more patients diagnosed in the 40-49 and 50-59 age groups than in other age groups in the period 2004-2005, with equal numbers in both the 40-49 and 50-59 age (33.7% of all cases each)

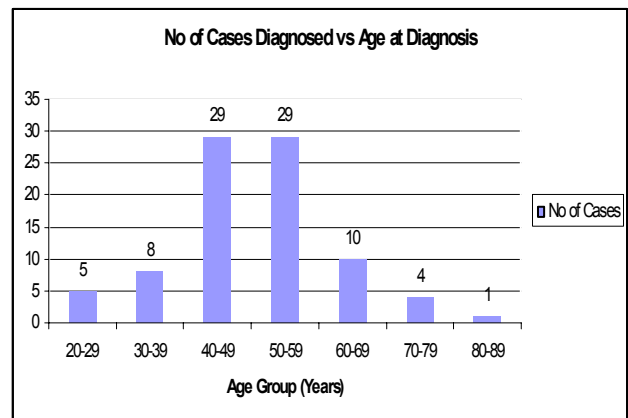


Figure 1. Number of cases diagnosed by age group in 2004-2005. The numbers in each group are indicated above the bars in the figure.

3.3 Proportions of tumours with different molecular markers

Because this was a retrospective analysis, results from the determination of molecular markers in tumours were only available for a proportion of the tumours. Of those where results were available, the proportions of stained tumours that gave a positive reaction for the different markers is shown in Table 2.

Marker	No. of Tumours Examined	No. Positive	Percent Positive
ER	59	25	42%
PR	58	25	43%
Her2	56	36	64%
p53	36	15	42%
Ki67	35	03	09%
Bcl2	36	12	33%
pS2	35	10	29%

Table 2. Proportions of positive tumours among those examined for different molecular markers, 2004-2005

3.3. Relationship between the level of markers and tumour stage

No clear trends or statistically significant relationships could be discerned between the level of expression of the different tumour molecular markers and tumour stage in the patients at diagnosis. Hence the details of these data are not presented.

3.4 Relationship between the level of markers and tumour grade

The relationship between the grade of tumour and the different tumour markers was also examined. Figure 2 shows the relationship between the level of detection of ER and the grade of the tumour. This relationship was statistically analysed by comparing the proportions of tumours expressing the marker at any level, and marker-negative tumours, in tumours of different grades. The pooling of the tumours that expressed markers at any level for each grade of tumour was done to increase the numbers in a given category for statistical analysis. Although the relationship did not reach acceptable statistical significance, there appeared to be a tendency for the higher grade tumours to be ER negative (Fisher's exact test $p=0.097$).

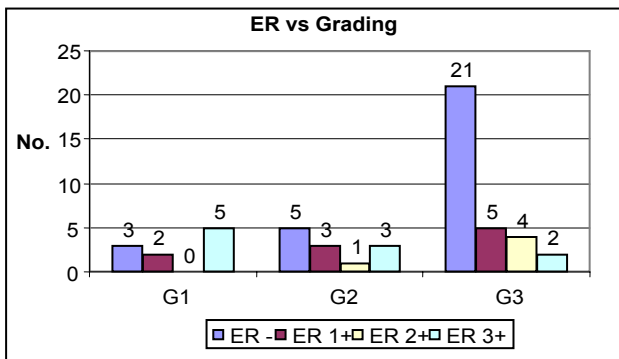


Figure 2. Relationship between ER expression levels and the grade of tumour. The numbers in each group are indicated above the bars in the figure.

A tendency for a similar relationship between the detection of PR and the grade of the tumour, although not reaching statistical significance, was also observed (Figure 3, $p=0.112$ by Fisher's exact test).

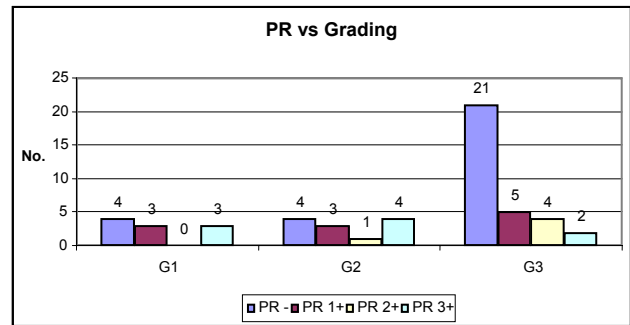


Figure 3. Relationship between PR expression levels and the grade of tumour. The numbers in each group are indicated above the bars in the figure.

For p53 the proportions of positive and negative marker reactions in tumours of different grades were not significant by the Fisher's exact test ($p=0.889$) and no obvious trend could be discerned (Figure 4). Although a large proportion of G3 stage tumours were p53 negative, this was also the case for G1 stage tumours.

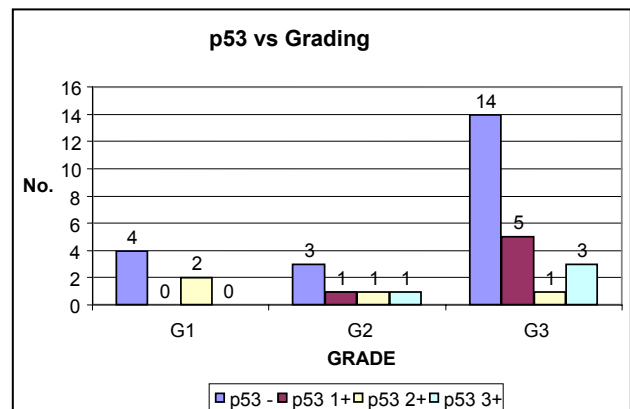


Figure 4. Relationship between p53 expression levels and the grade of tumour. The numbers in each group are indicated above the bars in the figure.

Additionally, no clear trend could be discerned, or statistically significant relationship established, between the expression of Bcl2 or Her2 and the grade of the tumour (Figures 5 & 6). With Ki67, the three tumours where the antigen was detected (two at the 3+ level and one at the 2+ level) were all of tumour grade 3.

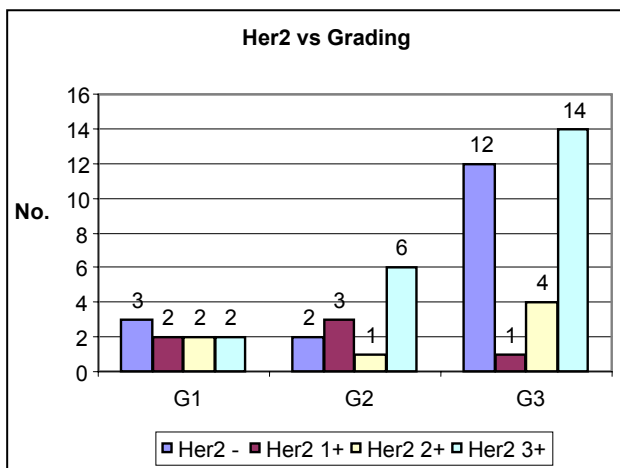


Figure 5. Relationship between Her2 expression levels and the grade of tumour. The numbers in each group are indicated above the bars in the figure.

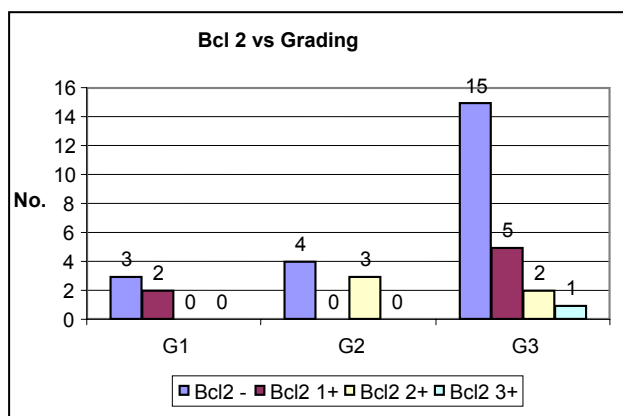


Figure 6. Relationship between Bcl2 levels and the grade of tumour. The numbers in each group are indicated above the bars in the figure.

3.5 Relationship between ER and pS2 expression in the tumours

Because pS2 is expressed after ER mediated estrogen signalling, a correlation between its expression and that of ER would be expected. It was observed that of 13 ER +ve tumours, 5 were pS2 +ve and of 22 ER -ve tumours, only 5 were pS2 +ve. The relationship between pS2 and ER expression was not statistically significant by Fisher's exact test ($p=0.44$). However, 49% of the tumours were both pS2 and ER -ve, while only 14% were pS2 +ve and ER -ve.

4. Discussion

4.1 Incidence of breast cancer in Brunei

Data from Singapore also show a higher incidence rate in Chinese females compared to Malay females [14]. For the 1998-2002 period in Singapore, the incidence rate for the Chinese female population was 72.7 per 100,000 per year with a 95% CI of 70.64 to 74.8 compared to an incidence rate for the Malay female population of 46.4 per 100,000 per year with a 95% CI between 42.5 and 50.4. While the incidence rates for Chinese in Singapore is comparable to that seen in Brunei in our study, the incidence rate for Malays in Singapore is significantly higher than in Brunei. While this may possibly be due to sociological differences related to seeking medical treatment for breast cancer in the two countries, it may also reflect geographical and other socio-economic factors that are reported to affect the incidence of breast cancer [2, 15]. The differential factors that tend to reduce the incidence of breast cancer among Bruneian Malays merit further investigation.

The differences between the Chinese and Malay populations observed in this study is consistent with genetic or culture associated behavioral factors or both playing a role in the development of breast cancer [3]. It is reported for example that the incidence of breast cancer in the USA greater for Caucasians than for African Americans and is least for the Asian/Pacific Islanders/Amerindians [16]. More detailed studies are needed to determine the ethnicity-related factors affecting breast cancer incidence in Brunei.

4.2 Age at diagnosis

The Second Report of the National Cancer Registry of Malaysia also reported a similar pattern of age-dependence for breast cancer incidence in Malaysia in 2003, as we find in Brunei in 2004/5. About 33 % of cases were diagnosed in the 40-49 age group and 31% in the 50-59 age group in Malaysia in 2003 [17]. In Singapore, the Singapore Cancer Registry report no.6 (2004) reported that over 54% of all breast cancer cases was diagnosed at 50 years of age or later [14]. On the other hand, the median age of diagnosis of breast cancer in the USA is 61 years [16].

The findings therefore suggest that breast cancer arises more in a slightly younger age group in the South East Asian populations, including Brunei Darussalam, than in the USA. The difference may be related to genetic, cultural, behavioural, and environmental factors and merits further investigation.

4.3 Relationship between the molecular markers and staging and grading

The prognosis is poorer for the more highly staged tumours [13] and for tumours of a higher grade (less differentiated tumours) [7]. The present study could not establish a clear relationship between the staging and any of the molecular markers. On the other hand, the results suggest that the expression of both ER and PR may tend to be inversely related to grade of the tumour which is consistent with other observations [9]. Analysis of a larger number of samples in Brunei for ER and PR will be needed to demonstrate a statistically significant relationship between the expression levels of these two markers and tumour grade. Such a finding would assist prognosis.

Mutation in the p53 gene leads to an accumulation of dysfunctional p53 protein that is detectable immunohistochemically [18]. Therefore it might be expected that more aggressive tumours of higher grade and stage might have more p53 detectable by immunohistochemistry. However there are conflicting reports in the literature with either a positive [19] or no [20] correlation of p53 detection with grade/stage of breast cancers.

The detection of Ki67 only in the G3 tumours is consistent with it being a marker for dividing cells. It has been reported that the expression of Ki67 in >20% of nuclei (approximately > Grade 1 according to the classification used in the present study) correlates significantly with histological grade and the absence of ER and PR, and that it is a simple, inexpensive and reliable measure of proliferation rate in breast cancer compared to flow cytometric measurement of DNA content [21].

Our findings did not demonstrate a relationship between Bcl-2 or Her 2 and tumour grade or stage. However the detection of Her 2 in the tumours is important for considering Herceptin therapy.

While not statistically significant, there was a tendency for the absence of the pS2 marker to be correlated with the absence of ER, consistent with pS2 being expressed downstream of estrogen signalling. However 5/22 ER negative tumours showed detectable pS2, and this may be due to the sensitivity of ER detection or the dysregulation of gene expression associated with malignant transformation. The pS2 positive fraction of ER positive tumours has been reported to have a better prognosis [9].

The expression of molecular markers in tumour cells is confounded by the gross genomic instability of tumours, which can result in highly dysregulated expression of proteins. Variable sensitivity of detection in immunocytochemistry is also possible. Gene expression profiling using nucleic acid hybridisation techniques is considered to be potentially of great value clinically in predicting sensitivity of tumours to new and established drugs. The findings reported here provide a baseline for more detailed investigations, and investigations using more sensitive techniques, on the use of molecular tumour markers in improving the treatment of breast cancer.

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