The Role of Ki-67 in HR+/HER2- Breast Cancer: a Real-world Study of 956 Patients

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Research Article

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Abstract

Purpose: This retrospective study aimed to determine the most suitable cut-off value of Ki-67 expression and further explore the prognostic effect of Ki-67 in hormone receptor positive and human epidermal growth factor receptor 2 negative (HR+/HER2-) breast cancer;

Methods: We assessed the Ki-67 expression of 956 patients with HR+/HER2 breast cancer diagnosed in General Hospital of Ningxia Medical University from 2015 to 2019 by immunohistochemistry, The disease-free survival (DFS) was defined as the time from postoperative to the first local recurrence, distant metastasis or death of the disease.

Results: 22.5% was used as the cut-off for low/high Ki-67 expression in HR+/HER2- breast cancer; compared with the commonly used threshold value of Ki-67(14%) in clinic at present, the consistency of the two value is moderate (Kappa = 0.484, $P < 0.001$). The expression of Ki-67 was increased with the increase of grade. (Median: G1:10%; G2:20%; G3:40%. Mean: G1:13%; G2:23%; G3:39%, $P < 0.001$). During the follow-up that 61 patients developed recurrence or metastasis which Ki-67 low expression was found in 21 cases and high expression in 40 cases, the patients with Ki-67 $\geq 22.5\%$ had a 2.937 higher risk of early recurrence and metastasis than the patients with Ki-67 $< 22.5\%$, the DFS in patients with high Ki-67 expression was lower than the patients with low Ki-67 expression (96% VS 89%, $P < 0.05$). The common position of distant metastasis were bone, liver and lung, and rare metastases were adrenal gland, bone marrow and pericardium. Recurrence and metastasis mostly occurred in the second and third year after operation, and multi-site metastasis was more common.

Conclusions: The cut-off value of Ki-67 expression in HR+/HER2- breast cancer is 22.5%; The patients with Ki-67 $\geq 22.5\%$ had a 2.937 higher risk of early recurrence and metastasis than the patients with Ki-67 $< 22.5\%$.

Introduction

According to the Global Cancer Statistics report in 2020, there are about 2.26 million new patients with breast cancer worldwide, accounting for 11.7% of all new cancer cases, surpassing lung cancer as the most common tumor in the world for the first time^[1]. There are four molecular subtypes with different characteristics and prognosis in breast cancer, the most common type of breast cancer is HR + breast cancer in clinic, accounting for about 75%^[2]. HR+/HER2- breast cancer account for 65% of breast cancer in women under the age of 50 and 75% of breast cancer in elderly women^[3]. In HR+/HER2- breast cancer, gene expression analysis can be divided into two different subtypes-luminal A and luminal B(HER2-)^[4]. Ki-67, which used to distinguish between luminal A and luminal B (HER2-), is located on the long arm of human chromosome 10(10q25)^[5]. The positive rate of Ki-67 expression is different in different stages of the cell cycle, the expression is low in G1 and early S phase, increases gradually during mitosis, reaches the peak in M stage, decreases rapidly in late and terminal stage of mitosis, and does not express in G0 stage^[6]. In 2011, the International Breast Cancer working Group pointed out that there is still no unified

"gold standard" for the evaluation of Ki-67 proliferation index due to the lack of standardized evaluation methods of Ki-67 expression^[7]. Although the consistency of Ki-67 testing in the same laboratory may be high, but in different laboratories is still vary greatly because the consistency and repeatability of Ki-67 expression evaluation are low between observers. Different cut-off values may bring different treatment options to patients, and lack of chemotherapy or over-treatment may lead to different prognosis. Therefore, in order to guide the standardized treatment of breast cancer patients in our hospital, clinicians must understand the distribution characteristics of Ki-67 and determine the most suitable cut-off value of Ki-67 for HR+/HER2- breast cancer in our own laboratory.

Methods

Patients: The study, of 956 patients with HR+/HER2- breast cancer diagnosed by operation and pathology, from 25 to 80 years old between 2015 to 2019 in General Hospital of Ningxia Medical University, all patients are female. We collect and organize the following parameters: age, immunohistochemical, tumor stage (according to the TNM stage of AJCC 8th edition), histological grade, lymph node metastasis, vascular/nerve invasion, transfer time, etc. Patients with stage IV, neoadjuvant therapy, triple-negative, or HER2-positive breast carcinoma were excluded from the study.

Ki-67 evaluation: The Ki-67 index was detected by immunohistochemical in all patients, which was evaluated and determined by two experienced pathologists. The yellow deposition in the nucleus is Ki-67 positive cells, and the expression rate of Ki-67 is the percentage of Ki-67 positive staining cells in the total number of tumor cells. In the aspect of regional selection, for tumor cells with uniform distribution of positive cells, it is only necessary to randomly select 3 or more invasive cancer high-power visual field counts to obtain the average Ki-67 index; for tumor cells with uneven distribution of positive cells, it is recommended to evaluate 3 or more invasive cancer high-power visual fields in hot spots of positive cells.

Immunohistochemical standard: The expression of estrogen receptor (ER), progesterone receptor (PR), HER2 in all HR+/HER2- breast cancers were detected by immunohistochemical. The semi-quantitative method was used to judge, 10 high power visual fields were selected for each section, and 100 tumor cells were counted in each visual field, scored according to the staining intensity and the percentage of positive cells. The positive of ER and PR is defined as the staining of tumor cells $\geq 1\%$. The expression level of HER2 was divided into 0 ~ 3+, the cases with HER2 expression of 2 + were further detected by fluorescence in situ hybridization (FISH). HER2 negative was defined as (0), (1 +), or (2 +) negative by FISH test, HER2 positive was defined as (3 +), or (2+) positive by FISH test.

Follow-up: The follow-up by means of inpatient or outpatient medical records or telephone. The starting time of follow-up is the date of operation, and the deadline is December 31, 2021. The end point is the deadline for disease progression, death, or loss of follow-up. DFS is defined as the time from postoperative to the first local recurrence, distant metastasis or death of breast cancer. Recurrent and metastatic lesions need to be confirmed by pathological puncture, if the lesions cannot be punctured it must be diagnosed after multidisciplinary discussion according to imaging examination.

Statistical analysis: The data were analyzed by SPSS 25.0 and GraphPad Prism 8.0.1. The receiver operating characteristic curve (ROC) was used to determine the cut-off value of Ki-67 expression, The relationship between Ki-67 expression and pathological were analyzed by χ^2 test, Kaplan-Meier method was used to draw the survival curve, significant level was set as 0.05.

Result

1. The expression range of Ki-67 in HR+/HER2- breast cancer of 956 patients is 1%-90%, the most common range is 10%-30%, the median of Ki-67 expression is 20%, and the average is 25%. Figure 1 and Fig. 2
2. With the statistical analysis of ROC curve, the cut-off value of Ki-67 expression is 22.5%. All patients were divided into two groups according to 22.5%, 585 cases (61.2%) $Ki-67 < 22.5\%$, 371 cases (38.8%) $Ki-67 \geq 22.5\%$. Compared with the commonly used threshold value of Ki-67(14%) in clinic at present, the consistency of the two value is moderate (Kappa = 0.484, $P < 0.001$). Figure 3
3. It was found that histological grade, PR expression, T stage, N stage and vascular nerve invasion were all correlated with the expression of Ki-67. Table 1 and Fig. 4

Table 1
Correlation of Ki-67 with pathological for the different cut-off values

Pathological	Ki-67		χ^2	<i>P</i>	Ki-67		χ^2	<i>P</i>
	< 14%	≥ 14%			< 22.5%	≥ 22.5%		
Age(years)			1.458	0.227			8.508	0.004
≤ 40	40	98			69	69		
> 40	280	538			516	302		
Grade			119.975	< 0.001			6.019	0.049
G1	101	54			82	73		
G2	196	390			363	223		
G3	23	192			140	75		
PR*expression			10.046	0.002			27.05	< 0.001
≤ 20%	56	170			105	121		
> 20%	264	466			480	250		
T stage			6.468	0.039			7.811	0.020
T1	201	336			352	185		
T2	119	282			233	168		
T3	5	13			8	10		
N stage			4.411	0.036			6.889	0.009
N0	179	310			319	170		
N+	141	326			266	201		
Vehicle			5.969	0.015			5.704	0.017
Yes	24	81			53	52		
No	296	555			532	319		
*20% is used as the cut-off value of PR expression [8].								

4. Survival analysis was based on all patients for a median of 37 months (4-77months), during the follow-up that 61 patients developed recurrence or metastasis which Ki-67 low expression was found in 21 cases and high expression in 40 cases, the patients with Ki-67 ≥ 22.5% had a 2.937 higher risk of early recurrence and metastasis than the patients with Ki-67 < 22.5%, the DFS in patients with high Ki-67

expression was lower than the patients with low Ki-67 expression (96% VS 89%, $P < 0.05$). The common position of distant metastasis were bone, liver and lung, and rare metastases were adrenal gland, bone marrow and pericardium. Recurrence and metastasis mostly occurred in the second and third year after operation, and multi-site metastasis was more common. Figure 5 and Fig. 6

Discussion

Ki-67 index is a common parameter for molecular type of HR+/HER2- breast cancer by immunohistochemistry instead of gene detection. In order to determine the threshold value of Ki-67 in HR+/HER2- breast cancer, we can detect multiple genes in all samples, and then compare the results of gene detection with immunohistochemical to get the best threshold value, but this method is expensive and difficult in clinical. The cut-off value that distinguishes the low/high Ki-67 expression in HR+/HER2- breast cancer is constantly change. The consensus of experts at the 2009 St Gallen International Breast Cancer Conference recommended that Ki-67 should be used as a proliferative marker for the selection of appropriate systemic treatment, classified according to three levels: low level ($\leq 15\%$), medium level (16–30%) or high level ($> 30\%$)^[9]. Through the study of PAM50 gene expression profile and comparison of immunohistochemical results, Cheang found that the best cut-off value to distinguish Luminal A and Luminal B (HER2-) breast cancer was 13.25%^[10]. Based on this result, the 2011 St Gallen meeting determined the cut-off value of Ki-67 to distinguish these two subtypes at 14%^[11]. The 2013 St Gallen meeting raised the cut-off value to 20%^[12]. At 2015, the expert group of the St Gallen meeting suggested that Ki-67 should be explained according to the values of the local laboratory^[13]. At the latest St Gallen meeting in 2021, 42.4% of the experts suggested the threshold for Ki-67 to recommend adjuvant chemotherapy in ER+, HER2-, node-negative breast cancer is 30%^[14]. Many studies have taken different threshold to explore the effect of Ki-67 expression on the prognosis, which including 14%^[15], 15%^[16], 22%^[17], 25%^[18]. Some studies have pointed out that Ki-67 as a prognostic marker of intracavitary breast cancer, 20% is more reliable than 14%^[19,20]. Meta analysis shows that the threshold value of Ki-67 between 5% and 30%^[21]. The guidelines and norms for breast cancer diagnosis and treatment of the Chinese Anti-Cancer Association point out that the value of Ki-67 proliferation index may be different in different pathological centers, 20%-30% can be used as the cut-off value of Ki-67^[22]. In previous studies, we evaluated the distribution of Ki-67 expression with HR+/HER2- breast cancer in the pathology laboratory of our hospital is high consistent and reliable in recent 5 years. Based on this, we further determined the cut-off value of Ki-67 expression is 22.5% through the analysis of ROC in this study, that means the patients with $Ki-67 \geq 22.5\%$ have a poor prognosis and are more likely to have early recurrence and metastasis. So we should use the Ki-67 threshold in our own laboratory instead of continuing to use 14%, which allows some patients to avoid chemotherapy.

HR+/HER2- breast cancer is a heterogeneous tumor, they are differences in PR expression, histological grade, Ki-67 proliferation and so on, and these characteristics are highly correlated^[23]. Studies has shown that the expression of Ki-67 is related to the clinicopathological features of breast cancer, such as tumor

size, histological grade, PR status, vascular nerve invasion and so on^[19, 24]. In this study, we found that histological grade, PR expression, T stage and vascular/nerve invasion are all related to Ki-67 expression. The consensus of experts at the 2017 St Gallen conference pointed out for the first time that histological grade can be used to distinguish the molecular type of HR+/HER2- breast cancer^[25]. Histological grade is mainly evaluated from three aspects: the degree of glandular duct formation, nuclear pleomorphism and mitosis count. The expression of Ki-67 increases gradually during mitosis, so the tumor grade may be indirectly related to the expression of Ki-67 according to mitotic cell count. Liang Qin found that Ki-67 is related to tumor grade, and can predict histological grade to some extent^[26]. Mohammed found that the expression level of Ki-67 was positively correlated with histological grade, the higher tumor grade, the higher Ki-67 expression, Ki-67 can predict tumor invasiveness and higher histological grade^[25]. Professor Fu Li pointed out that the positive rate of Ki-67 in ER + breast cancer varies widely, the expression of Ki-67 in high differentiated and G1 can be less than 5% but more than 20% in high histological grade breast cancer^[27]. It has been reported that luminal type breast cancer is at opposite ends in histological grade, G1 conforms to luminal A, G3 conforms to luminal B type, and G2 is between them^[23]. In this study, we analyzed the relationship between Ki-67 expression and histological grade and the results showed that the expression of Ki-67 is closely related to histological grade, the expression of Ki-67 was increased with the increase of grade. Both histological grade and Ki-67 value can distinguish the molecular type of luminal breast cancer, but the study found that the consistency between them is low, the consistency can be improved when these two factors are combined^[28]. It is reported that the risk of recurrence of HR+/HER2- breast cancer has remained relatively stable for many years, at least half of the patients with recurrence and metastasis occurred 5 years after diagnosis, and even some patients developed recurrence and metastasis for more than 10 years^[23]. In order to improve the survival rate of patients, clinicians need to identify high-risk patients with early recurrence and metastasis and strengthen treatment and follow-up. Our study found that the patients with $Ki-67 \geq 22.5\%$ had a 2.937 higher risk of early recurrence and metastasis than the patients with $Ki-67 < 22.5\%$. Studies have found that the common sites of recurrence and metastasis of HR+/HER2- breast cancer include bone, lymph nodes, pleura or lung, liver and skin^[23]. We found the most common metastatic site of HR+/HER2- breast cancer is bone (vertebra is the most common), followed by viscera (liver, lung), regional lymph nodes, local chest wall skin, and multi-site metastasis is more common, which is basically consistent with the literature report. In this study, we also found that three patients had rare metastases of adrenal gland, bone marrow and pericardium, which occurred at the same time or successively with the common metastatic sites mentioned above.

Conclusion

We suggest the cut-off value of Ki-67 expression in the pathological center of our hospital should be raised to 22.5%. The patients with $Ki-67 \geq 22.5\%$ are more likely to have early recurrence and metastasis, so postoperative treatment and follow-up should be strengthened for patients with high expression of Ki-67.

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Figures

Figure 1

The different expression of Ki-67 in breast cancer (SP x400)

A: Ki-67 expression is 5% B: Ki-67 expression is 20%

C: Ki-67 expression is 30% D: Ki-67 expression is 60%

Figure 2

The different levels of Ki-67 in 956 cases of tumor tissues.

Figure 3

The ROC curve of Ki-67 expression (Area under ROC curve: 0.689; Sensitivity: 63.02%; Specificity: 65.57%; Youden Index: 0.286; 95% CI: [0.626-0.754]; $P < 0.001$)

Figure 4

The expression of Ki-67 was increased with the increase of histological grade. (Median: G1:10%; G2:20%; G3:40%. Mean: G1:13%; G2:23%; G3:39%, $P < 0.001$)

Figure 5

Kaplan-Meier survival curve with the expression of Ki-67

Figure 6

The distribution of common metastatic sites in HR+/HER2- breast cancer (adrenal gland, bone marrow and pericardium combined with multiple site metastasis)