Accurate prognosis of patients with luminal type A and HER-2 overexpressing breast cancer via the partitioning calculation method of the Ki-67 index

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Abstract. The aim of the present study was to perform the accurate prognosis of different molecular types breast cancer (BC) by using the partitioning calculation method of the Ki-67 index. Partitioning calculation was used to judge the Ki-67 index and the molecular type was classified according to the 2019 Chinese Society of Clinical Oncology guidelines in 199 cases of BC. The association between the Ki-67 index and overall survival (OS), disease-free survival (DFS) and molecular types was analyzed using the Chi-squared test. Survival analysis was performed using the Kaplan-Meier method with statistical SPSS 18.0 software. The results revealed that the 5-year survival rate of patients with BC was 18.60% (8/43), the 6-year survival rate was 38.24% (26/68), while the 10-year survival rate of patients with BC was 73.08% (133/182); the results indicated that almost 80% patients succumbed during the 5 years following diagnosis. Marked differences were found between the OS of patients with BC and the Ki-67 index (between 50 to 54%) (χ^2=4.83, P=0.028; χ^2=4.60, P=0.032 respectively). All 14 cases of luminal A subtype BC with a Ki-67 index ≤4% survived at the end of follow-up time (69-156 months). At the same time, there was a marked difference between two groups of patients with overexpression HER-2 while using 50% as a cut-off point of Ki-67 (χ^2=5.54, P=0.019). On the whole, the present study demonstrates that the partitioning calculation of the Ki-67 index can be used to judge the prognosis of patients with BC accurately. The prognosis of patients with different molecular subtypes of BC may be associated with the different Ki-67 index.

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Abbreviations: BC, breast cancer; CSCO, Chinese Society of Clinical Oncology; OS, overall survival; DFS, disease-free survival; HER-2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry; pCRs, pathological complete responses; TNBC, triple-negative breast cancer; AJCC, American Joint Committee on Cancer

Key words: Ki-67 index, partitioning calculation, breast cancer, prognosis

Introduction

A limited number of biomarkers have been used to predict the prognosis and monitor the outcomes of patients with breast cancer (BC), such as lactate dehydrogenase (LDH), which is an independent prognostic marker for BC and the Ki-67 index, which is of utmost importance to the prognosis and molecular typing of patients with BC (1). Patients with BC with a high Ki-67 index exhibit more pathological complete responses (pCRs) regardless of the ER (estrogen receptor), progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER-2) status. Ki-67 even can be used to predict the pCRs of patients treated with neoadjuvant therapy from Asia and Europe, but not those from the USA (2). Even young women with BC have a higher Ki-67 index than that of women with BC >60 years of age (3).

In 2019, it was reported in the Guidelines of the Chinese Society of Clinical Oncology (CSCO) that the critical value of Ki-67 should be determined according to the practical situation of each laboratory (4). As recognized by the majority of Chinese experts, a Ki-67 index of <15% indicates a low expression and one of >30% is suggestive of a high expression. When the Ki-67 index is in the range between 15-30%, a secondary pathology consultation is suggested or clinical decisions can be made according to the values of other indices (4). During the practical pathological diagnosis, it is difficult to accurately calculate the critical value of Ki-67 with poor repeatability. The results obtained by different laboratories are derived from technicians who are familiar with the operation to different degrees and trained/untrained diagnosticians. Due to these differences, the results are not exactly the same. Sometimes, the definition of the Ki-67 index remains controversial, particularly regarding whether pathologists should evaluate the Ki-67 index in the 'hot-spots' area in the infiltrated tumor or whether they should report average values of

Ki-67 (5). Notably, after the critical value of the Ki-67 index has been reached, it is difficult to standardize the operation or accurately calculate this index. The accurate estimation of the Ki-67 index can be performed using a scientific method. The potential to achieve the accurate prognosis of patients with BC with the Ki-67 index is dependent on whether this index is associated with the molecular subtype of BC, the possibility of patients with different molecular types to be applicable to the same Ki-67 index, and requires this index to possess an interval value, facilitating clinical pathologists to operate and increase the critical application value. These topics are explored in the present study.

Patients and methods

Patient data and specimens. The paraffin-embedded specimens of patients with BC hospitalized in the 989th Hospital of the PLA Joint Logistic Support Force Hospital (Luoyang, China) during the period January, 2005 to July, 2013 were collected for the present retrospective study. Follow-up data were obtained from 199 patients with BC with complete information, including 1 male (0.5%) and 198 females (99.5%) with an age range of 25-84 years (average age, 48 years). The number of patients with an age ≤30 years was 1, that of patients with an age range of 30-60 years was 162, and that of patients with an age ≥60 years was 36. A total of 104 patients exhibited no lymphatic metastasis (N0), 53 patients presented with 1-3 lymphatic metastases (N1), 31 cases had 4-9 lymphatic metastases (N2), and 11 patients had ≥10 lymphatic metastases (N3). As regards the American Joint Committee on Cancer (AJCC) pathological staging (pTNM) (6), 34 patients were classified as stage IA, 72 cases as stage IIA, 45 cases as stage IIB, 35 cases as stage IIIA, 1 as stage IIIB, 11 cases as stage IIIC and 1 as stage IV. None of the patients accepted chemotherapy or radiotherapy prior to the operation. The specimens were fixed using 10% neutral formalin along with paraffin embedding. The following inclusion criteria were used: i) The patients received a definitive diagnosis via an examination following a BC radical mastectomy; ii) no chemotherapy or radiotherapy were provided prior to the surgery; iii) complete follow-up visit information was provided; iv) patients with BC metastasis or recurrence. The following exclusion criteria were used: i) A lack of complete follow-up visit information; ii) patients who received radiotherapy or chemotherapy prior to the surgery; iii) the patients who were diseased not due to BC metastasis or recurrence; iv) patients whose quality of life was severely affected by other causes or who had succumbed to the disease due to the concurrence of other tumors (Fig. 1).

Written informed consent was obtained from the 989th Hospital of the PLA Joint Logistic Support Force database for the collection of data and for the use of personal data for research purposes, and written informed consent was obtained from the patients or their immediate family. The present study was ethically approved by the Ethics Committee of the 989th Hospital of the PLA Joint Logistic Support Force review board on May 14, 2020 (Approval no. 20200508). The patient information was obtained from the medical records of 989th Hospital and the follow-up data were obtained from the patients or their immediate family members.

Immunohistochemical staining. The SP immunohistochemistry (IHC) method was used to cut the selected paraffin blocks into a thickness of 2-3 μm. Rabbit anti-human monoclonal ER (clone no. SP1, cat. no. Kit-0012, 1:300), rabbit anti-human monoclonal PR (clone no. SP2, cat. no. Kit-0013, 1:300), rabbit anti-human monoclonal HER-2 (clone no. MXR001, cat. no. Kit-0043, 1:200), rabbit anti-human monoclonal antibody Ki-67 (clone no. SP6, cat. no. RMA0542, 1:500), secondary antibodies (ready-to-use sheep anti-rabbit IgG polymer, cat. no. KIT 5010) and color-producing reagents (DAB staining solution) were all purchased from Fuzhou Maixin Biotech Co., Ltd. The operating steps followed the conventional IHC method and PBS was used to replace the primary antibodies with the negative control. Briefly, 2-3-μm-thick sections were mounted on poly-lysine treated glass slides. Endogenous peroxidase activity was blocked with 3.0% H_2O_2 for 15 min at room temperature. The sections were placed in a pressure cooker for 15 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval. The sections were then incubated with primary antibodies at 4˚C overnight. The following day, the sections were washed and incubated for 1 h at room temperature with the secondary antibodies. Peroxidase activity was visualized with DAB and observed under a light microscope (Olympus Corporation) at a low magnification. The appearance of brown particles in the membrane or the nucleus was considered as the positive criterion.

The following result interpretation criterion was used: ER, PR and Ki-67 were all found positive in the cell nucleus. In the case that the presence of brown particles was observed in >1% of BC cell nuclei, ER and PR were classified as positive (Fig. 2A and B). The presence of yellow or brown particles in the cell nucleus was considered to indicate positive Ki-67 staining (Fig. 2C-E).

The following HER-2 positive criteria were used: 0+ for unstained or ≤10% of invasive cancer cells presenting incomplete and weak cell membrane staining; 1+ for >10% of invasive cancer cells presenting incomplete and weak cell membrane staining (Fig. 2F); 2+ for >10% of invasive cancer cells presenting incomplete and/or moderate cell membrane staining or ≤10% of invasive cancer cells presenting strong and complete cell membrane staining (Fig. 2G); 3+ for >10% of invasive cancer cells presenting strong, complete, and uniform cell membrane staining (Fig. 2H) (7). In the presence of HER-2 2+ staining, fluorescence *in situ* hybridization detection was implemented according to the instructions of the manufacturer (Amoy Dx®HER-2 detection kit, Amoy Diagnostics Co., Ltd.) to further determine the status of HER-2. Briefly, 4-μm-thick sections were dewaxed and hydration, and then placed in boiled pre-treatment solution (pH 7.0) for 20 min. This was followed by washing with deionized water for 1 min and washing with 2X SSC solution (pH 7.0) for 1 min. The sections were then treated with protease K at 37˚C for 15 min, washed with 2 X SSC solution (pH 7.0) for 1 min, dehydrated and dried. A total of 8 μl hybridization buffer and 2 μl of the pre-labelled HER-2 probe were added to the microcentrifuge tube and centrifuged at 1,000 x g for 3 sec at room temperature. Subsequently, 10 μl of the probe hybridization mixture was applied, sealed and denatured at 78˚C for 3 min. The slides were then incubated in a humidified chamber protect from light at 37˚C for 16 h. The slides were then washed with 2X SSC/0.3% NP-40 at 46˚C

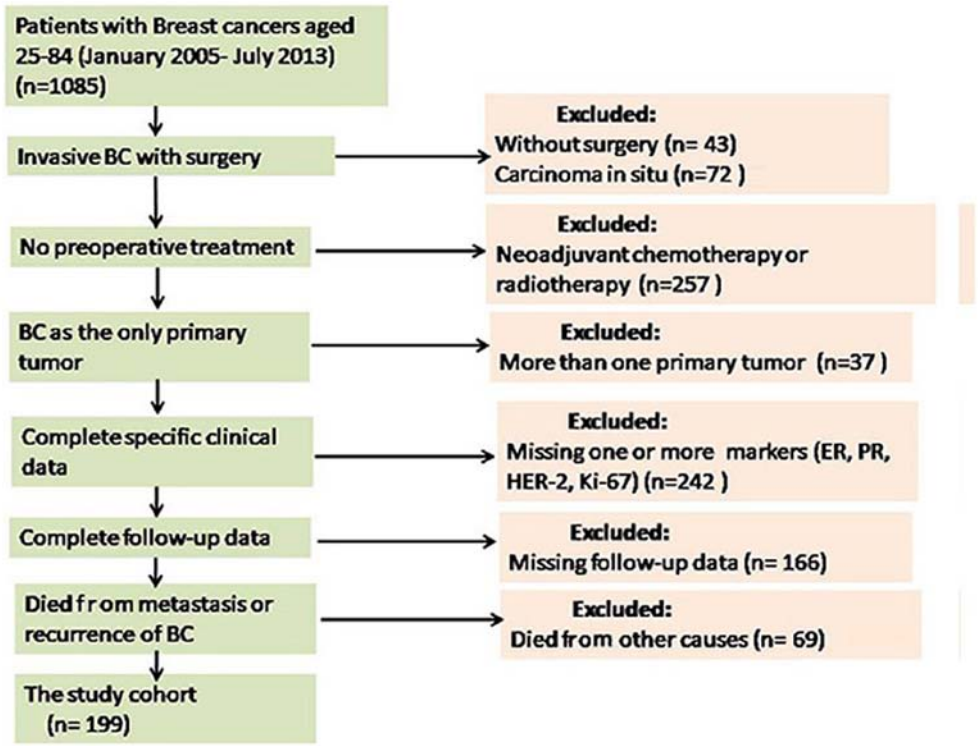

Figure 1. Flow chart of the inclusion and exclusion criteria for the patients in the present study. A total of 199 patients with breast cancer were enrolled. BC, breast cancer; PR, progestogen receptor; ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2.

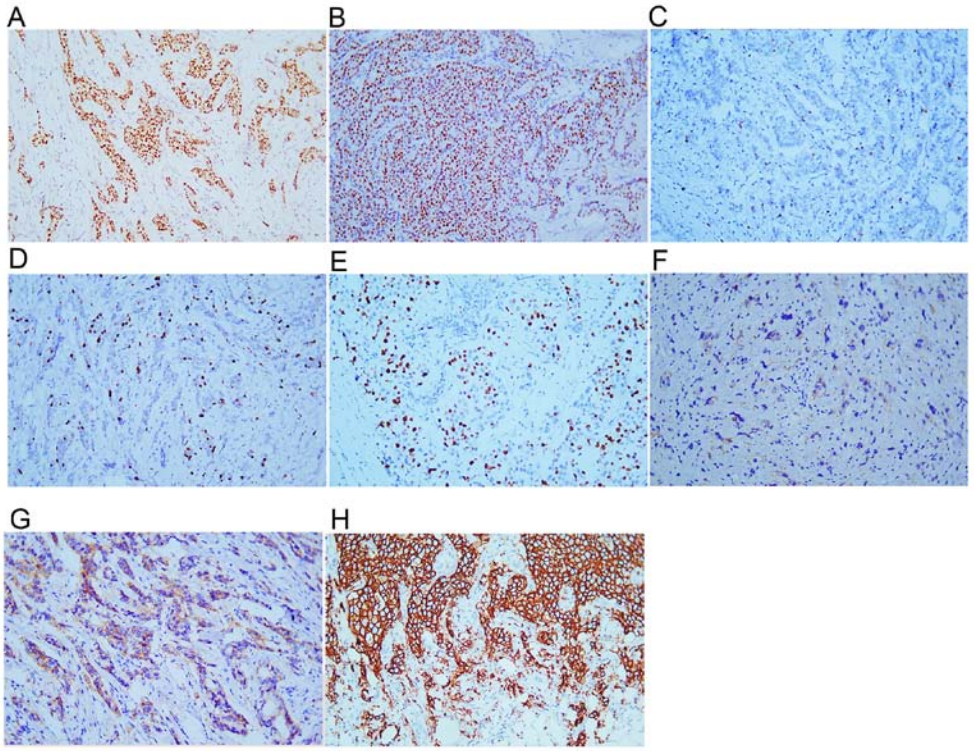

Figure 2. Immunohistochemical staining results of ER, PR, HER-2 and Ki-67. Tumor cells positive for (A) ER, (B) PR, (C) Ki-67 index <1%, (D) Ki-67 index 15%, (E) Ki-67 index >30%, (F) HER-2 (1+), (G) HER-2 (2+), (H) HER-2 (3+). Magnification, x200. PR, progestogen receptor; ER, estrogen receptor; HER-2, human epidermal growth factor receptor 2.

for 2 min, placed in gradient alcohol ethanol for 5 min, and finally air-dried. A total of 10 μl DAPI was then immediately added and the sections were observed under a fluorescence microscope (Olympus Corporation). When the experiments

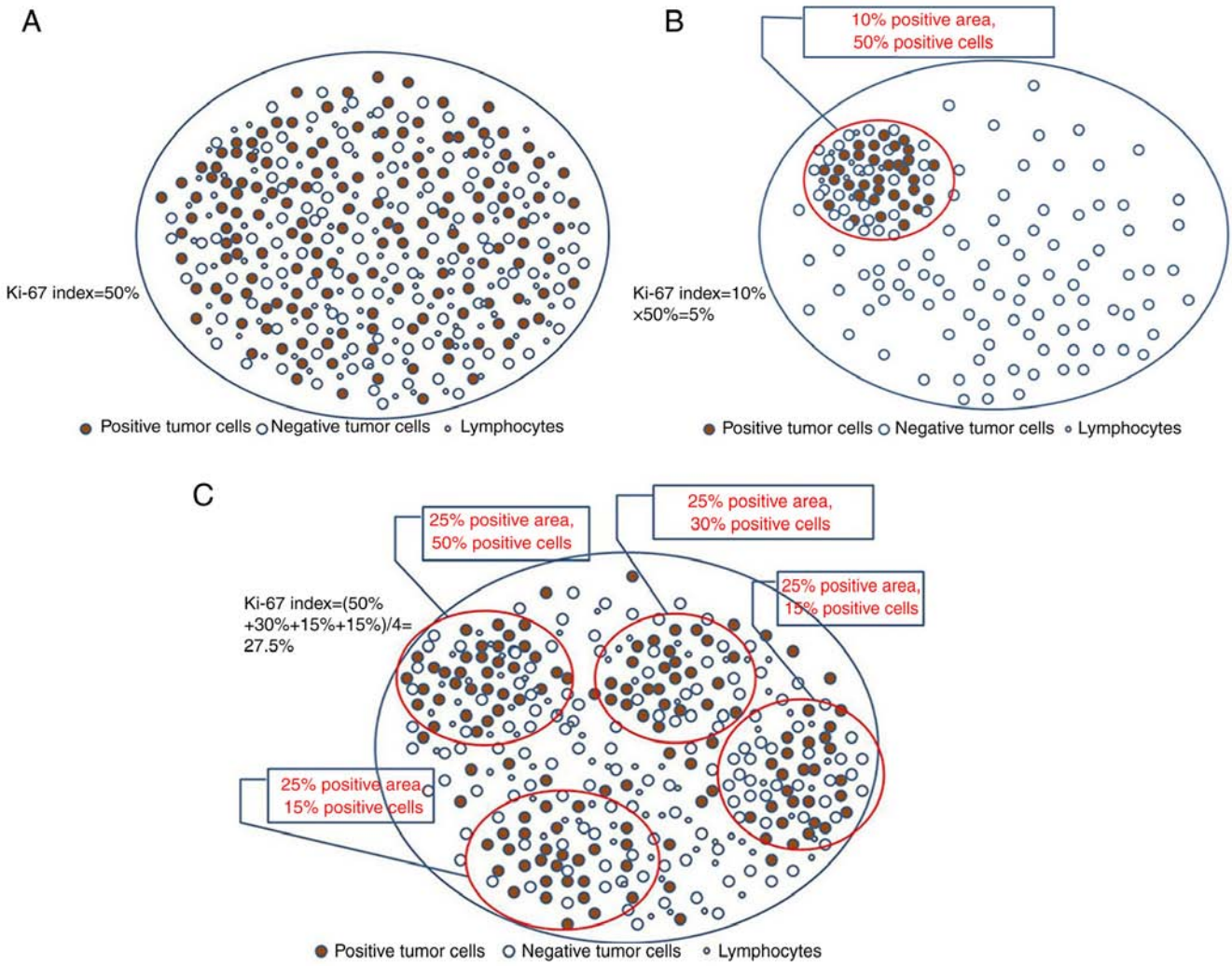

Figure 3. Schematic diagram of the calculation of the Ki-67 index. (A) The staining of Ki-67 was uniform, a total of 1,000 tumor cells were counted under a medium or low-power lens (20-40X), and the Ki-67 index was then calculated. (B) The staining of was Ki-67 non-uniform, Ki-67 index=percentage of positive tumor cell region in the whole section x percentage of positive tumor cells in the positive region. (C) The staining of Ki-67 was non-uniform, the positive tumor cells presented focal distribution on the whole section, and this section was then divided into several regions to calculate the Ki-67 index in each region, Ki-67 index=∑ (percentage of positive tumor cells 1+ percentage of positive tumor cells 2+ percentage of positive tumor cells 3…+percentage of positive tumor cells n)/∑ (1+2+3+……n) x100.

were completed, at least 20 infiltrating cancer cells in ≥2 representative areas were counted to determine the status of HER-2. The HER-2/CEP17 ratio ≥2.0 and the mean HER-2 copy number/cell ≥4.0 indicate a positive expression of HER-2. In the case of an HER-2/CEP17 ratio ≥2.0 and a mean HER-2 copy number/cell <4.0, it is recommended to increase the number of cells, and once the result remains unaltered, it is judged as negative. In the case of an HER-2/CEP17 ratio <2.0 and a mean HER-2 copy number/cell ≥6.0, it is recommended to increase the count of cells, and if the result remains unaltered, it is judged positive. In the case of an HER-2/CEP17 ratio <2.0, mean HER-2 copy number/cell ≥4.0 and <6.0, and if the result of IHC HER-2 is not 3+, the signal of another 20 tumor cells was re-counted. If the results changed, the two results were comprehensively analyzed according to the results of IHC.

Interpretation method for the Ki-67 index. The whole section was previewed under a low power lens of the microscope prior to the interpretation to assess whether the Ki-67 staining was uniform. If the staining was uniform, a total of 1,000 tumor cells were counted under a medium and high-power lens (20-40 X objective lens), and subsequently, the Ki-67 index was calculated (Fig. 3A).

If the staining was non-uniform, the following two possible outcomes were considered: The positive tumor cells only accounted for one part of the total pathological section and the Ki-67 index was obtained by multiplying the percentage of positive tumor cells in this region by the percentage of positive tumor cells in the whole pathological section, namely, Ki-67 index=percentage of positive tumor cell regions in the total pathological section x percentage of positive tumor cells in the positive region (Fig. 3B); secondly, the presence of focal distribution of the positive tumor cells on the total pathological section was considered, which was used to divide this section into several regions in order to estimate the Ki-67 index in each region. In the end, the sum of Ki-67 indices in all regions was divided by the number of regions to obtain the Ki-67 index.

The following formula was used: Ki-67 index=∑ (percentage of positive tumor cells 1+ percentage of positive tumor cells 2+ percentage of positive tumor cells 3…+percentage of positive tumor cells n)/∑(1+2+3+……n) x100 (Fig. 3C). The staining intensity could not be interpreted and tumor cells with an unclear contour were not counted.

Molecular typing. The molecular typing was implemented according to the suggestions provided in the 2019 CSCO Guidelines (low expression if the critical value of Ki-67index <15%, and high expression if it was >30%) (4). When the $ER^+/HER\text{-}2^-$ status and the Ki-67 index were within 15-30%, the molecular typing of BC was conducted according to the percentage of cells with a positive PR expression. Subsequently, the PR index >20% was considered as the cut-off value in accordance with the 2019 CSCO Guidelines. If the PR index was >20%, BC was classified as luminal type A, whereas if the PR index was <20%, the BC was classified as luminal type B.

Statistical analysis. The survival data of patients with BC were statistically analyzed using SPSS 18.0 software (SPSS, Inc.). The Chi-square (χ^2) test was used to assess the Ki-67 index, the OS and the DFS of patients with BC, as well as the association between the different molecular types. The Kaplan-Meier survival analysis method (with the log-rank test) was adopted. A value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinicopathological features of patients with BC. The median age of the 199 patients with invasive ductal carcinoma was 48 years (25-84 years old), of whom those at stage SBRI accounted for 21.2% (42/199), those at stage II for 57.8% (115/199), and those at stage III for 21.2% (42/199). The patients with lymphatic metastasis comprised 47.7% (95/199) of the total sample size and those without lymphatic metastasis accounted for 52.3% (104/199). According to the AJCC staging (6), the percentages of patients at stage I, II, III and IV were 17.1% (34/199), 58.8% (117/199), 23.6% (47/199) and 0.5% (1/199), respectively (Table I). The median follow-up time lasted 82 months (12-157 months). The statistical analysis indicated that the 5-, 6-, 7- and 10-year OS rates of the patients with BC were 18.60% (8/43), 38.24% (26/68), 60.68% (71/117) and 73.08% (133/182), respectively. The data indicated that >80% of the patients with BC succumbed to the disease within 5 years, whereas their survival rate was 100% following 10 years. The OS rates of the patients with BC in the different age range groups differed significantly ($\chi^2=211.1$, $P<0.0001$) (Fig. 4).

Associations of the Ki-67 index with the OS and DFS of patients with BC. The number of patients with a Ki-67 index ≤1% was 16 (8.0%) and that of patients with a Ki-67 index ≥90% was 9 (4.5%). The median percentage was 26.53% and the average value was 29.6±23.9%. The results of statistical analysis indicated that when the Ki-67 index was in the range of 50-54%, the OS of the patients with BC was questionable and the two groups exhibited significant differences (Table II and Fig. 5), suggesting that the Ki-67 index within the interval

Table I. The clinicopathological characteristics of 199 patients with breast cancer in the present study.

Characteristic	No. of patients	%
Sex		
Male	1	0.5
Female	198	99.5
Age, years		
≤30	1	0.5
30-60	162	81.4
>60	36	18.1
Tstage		
T1b	13	6.5
T1c	40	20.1
T2	126	63.3
T3	18	9.0
T4	2	1.0
N stage		
0	104	52.3
N1	53	26.6
N2	31	15.6
N3	11	5.5
AJCCstage		
IA	34	17.1
IIA	72	36.2
IIB	45	22.6
IIIA	35	17.6
IIIB	1	0.5
IIIC	11	5.5
IV	1	0.5
SBR grade		
I	42	21.1
II	115	57.8
III	42	21.1

AJCC, American Joint Committee on Cancer; SBR, Scarff-Bloom-Richardson.

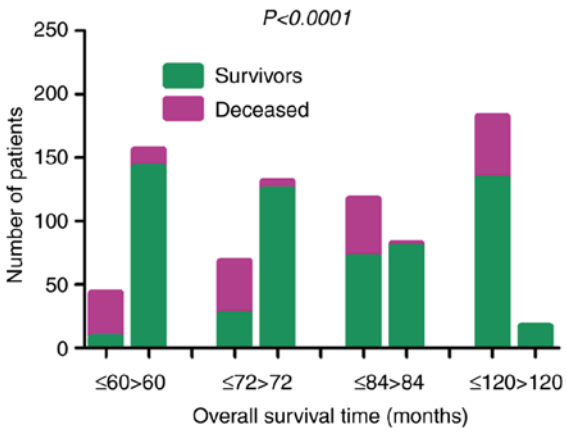

Figure 4. Survival rates of the patients in the present study. The data indicated that over 80% of the patients with BC succumbed to the disease within 5 years, whereas their survival rate was 100% following 10 years.

Table II. Association between the Ki-67 index and the overall survival of patients with breast cancer.

Ki-67 index	Patients who succumbed (n, %)	Patients who survived (n, %)	Survival rate (%)	χ^2 value	P-value
≤50%	36 (21.82)	129 (78.18)	78.18	4.83	0.028
>50%	13 (38.24)	21 (61.76)	61.76		
≤54%	37 (22.02)	131 (77.98)	77.98	4.60	0.032
>54%	12 (38.71)	19 (61.29)	61.29		

Table III. Molecular subtype of the 199 patients with breast cancer according to the 2019 CSCO guidelines.

Molecular subtype	Patients who succumbed (n, %)	Patients who survived (n, %)	Total (n)
Luminal A	9 (16.7)	45 (83.3)	54
Luminal B HER-2(-)	6 (21.4)	22 (78.6)	28
Luminal B HER-2(+)	9 (23.1)	30 (76.9)	39
Overexpression of HER-2	13 (33.3)	26 (66.7)	39
Triple-negative	12 (30.8)	27 (69.2)	39

CSCO, Chinese Society of Clinical Oncology; HER-2, human epidermal growth factor receptor 2.

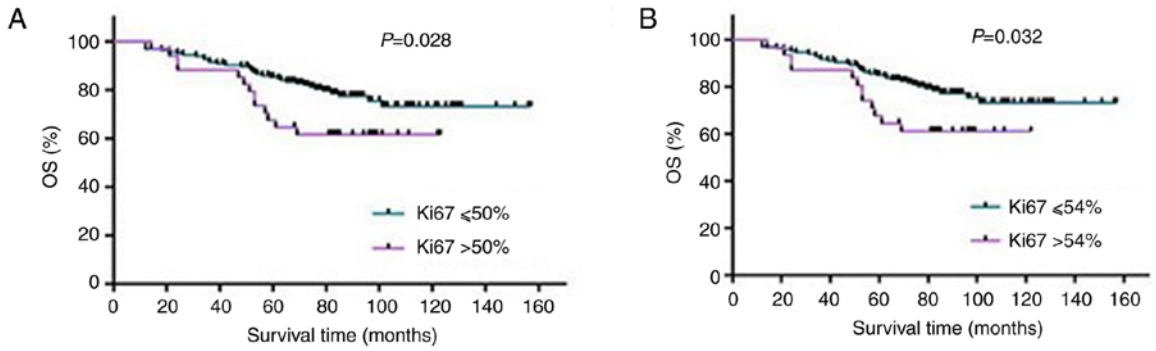

Figure 5. Kaplan-Meier curves for the OS rates of patients with breast cancer according to the different Ki-67 index. (A) Ki-67 index, 50%; (B) Ki-67 index, 54%. OS, overall survival.

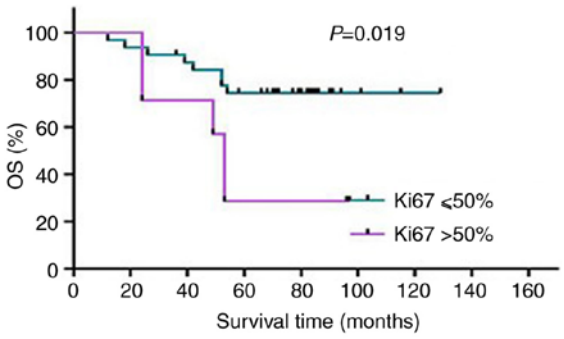

Figure 6. Kaplan-Meier curve for the OS rate of patients with BC overexpressing human epidermal growth factor receptor 2 according to a Ki-67 index of 50%. When 50% was used as the cut-off value for the Ki-67 index, the mortality rate of the patients with BC was significantly higher in the group with a Ki-67 index >50% than in the group with an index≤50%. BR, breast cancer; OS, overall survival.

of 50-54% was meaningful for the assessment of the OS of patients with BC.

Following the termination of the follow-up time period, 24 cases among the 199 patients with BC experienced single-organ and/or multi-organ metastasis (lung, brain and bone) or chest wall *in situ* recurrence; a total of 175 patients succumbed to the disease or were under a progression-free survival status after the follow-up time.

Prognosis of patients with BC with different molecular types via the Ki-67 index. The molecular subtyping of the 199 patients with BC was implemented in accordance with the 2019 CSCO standard (4). The results indicated that 54 patients were classified as luminal type A; 67 cases were classified as luminal type B, including 28 patients with luminal B HER-2 (-) and 39 with luminal B HER-2 (+) type; 39 patients were classified as the HER-2 overexpression type and 39 had triple-negative BC (TNBC) type (Table III). Of note, the patients with luminal type A BC and a Ki-67 index ≤4% survived and their follow-up time period lasted 69-156 months, suggesting that the survival time of patients with luminal type A BC and a low Ki-67 expression was long (data not shown).

Among the patients with HER-2-overexpressing BC, 50% was considered as the cut-off value of Ki-67, and their mortality rate was 71.43% (5/7) when the Ki-67 index was >50%, which was significantly different from the group with a Ki-67 index ≤50% (χ^2=5.54, P=0.019). The data indicated that this cut-off value for the Ki-67 index was significant for the prognosis of patients with HER-2-overexpressing BC; specifically, the patients with HER-2-overexpressing BC and a Ki-67 index >50% had a poor prognosis (Fig. 6).

Discussion

Ki-67, which has been widely applied in clinical pathology, is a nucleoprotein discovered and determined in Hodgkin's lymphoma in the 1980s (8). Its application to BC is based on the following two aspects: i) It is used for molecular typing and to treatment guidance; its use is particularly crucial for distinguishing luminal type A from luminal type B, since patients with luminal type A only require endocrinotherapy, while patients with luminal type B obtain an optimal clinical effect by using the systematic chemotherapy combined with endocrinotherapy (9); ii) it is applied to the prognosis of patients with BC and several studies have shown that patients with BC and a high Ki-67 index exhibit a poor prognosis and are more susceptible to recurrence (10,11). However, the results regarding the application of the Ki-67 index in BC at home and abroad are inconsistent and even mutually contradictory. It has been demonstrated that patients with BC and a high Ki-67 index will acquire improved pCRs and will not require the assessment of their hormone receptor levels (ER or PR) and HER-2 status (2). However, it has also been shown that the Ki-67 index is unrelated to the prognosis of patients with BC; therefore, the lower the index, the poorer the prognosis (12). According to the study by Kadivar and Aram (13), other clinicopathological features, such as histological grade, the presence or absence of lymphatic metastasis, the tumor volume, and vascular invasion should be combined when the Ki-67 index is used to assess the influence of clinical treatment on patients with BC. The emphases of the present study is based on the ability to effectively solve these issues, to contribute to improve the operability and accuracy of the Ki-67 index when used in BC, and accurately measure the Ki-67 index and apply it to the prognosis of patients with BC. In addition, the unspecific grouping by the 'high Ki-67 index' and 'low Ki-67 index' should be avoided as much as possible, as the distribution interval of 'high and low Ki-67 index' can be too large to operate in practice; for example, the low Ki-67 index can range from 0 to 28.6% (14,15).

Another difficulty faced by pathologists is the accurate estimation of the Ki-67 index. In order to optimize the counting method and reduce the differences among observers to the greatest extent, the international Ki-67 in the Breast Cancer Working Group has provided certain suggestions; namely, the cells should be counted at least under three high power fields (40 X objective lens) or at least 500-1,000 cancer cells should be counted, including the invasion edge and hotspot area of the tumor (16). Although this method is both time- and labor-consuming, it is still the most economical and practical method, earning considerable acceptance from pathologists (17). The Ki-67 counting by computer-aided means has also been reported (18). In the present study, the Ki-67 index was calculated using the artificial partitioning calculation method, which enabled more accurate calculations. Specifically, the Ki-67 index was calculated by two senior pathologists in each case, and the average number was finally calculated as the Ki-67 index of each patient. With simple and convenient operation, this method can not only reduce the errors among different observers, but can also consider the non-uniform staining of tumor cells.

Following the counting of the percentage of Ki-67-positive cells in the 199 BC samples and statistical analysis, an interval of the Ki-67 index was noted for the prognosis of patients with BC; when the Ki-67 index was within the range of 50-54%, it was valuable for assessing the OS of patients with BC. Previous research has indicated that when 10% is considered as the cut-off value for Ki-67, the OS and recurrence-free survival of patients with BC in the low PR expression group (<20%) are both higher than those in the high PR expression group (>20%), irrespective of the group classification [high-expression (≥10%) group or low-expression (<10%) group]; notably, the patients presenting with a high Ki-67 expression and a low PR expression exhibit the poorest prognosis (19). When 14% is considered as the cut-off value for Ki-67, the pCR rate of patients with luminal type BC, a high Ki-67 expression (≥14%), and low PR expression (<50%) following neoadjuvant chemotherapy is relatively high (20). These conclusions have indicated that the Ki-67 index is meaningful for the prognosis of patients with BC; however, the cut-off value is inconsistent, which is closely related to the grouping conditions of patients, the interpretation method of the Ki-67 index, and notably, the standardization of the Ki-67 interpretation.

In the present study, the results indicated that the patients with luminal type A BC and a low Ki-67 index (≤4%) exhibited a favorable prognosis, and all patients survived through the 13-year follow-up time period. The mean OS of the patients with HER-2-overexpressing BC was significantly different when 50% was considered as the cut-off value of Ki-67. However, Jain *et al* (21) suggested the use of 35% as the cut-off value to predict the pathological response of patients with BC to neoadjuvant chemotherapy. Moreover, it has been demonstrated that when the Ki-67 index is within 10-20%, high intergroup associations manifest among different respondents despite the presence of group differences (22). It is suggested that the clinical use of 'low Ki-67 expression' and 'high Ki-67 expression' may be more meaningful. When the hotspot areas are counted, the number of observers reported with a high Ki-67 expression is apparently higher than that of the average level. When the Ki-67 staining is weak, the observers are also prone to a high Ki-67 expression (23). Accordingly, in the study by Zhu *et al* (24), the cut-off value of Ki-67 was 30% and was used as an independent prognostic factor affecting the OS and DFS of patients with TNBC. The latter patients with Ki-67 >30% had a poor prognosis (24). Therefore, the prognosis of patients with BC could be predicted more accurately by using the different Ki-67 indices for the different molecular subtypes of BC. Concomitantly, as demonstrated in a previous study, the activity of LDH and catalase (CAT) in BC tissues aided the identification of the characteristics of cancer aggressiveness (25). In practice, every biomarker which was used to predict the prognosis of patients with BC has limitations, such as Ki-67, LDH and CAT. Thus, a few markers need to be used jointly to determine the prognosis of patients with BC objectively. For the Ki-67 index, the different cut-off values

should be used to predict the survival of patients with different molecular subtypes of BC. At the same time, this conclusion may have a limitation as the data obtained herein were drawn from patients in China; thus, further multicenter studies are warranted to draw more objective conclusions.

In conclusion, the present study provided a systematic exploration of the interpretation of the Ki-67 index based on the partitioning calculation method and assessed its clinical value for the prognosis of patients with BC. The results indicated that the Ki-67 index obtained through the partitioning calculation method was very meaningful for assessing the OS of patients with BC. Different cut-off values of Ki-67 should be adopted for providing information for patients with BC with different molecular subtypes. The cut-off value of Ki-67 should fall into a certain interval, but should not be used at a fixed point.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CW was involved in the conception and design of the study. XL and NF were involved in the development of the study methodology. NM and FL were involved in the acquisition of data. YW and YC were involved in data analysis. CW and TY were involved in data interpretation. CW, TY, YC and NF were involved in the writing and reviewing/revision of the manuscript. NF and CW supervised the study. XL, NF and YC confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the 989th Hospital of the PLA Joint Logistic Support Force database for the collection of data and for the use of personal data for research purposes, and written informed consent was obtained from the patients or their immediate family. The present study was ethically approved by the Ethics Committee of the 989th Hospital of the PLA Joint Logistic Support Force review board on May 14, 2020 (Approval no. 20200508).

Patient consent for publication

Written informed consent was obtained from all patients or their immediate family members for the publication of their data and any related images.

Competing interests

The authors declare that they have no competing interests.

References

1. Jurisic V, Radenkovic S and Konjevic G: The actual role of LDH as tumor marker, biochemical and clinical aspects. Adv Exp Med Biol 867: 115-124, 2015.
2. Chen XY, He C, Han DD, Zhou M, Wang Q, Tian J, Li L, Xu F, Zhou E and Yang K: The predictive value of Ki-67 before neoadjuvant chemotherapy for breast cancer: A systematic review and meta-analysis. Future Oncol 13: 843-857, 2017.
3. Erić I, Petek Erić A, Kristek J, Koprivčić I and Babić M: Breast cancer in young women: Pathologic and immunohistochemical features. Acta Clin Croat 57: 497-502, 2018.
4. Li JB and Jiang ZF: Update and interpretation of 2019 guideline of Chinese Society of clinical oncology (CSCO): Breast cancer. Chin J Surg Oncol 11: 155-160, 2019.
5. Shet T: Ki-67 in breast cancer: Simulacra and simulation. Indian J Cancer 57: 231-233, 2020.
6. Teichgraeber DC, Guirguis MS and Whitman GJ: Breast cancer staging: Updates in the AJCC cancer staging manual, 8th edition, and current challenges for radiologists, from the AJR special series on cancer staging. AJR Am J Roentgenol 217: 278-290, 2021.
7. Guideline Recommendations for HER2 Detection in Breast Cancer Group: Guidelines for HER2 detection in breast cancer, the 2014 version. Zhonghua Bing Li Xue Za Zhi 43: 262-267, 2014 (In Chinese).
8. Gerdes J, Schwarting R and Stein H: High proliferative activity of Reed Sternberg associated antigen Ki-1 positive cells in normal lymphoid tissue. J Clin Pathol 39: 993-997, 1986.
9. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B and Senn HJ; Panel members: Personalizing the treatment of women with early breast cancer: Highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer. Ann Oncol 24: 2206-2223, 2013.
10. Nishimura R, Osako T, Nishiyama Y, Tashima R, Nakano M, Fujisue M, Toyozumi Y and Arima N: Prognostic significance of Ki-67 index value at the primary breast tumor in recurrent breast cancer. Mol Clin Oncol 2: 1062-1068, 2014.
11. Pathmanathan N, Balleine RL, Jayasinghe UW, Bilinski KL, Provan PJ, Byth K, Bilous AM, Salisbury EL and Boyages J: The prognostic value of Ki67 in systemically untreated patients with node-negative breast cancer. J Clin Pathol 67: 222-228, 2014.
12. Kurozumi S, Matsumoto H, Hayashi Y, Tozuka K, Inoue K, Horiguchi J, Takeyoshi I, Oyama T and Kurosumi M: Power of PgR expression as a prognostic factor for ER-positive/HER2-negative breast cancer patients at intermediate risk classified by the Ki67 labeling index. BMC Cancer 17: 354, 2017.
13. Kadivar M and Aram F: Assessment of Ki67 in breast cancer: A comparison between the Eye-10 method, stepwise counting strategy, and international system of Ki67 evaluation. Iran J Pathol 15: 13-18, 2020.
14. Urruticoechea A, Smith IE and Dowsett M: Proliferation marker Ki-67 in early breast cancer. J Clin Oncol 23: 7212-7220, 2005.
15. Stuart-Harris R, Caldas C, Pinder SE and Pharoah P: Proliferation markers and survival in early breast cancer: A systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 17: 323-334, 2008.
16. Fulawka L and Halon A: Ki-67 evaluation in breast cancer: The daily diagnostic practice. Indian J Pathol Microbiol 60: 177-184, 2017.
17. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, *et al*: Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst 103: 1656-1664, 2011.
18. Ašoklis R, Kadziauskienė A, Paulavičienė R, Petroška D and Laurinavičius A: Quantitative histopathological assessment of ocular surface squamous neoplasia using digital image analysis. Oncol Lett 8: 1482-1486, 2014.
19. Kang YJ, Lee HB, Kim YG, Han J, Kim Y, Yoo TK, Lee ES, Moon HG, Noh DY and Han W: Ki-67 expression is a significant prognostic factor only when progesterone receptor expression is low in estrogen receptor-positive and HER2-negative early breast cancer. J Oncol 2019: 7386734, 2019.

20. Silva LRD, Vargas RF, Shinzato JY, Derchain SFM, Ramalho S and Zeferino LC: Association of menopausal status, expression of progesterone receptor and Ki67 to the clinical response to neoadjuvant chemotherapy in Luminal breast cancer. Rev Bras Ginecol Obstet 41: 710-717, 2019.
21. Jain P, Doval DC, Batra U, Goyal P, Bothra SJ, Agarwal C, Choudhary DK, Yadav A, Koyalla VPB, Sharma M, *et al*: Ki-67 labeling index as a predictor of response to neoadjuvant chemotherapy in breast cancer. Jpn J Clin Oncol 49: 329-338, 2019.
22. Li XR, Liu M, Zhang YJ, Wang JD, Zheng YQ, Li J, Ma B and Song X: Evaluation of ER, PgR, HER-2, Ki-67, cyclin D1, and nm23-H1 as predictors of pathological complete response to neoadjuvant chemotherapy for locally advanced breast cancer. Med Oncol 28 (Suppl 1): S31-S38, 2011.
23. Røge R, Nielsen S, Riber-Hansen R and Vyberg M: Ki-67 proliferation index in breast cancer as a function of assessment method: A NordiQC experience. Appl Immunohistochem Mol Morphol 29: 99-104, 2021.
24. Zhu X, Chen L, Huang B, Wang Y, Ji L, Wu J, Di G, Liu G, Yu K, Shao Z and Wang Z: The prognostic and predictive potential of Ki-67 in triple-negative breast cancer. Sci Rep 10: 225, 2020.
25. Radenkovic S, Milosevic Z, Konjevic G, Karadzic K, Rovcanin B, Buta M, Gopcevic K and Jurisic V: Lactate dehydrogenase, catalase, and superoxide dismutase in tumor tissue of breast cancer patients in respect to mammographic findings. Cell Biochem Biophys 66: 287-295, 2013.