Factors affecting estrogen receptor status in a multiracial Asian country: An analysis of 3557 cases

C.H. Yip a,*, N. Bhoo Pathy b,c,d, C.S. Uiterwaal b, N.A. Taib a, G.H. Tan a, K.S. Mun e, W.Y. Choo f, A. Rhodes g

a Department of Surgery, University Malaya Cancer Research Institute, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
b Julius Center for Health Sciences and Primary Care, University Medical Center, PO Box 85500, 3508 GA Utrecht, The Netherlands
c Julius Centre University of Malaya, Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
d Ministry of Health, Malaysia
e Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
f Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
g Faculty of Health and Life Sciences, University of the West of England, Bristol, BS16 1QY, United Kingdom

Keywords:
Breast cancer
Middle income country
ER positivity

Abstract
Estrogen receptor (ER) positive rates in breast cancer may be influenced by grade, stage, age and race. This study reviews the ER positive rates over a 15-year period at the University Malaya Medical Centre, Kuala Lumpur, Malaysia. Data on ER status of 3557 patients from 1994 to 2008 was analyzed. ER status was determined by immunohistochemistry with a cut-off point of 10%. ER positivity increased by about 2% for every 5-year cohort, from 54.5% in 1994–1998 to 58.4% in 2004–2008. Ethnicity and grade were significantly associated with ER positivity rates: Malay women were found to have a higher risk of ER negative tumors compared with Chinese women. Grade 1 cancers were nine times more likely to be ER positive compared with grade 3 cancers. In summary, the proportion of ER positive cancers increased with each time period, and ethnicity and grade were independent factors that influenced ER positive rates.

Introduction
Estrogen receptor (ER) status is an important predictive and prognostic factor in breast cancer.1 Epidemiologic studies have shown that the percentage of ER positive breast cancers has been increasing over time.2,3 The reason for this increase is unclear, but may be due to environmental factors.4,5 The risk factors responsible for ER or progesterone (PR) positive and ER or PR negative breast cancers appear to be different. ER positive breast cancers are associated with age at diagnosis and the use of postmenopausal hormone replacement therapy, but not with family history, benign breast disease, alcohol use, or height.6 Ethnicity also appears to be a factor in the occurrence of ER positive breast cancers.7–9

The objective of this study is to analyze the trend in the proportion of ER positive breast cancers over a 15-year period at the University Malaya Medical Centre (UMMC), which is a tertiary hospital in Malaysia, a multiracial middle income country in Southeast Asia. Four factors, that is, ethnicity, age, stage and grade, were studied in relation to the rate of ER positive breast cancers.

Methods
The breast cancer registry at the UMMC was started in 1993, and data on demography, stage, pathological characteristics, treatment and outcome was prospectively entered into a database. From this hospital registry, data on ER status of 3557 patients over a 15-year period, from 1994 to 2008, was analyzed. Malays, Chinese and Indian ethnicities were included; other races were excluded. Non-epithelial cancers such as sarcomas and lymphomas were also excluded. The patients were divided into three 5-year study period cohorts, 1994–1998, 1999–2003 and 2004–2008. The ER status was retrieved from histopathology reports of the patients.

Routine ER immunohistochemistry was performed in the histopathology laboratory of the Department of Pathology, using the EnVision method (Dako Ltd, Denmark). The ER antibodies used over the course of the study were the 1D5 clone (Dako Ltd, Denmark) and the clone SP1 (LabVision Products, Thermo Scientific Fisher, Fremont, California, USA). Unstained tissue sections, approximately 4 microns.
of ER was only carried out from 1996 onwards, and even then, there were some cases where it was inadvertently omitted. The percentage of patients with tumors of known ER status increased from 54.2% in the first cohort (1994–1998) to 95.3% in the most recent cohort (2004–2008). Furthermore, Table 1 shows that ER positive rates were higher in later cohorts.

The rise in ER positive rates over time was formally tested. There was a statistically significant association between calendar time (year) at diagnosis and the chance of being ER positive (OR 1.026, 95% CI 1.004–1.048, \( P = 0.02 \)), meaning that for every later calendar year that a diagnosis was made there was a 2.6% higher chance of being ER positive. After adjustment for age, race, stage and grading, this association did not change (OR 1.036, 95% CI 1.010–1.064, \( P = 0.007 \)), so independent of these factors, for every calendar year later that the diagnosis was made, there was a 4% higher chance of being ER positive.

The association between ER determination rates over time, and whether the change of these rates over time was explained by differences within groups for age, ethnicity, stage and grade was analyzed. There was a statistically significant trend between ER determination and calendar year. With every subsequent year that a diagnosis was made there was a 37% higher chance of ER status being determined. Adjustment for age, ethnicity, stage and grade did not explain this association, rather it became somewhat stronger (OR 1.374, 95% CI 1.335–1.414, \( P < 0.0001 \)) and OR 1.584, 95% CI 1.483–1.691, \( P < 0.0001 \). Table 2 summarizes the association between demographic, clinical, and pathological characteristics with ER positivity.

### Table 1

<table>
<thead>
<tr>
<th>Cohort/Year</th>
<th>Total Number (N)</th>
<th>ER Status Known (% with ER status)</th>
<th>ER ‘n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994–1998</td>
<td>515</td>
<td>279 (54.2%)</td>
<td>152 (54.5%)</td>
</tr>
<tr>
<td>1999–2003</td>
<td>1198</td>
<td>1041 (86.9%)</td>
<td>587 (56.4%)</td>
</tr>
<tr>
<td>2004–2008</td>
<td>1844</td>
<td>1757 (95.3%)</td>
<td>1018 (58.4%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3557</td>
<td>3077 (86.5%)</td>
<td>1757 (57.4%)</td>
</tr>
</tbody>
</table>

* Calculated based on number of known ER positive tumors for each cohort.

### Table 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Univariable</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 years</td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>≥40 years</td>
<td>437</td>
<td>52.2</td>
<td>1.28 (1.04–1.57)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>2625</td>
<td>58.2</td>
<td>1.28 (1.04–1.57)</td>
</tr>
<tr>
<td>Chinese</td>
<td>611</td>
<td>59.4</td>
<td>1.04 (0.82–1.31)</td>
</tr>
<tr>
<td>Indian</td>
<td>401</td>
<td>55.1</td>
<td>1.04 (0.82–1.31)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–II</td>
<td>2386</td>
<td>59.1</td>
<td>0.73 (0.61–0.86)</td>
</tr>
<tr>
<td>III–IV</td>
<td>676</td>
<td>51.2</td>
<td>0.73 (0.61–0.86)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>233</td>
<td>82.4</td>
<td>8.72 (6.06–12.55)</td>
</tr>
<tr>
<td>2</td>
<td>1213</td>
<td>68.2</td>
<td>3.99 (3.33–4.79)</td>
</tr>
<tr>
<td>3</td>
<td>916</td>
<td>34.9</td>
<td>Reference</td>
</tr>
</tbody>
</table>

Results

Table 1 shows that a total of 3557 cases were identified during the 15-year period from 1994 to 2008; 88.5% of these cases had their estrogen status evaluated (known ER status). Routine testing
In univariable analysis, ER positive rates where lower for women younger than 40 years of age than for women 40 years of age or older (52.2% versus 58.2%, $P = 0.05$). This represents a 28% higher chance of being ER positive for women 40 years and older than for women younger than 40 years. Malay women had a statistically significant 26% lower chance of being ER positive than Chinese women (52.0% versus 59.4%, $P < 0.05$), while Indian women had a 16% lower chance than Chinese women (55.1% versus 59.4%, $P = 0.11$). Stage III–IV tumors were associated with a 27% lower chance of being ER positive compared to stage I–II tumors (59.1% versus 51.2%, $P < 0.05$). Having low grade tumors was associated with a higher chance of ER positivity. Compared to grade 3, grade 2 tumors had about a 4 times higher chance of being ER positive and grade 1 tumors had an 8.7 times higher chance (34.9%, 68.2% and 82.4%, respectively).

In the multivariable analysis, the associations between ER positivity and age and stage disappeared, whereas with ethnicity and grade the associations remained largely unchanged.

**Discussion**

Knowing a breast tumor’s ER status is essential for optimal management of breast cancer. ER positive tumors are associated with a better overall survival compared with ER negative tumors. Tamoxifen has been shown to improve overall survival in ER positive breast cancer, and has been thought to be one of the reasons for improving survival rates in breast cancer.

In low and middle income countries (LMCs), estimation of hormonal receptor status has not been routinely performed because of cost constraints. In addition, quality control of immunohistochemistry procedures is an important issue in LMCs. It is possible that the changing estrogen receptor positivity rate seen in our studies and other studies is related to changes in technical practice and standards over the intervening years. However, the differing antibodies and reagents that the University of Malaya pathology laboratory has used over the study period, to determine the estrogen receptor status of breast carcinomas, reflect those used by the majority of Western laboratories during the same time period (1994–2008) and so should reflect comparative values. Moreover, in order to ensure the quality and reproducibility of its immunocytochemical assays and to compare its results to those achieved in other parts of the world, the University of Malaya subscribes to the UK National External Quality Assessment Scheme (UK NEQAS) for Immunohistochemistry. Rhodes et al. found that while differences in methodology could cause interlaboratory variation in results for estrogen receptors, when testing the same cases, minor adjustment of the assay, through participation in an appropriate quality assurance program such as UK NEQAS, brought about amelioration of these differences.

While the definition of ER positivity has varied in studies, currently, at the University of Malaya for research and treatment purposes, a value of 10% is accepted as the cut-off point for a negative finding. Whilst recent American Society of Clinical Oncology (ASCO) 2010 guidelines recommend a cut-off point >1% to define ER positivity, earlier data from Europe utilizing a 10% cut-off point showed a higher rate of positivity among a Western population during the same time period as the earlier cohorts in the present study.

In the early study periods, estimation of ER status was not routine, in part due to budget constraints. In more recent years, when evidence of the efficacy of tamoxifen only in ER positive cancers became apparent, there was pressure to provide the test on a more routine basis: in the most recent cohort (2004–2008), it was carried out in over 90% of cases.

In 1994, Pujol et al. reported the incidence of ER positive breast cancer had been shown to increase with time in several studies. Li et al. reported the incidence rate of hormone receptor-negative tumors remained fairly constant in the United States between 1992 and 1998, despite the overall increase in incidence of breast cancer. This suggests that the increase in breast cancer incidence could be due to the increase in hormone-receptor positive breast cancers.

It is well-known that estrogens play an important role in the etiology of breast cancer, as is seen in the established reproductive risk factors, such as early menarche and late menopause, suggesting that prolonged estrogen exposure could play a role. Exposure to exogenous estrogens, as in hormone replacement therapy, is also a risk factor. Environmental exposure includes chemicals such as organochlorine pesticides and polychlorinated biphenols which mimic estrogens. These chemicals can contaminate the food, water and air, and can accumulate in human breast fat.

In our study, the proportion of ER positive breast cancers increased by about 2% every 5 years, which is consistent with findings of an increase of 2.1% between 1992 and 1998 reported by Li et al. Pujol et al. reported a similar increase in ER positive breast cancers of 5% over a 20-year period from 1973 to 1992, and concluded that this increase was unlikely to be related to changes in technology, age, size of tumor or lymph node status, and that this rise may be related to changes in tumor biology or hormonal events that influence breast cancer genesis and growth. Our findings show that there was a clear increase of ER positivity over time in our study population. This increase was not explained by age, ethnicity, stage or grade of women with known ER status. However, there was also a statistically significant increase over time of the proportion of women who were evaluated for ER status. Therefore, it could be that the observed increase of ER positivity over time was biased by differential selection of women for status determination.

In countries where there is a population-based mammography screening program, there is likely to be an increase in the number of ER positive cancers as screen-detected cancers are generally slower growing and hence more likely to be ER positive. In most LMCs like Malaysia, where there is no population-based mammography screening program, the proportion of ER positive cancers reported is likely to remain lower than in high-resource countries.

Ethnicity and age have been shown to also be a factor for ER positivity. Studies in the United States showed that African Americans, Asians, Native Americans and Hispanic Whites were more likely to have ER negative tumors than non-Hispanic Whites. Although the reason for the differences in hormonal receptor status is unknown, diet and lifestyle differences between the ethnic groups may play a role. There also appears to be a geographical difference in the proportion of ER positive breast cancer, with lower rates reported in developing countries. The ER positive rates in Asia are an average of 60% compared with 70% in Western countries. This may be related to the younger age of diagnosis in Asian countries. However, even when age is taken into account, the ER positive rates remain lower in postmenopausal Asian women than reported data from Western studies.

Studies in LMCs may face additional resource limitations that impact tissue sampling and processing which can effect ER positivity rates. Poor tissue handling or processing can result in false negative results.

This study of women residing in Malaysia, who had tumor classification at the same university laboratory, shows that Chinese women are significantly more likely to have ER positive cancers compared to Malay women. The proportion of ER positive breast cancer in Chinese women in our study was similar to a study by Chow et al. on Chinese women in Hong Kong which reported an ER positive rate of 53% in premenopausal women and 61.6% in postmenopausal women.
However, a study comparing the ER status in tumors of Vietnamese women and Australian Caucasian women found that when the laboratory methods were standardized, the ER expression in Vietnamese women was no different from the Australian Caucasian counterpart, suggesting that ER expression in breast cancer among women from other countries in Asia is also higher than previously believed. Recent studies (2008) in Nigeria and Kenya also found ER status to be higher than previously reported for African ancestry. Studies done in the United States, prior to 2002, among Asians, Africans and other non-Whites living in the United States, found the rate of ER expression to be lower in these ethnic groups compared to Caucasians, assuming the variables were standardized across studies.

Besides ethnicity, age has also been significantly associated with ER positivity in studies published in 2000 and 2002, with post-menopausal women having higher rates of ER positive tumors. Despite being associated with a higher grade and stage, ER status is an independent factor for survival, with ER positive cancers having better overall survival. In this study, ER positive cancers were significantly associated with early stage breast cancer (stage I–II) and a lower grade. There has also been described in a previous study (2005) from Malaysia that Malay women present with larger tumors and later stage of disease than Chinese and Indian women, and this may explain the lower proportion of ER positive cancers in these populations. A previous study (2008) in our hospital had shown that ER positive tumors were an independent factor for better overall survival.

In our study, age, ethnicity, stage and grade were strong determinants of ER positivity and of these, ethnicity and grade were independent predictors. It is hard to explain the lower rate of ER positive breast cancers in Malay women, as it appears to be independent of the stage of disease at diagnosis. In Malaysia, even within the same country, the 3 main races (ie, Indian, Chinese and Malay), have maintained their distinct cultures, reproductive practices and diets, with very few intermarriages. Malay women lead a more traditional lifestyle, are more likely to be rural dwellers, marry earlier and have children earlier, have more children and are more likely to breastfeed their children. A recent study (2010) in Egypt showed that the urban ER positive rate was 2–4 times higher than the rural ER positive rate. The incidence of breast cancer in Malay women is significantly lower than that in Chinese and Indian women, and it may be that the rate of ER positive breast cancer increases with the overall incidence of breast cancer. In fact, the increase in the incidence of breast cancer in high income countries (1992–1998) is believed to be due to the increase in the incidence of ER positive breast cancer.

Grade is another independent factor associated with ER positivity; in this study, grade 1 cancers were shown to be almost 9 times more likely to be ER positive compared with grade 3 breast cancers. Because ER negative tumors are more aggressive, it is not surprising that it is also more likely to be of a higher grade.

Conclusion

In this study in Malaysia, which is a typical middle income country, among the three main Asian races (Chinese, Malays and Indians) ER positive rates were related to age, grade, stage and ethnicity of the patient, but only ethnicity and grade were independent determinants of tumors being ER positive. There was an increase over time (calendar year) of ER positivity that is not explained by these determinants.

The increasing ER positivity has also been reported in several high income countries, where the proportion of ER positive cancers increased with time. It is envisaged that within the next 30 years, as Malaysia undergoes further development toward a high income country designation, the proportion of ER positive breast cancers will continue to increase and be similar to that seen in high income countries currently.

Contributors

CHY conceived and designed the study and was lead manuscript writer, GHT and NAT contributed to data collection, NBP, WYC and CSU analyzed the data and contributed to the writing, KSM and AR wrote and edited the technical details of the laboratory assessment of the ER status. All authors reviewed and approved the final manuscript.

Conflict of interest and funding

All the authors reported no conflict of interest. None of the authors received funding for the study.

References


