

Hormone Receptors and HER-2 Status as Surrogates for Breast Cancer Molecular Subtypes Prognosticate for Disease Control in Node Negative Asian Patients Treated with Breast Conservation Therapy

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Abstract

Introduction: Our study investigates whether an approximation of breast cancer molecular subtypes using the hormone receptors and HER-2 status prognosticates for disease control after breast conservation therapy (BCT) in node-negative Asian breast cancer patients. **Methods and Materials:** We retrospectively reviewed 541 women with node-negative breast cancers treated with BCT between 1989 and 2007. Hormone receptors and HER-2 status were obtained from patients' histological report. All patients received radiotherapy. Thirty-six percent and 68% of women received chemotherapy and hormonal treatment respectively. **Results:** Median follow-up of patients is 72 months. Five-year local recurrence free survival (LRFS) is 97.2% for the cohort but differs between subtypes: luminal A, 0.8%; luminal B, 1.4%; HER-2, 3.6% and basal-like, 12.7% ($P = 0.047$). The 5-year distant disease free survival (DDFS) is 96.4% for the cohort but differs between subtypes: luminal A, 98.2%; luminal B, 92.6%; HER-2, 89.5% and basal-like, 91.5% ($P = 0.019$). The 5-year disease free survival (DFS) is 94.4% for the cohort but differs between subtypes: luminal A, 97.4%; luminal B, 92.7%; HER-2, 86.3% and basal-like, 85.0% ($P = 0.007$). Univariate analysis with luminal A as baseline revealed an association of the other 3 subtypes with decreased DFS ($P = 0.007$), Hazard Ratio (HR) of 2.2, 4.4 and 3.3 to Luminal B, HER-2 and basal subtypes, respectively. On multivariate analysis, HER-2 subtype (AHR = 3.3, 95% CI, 1.1 to 9.8, $P = 0.036$) and basal-like subtype (HR = 3.5, 95% CI, 1.2 to 9.9, $P = 0.019$) prognosticate adversely for DFS. **Conclusion:** The combination of hormone receptors and HER-2 status can be used as surrogates for molecular subtypes in Asian breast cancer patients with node-negative disease to prognosticate LRFS, DFS and DDFS.

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Introduction

Breast cancer is a major cause of morbidity and mortality in women worldwide.¹ Asian women have a lower incidence of breast cancer historically but over the last 40 years, breast cancer incidence in countries such as China, Korea and Japan has increased. In Singapore, not only is breast cancer the most common cancer in women, it is also the most rapidly increasing one as well, having more than doubled its incidence over the past 25 years.²

Breast cancer is a heterogeneous disease with a wide range of presentation and response to treatment which presents a significant challenge to its diagnosis and management. Both external environmental factors such as lifestyle and childbearing habits as well as internal genetic features

including racial and ethnic make-up of the patients are known to affect the incidence and evolution of this disease.³ While the underlying cellular and molecular mechanisms is not understood in complete detail, emerging evidence points to the existence of various molecular subtypes to account for this variety in clinical behaviour, at least among Caucasian women.⁴⁻⁸

These molecular subtypes were first demonstrated in Caucasian women using gene expression profiles which divide breast cancer into a few distinct groups, including the luminal subtypes (A, B and C), human epidermal growth factor receptor 2 overexpressing (HER-2), basal-like and normal breast subtypes.^{4,5} A recent study using similar molecular profiling techniques has established that these

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subtypes are also seen in a predominantly Asian-Chinese population.^{9,10}

These molecular subtypes have been shown to correlate with disease control and survival.^{5,6}

We conducted a retrospective study to investigate whether an approximation of breast cancer molecular subtypes using hormone receptors and HER-2 status prognosticates for disease control after breast conservation therapy in node-negative Asian breast cancer patients.

However, it is expensive and impractical to perform gene expression profiling for the large number of patients that would be required to demonstrate the difference in outcomes of this specific group of patients generally thought to have a good prognosis. Therefore, we have decided to use more readily available immunohistochemical (IHC) parameters, specifically the estrogen receptors (ER), progesterone receptors (PR) and HER-2 status of a tumour as surrogates to approximate its molecular subtype.¹¹⁻¹³

Materials and Methods

We reviewed 763 women referred for adjuvant radiotherapy following breast conservation surgery (BCS) for invasive breast cancer between April 1989 and December 2007. Of these, 541 patients (71%) with node negative disease form the basis of our analysis. Patients with prior malignancy, synchronous bilateral breast cancer or have received neoadjuvant chemotherapy were excluded. Clinicopathological details of these patients are listed in Table 1. This study received ethics review board approval.

The median age at diagnosis was 49 years old (range, 26 to 80 years). Invasive ductal carcinoma of the breast was the most commonly found histology, comprising 85% of this cohort. All of the included patients had node negative disease and 72.6% of these patients had tumours of size 2 cm or less.

Of these patients, 69.1% were ER or PR positive on IHC. Positive hormone receptor status was described as unequivocal nuclear staining of a minimal 2+ intensity in at least 10% of the tumour cells. Among these female patients, 20.5% were found to have HER-2 overexpression on the basis of either scoring 3+ on IHC or found to have HER-2 amplification on fluorescence in-situ hybridisation (FISH).

Patients were categorised based on IHC of their tumour in the following manner: luminal A (ER+ or PR+ and HER-2-), luminal B (ER+ or PR+ and HER-2+), HER-2 (ER- and PR- and HER-2+), and basal-like (ER- and PR- and HER-2-).¹⁴ Accordingly, 247 (45.7%) women were categorised as luminal A, 76 (14.0%) as luminal B, 34 (6.3%) as HER-2 subtype and 56 (10.4%) as basal subtype. One hundred and twenty-eight (23.7%) women were not classifiable on this basis for the lack of one or more of these IHC features.

Table 1. Patient's Clinicopathological Characteristics

	n	%
Age		
Median (years)		49
Range (years)		26 - 80
Race		
Chinese	484	89.5%
Malay	24	4.4%
Indian	20	3.7%
Others	13	2.4%
T-stage		
T1	390	72.1%
T2	149	27.5%
T3	2	0.4%
AJCC stage grouping		
Stage I	390	72.1%
Stage IIA	258	27.5%
Stage IIB	2	0.4%
Histological tumour type		
Infiltrative Ductal Carcinoma	459	84.8%
Infiltrative Lobular Carcinoma	23	4.3%
Others	59	10.9%
Histological grade		
Grade 1	134	24.8%
Grade 2	191	35.3%
Grade 3	166	30.7%
Unknown	50	9.2%
Peri-tumoural vascular invasion		
Positive	71	13.2%
Negative	391	72.5%
Unknown	77	14.3%
Oestrogen receptor status		
Positive	339	62.8%
Negative	144	26.7%
Unknown	58	10.6%
Progesterone receptor status		
Positive	315	59.1%
Negative	160	30.2%
Unknown	59	10.7%
HER-2 over expression		
Positive	111	20.6%
Negative	302	55.9%
Unknown	127	23.5%
Patients with chemotherapy	194	35.8%
Patients with hormone therapy	369	68.3%

Treatment

Preoperatively, all patients underwent mammographic and ultrasonographic assessment and localisation of the breast primary. Staging investigations include chest x-ray, abdominal ultrasound and bone scan.

All patients underwent BCS to obtain clear margins and either axillary clearance up to level II or sentinel lymph node biopsy (SLNB) to confirm axillary node negativity.

Chemotherapy is infrequently used in these node negative patients. Indications for its use include hormonally insensitive disease, triple negatives disease, tumour size larger than 1 cm and high-grade disease. Chemotherapy consisted of 6 cycles of cyclophosphamide, methotrexate and fluorouracil in the earlier years while the more recent anthracycline-based chemotherapy usually consists of 4 cycles of doxorubicin and cyclophosphamide or 6 cycles of fluorouracil, epirubicin and cyclophosphamide.

Only 2 patients received adjuvant trastuzumab: 1 patient classified as HER-2 and the other as luminal B subtype. An additional 6 patients received trastuzumab when they relapsed with HER-2 positive disease.

Hormonal therapy was administered to patients who were ER or PR positive, usually Tamoxifen 20 mg once a day for a total of 5 years. In recent years, post-menopausal women were given an aromatase inhibitor up front in place of Tamoxifen or as part of a switching strategy after 2 to 3 years on Tamoxifen. ER and PR negative patients were not given hormonal therapy.

All patients received postoperative radiotherapy to the breast using tangential photon, delivering 50Gy in 25 fractions followed by electron boost of 10Gy over 5 fractions to the tumour bed. Patients with tumours in the upper half of the breast received supraclavicular fossa radiation to 50Gy in 25 fractions. The axillary fossa and the internal mammary chain is not routinely treated.

End Points

The primary end point is the actuarial rate of ipsilateral breast tumour recurrence (IBTR), defined as the interval between diagnosis and date of recurrence in the ipsilateral breast with or without simultaneous recurrent axilla or supraclavicular disease.

The secondary end points include actuarial rates of disease free survival (DFS) and distant disease free survival (DDFS). DFS is defined as the interval between diagnosis and recurrence of disease at any site. DDFS is defined as the interval between diagnosis and the recurrence of any distant disease.

Statistical Analysis

Patients were censored on the dates of their last follow-up or the dates of the defined event. The survival rates

were calculated using the Kaplan–Meier method and comparison of the local, distant and disease-free survival rates were performed using log-rank test for univariate analysis. Multivariate analyses of the independently significant prognostic factors were performed using the Cox proportional hazards regression model. All statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, USA). All statistical tests were 2-sided.

Results

Treatment

All patients underwent BCS and were proven node negative by axillary clearance or SLNB.

Of the women in this cohort, 35.8% received chemotherapy although this was not distributed evenly: only 10.2% of women with tumours <1 cm received chemotherapy compared to 44.6% of those with tumours >1 cm. Across molecular subgroups, chemotherapy was also not administered uniformly: 30.8% and 38.2% of patients with luminal A and B respectively received chemotherapy whereas 73.5% and 62.5% of HER-2 and basal subtypes received chemotherapy ($P < 0.0001$).

Hormonal therapy was administered to 68.3% of all patients including 90.1% of ER/PR positive patients.

All patients completed radiotherapy as planned. Patients not receiving chemotherapy commenced radiotherapy at a median of 5 weeks after surgery, compared to 17 weeks for those who received chemotherapy. Radiotherapy was completed in 6 weeks for 93% of patients.

Distribution of Prognostic Factors between Molecular Subtypes

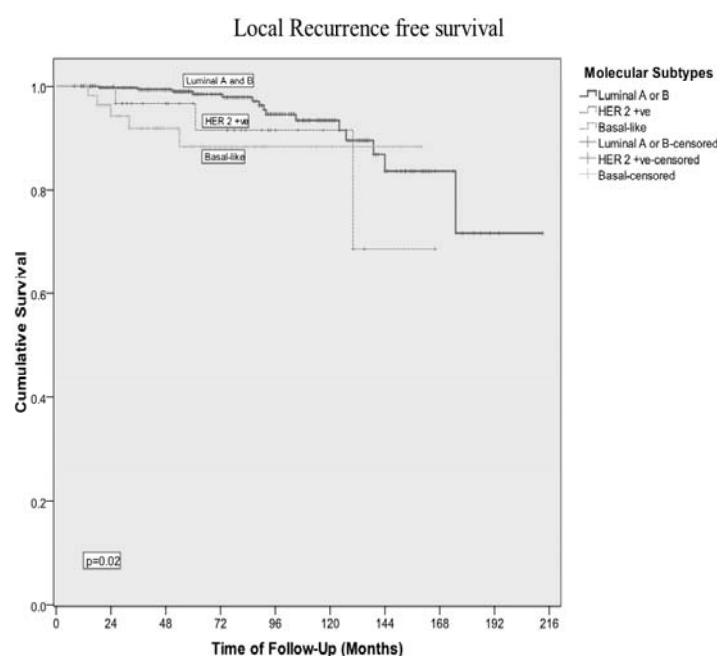
In our cohort, women with HER-2 and basal subtype breast cancers tend to present with higher grade tumours ($P < 0.0001$). Women with HER-2 subtype tumours also tend to present at a younger age ($P = 0.03$). In addition, there was a trend for HER-2 tumours to be larger in size and hence of a higher T-stage although this did not reach significance. There was no difference between the tumour subtypes with regards to margin positivity or lymphovascular invasion in our study population (Table 2).

Local Recurrence

The median follow-up of this group of patients was 72 months (range, 8 to 228 months). At the time of analysis, there were a total of 27 local failures, giving a 5-year local recurrence free survival of 97.2% for the whole population. The cumulative 5-year local recurrence rate was significantly different between the different molecular subtypes: 0.8%, 1.4%, 3.6% and 12.7% ($P = 0.047$) for luminal A, luminal B, HER-2 and basal subtypes respectively. By considering luminal A and luminal B subtypes together as baseline,

Table 2. Patient's Clinicopathological Characteristic Stratified by Molecular Subtypes

Age (years)	Cohort	Luminal A	Luminal B	HER-2	Basal	P
≤ 40	16.2%	14.6%	10.5%	32.4%	21.4%	
> 40 – 55	58.8%	57.1%	75.0%	50.0%	50.0%	
≥ 55	24.9%	28.3%	14.5%	17.6%	28.6%	0.006
Mean (years)	49.1	50.3	48.0	45.7	49.7	0.03
Tumour size						
<1 cm	26.7%	31.0%	18.7%	12.5%	27.3%	
1.1 – 2 cm	47.3%	47.1%	49.3%	50.0%	43.6%	
>2 cm	26.0%	21.9%	32.0%	37.5%	29.1%	0.084
T-stage						
T1	73.9%	77.5%	70.7%	62.5%	69.1%	
T2	25.6%	22.5%	29.3%	37.5%	27.3%	
T3	0.5%	0%	0%	0%	3.6%	0.057
Grade						
1	28.9%	39.3%	22.2%	3.3%	5.9%	
2	37.5%	41.5%	43.1%	16.7%	23.5%	
3	33.6%	19.2%	34.7%	80.0%	70.6%	<0.0001
Margins status						
Positive	20	9	6	2	3	0.487
LV invasion status						
Positive	14.5%	12.7%	14.7%	21.9%	18.2%	0.242

Fig. 1. Considering luminal subtypes together as base line, HER-2 and basal-like subtypes are significant prognostic factor for local recurrence ($P = 0.02$).

HER-2 ($HR = 2.2$, $P = NS$) and basal subtypes ($HR = 3.3$, 95% CI, 1.2 to 9.6, $P = 0.015$) are significant prognostic factor for local recurrence ($P[\text{HER-2 and basal subtypes combined}] = 0.02$) (Fig. 1).

Distant Disease Free Survival

Twenty-three patients had developed metastases at the time of analysis, giving a 5-year DDFS of 96.4% for the whole population. The cumulative 5-year DDFS was significantly different between the different molecular subtypes: 98.2%, 92.6%, 89.5% and 91.5% ($P = 0.019$) for luminal A, luminal B, HER-2 and basal subtypes, respectively (Fig. 2A). On univariate analysis with luminal A subtype as base line, the other subtypes were all significantly associated with increased unadjusted hazard ratios of 3.6, (95% CI, 1.1 to 11.3, $P = 0.031$) for luminal B, 6.0, (95% CI, 1.6 to 22.6, $P = 0.009$) for HER-2 subtype and 4.2, (95% CI, 1.1 to 16.0, $P = 0.033$) for basal subtype.

Patients who developed local recurrence were at higher risk of developing distant metastases. The actuarial distant disease free survival at 5 years for patients without local recurrence was 97.2% compared to 85.3% ($HR = 4.3$, 95% CI, 1.7 to 11.1, $P = 0.001$) (Fig. 2B) in those patients with local recurrence. The median time interval between

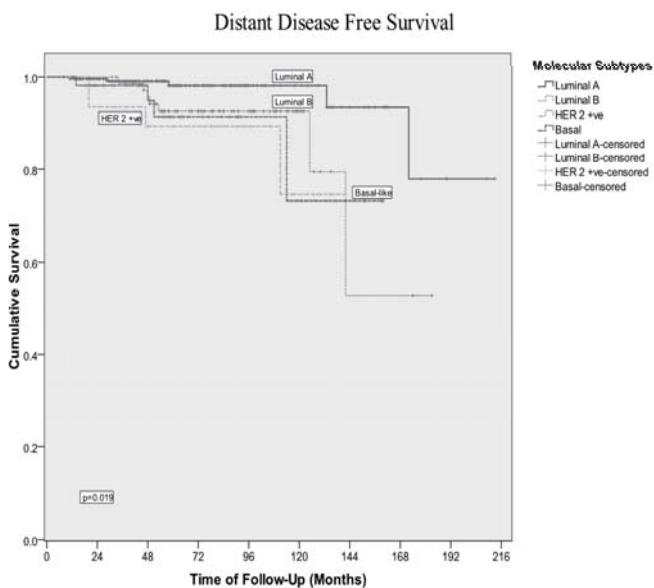


Fig. 2A. Five-year DDFS differs significantly between subtypes: luminal A 98.2%; luminal B 92.6%; HER-2 89.5%; basal-like 91.5% ($P = 0.019$).

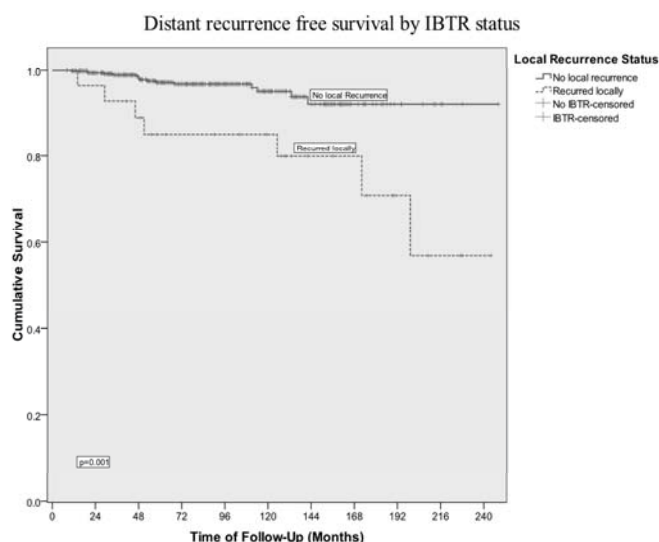


Fig. 2B. Five-year DDFS for patients without local recurrence is 97.2% compared to 85.3% (HR = 4.3, 95% CI, 1.7 to 11.1, $P = 0.001$).

developing local recurrence to distant metastases was 27 months.

Disease Free Survival

Five-year DFS was significantly different between the different molecular subtypes ($P = 0.007$). The 5-year cumulative incidence of any disease recurrence for the entire study cohort was 6.7% (95% CI, 3.5% to 7.6%). For patients with luminal A subtype, the 5-year cumulative incidence of disease recurrence was 3.6% (95% CI, 0.2% to 6.9%), compared with 7.3% (95% CI, 1.2% to 13.4%) for luminal B, 13.7% (95% CI, 1.2% to 26.2%) for HER-2, and 15.0%

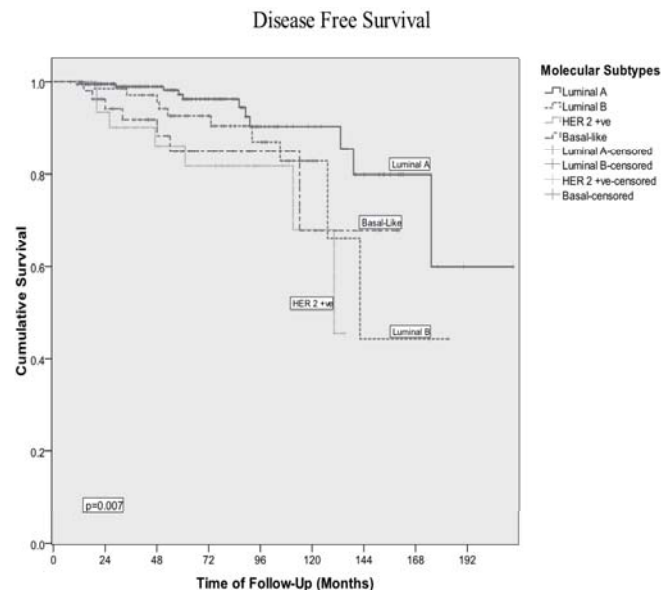


Fig. 3. For patients with luminal A subtype, the 5-year cumulative incidence of disease recurrence was 2.6% (95% CI, 0.2% to 6.9%), compared with 7.3% (95% CI, 1.2% to 13.4%) for luminal B, 13.7% (95% CI, 1.2% to 26.2%) for HER-2, and 15.0% (95% CI, 3.4% to 26.6%) for basal-like patients ($P = 0.007$).

(95% CI, 3.4% to 26.6%) for basal subtypes (Fig. 3).

Univariate analysis with luminal A subtype as baseline revealed an association of the other 3 molecular subtypes with increased risk of disease recurrence ($P = 0.007$). Luminal B, HER-2 and basal subtypes presented a hazard ratio of 2.2 (95% CI, 1.0 to 5.3, $P = 0.067$), 4.4 (95% CI, 1.7 to 11.5, $P = 0.003$) and 3.3 (95% CI, 1.3 to 8.6, $P = 0.014$) respectively. On multivariate analysis, age (Adjusted Hazard Ratio, AHR=0.95, 95% CI, 0.91 to 0.99, $P = 0.010$), HER-2 subtype (AHR = 3.3, 95% CI, 1.1 to 9.8, $P = 0.036$) and basal subtype (AHR=3.5, 95% CI, 1.2 to 9.9, $P = 0.019$) remained as significant factors for prognosticating DFS after controlling for grade of disease (Table 3).

At the time of analysis, only 24 patients had died, giving a 5-year overall survival (OS) of 98.7% (95% CI = 99.7% to 97.7%, $P = 0.050$). Basal subtype predicts for decreased OS at 5 years of 96% (HR = 5.6, 95% CI 1.4 to 23.0, $P = 0.015$).

Discussion

First demonstrated using molecular profiling, the existence of distinct molecular subtypes of breast cancer has given us the genetic underpinnings to partially account for the differences in natural history and treatment responses of breast cancer. These subtypes are distributed differently between women of different races which may account for the differences in their presentation and response to treatment. For example, basal subtype breast cancer is found in increased prevalence among women of African

descent and this group of patients is marked by a poorer clinical outcome.³ Such clinicopathological correlation is less well established in Asian women.

Our study demonstrates that using routinely available IHC parameters, we can divide Asian breast cancer patients into distinct groups that approximate these molecular subtypes. Our study is unique in concentrating on the subset of lower risk, node negative Asian patients treated exclusively with BCS followed by radiotherapy. Despite these patients' relatively good prognosis, the differences in their rates of locoregional control and survival are statistically significant and clinically relevant.

The success of population-based mammographic screening programme and increased breast cancer awareness has led to a rapid increase in the group of early stage, node negative breast cancer patients. Singapore started its first nationwide breast screening program in 2002. It has been shown that screen detected cancers are smaller, of lower stage and grade and more likely to be treated with BCS.¹⁵ This is the reason for focusing on this particular group of patients in our study.

There is no consensus yet on the molecular subtyping of breast cancer and the schema employed in this study represents a work in progress. Other schemas that utilises the IHC staining of cytokeratins (CK) as surrogates for molecular subtypes exists, although routine staining for CKs is not widely practiced. El-Rehim showed that basal-like tumours which stained for CK 5/6 and CK 14 had overall poorer prognosis as compared to those staining positively for luminal CK 7/8, 18 and 19.¹²

Although there is significant correspondence between the IHC groupings and molecular subtypes, this relationship is by no means perfect.^{7,16} For example, Carey et al⁷ demonstrated that only 30% to 50% of luminal B tumours are HER-2 positive whereas we have included all patients positive for hormone receptors and HER-2 as luminal B. Despite significant overlap in their biological and clinical characteristics, triple negative and basal-like breast cancers are not synonymous. It is likely that, within what is already a very heterogenous disease, triple negative breast cancers may be made up of further subtypes of disease.¹⁷ Until their true relationship is defined or until such time that 'gold standard' microarrays are routinely performed, we have elected to classify TN breast cancers as belonging to basal-like. Furthermore, the cut-off for determining positivity of hormonal and HER2 status differs between studies and across time and is subjected to differences in the reagents used and the qualitative methods of their determination.

Recent data support the use of this approximation in Asian patients. Using a customised Multiple Signature Microarray (MSA) containing expression signatures for 3 breast cancer molecular subtypes (luminal/ ER positive,

Table 3. Factors influencing disease free survival (Univariate and Multivariate analysis)

Variable	Univariate analysis	Multivariate analysis		
	P	P	HR	95% CI
Age	0.001	0.010	0.95	0.91 – 0.99
Grade 1,2 vs 3	0.022	NS	1.2	0.53 – 2.6
Molecular subtypes	0.007			
Luminal A			1.00	1.00
Luminal B		NS	2.16	0.85 – 5.5
HER-2 positive		0.036	2.22	1.08 – 9.84
Basal-like		0.019	3.48	1.22 – 9.93

HER2 and 'Basaloid'), Tan et al¹⁰ showed 90% concordance with ER IHC and 76% correlation with HER2 FISH. When validated against the Affymetrix genome-wide arrays, the MSA demonstrated similar high concordance of 95%. Thihe et al¹⁸ demonstrated that 549 of 643 (84%) patients with triple negative breast cancers are typed as basal-like using a triple panel of CK14, EGFR and 34bE12.

Our results provide further evidence relating molecular subtypes with clinical outcomes. In one of the earlier studies, Carey et al⁷ showed that breast cancer-specific survival among North Carolina patients was significantly worse for basal-like and HER2+/ER subtypes compared with luminal A. Parise et al⁸ showed that 5-year survival was significantly worse for HER2-overexpressing and triple negative subtypes as compared with ER positive subtypes.

Using a similar IHC approximation of molecular subtypes, Nguyen et al¹⁹ demonstrated an increased risk of local recurrence of breast cancer in women with HER-2 and basal-like subtypes. In addition, all other subtypes were found to have an increased risk of distant metastases compared to the luminal A subtype.

Using quantitative assays of ER, PR, HER-1 and HER-2 to divide 787 breast cancer patients into different subtypes using a similar schema, Del Casar et al²⁰ showed that, compared to ER positive luminal subtype, HER-2 positive and basal-like patients had poorer relapse free survival and OS. In the group of patients who had node-negative disease, significantly poorer relapse-free and OS among the HER-2 positive and basal-like tumours were observed.

In Asia, Nakajima et al²¹ used IHC to similarly divide a group of Japanese breast cancer patients into the aforementioned molecular subtypes. In this study, HER-2 and basal subtypes had poorer 4-year disease-free survival (70.6% and 76.2%, respectively) compared to luminal A (94.2%).

Despite the short follow-up in our cohort of node negative patients, significant differences in their local and distant recurrence rates are discernible with highly significant impact on the DFS between patients in different prognostic groups. Our results are in accordance with previous reports indicating the higher risk of HER-2 positive subtype and basal subtype breast cancer of local and distant relapse. While the uneven distribution of tumour grade and age at disease presentation may account for the differences in the DFS between these subtypes, MVA confirms the independent prognostic significance of the HER-2 and basal subtypes even after taking into account the higher propensity of these 2 subtypes to present at a younger age and with higher grade tumour.

Conclusion

The combination of hormone receptors and HER-2 status enables Asian women with node negative breast cancer to be divided into distinct prognostic groups with different rates of local and distant recurrences. Using these phenotypic features as surrogates for the underlying genotypes may aid in prognostication and predicting treatment outcomes for both Asian and Caucasian women despite differences in breast cancer characteristics between these 2 ethnic groups. Further investigations are needed to correlate genotypes and clinical outcomes in a more direct fashion such as microarrays to provide more specific treatment guidance.

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