

RESEARCH ARTICLE

Roles of Ki67 in Breast Cancer - Important for Management?

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Abstract

Background: The three standard biomarkers used in breast cancer are the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). The Ki-67 index, a proliferative marker, has been shown to be associated with a poorer outcome, and despite absence of standardization of pathological assessment, is widely used for therapy decision making. We aim to study the role of the Ki-67 index in a group of Asian women with breast cancer. **Materials and Methods:** A total of 450 women newly diagnosed with Stage 1 to 3 invasive breast cancer in a single centre from July 2013 to Dec 2014 were included in this study. Univariable and multivariable logistic regression was used to determine the association between Ki-67 (positive defined as 14% and above) and age, ethnicity, grade, mitotic index, ER, PR, HER2, lymph node status and size. All analyses were performed using SPSS Version 22. **Results:** In univariable analysis, Ki-67 index was associated with younger age, higher grade, ER and PR negativity, HER2 positivity, high mitotic index and positive lymph nodes. However on multivariable analysis only tumour size, grade, PR and HER2 remained significant. Out of 102 stage 1 patients who had ER positive/PR positive/HER2 negative tumours and non-grade 3, only 5 (4.9%) had a positive Ki-67 index and may have been offered chemotherapy. However, it is interesting to note that none of these patients received chemotherapy. **Conclusions:** Information on Ki67 would have potentially changed management in an insignificant proportion of patients with stage 1 breast cancer.

Keywords: Ki-67 index - invasive breast cancer - indications for chemotherapy

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Introduction

Breast cancer is the commonest cancer in women worldwide. Currently the three standard biomarkers to determine prognosis and prediction to response to therapy is the estrogen receptor (ER), progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2).

The use of Ki-67 index, which is a proliferative marker, as a predictive and prognostic marker in breast cancer, has been widely investigated. (Inwald et al., 2013). In general, the Ki-67 index is defined as the percentage of total number of tumour cells with nuclear staining. (Urruticoechea et al., 2005). Baseline Ki-67 index has been found to be higher in patients with triple negative breast cancer (where ER, PR and HER2 are not expressed) (Keam et al., 2011) while patients with ER positive and/or PR positive tumours had a lower Ki-67 index. (Trihia et al., 2003). Based on gene expression profiling, breast cancer can be divided into four molecular subtypes, Luminal A, Luminal B, HER2 over-expressing and Basal-like. However gene expression profiling is expensive and not practical for routine clinical

practice, and surrogates for the molecular subtypes based on immunohistochemistry (IHC) evaluation of ER, PR and HER2 have been used. While the IHC surrogates for HER2 over-expressing subtype (ER negative, PR negative and HER2 positive) and basal-like subtype (ER, PR and HER2 negative) are well accepted, the IHC surrogates for Luminal A and Luminal B are less well-defined with various combinations of ER, PR and HER2 suggested. The panel of experts at the St Gallen Consensus in 2011 (Goldhirsch et al., 2011) and 2013 (Goldhirsch et al., 2013) have divided the Luminal B group into a HER2 negative and a HER2 positive subtype, where the Luminal B HER2 negative subtype is a subgroup with ER positive, HER2 negative and at least one of the following: 1. Ki-67 index high (defined as 14% or more) and PR negative or low (defined as <20% of cells stained positive). (Table 1)

The cut-off value of 14% was based on the work of Cheang et al who subtyped 357 patients with invasive breast cancer by gene expression profiling and together with IHC determination of hormone receptor status, HER2 status and Ki-67 index, used receiver operating

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characteristics (ROC) curves to determine that the best cut-off for Ki-67 index to determine between Luminal A and Luminal B was 13.25%. (Cheang et al., 2009).

However the role of Ki-67 index to determine adjuvant therapy in early breast cancer is not widely practised, and Ki-67 index is not included in most guidelines on pathology reporting of breast cancer. This is because there is currently no standardized methodology to determine the Ki-67 index hence leading to a broad range of recommendations regarding the minimum number of cells counted to ascertain the percentage. Further variation in the method of counting ie computer aided versus human analysis leads to inter and intraoperator and laboratory variances (Dowsett et al., 2011). Because of the current interest in Ki-67 index as a prognostic and predictive marker, we aim to study the role of Ki-67 index by investigating its association with age, ethnicity, size, grade, lymph node status, breast cancer subtype, and mitotic index in a cohort of Asian women with breast cancer. We also aim to determine the proportion of patients who potentially would have their management changed based on Ki-67 index.

Materials and Methods

Patient population

Four-hundred and fifty consecutive women newly diagnosed with Stage 1-3 breast cancer in the Subang Jaya Medical Centre (SJMC) from 1st July 2013 to 31st December 2014 were included. The SJMC is a private hospital in an urban setting in Klang Valley in Malaysia. We excluded women with Stage 4 breast cancer because the numbers were low. The study is approved by the institutional ethics committee.

Immunohistochemistry

ER, PR and HER2 were determined by immunohistochemistry (IHC) staining of the tumour. ER and PR were considered negative if staining of the tumour cell nuclei was 1% or less, regardless of staining intensity. HER2 was assessed through IHC and based on the staining intensity and percentage, which was classified as 0 (negative) 1+, 2+ and 3+ according to the criteria set by DAKO. Only a report of negative or 1+ was taken as HER2 negative. Silver in situ hybridization was carried out when there was a report of 2+, and a ratio of HER2 gene to chromosome 17 of >2.2 confirmed that HER2 was positive.

Ki-67 index was recorded as the percentage of positively staining malignant cells using the anti-human Ki-67 monoclonal MIB1 which is one of the most commonly used antibodies and considered as the gold standard. The Ki-67 percentage score or Ki-67 index is defined as the percentage of positively stained tumour cells among the total number of malignant cells assessed, and routinely 100 malignant cells in a representative section of the tumour were counted.

All the four biomarkers were evaluated by two pathologists in a single pathology laboratory, hence reducing any inter-observer or inter-laboratory variation

Statistical analysis

Univariable and multivariable logistic regression analysis was used to determine the association between Ki-67 index (positive was taken as 14% and above) and each of the following variables - age (continuous), ethnicity (Chinese, Malays, Indians and Others), grade (1 to 3), mitotic index (0-7, 8-14, 15 and above), ER (positive or negative), PR (positive or negative) HER2 (positive or negative), lymph node status (N0 = no nodes involved, N1=1-3 lymph nodes involved, N2=4-9 lymph nodes involved, N3=10 or more lymph nodes involved), Stage (1, 2 or 3), and size (2cm or less, >2 cm). All analysis was done using SPSS Version 22.

Results

The median age at diagnosis was 51 years. The median tumour size was 20mm. The majority of the patients were Chinese (89.5%) and most patients presented with Stage 1 and 2 disease (40.2% and 38.7% respectively). Most patients presented with Grade 2 (48.4%), ER positive (70.7%), PR positive (63.8%), and HER2 negative (75.1%) tumors. Using the definition of molecular subtypes as defined by the St Gallen's Consensus (Table 2), the majority of patients (37.2%) had Luminal A breast cancer while 16.3% had triple negative breast cancer.

Ki-67 index absolute values ranged from 1 to 80, with a mean of 16 and a median of 10. The median values were significantly higher in women less than 40 years old, higher grade, higher mitotic index, ER negative, PR negative and HER2 positive. There did not seem to be a significant association with size or number of involved axillary lymph nodes. (Table 3) There was a trend for median values to be higher in Indians compared to Malays and Chinese although this was not significant.

Using 14% as the cut-off value for Ki-67 index, 43.4% of patients were Ki-67 index positive. There was no association between ethnicity and Ki-67 index although Indian patients were more likely to have a positive Ki-67 index. In univariable analysis, Ki-67 index positivity was significantly associated with a younger age, higher grade, ER and PR negativity, HER2 positivity, larger tumour size, a high mitotic index and positive lymph nodes. However in multivariable analysis, only tumour size, grade, PR and HER2 remained significant. Mitotic index was not found to be significant in the multivariable analysis, and a step-wise adjustment indicated that the initial association between mitotic index and Ki-67 index was completely explained by the underlying differences in tumour grade. (Table 4)

In the latest Malaysian Clinical Practice Guidelines for Management of Breast Cancer, adjuvant chemotherapy is indicated in women with one or more positive lymph nodes, ER and PR negative tumours, HER2 positivity, tumour larger than 2 cm or in grade 3 tumours. Ki-67 positivity is not considered a criteria in decision-making for chemotherapy. Using this criteria, of the 450 patients, 77.6% would have required chemotherapy. Notably, out of 102 Stage 1 patients who have ER positive PR positive HER2 negative tumours and non-Grade 3, only 5 (4.9%) had a Ki-67 index of more than 14%. It is interesting to

Table 1. Breast Cancer Subtypes (St Gallen Consensus)

Breast Cancer Subtype	Definition
Luminal A	ER positive, PR positive, HER2 negative Ki-67 index low (defined as <14%) Luminal B (HER2 negative)
Luminal B	ER positive, HER2 negative and at one of: Ki-67 index high (defined as 14% and above) PR negative or low (defined as <20%) Luminal B (HER2 positive)
HER2 over-expressing	ER positive, HER2 over-expressed or amplified, any Ki-67 index, Any PR
Triple negative	HER2 over-expressed or amplified, ER and PR negative ER and PR negative, HER2 negative

*Reference: Goldhirsch et al 2013 (Reference 16)

Table 2. Characteristics of 450 Patients with Stage 1-3 Breast Cancer

	No	%
Ethnicity		
Chinese	403	89.6
Malay	23	5.1
Indian	16	3.6
Others	8	1.8
Stage		
1	192	42.7
2	182	40.4
3	76	16.9
Grade		
1	47	10.9
2	211	48.7
3	175	40.4
Size		
2 cm or less	257	57.1
>2cm	193	42.9
Mitotic index		
1 to 7	252	59.4
8 to 14	109	25.7
15 and above	63	14.9
ER		
Negative	133	22.6
Positive	317	70.4
PR		
Negative	161	35.8
Positive	289	64.2
HER2		
Negative	341	75.8
Positive	109	24.2
Lymph nodes		
0	274	61.9
1 to 3	96	1.7
4 to 9	39	8.8
10 and more	34	7.7
Stage		
1	192	42.7
2	182	40.4
3	76	16.9
Molecular subtype		
Luminal A	174	38.7
Luminal B HER2 negative	92	20.4
Luminal B HER2 positive	59	13.1
HER2 over-expressing	56	11.1
Triple negative breast cancer	75	16.7

Table 3. Differentiation of Ki-67 index by Analysis of Variance (ANOVA) in 450 Patients

	Absolute median Ki-67 index	SD	P Value
Ethnicity			
Chinese	10	15.2	0.59
Malays	10	15.3	
Indians	15	15	
Others	12.5	12.5	
Age			
<40	19	19.3	0.00*
40 and above	10	14.9	
Size			
2 cm and below	10	14.5	0.07
>2 cm	15	16.1	
Axillary lymph nodes involved			
N0	10	15.1	0.11
N1	11.5	15.2	
N2	12.5	17.5	
N3	18	15.9	
Grade			
1	5	3.4	0.00*
2	8	12.7	
3	20	17.3	
ER			
Negative	20	19.8	0.00*
Positive	9.5	11.1	
PR			
Negative	18	18.8	0.00*
Positive	8	11	
HER2			
Negative	10	15.4	0.00*
Positive	17	14.5	
Mitotic index			
0.7	8	12.9	0.00*
8-14	15	13.9	
≥15	21	19.2	

* Statistically Significant

note that none of these patients received chemotherapy.

Discussion

The ASCO-CAP (American Society of Clinical Oncologists - College of American Pathologists) guidelines clearly lay out what is considered to be ER, PR and HER2 positive and that quality assurance is of the utmost importance. (Hammond et al., 2010; Wolff et al.,

Table 4. Association Between Demographic / Tumor Characteristics with Ki67 Status in 450 Patients with Stage I – stage III Breast Cancer

	Ki-67 negative	Ki-67 positive	Univariable odds ratio for positive Ki-67 (95% CI)	Multivariable odds ratio for positive Ki-67 (95% CI)
Median age (years)	51	51	0.98 (0.87-1.00)*	0.98 (0.96-1.00)
Ethnicity				
Chinese	236	167	1.00	1.00
Malays	12	11	1.29 (0.57-3.01)	0.90 (0.34-2.40)
Indians	8	8	1.41 (0.52-3.84)	1.16 (0.33-4.06)
Others	4	4	1.41 (0.35-5.73)	1.20 (0.26-5.53)
Median tumour size (cm)	2.0	2.25	1.29 (1.11-1.49)*	1.23 (1.03-1.47)*
Axillary lymph nodes involved				
N0	173	101	1.00	1.00
N1	48	48	1.71 (1.7-2.74)*	1.55 (0.88-2.73)
N2	19	20	1.80 (0.92-3.54)	1.37 (0.62-3.05)
N3	14	20	2.45 (1.18-5.06)*	1.29 (0.53-3.14)
Grade				
1	45	2	0.02 (0.05-0.09)*	0.05 (0.01-0.23)*
2	150	61	0.20 (0.13-0.31)*	0.25 (0.12-0.49)*
3	58	117	1.00	1.00
ER				
Negative	42	91	1.00	1.00
Positive	218	99	0.21 (0.14-0.32)*	0.85 (0.40-1.81)
PR				
Negative	54	107	1.00	1.00
Positive	206	83	0.20 (0.13-0.31)*	0.42 (0.21-0.86)*
HER2				
Negative	226	115	1.00	1.00
Positive	34	75	4.33 (2.73-6.89)*	3.01 (1.74-5.20)*
Mitotic index				
0-7	181	71	1.00	1.00
8-14	47	62	3.36 (2.11-5.37)*	0.58 (0.28-1.20)
≥15	19	44	5.90 (3.23-10.80)*	0.35-2.01)

* Statistically significant

2014) The role of Ki-67 index as a prognostic marker is well-studied though significant issues remain regarding its predictive utility and validity, and these issues have to be addressed before it can be used routinely in clinical practice.

In spite of it not being a recommended routine marker in breast pathology, it continues to be reported in several centres, and is used as a marker for decision making in guidelines such as the St Gallens consensus. (Goldhirsch et al., 2013) The Ki-67 index is also one of the variables included in the web-based online prognostic model, PREDICT. PREDICT was developed in the United Kingdom, and had been recommended to aid prognostication and selection of patients with early breast cancer for adjuvant chemotherapy. (Wishart et al., 2010) However, the added prognostic value of Ki-67 index in the PREDICT program is yet to be externally validated due to a lack of information on Ki-67 index in the appropriate population. A recent publication cautions oncologists not to rely on the use of Ki67 for patient with early breast cancer and only 1-3 positive lymph nodes (Andre et al., 2015).

The histopathological factors associated with Ki-67

index positivity and higher Ki-67 absolute value, such as a higher grade, ER and PR negativity, HER2 positivity and high mitotic index seen in this study is also comparable to other studies. (Trihia et al., 2003; Wiesner et al., 2009; Inwald et al., 2013). A larger tumour and lymph node positivity, which are time-dependent factors, are also associated with a positive Ki-67 index and higher absolute Ki-67 index values. In multivariable analysis, lymph node status, ER status and mitotic index were no longer significantly associated with Ki-67 index.

A meta-analysis of 46 studies including 12 155 patients evaluated the impact of Ki-67 index on disease-free and overall survival, and found that Ki-67 index positivity (according to the cut-off points as defined by each study) was associated with a higher probability of relapse in all patients. What is interesting is the wide variation in the cut-off value in the studies, which ranged from 3.5% to 32%, with the majority of studies using 10% as an arbitrary cut-off value, because that was the median value of Ki-67 index. (de Azambuja et al., 2007). Spyrtos et al compared five different cut-offs (10%, 15%, 17%, 20% and 25%) with other proliferative markers such as mitotic index and grade, and decided that a Ki-67 index

cut-off of 25% was optimal to identify patients sensitive to chemotherapy protocols, and with a cut-off of 10%, few tumours with low proliferation would be misclassified. (Spyratos et al., 2002). The PREDICT model defined a positive Ki-67 index as being more than 10%. (Wishart et al., 2010). However the best evidence for the cut-off value is from a study by Cheang et al., who used gene expression profiling to determine that the best Ki-67 index cut-off to differentiate between Luminal A and Luminal B HER2 negative was 13.25%. (Cheang et al., 2009). Notably, the median value of Ki-67 index in this study was 15, and hence the cut-off value of 14% as advised by the St Gallens Consensus is highly likely to identify the more proliferative tumours (Goldhirsch et al., 2013).

A recent study had demonstrated that the IHC4 (based on ER, PR, HER2 and Ki-67 index) is equivalent to genomic profiling (ie Oncotype DX and Mammoprint) in predicting outcome in estrogen-receptor positive breast cancers (Cuzick et al., 2011). However calculation of the IHC4 required quantitative estimates of the ER, PR and Ki-67 index in percentages. The IHC4 score classifies tumours into low, intermediate and high-risk. It is important to note that these recurrence scores were designed for ER positive, HER2 negative and lymph node negative tumours, i.e. the group where the need for chemotherapy is equivocal. Compared with the cost of genomic profiling, it has been suggested that testing of Ki-67 index would be resource-saving and economical especially if done concurrently with other biomarkers which are already in standard clinical practice.

Besides being a prognostic marker, Ki-67 index has been shown to be predictive of the response to neoadjuvant chemotherapy, whereby a higher Ki-67 index is associated with higher pathologic complete response rate. It has been suggested that tumours with a Ki-67 index above 25% may be better candidates for neoadjuvant chemotherapy. (Nishimura et al., 2010). Measurement of Ki-67 index before and after a 2-week course of pre-surgical hormone therapy found that higher levels of Ki-67 index after the course of hormone therapy was statistically significantly associated with a lower recurrence-free survival (Dowsett et al., 2007).

Decision-making on adjuvant therapy depends on institutional guidelines, as well as international guidelines. Women decide on adjuvant therapy based on their perception of risks and benefits of adjuvant therapy with guidance from their treating physician. It has been suggested that women with a positive K-67 index should be offered chemotherapy because of poorer outcomes. However it is noted that if Ki-67 positivity is used for decision-making, only 4.9% of the "good" prognosis patients, i.e. node negative, 2 cm or smaller, ER and/or PR positive, HER2 negative, and non-Grade 3, had Ki-67 index levels 14% and above, and potentially would have been advised for adjuvant chemotherapy. However, none of these patients in the current study received adjuvant chemotherapy. This indicates that the oncologists do not regard Ki-67 index positivity as a sole criteria for adjuvant chemotherapy administration.

In conclusion, The absence of standardization of Ki-67 index requires further research before it can be accepted

as a routine prognostic or predictive marker. (Jonat and Arnold, 2011) Further analysis using validated methods is necessary before its widespread adoption and only a complete standardization of tissue handling and processing will improve the value of Ki-67 index as a clinically useful marker (Dowsett et al., 2007).

Despite all these reservations, the role of Ki-67 index in decision making seems to be established in several treatment guidelines and prediction models. Based on the current study, it appear that in the absence of other pathologically adverse features, information on Ki-67 index would have little impact on chemotherapy decision-making.

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