Cell Proliferation Measured by Ki67 Staining and Correlation to Clinicopathological Parameters in Operable Breast Carcinomas from Vietnamese and Swedish Patients

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Abstract: *Background*: Cell proliferation measured by Ki67 has recently been shown to be a prognostic and predictive factor in breast cancer. The aim of this study was to compare cell proliferation determined by Ki67 expression with different clinicopathologic parameters among Vietnamese and Swedish women with breast cancer.

Materials and Methods: The study was based on series of breast cancer from Vietnamese patients treated in the National Cancer Hospital in Hanoi, Vietnam and from Swedish patients treated in the Karolinska Hospital, Stockholm, Sweden. Cell proliferation was measured by Ki67 staining in an automated procedure and was expressed as percentage of stained tumor cell nuclei.

Results: The distribution and mean of Ki67 indices from Vietnamese patients were similar to those estimated from Swedish patients, 27.7% (±17.1%) vs. 26.9% (±23.1%). There were no differences between the two series of patients with respect to proliferation index and age, tumor size and lymph node status. The mean Ki67 indices were higher in high grade tumors in both series. In addition, Swedish patients had significantly higher Ki67 indices in tumors associated with other poor prognostic factors as compared to Vietnamese, 52.8% vs. 31.9% in ER(-) tumors, 39.6% vs. 30.7% in PgR(-) tumors and 40.1% vs. 28.3% in *HER2* amplified tumors, respectively.

Conclusions: The cell proliferation index in breast cancers was similar in the Vietnamese and Swedish series. High proliferation was associated with poor prognostic factors such as high grade, hormone receptor negativity and *HER2* amplification.

Keywords: Breast cancer, Immunohistochemistry, Ki67 staining, biomarkers, comparative study.

INTRODUCTION

There are several routinely investigated prognostic and predictive factors in patients with breast cancer such as tumor size, lymph node status, histological grade, hormone receptors and HER2 (human epidermal growth factor receptor-2) status. Postoperative treatment is today often based on hormone receptors and HER2 status [1, 2].

Ki67 is a nuclear protein expressed in G_1, S, G_2 and M phases but not in resting (G_0) cells though the exact role of this protein in cell cycle regulation is still unknown [3]. The cell proliferation in tumors is today measured by immunohistochemical staining for Ki67 in paraffin sections after antigen retrieval with different antibodies, such as MIB-1 and K-2 [3-5]. The percentage of stained nuclei reflects the fraction of growing cells.

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The rate of cancer cell proliferation measured by Ki67 staining has been shown to be a good independent marker of prognosis and treatment response, although there is no consensus on optimal cut-off point [6, 7]. In univariate analysis, Klintman *et al* found that a proliferation rate over 20% was correlated to worse prognosis in premenopausal patients with node-negative breast cancer [8]. Other studies reported that both low and high levels of proliferation (using cut-offs at either 10% or 15%) contributed independently to prognostic information in breast cancer [5, 9]. It has also been reported that the rate of proliferation measured by Ki67 staining using 15% and 30% as cut-off points was an independent prognosticator for survival after adjustment are made for age, tumor size, histological grade and nodal status [10]. In a review article it was stated that the Ki67 index had prognostic information both in uni-and multivariate analyses in spite of the fact that the cut-off index used varied between 0-30% [11].

Analysis of Ki67 expression has been shown to be an important marker for neoadjuvant therapy [2, 12].

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Patients with Ki67 levels of less than 15% benefited from endocrine therapy in contrast to those with higher Ki67 indices [13]. Recently, the St Gallen international breast cancer conference recommended the use of proliferation, such as Ki67 to determine optimum treatment for early breast cancer [14, 15].

There are only occasional reports on the rate of cell proliferation in breast cancers among various ethnical groups, such as Asian and African patients. A large proportion of breast cancers from Japanese women had a Ki67 index of less than 1% [16]. In contrast, rates of proliferation above 15% have been reported in samples of Japanese and Chinese breast cancer patients [17, 18]. A majority of breast cancers from Western patients had proliferation indices over 15% [6, 19]. The frequency of Ki67 expression above 20% in Afro-American was lower than that in Caucasian patients [20]. We have previously found differences in hormone receptor content and *HER2* status in breast cancers in a comparative study of Vietnamese and Swedish patients [21, 22]. The aim of this current study was to compare cell proliferation as determined by Ki67 expression and its correlation with prognostic and predictive factors in Vietnamese and Swedish patients.

MATERIALS AND METHODS

Material Selection

Randomly selected tissue blocks from 237 primary invasive breast cancers in Vietnamese patients operated on in the period 2002-2003 were selected from the tissue bank of the pathology department of National Cancer Hospital in Hanoi, Vietnam [21]. Two hundred thirty seven age groups matched primary invasive carcinomas from Swedish patients operated at the Karolinska Hospital between 2007 and 2008 were used as comparison. The Vietnamese patients were all treated with mastectomy or partial mastectomy with complete axillary lymph node dissection. The Swedish patients were in a majority of cases operated with partial mastectomy and sentinel node resection. In both series, clinical parameters including age at diagnosis, menopausal status, tumor size and axillary lymph node status, were recorded. The tumor grading was performed according to Elston-Ellis criteria for both Swedish patients and Vietnamese patients [23]. In patients with multiple tumors, the highest grade of tumor was chosen. Breast cancer specimens of patients with neoadjuvant endocrine treatment or chemotherapy or/and inflammatory cancer were excluded in this study. All tumor slides were reviewed before assessing the biomarkers.

Immunohistochemical Staining

Four µm-thick sections of tissue paraffin blocks from Vietnamese patients were assessed by Ki67 staining using an immunohistochemistry automated staining procedure (Venata Medical System, Inc. Tucson, AZ). The protocol consisted of a pre-treatment with cell conditioning solution (CC1), for 60 minutes, pH 8.0 followed by incubation with anti-Ki67 (30-9) rabbit monoclonal primary antibody in 16 minutes (Ventana Catalog number 950-124). The antigen-antibody complexes were detected using an iVIEW™ DAB detection kit (Ventana). Positive and negative controls were run in all assays. The tissue sections from the Swedish patients were also stained using an automated either Ventana or Bond Max system. The monoclonal mouse antibody with Ki67 was used as clone MIB-1 from Dako Company. In a limited study, we compared staining with the two different antibodies on breast cancers from Vietnam. The rates of cell proliferation between these antibodies were almost identical (data not shown).

Tumor Biomarker Scoring

The Ki67 proliferation indices were assessed independently by two investigators (VHT, LS) using light microscopy. The numbers of stained cancer cell nuclei were scored in 400 cancer cells and the ratios of stained to total cells expressed as percentage were defined as Ki67 indices. The ER and PgR status, *HER2* gene expression were assessed as previously described [21, 22]. We calculated the frequencies of tumors with three levels of Ki67 index; low (≤15%), intermediate (16-30%), and high (>30%). We also compared the rate of cell proliferation between Vietnamese and Swedish breast cancers according to the four subtypes as recent suggested by International Expert consensus panel [26].

Statistical Analysis

All data was stored using the SPSS software program (SPSS version 19.0, IBM. Chicago, IL, USA). The Ki67 index of Vietnamese and Swedish patients were compared each with respect to mean, median, and fraction of cut-off values (≤15%, 16-30%, and >30%) age, tumor size, histologic type, Elston-Ellis grade, axillary node, ER status, PgR status, and *HER2* gene amplification. We used t-test for comparing the means with confident interval 95%, Wilcoxon rank-sum test for the median, and Pearson's chi-square test (or Fisher's exact test when appropriate) used in relation

with the fraction. Statistical significance was defined in those cases in which a p-value (two-side test) was less than or equal to 0.05. STATA version 10.1 (StataCorp 2009, college Station, TX, USA) was used for analyses.

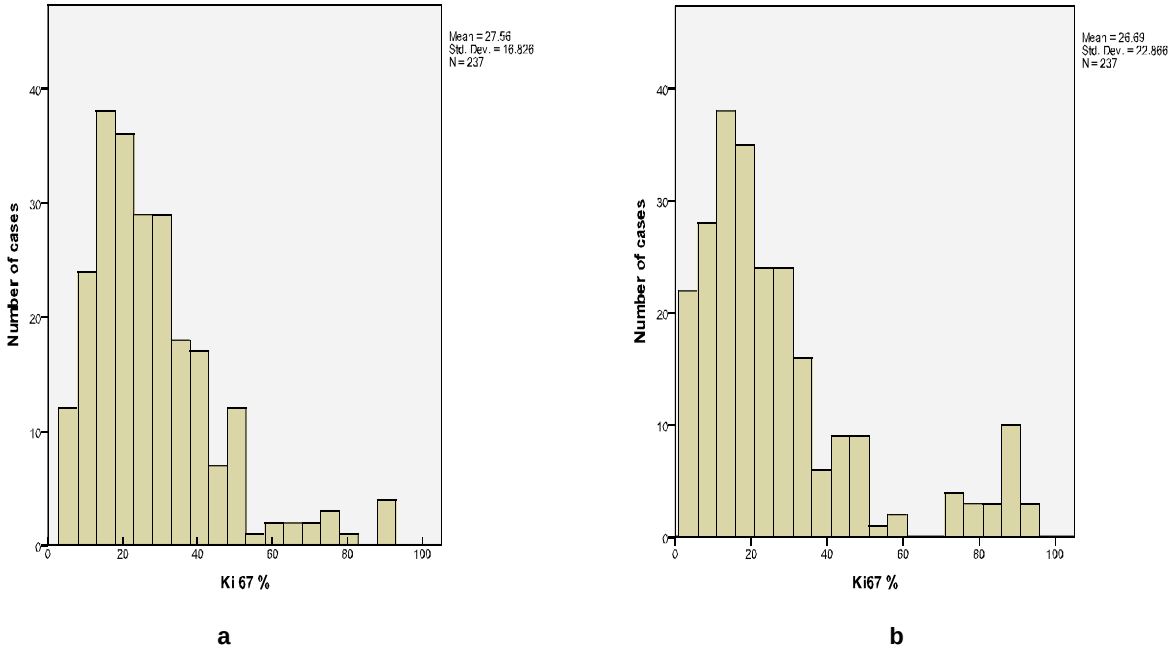
RESULTS

The demographic details of the two series of breast cancer patients are presented in Table **1**. It can be seen that the mean age at diagnosis was 47.7 years (range 26-72) for Vietnamese patients and 51.3 years (range 24-89) for Swedish patients. Vietnamese patients had more advanced stages and more often tumors which were hormone receptor negative and *HER2* gene amplified than Swedish patients. However, histologic subtype, tumor grade and axillary node status were comparable between the two series.

The distribution of Ki67 indices was similar for the Vietnamese and the Swedish patients which is shown in Figure **1**. The Ki67 index values varied markedly but had a mean of 27.7% (±17.1%), median of 24% (range 3-90%) for Vietnamese patients and a mean of 26.9% (±23.1%), median of 20% (range 1-95%) for Swedish patients. We found that 24%, 43% and 33% of the Vietnamese patients, and 37%, 35%, and 28% of the Swedish patients had tumors with low, intermediate and high Ki67 indices, respectively.

Table 1: Characteristics of Study Population

Parameters	Vietnamese (n= 237) n (%)	Swedish (n= 237) n (%)	P-value
Mean age (yrs)	47.7 (range 26-72)	51.3 (range 24-89)	
Tumor size			
≤2 cm	39 (16)	102 (43)	<0.001
>2 cm	196 (83)	135 (57)	
Unknown	2 (1)		
Histologic type			
Ductal*	217 (92)	202 (85)	0.031
Others	20 (8)	35 (15)	
Elston-Ellis grade			
I	66 (31)	44 (19)	
II	109 (51)	112 (47)	0.001
III	37 (18)	80 (34)	
Axillary node			
No metastasis	129 (54)	142 (60)	
Metastasis	106 (45)	91 (38)	0.185
Unknown	2 (1)	4 (2)	
ER status			
Negative	87 (37)	45 (19)	<0.001
Positive	143 (60)	192 (81)	
Unknown	7 (3)		
PgR status			
Negative	125 (53)	83 (35)	<0.0001
Positive	105 (44)	154 (65)	
Unknown	7 (3)		
HER2 gene			
Not amplified	141 (60)	198 (84)	<0.0001
Amplified	95 (40)	39 (16)	



a **b**

Figure 1: Distribution of Ki67 level in operable breast cancers from Vietnamese (A) and Swedish (B) group.

a: Ki67 index distribution in Vietnamese patients, median: 24%.

b: Distribution of Ki67 index in Swedish patients, median: 20%.

The comparison between the mean Ki67 index and various clinicopathologic parameters in the two series of patients is shown in Table **2**. The mean Ki67 index for patients under or equal to 50 years of age was 28.8% (±18.6%) for Vietnamese and 29.6% (± 25.0%) for Swedish patients. Among patients over 50 years of age, the Ki67 index was higher for Vietnamese patients compared to their Swedish counterparts but this difference was not statistically significant. No differences were observed when the mean Ki67 indices of Vietnamese and Swedish tumors were correlated with tumor size, tumor type, and nodal status. In contrast, grade I and grade II tumors from Vietnamese patients had a higher mean Ki67 index of 25.1% and 28.1% as compared to 12.8% and 20.2% in those from Swedish patients ($p<0.001$) (Table **2**). Grade III tumors from Vietnamese and Swedish patients had high Ki67 indices of 39.5% vs. 44.3%, respectively.

In addition, it was found that the mean Ki67 indices in either ER(-) or PgR(-) tumors were significantly lower, 31.9% (±19.1%) and 30.7% (±17.7%) in Vietnamese patients vs. 52.8% (±30.8%) and 39.6% (±30.2%) in tumors from Swedish patients. However, in the respective series the rate of proliferation was higher in hormone receptors negative tumors than that in hormone receptor-positive cases. The mean Ki67 index in *HER2*-amplified Vietnamese tumors was significantly lower than in their counterpart Swedish tumors, 28.3% (±14.4%) vs. 40.1% (±26.0%), respectively. There was no difference in proliferation rate in *HER2* non-amplified tumors between the two series of patients.

The correlation between tumors with low (≤15%), intermediate (16-30%), high (>30%) rate of proliferation and various clinicopathologic parameters was also analyzed. The Table **3a** shows that the distribution of Ki67 indices among low, intermediate and high cell proliferation rates was similar for Vietnamese patients below and above 50 years. The distribution of proliferation was similar between tumors of 2 cm or less in diameter and larger tumors. Only 5% of the high grade tumors had a proliferation below 15% while 49% had a proliferation above 30%. Tumors with grade I or II were more evenly distributed among the three classes of proliferation rate.

Patients with positive axillary nodes had tumors with low proliferation rate more seldom than tumors from node negative patients (Table **3a**). Thirty percent and 36% of estrogen and progesterone receptor-positive tumors had a proliferation below or equal to 15%, while only 16% and 15% of tumors without detectable ER and PgR, respectively were found in this interval of proliferation. It can also be seen from Table **3a** that ER(-) and PgR(-) tumors more often, 44% and 39%, were represented in the category with the highest proliferation rate as compared to 26% and 25% for

Table 2: Comparison of Ki67 Index (%) between Two Populations According to Biologic Factors

Parameters		Vietnamese	Swedish	P-value
All	n	237	237	
	mean (±SD)	27.7 (±17.1)	26.9 (± 23.1)	0.676
	(range)	(3-90%)	(1-95%)	
	median	24	20	
Age (yrs) ≤50	n	147	147	
	mean (±SD)	28.8 (±18.6)	29.6 (±25.0)	0.756
	median	24	22	
Age (yrs) >50	n	90	90	
	mean (±SD)	25.9 (±14.2)	22.3 (±18.8)	0.149
	median	24	18	
Tumor size ≤2 cm	n	39	102	
	Mean (±SD)	27.0 (±17.8)	26.2 (±24.6)	0.853
	median	22	18	
Tumor size >2 cm	n	196	135	
	mean (±SD)	27.7 (±17.0)	27.4 (±22.0)	0.888
	median	24	22	
Ductal carcinoma	n	223	202	
	mean (±SD)	28.5 (±17.1)	28.9 (±24.2)	0.882
	median	25	21	
Elston-Ellis grade	Grade I (n) mean (±SD) Grade II (n)	66 25.1 (±14.9) 109	44 12.8 (±9.3) 112	<0.001
	mean (±SD) Grade III (n)	28.1 (±16.8) 37	20.2 (±13.9) 80	<0.001
	mean (±SD)	38.5 (±19.4)	44.3 (±28.0)	0.257
Axillary node (-)	n	129	142	
	mean (±SD)	27.3 (±17.7)	27.6 (±23.3)	0.905
	median	24	20	
Axillary node (+)	n	106	91	
	mean (±SD)	28.0 (±16.4)	25.6 (±22.9)	0.394
	median	24	20	
ER(-)	n	87	45	
	mean (±SD)	31.9 (±19.1)	52.8 (±30.8)	<0.001
	median	27	46	
ER(+)	n	143	192	
	mean (±SD)	25.1 (±15.6)	20.8 (±15.7)	0.013
	median	22	17	
PgR(-)	n	125	83	
	mean (±SD)	30.7 (±17.7)	39.6 (±30.2)	0.008
	median	25	30	
PgR(+)	n	105	154	
	mean (±SD) median	24.0 (±16.0) 20	20.0 (±14.1) 16	0.034
HER2(-)	n	141	198	
	mean (±SD)	27.3 (±18.8)	24.2 (±21.6)	0.170
	median	22	18	
HER2(+)	n	95	39	
	mean (±SD)	28.3 (±14.4)	40.1 (±26.0)	0.001
	median	27	35	

Table 3a: Correlation between Clinicopathologic Parameters and Ki67 Index of Vietnamese Population (%)

Variables		Patient No	≤15%	16-30%	>30%	P-value
Age (yrs)	≤50	147	23.8	40.8	35.4	0.558
	>50	90	24.4	46.7	28.9	
Tumor size	≤2cm	39	30.8	28.2	41.0	0.125
	>2cm	196	23.0	45.9	31.1	
Histologic type	Ductal**	223	22.0	43.0	35.0	
	Others	14	57.1	42.9	0	0.003*
Elston-Ellis grade	I+II	175	23.4	44.0	32.6	
	III	37	5.4	45.9	48.6	<0.001
Axillary node	(-)	129	28.7	38.7	32.6	
	(+)	106	18.9	48.1	33.0	0.174
ER status	(-)	87	16.1	40.2	43.7	0.008
	(+)	143	30.1	44.0	25.9	
PgR status	(-)	125	15.2	45.6	39.2	0.001
	(+)	105	36.2	39.0	24.8	
HER2 gene	(-)	141	26.2	45.4	28.4	0.377
	(+)	95	21.1	38.9	40.0	

*by Fisher test, **ductal/ ductal component.

Table 3b: Correlation between Clinicopathologic Parameters and Ki67 Index of Swedish Population (%)

Variables		Patient No	≤15%	16-30%	>30%	P-value
Age (yrs)	≤50	147	35.4	30.6	34.0	0.021
	>50	90	40.0	42.2	17.8	
Tumor size	≤2cm	102	45.1	30.4	24.5	0.087
	>2cm	135	31.1	38.5	30.4	
Histologic type	Ductal*	202	35.1	32.7	32.2	0.002
	Others	35	48.6	48.6	2.9	
Elston-Ellis grade	I+II	156	50.6	35.9	13.5	<0.001
	III	80	10.0	33.7	56.3	
Axillary node	(-)	142	36.6	33.1	30.3	0.638
	(+)	91	38.5	38.5	23.1	
ER status	(-)	45	13.3	20.0	66.7	<0.001
	(+)	192	42.7	38.5	18.8	
PgR status	(-)	83	25.3	26.5	48.2	<0.001
	(+)	154	43.5	39.6	16.9	
HER2 gene	(-)	198	41.9	34.8	23.2	<0.001
	(+)	39	12.8	35.9	51.3	

*ductal/ ductal component.

ER(+) and PgR(+) tumors, respectively. There was a similar distribution of *HER2*-amplified and non-amplified tumors among the three classes of proliferation.

The correlation between various clinicopathologic parameters and cell proliferation for tumors from Swedish patients is shown in Table **3b**. For patients under 50 years of age there was almost an equal distribution between low, intermediate and high proliferation. In contrast, 18% of the tumors from patients with age over 50 years showed high proliferation as compared to 40% and 42% for low and intermediate proliferation, respectively.

Tumors which were 2 cm in diameter or less had low proliferation in 45% of the cases as compared to 25% which had high proliferation. For tumors larger than 2 cm in diameter there was a relatively even distribution between low, intermediate and high proliferation level. Table **3b** also shows that 51% of grade I/II tumors had low proliferation, but that only 14% had a high proliferation. High grade tumors showed a low proliferation in 10% of the cases whereas 56% were classified as highly proliferative tumors. Axillary node status did not correlate to proliferation rate (Table **3b**).

The ER and PgR status showed a significant correlation to cell proliferation. Thus, ER(+) or PgR(+) tumors showed a proliferation over 30% in 19% and 17% of the cases, respectively (Table **3b**). In contrast, 67% of ER(-) and 48% of PgR(-) tumors had proliferation rate of over 30%. There was a significant correlation between *HER2* status and cell proliferation rate. *HER2* non-amplified tumors had in 77% of the cases a proliferation of below 30% (Table **3b**), whereas, the majority (51%) of *HER2* amplified tumors had a proliferation over 30%.

Table **4** shows that the Luminal A subtype represents 10.6% and 31.6% of Vietnamese and Swedish patients, respectively. Luminal B1 tumors were found in 33.5 and 41.3% in Vietnamese and Swedish patients. Luminal B2, triple negative and *HER2* enriched tumor were all more common in Vietnamese than Swedish patients. The triple negative and *HER2* subtypes were dominated by highly (>30%) proliferative tumors in both series of patients.

Table 4: Distribution of Subtypes between Vietnamese and Swedish Breast Cancers (%)

Variables	Vietnamese	Swedish
Luminal A	10.6	31.6
Luminal B1	33.5	41.3
B2	23.0	8.9
Triple negative	13.6	10.6
HER2 enriched	19.3	7.6

DISCUSSION

Several methods are used to measure cell proliferation. Mitotic index is the oldest technique to assess cell proliferation on routinely stained histological slides but it is time consuming and has a relatively low reproducibility. Thymidine or bromodeoxyuridine labelling have seldom been used routinely since they require fresh tumor cells and are laboratory-intensive. Flow cytometry, which is a rapid and reproducible technique, can be performed on both fresh and paraffin-embedded tissue [24]. However, the equipment required for this technique is expensive and it requires relatively large amounts of tumor tissue. Immunohistochemistry is today used in many laboratories to measure cell proliferation with an antibody raised against the nuclear Ki67 antigen. The technique is reliable, relatively inexpensive and can be performed on routine sections of paraffin-embedded tumor tissue [3, 4]. It is also applicable to tumor cells procured through fine needle aspiration [9]. We chose immunostaining to study the rate of cell proliferation in tumor tissues from the pathology archives in the Vietnam National Cancer Hospital, Hanoi and compared it to that found in a series of breast cancers from the Karolinska Hospital, Stockholm. Since the two series of patients were operated over different time periods a valid comparison of survival could not be made.

The distribution of the Ki67 index varied markedly in both series of samples taken from Vietnamese and Swedish breast cancer patients with a mean of 27.7% (±17.1%), median of 24% for Vietnamese, and a mean of 26.9% (±23.1%), median of 20% for Swedish patients (Table 2). Thus, we found no significant difference between these two populations with respect to distribution of either mean or median values for Ki67 indices between these series. A proliferation frequency above 15% was found in 76% of Vietnamese and 63% of Swedish tumors. To our knowledge, this is the first study comparing the proliferation rate of breast cancer patients between Asian and Western populations. The previous reports on the rate of cell proliferation in breast carcinomas from Asian patients have given discrepant results. For example, in a large series of breast cancers from Japanese patients it was found that 68% of the cases had a proliferation index over 15% and mean of 20% [12]. Whereas, only 44.5% of Chinese patients had a Ki67 index above 15% with a mean of 27% [18]. Others have, however, reported relatively low Ki67 values in breast cancers from other Asian countries. Thus, Park *et al.* found that Korean

patients in a majority of cases (78%) had a proliferation rate under 14% [25]. In agreement with this, one study showed that only 54% of Japanese tumors had a proliferation above 1% [16]. The discrepancies between these studies are probably due to differences in conditions for fixation and staining including choice of antibody. In addition, the microscopic evaluation of the staining can be a source of discrepancy since factors such as choice of tumor area, number of tumor cells counted and level of staining intensity for scoring will have an impact on the index calculated [7].

Our results on the cell proliferation rate for breast cancers from Swedish patients were consistent with other reports for patients from Western countries. Thus, several authors have found mean values between 23.1% and 27% which are similar to the findings in our study [4, 19]. However, a series of breast cancers with somewhat lower mean Ki67 values between 11% and 20% have also been published [5, 6, 10]. Again, it seems unlikely that these differences are true variations among the various patient series but rather represent differences in fixation, staining and evaluation as discussed above.

We also correlated the Ki67 index in Asian and Western breast cancer patients with various clinicopathologic parameters. The mean values of Ki67 indices were used in this comparison. We found that older (>50 yrs) patients from both Vietnam and Sweden had similar mean Ki67 values (25.9% vs. 22.3%) and younger patients (≤50 yrs) had mean values of 28.8% and 29.6%, respectively. The mean of Ki67 fraction in cancers from Vietnamese patients was similar to that from Swedish patients when correlated with tumor size, ductal subtype, and nodal status. Mean Ki67 indices were higher in poor prognosis tumors such as ER(-), PgR(-), and *HER2*(+) tumors. Thus ER(-) tumors from Vietnamese patients had a mean Ki67 index of 31.9%, as compared to 25.1% for ER(+) tumors. Similarly, for Swedish patients ER(-) tumors had a mean index of 52.8% vs. 20.8% for the ER(+) tumors. These findings for Swedish patients are in agreement with a previous study from Germany [4]. Our results also show that there was a significant difference in mean Ki67 index values in PgR(-) tumors between Vietnamese and Swedish patients, 30.7% vs. 39.6%, respectively. The mean Ki67 index was 28.3% for Vietnamese patients as compared to 40.1% for Swedish patients in *HER2* amplified tumors. The Ki67 index was thus lower in tumors of similar size from Vietnamese patients than that from Swedish patients when these weaker prognostic markers were analyzed.

There is no consensus on cut-off levels to identify tumors with either a good or bad prognosis. Such a separation is mandatory to allow a more individualized treatment with endocrine therapy or chemotherapy. Some studies have used 10%, 15% or 20% as cut-off points, whereas others have applied median value [4, 5, 8, 9, 12]. Recently, two reports from the St Gallen Conference addressed this issue. In 2009, it was suggested that breast carcinoma should be divided into three groups with low (≤15%), intermediate (16-30%) and high (>30%) rate of proliferation to aid the selection of patients for endocrine treatment and chemotherapy [14]. This recommendation was modified in 2011 when the Panel chose to use a Ki67 index of 15% as optimal cut-off point between low and high proliferation when selecting patients for chemotherapy [15]. Furthermore, previous studies from Sweden showed that 15% of Ki67-stained cells were an optimal cut-off point for predictive as well as prognostic information [9, 15]. A value of 15% was also considered as an optimal cut-off for prognosis to Chinese patients [18]. It was therefore of interest to correlate the various clinicopathological parameters for both Vietnamese and Swedish patients with three levels low, intermediate and high Ki67 proliferation.

We found that there was no significant difference between young (≤50 years of age) and old (>50 years of age) Vietnamese patients, with respect to distribution between three Ki67 intervals. Thus, it is possible that the Ki67 proliferation rate can be an age-independent prognostic factor in Vietnamese patients. A similar finding has been reported in Norwegian breast cancer patients [5]. In contrast, a majority of Swedish patients over 50 years of age had low to intermediate Ki67 values which has also been reported for other groups of Western patients [4, 8].

Tumor size of ≤2 cm or >2 cm showed a relatively even distribution between low, intermediate and high levels of proliferation in both Vietnamese and Swedish patients although there was a tendency that small tumors in the Swedish series more often had a proliferation level under 15%. There are several reports that small tumors more often have a lower Ki67 value than large tumors [6, 19]. However, there are also studies which are in agreement with our results [5, 16, 20].

Published results were contradictory regarding the association between proliferation and lymph node status. Our two population series showed similar frequencies of low, intermediate and high Ki67 values

for tumors with or without lymph node engagement. Similar results have been published by others [5, 16]. In contrast, associations between high Ki67 values and positive lymph nodes have been reported previously [4, 6, 12]. These discrepancies are difficult to explain but may result from both differences in the study cohorts and cut-off levels as well as the technique used for Ki67 evaluation.

Tumor grading according to Elston-Ellis includes analysis of mitotic number. It is therefore not surprising to find that tumors from both Vietnamese and Swedish patients had higher Ki67 values in grade III tumors as compared to grade I or II. Similar results have been reported by several authors [4, 5, 8, 18, 19].

A significant inverse relationship between the Ki67 index and hormone receptor positivity has been reported previously for both Asian and Western patients [4, 8, 17, 19]. For both the Vietnamese and the Swedish patients in the current study, ER(-) tumors more often had higher Ki67 indices as compared to ER(+) tumors. This difference was most pronounced for the Swedish patients. A similar correlation was seen for tumors with detectable or non-detectable PgR. The finding that a relatively large proportion of receptor-positive tumors are classified as highly proliferative suggests that endocrine therapy alone in such patients could be questioned.

Different results have been reported on the association between *HER2* status and cell proliferation. A rate of cell proliferation above 30% was found in tumors with *HER2* amplification as compared to those without amplification, 40% vs. 28% of Vietnamese patients and 51% vs. 23% of Swedish patients, respectively. The difference was statistically significant only in the Swedish series. A correlation between high index of proliferation and *HER2* positivity was reported in a number of studies [4, 8, 11, 19]. Thus, it seems clear that high proliferation rate is correlated with HER2 protein overexpression. There is only one contradictory report from Japan which described that Ki67 expression was not correlated to HER2 status [16]. Again, the finding that a proportion of *HER2* (+) tumors have a low (≤15%) rate of proliferation may question their classification as highly aggressive cancers.

The recently presented genetic/immunohisto-chemical subtyping of breast cancers in Luminal A-, Luminal B-, triple negative-, and *HER2*- type has been claimed to be useful for clinical management as well as providing an improved prognostic information [12, 25]. Tumors from Vietnamese and Swedish patients belonging to the poor prognosis subtypes, triple negative and *HER2* enriched were more often highly proliferative (>30%). The proliferation rates for the various subtypes in Swedish patients are in good agreement with previous results from Western patients. There were some differences between Swedish and Vietnamese patients. Thus the Luminal A type was less common and Luminal B, triple negative and *HER2* enrichment were more frequent among the Vietnamese tumors. . To our knowledge, this is the first comparative report on the rate proliferation in the immunohistochemical subtypes in tumors from Asian patients. It will be of interest to study the prognostic value of this subtyping in Vietnamese patients as well as of the proliferation rate will be an independent prognostic factor.

Our findings have shown that there was a correlation between rate of proliferation and cell markers for poor prognosis. It will be therefore be of great interest to evaluate the Ki67 fraction as an independent marker for overall prognosis as well as its use as a predictor for medical therapy. Such a study has been initiated for a series of Vietnamese patients. Furthermore, this study provides a baseline study cohort for comparative studies with other ethnicities and Vietnamese who have migrated to the Western countries.

CONCLUSIONS

The Ki67 index showed a distribution between 1-95% in primary carcinomas with a median of 24% and 20% from Vietnamese and Swedish patients, respectively. The mean Ki67 index was similar for the two populations. Poor prognostic and predictive biomarkers in both Vietnamese and Swedish patients related significantly to a high Ki67 fraction but a fraction of the tumors had a low rate of proliferation. These findings suggest that Ki67 can be used to add predictive and prognostic information both in Vietnamese and Swedish patients.

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DECLARATION OF INTEREST

No authors declared conflict of interest.

APPROVAL

This work was approved by the ethical committee of the Karolinska Institutet, 2011/2033-32 (Sweden) and the Hanoi Medical University, No 95/HMURB (Vietnam), respectively.

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