Ki-67 marker useful for classification of malignant invasive ductal breast cancer

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ABSTRACT

BACKGROUND

Breast cancer is an important health problem in the world. Uncontrolled cell proliferation represents a malignant characteristic of neoplasia such as breast cancer, and can be examined immunohistochemically by measuring the Ki-67 proliferative marker. The objective of this study was to determine the role of Ki-67 for classification of the degree of malignancy in women with invasive ductal breast cancer.

METHODS

A cross-sectional study was conducted on 20 women with invasive ductal breast cancer. The samples were immuno-histochemically tested for Ki-67 using anti-Ki-67 primary antibody. The Ki-67 proliferative index was determined by enumerating the proportion of Ki-67 positive nuclei among the total number of cells in ten areas observed at 400x magnification, using a 20% cut-off value to distinguish between low and high proliferative indices. Statistical analysis was by means of the chi-square test.

RESULTS

Seventy five persent of the high grade malignancies had a high Ki-67 proliferative index ($\geq$20%), while only 12.5% of the low grade malignancies had a high Ki-67 index ($\geq$20%). The difference in grade malignancy was statistically significant (p=0.022), whereas tumor size was not associated with a statistically significant difference in Ki-67 index (p=0.648).

CONCLUSION

The study showed that invasive ductal breast cancer with high Ki-67 index was significantly associated with high grade of malignacy. The high Ki-67 marker index can be used for classification of the grade of malignancy of invasive ductal breast cancer.

Keywords: Ki-67 proliferative index, grading**,** ductal invasive breast cancer

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Petanda Ki-67 mampu mengklasifikasi derajat keganasan kanker payudara duktal invasif

ABSTRAK

ABSTRAK
Kanker payudara merupakan masalah kesehatan penting dunia. Tidak terkendalinya proliferasi sel merupakan ciri ganas suatu neoplasia termasuk kanker payudara. Proliferasi sel dapat diperiksa secara imunohistokimia dengan petanda Ki-67. Penelitian ini bertujuan untuk menentukan peran petanda proliferasi Ki-67indeks untuk klasifikasi derajat keganasan pada perempuan dengan kanker payudara duktal invasif.

METODE
Rancangan penelitian potong silang digunakan pada 20 perempuan dengan kanker payudara duktal invasif. Sampel diuji secara imunohistokimiawi terhadap keberadaan Ki-67, dengan antibodi primer anti-Ki-67. Indeks Ki-67 dihitung berdasarkan proporsi jumlah sel Ki-67 positif dengan semua jumlah sel pada area yang diobservasi dengan pembesaran 400 x. Digunakan nilai cut-off 20% untuk membedakan indeks proliferasi rendah dan tinggi, dan analisis data dilakukan menggunakan uji chi-square.

HASIL
Tujuhpuluh lima persen dari karsinoma payudara duktal invasif dengan derajat keganasan tinggi mempunyai indeks Ki-67 yang tinggi ($\geq$20%), dan hanya 12,5% dari karsinoma payudara dengan derajat keganasan rendah mempunyai indeks Ki-67 tinggi ($\geq$20%). Perbedaan derajat keganasan tersebut bermakna secara statistik (p=0,022). Namun besar tumor tidak terkait dengan perbedaan indeks Ki-67 yang bermakna (p=0,648).

KESIMPULAN
Hasil penelitian menunjukkan bahwa kanker payudara duktal invasif dengan indeks Ki-67 yang tinggi secara bermakna terkait dengan derajat keganasan yang tinggi. Indeks petanda Ki-67 yang tinggi dapat digunakan untuk klasifikasi derajat keganasan kanker payudara duktal invasif.

Kata kunci: *Indeks proliferasi Ki-67, derajat keganasan, kanker payudara duktal invasif*

INTRODUCTION

Breast cancer is still an important health problem both in developed and developing countries. According to global cancer statistics, breast cancer is the most frequently diagnosed cancer and the major cause of death in females, accounting for 23% of all cancer cases and causing 14% of deaths from cancer.[1] In Indonesia, breast cancer has risen from second to first rank among the 10 most frequent cancers in females, replacing cervical cancer.[2] The most frequent type of breast cancer is ductal invasive breast cancer.[3]

The diagnosis of a tumor is based on its morphological features on histopathological examination. Histopathologically, the degree of differentiation of breast cancer is determined by 3 components, i.e. nuclear morphology (nuclear pleomorphism), differentiation of tubules, and frequency of mitoses.[3]

Cell proliferation is a concrete parameter that can be determined by examination of Ki-67, which indicates agressiveness of tumor growth.[4] For many years cell proliferation was determined by enumeration of the number of mitoses on routine histomorphological preparations. However, with this type of examination it is

difficult to distinguish between mitotic and pycnotic nuclei.

Currently many experts use immunohistochemistry as an early indicator of malignant tumor growth. Ki-67 is a nuclear protein antigen that stimulates the production of an antibody that is a marker for a tumor antigen found on breast cancer cells.[5] Identification of a tumor biomarker can be used for early detection of cancers, for monitoring the progress of the disease, and as prognostic and predictive markers for individualized patient management.[6]

Trihia et al. state that the Ki-67 assay is easy to perform immunohistochemically on tumor samples obtained by multiple core biopsies and on cytological specimens.[4] According to Urruticoechea et al., immunohistochemical assessment of Ki-67 as a cell proliferation marker is easy and economical to perform, highly reproducible, and able to detect Ki-67 in biopsied tissues.[7]

The specific tumor cell proliferation marker Ki-67 is an antigen expressed in all active phases of the cell cycle, from phases G1-S-G2 to M (mitosis), except in the resting phase (G0). In addition, Ki-67 can be used to enumerate the proportion of actively dividing tumor cells, to assess tumor growth, and to determine the grade of malignancy. From the results of the study by Trihia et al., it was concluded that detection of Ki-67 is a reliable method for objective assessment of mitosis to be used in the grading system, and for predicting the degree of histological differentiation. Ki-67 is also a prognostic parameter in addition to vascular invasion, tumor size, and lymph node involvement, and is a predictor of survival of breast cancer patients. Similarly, Barnard et al. found a positive correlation of Ki-67 score with mitotic index, and a weakly positive correlation with histologic tumor type.[8]

The use of the Ki-67 proliferation biomarker as predictive and prognostic markers for breast cancer has been widely studied.[9-11] Therefore the expert panel at the St. Gallen Consensus conference considered that in addition to the estrogen receptor (ER), progesterone receptor (PR) status, and the human epidermal growth factor receptor 2 (HER-2) status, the Ki-67 label index is an important prognostic parameter for breast cancer.[9] Using the Ki-67 assay, a tumor may be classified as a low-grade, intermediate, and high-grade malignancy, on the basis of a Ki-67 label index of $\leq 15\%$, 16%-30%, and $>30\%$, respectively.[9] In the study by Nishimura et al., it was shown that a higher Ki-67 index ($\geq 20\%$) was significantly correlated with a higher grade of malignancy.[12] Several studies have argued that, apart from the presence or absence of standards of pathological evaluation of Ki-67, the acceptance of Ki-67 as a standard marker should be further investigated.[6,13] The objective of this study was to determine the role of Ki-67 for classification of the degree of malignancy in women with invasive ductal breast cancer.

The objective of this study was to determine a relationship between Ki-67 proliferation marker index scores and degree of malignancy of invasive ductal breast cancer.

METHODS

Study design

This study used a cross-sectional design and was conducted from January 2008 until December 2011.

Study subjects

The study subjects comprised 20 women hospitalized at the Tomang Clinic and Royal Taruma Hospital with the diagnosis of invasive ductal breast cancer, on whom biopsies extirpation had been performed.

Immunohistochemistry

The hematoxylin-eosin (HE) stained sections prepared at the Department of Pathological Anatomy, Faculty of Medicine, Tarumanagara University, and diagnosed histopathologically as invasive ductal breast

cancer, were sent to the Stem Cell and Cancer Institute for immunohistochemical analysis of the Ki-67 proliferative marker, using anti-Ki-67 monoclonal antibody.[11] In the immunohistochemical assay, Ki-67 primary antibody was added to the sections, followed by chromogen to visualize the antigen-antibody reaction. In the negative control group the sections were counterstained with HE without addition of Ki-67 antibody. The assay was considered to be positive for Ki-67 if the cell nucleus had a brown color. Ki-67 negative nuclei did not express the brown color.

The Ki-67 proliferation marker index is expressed as percentages, determined from the number of brown-colored Ki-67 positive cell nuclei divided by the total cell population in the observation fields. For reading of the results, 10 high power fields were selected containing many Ki-67 positive cells.[12,14]

The immunohistochemical preparations of Ki-67 markers were evaluated according to abovementioned criteria and confirmed by 3 independent evaluators. In cases of differing results, the evaluators re-examined the preparations together for coming to a consensus.

Histopathological examination

The histopathological sections, stained routinely with HE, were evaluated morphologically according to WHO histologic typing.[3] Tumor aggressiveness was histopathologically categorized as well-differentiated, moderately differentiated, and poorly differentiated, using a modification of the Nottingham System of Bloom-Richardson. This categorization depended on a total score of 3 components, viz. number of tubules (glands), nuclear pleomorphism, and number of mitoses in 10 high power fields (400x magnification). Each component was evaluated and accorded a score from 1-3. The total score of the three components was 3-9. A score of 3-5 was categorized as grade 1 (well-differentiated), a score of 6-7 indicated grade 2 (moderately differentiated), and a score of 8-9 was categorized as grade 3 (poorly differentiated).[3] Tumor diameter was divided into 3 groups, namely ≤ 2 cm, >2-5 cm, and >5 cm, respectively.[3]

Ethical clearance

This study was approved by the Committee for Research Ethics of the Gading Pluit Hospital, Jakarta.

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 17.0 and Medcalc. The analysis was based on differences in Ki-67 scores at a cut-off point of 20%, for invasive ductal breast cancers with low and high grades of malignancy and a tumor diameter of 5 cm. The data were analyzed by means of the chi-square test.

RESULTS

The 20 samples were grouped according to Ki-67 index, grade of malignancy, tumor size, and age of the patient. Table I shows 10 samples (50%) with a Ki-67 index of <20% and 10 samples (50%) with a Ki-57 index of $\geq 20\%$. Tumors with a low degree of malignancy comprised 8 samples (40%), and those with high degree of malignancy comprised 12 samples (60%). Based on tumor size, tumor diameters of ≤ 5cm were found in 8 samples (40%), and tumor diameters of >5 cm were found in 12 samples (60%). The age of the patients was in the range of 31 up to 78 years, with mean age of 45.35 $\pm$ 12.19 years.

In the group with a high degree of malignancy, 75% showed a high ($\geq 20\%$) Ki-67 index compared to 12.5% in the group with a low degree of malignancy. The difference was statistically significant (p=0.022). (See also Figures 1 and 2). But the difference in Ki-67 index was not statistically significant between tumor sizes (p=0.648) (Table 1).
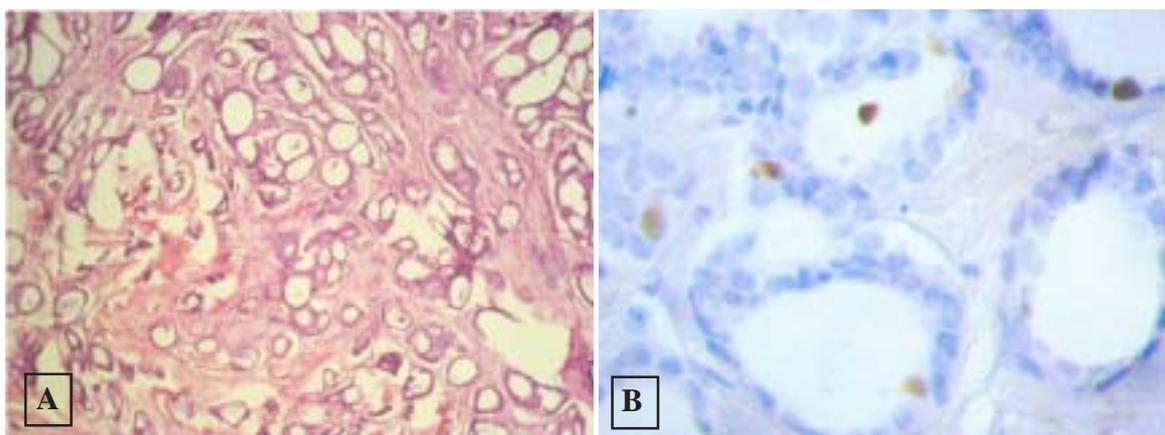
Figure 1. (A) Invasive ductal breast cancer, low-grade/well-differentiated (HE) (at x 100 magnification);
(B) Ki-67 = 4% <20% (brown-colored nuclei) (at x 400 magnification)

DISCUSSION

According to Trihia, for an objective determination of the number of mitoses, the Ki-67 proliferation marker assay may be used,[4] since it is correlated with the clinical picture. This statement is supported by the results of studies by Trihia et al., Urruticoechea et al., and Nishimura, showing that a high Ki-67 score indicates a poor prognosis, but a good response to chemotherapy. [4,7,17]

The lack of standards for the Ki-67 cut-off value, as a result of the use of different antibodies and the absence of standards for the minimum number of cells to be counted, explains the findings of different Ki-67 scores among the various studies. Nishimura et al. and Wiesner et al. use the Ki-67 cut-off value of 20%.[14,17] A Ki-67 index of 20% indicates a high level of cell growth and division.[12] Because a high grade of malignancy also shows a high Ki-67 score,[13,14] the Ki-67 index is considered to be of significant positive value for differentiating among tumors with low and high malignancy grades, by using the categories of low, medium, and high proliferation indices of respectively <20%, 20%-<50%, and 50%.[14] The assessment of the Ki-67 index according to the St. Gallen Consensus of 2009 is important to select prospective patients for chemotherapy as an adjunct to endocrine
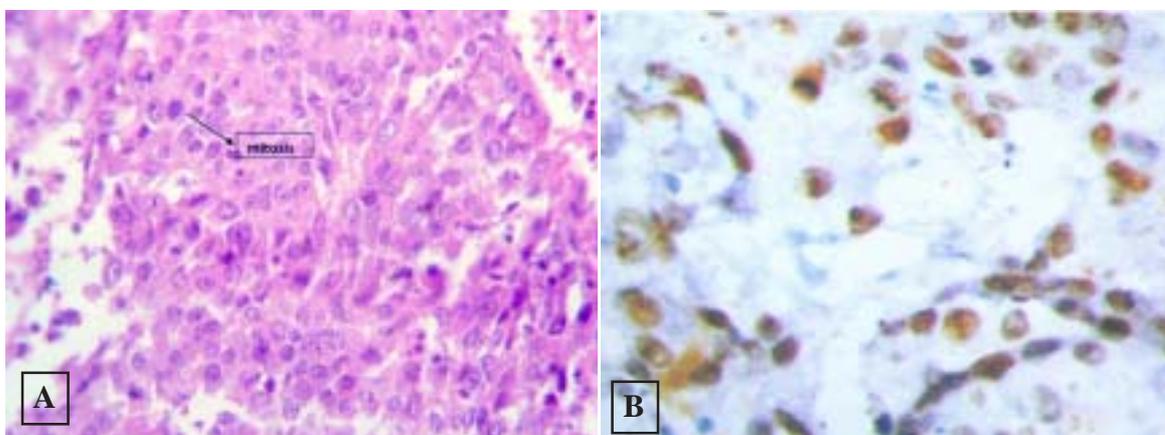

Figure 2. (A) Invasive ductal breast cancer, high-grade/poorly differentiated (HE). Solid, markedly pleomorphic nuclei; mitoses ++; (B) Grade 3: Ki-67 = 60% ≥20%. (both at x 400 magnification)

Table 1. Distribution of Ki-67 index by grade of malignancy and tumor size

Variables	Ki-67 indices		p
	< 20% (n=10)	≥ 20% (n=10)	
Grade of malignancy			
Low	7 (87.5%)	1 (12.5 %)	0.022
High	3 (25.0%)	9 (75.0 %)	
Tumor size			
≤ 5 cm	4 (50.0%)	4 (50.0 %)	0.648
> 5 cm	6 (50.0%)	6 (50.0 %)	

therapy. Tumors are classified as having low, intermediate, and high proliferation rates, on the basis of Ki-67 indices of <15%, 16%-30%, and >30%, respectively.[9] De Azambuja et al. use a cut-off value of 10%, whereas other investigators use the mean, median, and optimum cut-off value, or varying values, and it is these differing values that cause difficulties in determining a threshold value as a standard for daily practice,[11] but some authors use cut-off points that are dependent on objective clinical data. The use of the Ki-67 cut-off point of 10% is to exclude patients with slow proliferation rates from the chemotherapy protocol, so as to prevent excessive treatment for these patients. On the other hand, Nishimura et al. are of the opinion that it is better to administer adjunctive chemotherapy to patients with high cell proliferation rates and Ki-67 values of >25%.[18] In this study, the authors used the cut-off value of 20%, considering the small number of tumor samples and the fact that the cases were at an advanced stage. On the basis of the Ki-67 score, the samples were divided into two groups, i.e. the group with a low Ki-67 score of <20%, and the group with a high Ki-67 score of ≥20%. The samples with a Ki-67 index of <20% accounted for 50%, which was almost equal to the value of 46.5% obtained by Nishimura et al.[14] This was also the case with the samples with a Ki-67 index of 20%/>, with 10 cases (50%) in our study and 53.5% in the study by Nishimura et al.[17] Our study found 8 cases (40%) with a histopathological picture of well differentiated or low grade of malignancy, and 12 cases (60%)

of moderately to poorly differentiated or high grade malignancies. A total of 90% of invasive ductal breast cancers with a higher Ki-67 index (≥20%) also had a high degree of malignancy, while 75% of tumors with a high degree of malignancy also had a high Ki-67 index (≥20%). Similarly, 87.5% of tumors with a low grade of malignancy, had a low Ki-67 index (<20%). Statistically, there was a significant association between Ki-67 proliferation index and grade of malignancy of the tumors (p<0.05).

The majority of cases (7 or 70%) with a Ki-67 index of <20% showed a histopathologic picture of well-differentiated tumors of low degree of malignancy, whereas the remaining 3 cases (30%) were tumors with an intermediate or low level of differentiation. Samples with a Ki-67 proliferation index of 20% ≥ showed 9 cases (90%) with a higher proliferation rate and morphologically these showed a moderate to poor level of differentiation or high grade of malignancy. Only one case (10%) showed a histopathologic picture of well-differentiated tumors of low degree of malignancy. Loly found that invasive breast cancers with a poor degree of differentiation tended to have a Ki-67 expression of ≥10%.[20] The results of the present study also showed that the Ki-67 proliferation index was correlated with the degree of malignancy. High Ki-67 indices showed tumor aggressiveness, in agreement with the results obtained by Nishimura et al. and Trihia et al.[14] Our study also supports the necessity of assessing tumor proliferation markers as indicators of tumor growth, based on the cell divisions

occurring in all phases of the cell cycle, except in the G0 phase. The Ki-67 indices can be used as indicators of prognosis and as predictors of therapeutic reponse.[4,11,14,18]

Recently, Zabaglo published the results of a study comparing Ki-67 to the SP6 antibody, which is more suitable for analytic imaging, while both antibodies are also markers for monitoring tumor proliferation.[21]

The age of the patients in this study was in the range of 31 up to 78 years, with mean age of 45.35 ± 12.9 years, and the most frequent age was between 41 and 50 years, comprising 9 cases. The study results of Nishimura showed that the youngest age was <31 years and the oldest 95 years, with mean age of 56.2 years, while the most frequent age was >50 to ≤65 years. The differences in youngest and oldest ages between our results and those of Nishimura et al. were probably the result of differences in life span, and in genetic and hormonal factors. It is also possible that the patients in the study by Nishimura et al. were more conscious of any abnormalities of their body and immediately sought treatment.[14] The mean age of the patients in our study was lower, and the factors affecting development of disease are unclear. Possibly these comprise genetic and economic factors, because Loly et al. mentioned in their study that in West Sumatra the majority of patients come from the economically disadvantaged, with a peak at ages 40-50 years,[20] similar to our data. In this regard, a larger population is needed, to find factors that play a role in the development of breast malignancies at younger ages.

Tumors with a diameter between 3 and 12 cm (mean 5.925 cm) comprised 50% of samples, while in the study of Nishimura et al. the mean tumor diameter was 2.2 cm.[14] The majority of tumors were >5 cm in diameter, comprising 12 samples (60%). The results of our statistical analysis showed that tumor size did not correlate with Ki-67 score and degree of differentiation. This is in contrast with the findings obtained by Nishmura and Trihia, showing a significant correlation, with a higher Ki-67 index indicating a higher degree of malignancy of the tumors.[4,17] The different results may be due to the low number of samples; our data showed that Ki-67 index was more correlated with degree of tumor differentiation, depending on morphology and biological behavior of the tumors, as evaluated by number of mitoses.

The implication of this study is that immunohistochemical determination of the Ki-67 proliferation index should be performed in cases of breast cancer, to obtain clinically useful information on tumor aggressiveness as reflected in their proliferative rate, which is essential to the management of the patients on an individual basis. There are other assays of markers for monitoring tumor proliferation, namely the Ki-S2 and SP6 antibody assays, but these were not performed in our study due to limited funds.

CONCLUSIONS

Based on the results of this study, invasive ductal breast cancer with high Ki-67 index was significantly associated with high grade of malignancy. The high Ki-67 marker index can be used for classification of the grade of malignancy of invasive ductal breast cancer. Further studies are required on a larger number of samples, using a Ki-67 cut-off of say 10%, to be compared with the existing study results, in the expectancy of being useful to other investigators.

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