Int J Clin Exp Med 2019;12(5):6044-6048
www.ijcem.com /ISSN:1940-5901/IJCEM0078580

Original Article

Relationship between Ki67 and the efficacy of neoadjuvant chemotherapy: clinicopathological characteristics of luminal B breast cancer

Jiayu Wang[1*], Die Sang[2*], Binghe Xu[1], Peng Yuan[1], Fei Ma[1], Yang Luo[1], Qing Li[1], Pin Zhang[1], Ruigang Cai[1], Ying Fan[1], Shanshan Chen[1], Qiao Li[1]

[1]*Department of Medical Oncology, National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 17, Panjiayuannanli, Chaoyang District, Beijing 100021, China;* [2]*Department of Medical Oncology, San Huan Cancer Hospital, No. 352, Shilihe, Chaoyang District, Beijing 100122, China.* [*]*Equal contributors.*

Received April 26, 2018; Accepted January 10, 2019; Epub May 15, 2019; Published May 30, 2019

Abstract: Objective: To determine the correlation between Ki67 expression and the efficacy of neoadjuvant chemotherapy, and to assess the clinicopathological characteristics of luminal B breast cancer. Methods: The expression of Ki-67, ER, PR and HER2 in breast cancer tissue samples from 163 patients with NCT was detected using immunohistochemical techniques. The relationship between Ki67 and the clinicopathological characteristics of patients was determined using Spearman rank correlation analysis. Results: Based on the principle of maximizing the sum of sensitivity and specificity, 30% was calculated to be the optimum critical value of Ki67 as an indicator for PCR prediction and the area under the ROC curve was 0.729 (P = 0.000). Two cohorts of patient samples were divided in this trial according to the expression levels of Ki-67 (99 cases with low expression, Ki-67 ≤ 30%) and 64 cases with high expression, Ki-67 > 30%). The results demonstrated that the cohort of the high Ki67 expression group exhibited a greater PCR percentage than the low expression cohort after neoadjuvant chemotherapy (26.6%, 8.1%, P = 0.001). Moreover, the PCR rate of 137 (84.0%) of the patients with a Ki67 decrease trended apparently better than the non-decreasing group (17.5%, 3.8%, P = 0.076). The results also suggested that the expression of Ki67 was positively correlated with the PCR rate (r = 0.25, P = 0.001), breast tumor diameter (r = 0.158, P = 0.044), menstrual status (r = 0.187, P = 0.017), and was negatively correlated with ER (r = -0.242, P = 0.002) and age (r = -0.254, P = 0.001). Conclusions: The high expression of Ki67 responded well to neoadjuvant chemotherapy and the optimum critical value of Ki67 to predict PCR was 30%. There was a definite relationship between Ki67 levels and clinical pathological indicators, which pointed to the benefits of NCT.

Keywords: Luminal B breast cancer, Ki-67, NCT, ROC curve, clinicopathological characteristics, prediction

Introduction

The immunophenotype of luminal B breast cancer is characterized by ER (+) and (or) PR (+), HER2 (+) or (-) and Ki-67 > 14%. Prior to 2011, luminal A was reported to be the main molecular type of breast cancer [1, 2], while luminal B occupied a tiny proportion without taking Ki67 into consideration. The St Gallen international consensus meeting in 2011 [3] concluded that Ki67 should be considered to be one of the interpretation standards for breast cancer molecular types, particularly for the luminal B type. It goes without saying that great significance is attached to the therapy and study of the clinical characteristics of luminal B breast cancer.

Materials and methods

General information

This study was a retrospective observational study consisting of 163 luminal B breast cancer patients who received NCT in our hospital from January 2011 to October 2014. Eligibility criteria included clinical stage II or III based on the AJCC staging system (Version 7), confirmed pathological and immunohistochemistry characteristics such as ER, PR, HER2 and Ki67 determined by coarse needle biopsy from breast tissues before chemotherapy. In the cohort of patients, there were 156 invasive ductal carcinomas, 5 invasive lobular carcinomas and 2

mucinous adenocarcinomas. The median age of the patients was 48 years (range 23-71 years). Of these, 160 (98.2%) had an ECOG performance status of zero, and 3 (1.8%) had a PS of one. Patient consent was not required, for there was no direct intervention of treatment or care. This study was approved by the ethics committee of the Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (approval no. NCC2014ST-01).

Definition of interpretation standards and the main index for efficacy

The breast biopsy specimens were examined by pathological staff after automatic immunohistochemistry analysis, and then the specimens were embedded in paraffin and sectioned into 4 μm slices. ER and PR positivity was defined as ≥ to 1% positive immunohistochemical nuclear staining of tumor cells on a scale of 0-3+, while ER and PR negativity was defined as < 1% negative staining. HER2 immunohistochemistry and FISH[4] IHC+++ was interpreted to be positive, and FISH was required for IHC++. A gene copy number amplification > 2.2 was interpreted to be positive and IHC+ was interpreted to be negative. The interpretation of Ki-67 [4] was that the positive cells nuclei were stained from light yellow to brownish yellow. Ten visual fields were randomly chosen under light microscopy at a magnification of ×400. In each field of view, 500 cells were counted, and the proportion of Ki67 positive cells *vs* the total cell count was calculated. Ki67 was adopted for the interpretation of breast cancer molecular type at the St Gallen international meeting in 2011; a low expression of Ki67 is ≤ 14%, while a high expression is > 14% [3].

PCR was defined as no invasive or non-invasive tumor residuals and a complete disappearance of axillary lymph node metastasis after NCT. Based on the principle of maximizing the sum of sensitivity and specificity, the predicted optimum critical value of PCR was 30%. A low expression referred to a Ki67 ≤ 30%, while a high expression was > 30%.

NCT management and surgical treatment

A total of 133 patients were treated with intravenous paclitaxel 175 mg/m^2 + epirubicin 75 mg/m^2 on d1 and d2 and 12 patients were treated with intravenous paclitaxel 175 mg/m^2, d1 + carboplatin AUC = 4-5, d2. There were 57 cases of HER2+++ and 18 cases were treated with a TCH regimen. Trastuzumab was first administered at a dose of 8 mg/kg, d1 (and at 6 mg/kg for subsequent chemotherapy cycles) + intravenous paclitaxel, 175 mg/m^2, d2 + carboplatin, AUC = 4-5, d3, every 21 d. Surgery was performed after 2-6 cycles of neoadjuvant chemotherapy, and the median chemotherapy cycle number was 4. All patients had surgical treatment within 1 month after chemotherapy ceased; 144 patients underwent a modified radical mastectomy and 19 breast conserving surgery, with 6 cycles of chemotherapy being administered before and after surgery.

Statistical analysis

SPSS Statistics for Windows (Version 19.0. Armonk, NY: IBM Corp.) was used for data analysis. ROC curves that predicted PCR were plotted according to the Ki67 expression levels in 163 patients. The cut-off value of the Ki67 rate to predict PCR was calculated based on maximization of the sum of sensitivity and specificity. Spearman analysis was employed to determine whether there was a correlation between Ki67 and the clinicopathological characteristics. A value of r > 0 indicates a positive correlation and a coherent direction of change between the independent and dependent variables. Alternatively, r < 0 indicates a negative correlation and a reverse direction of change between the independent and dependent variables. The relationship between the Ki67 expression level and the efficacy of NCT were analyzed using a chi-square test. A *P*-value < 0.05 was considered to be statistically significant.

Results

The optimum critical value of Ki67 for PCR prediction was 30%, based on the principle of maximizing the sum of sensitivity and specificity, the area under the ROC curve was 0.729, the standard error of the area was 0.0054, and was statistically significant for Ki67 to predict PCR (*P* = 0.000). A higher expression of Ki67 predicted a greater possibility to achieve PCR (**Figure 1** and **Table 1**).

The relationship between the Ki67 expression level and the efficacy of NCT in breast cancer

In this study, different expression levels of Ki67 were positively correlated with PCR (r = 0.25, *P*

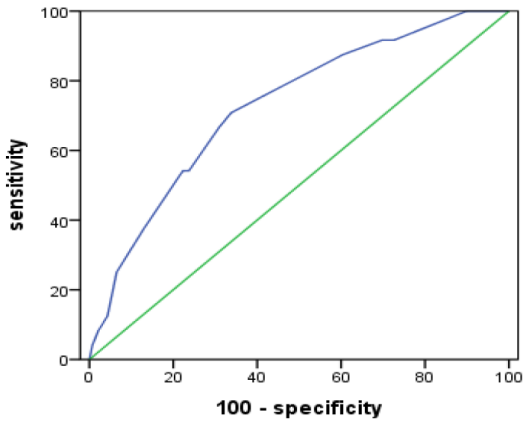

Figure 1. ROC curve of Ki67 for PCR prediction.

= 0.001). The results indicated that efficacy in the high expression group was better than in the low expression level group (26.6%, 8.1%) as shown in **Table 2**.

The relationship between Ki67 expression levels and the clinicopathological characteristics

The Ki67 expression level was negatively correlated with ER (r = -0.242, *P* = 0.002) and age (r = 0.254, *P* = 0.001) but positively correlated with breast tumors (r = 0.158, *P* = 0.44) and menstrual status (r = 0.187, *P* = 0.017) in our study. No significant correlation with PR, HER2 or the axillary lymph state was detected (*P* > 0.05, **Table 2**).

Discussion

NCT is one of the standard therapies used to treat locally advanced breast cancer, and PCR can significantly improve the disease-free survival time [5]. It has been reported that there is a higher long-term survival rate for PCR patients than non-PCR patients [6], and that PCR can be used as a substitute indicator of clinical benefit. The PCR rate after NCT for luminal type breast cancer ranged from 6-12% [7]. A combination of anthracycline and paclitaxel can increase the rate of PCR, and chemotherapy combined with anti-HER2 targeting drugs can further improve the prognosis of luminal B breast cancer with an overexpression of HER2. In the present study, 25 patients (15.3%) achieved PCR values that were higher than those previously reported, probably because Herceptin therapy improved the treatment efficacy of luminal B patients with an overexpression of HER2.

Ki67 has been reported to be closely associated with recurrence and metastasis [8-11], as a proliferating cell nuclear antigen, and as a reliable indicator for the prognosis of malignancy. A higher proliferation activity means a worse prognosis and stronger sensitivity to chemotherapy. The Ki67 rate of all patients was > 14% in the current study. According to the 30% Ki67 critical value, the PCR rates were 26.6% and 8.1% in the high and low expression groups, respectively. The difference was significant suggesting that a higher expression level of Ki67 could predict greater sensibility to chemotherapy, a finding consistent with previously reported studies [12, 13].

Our research has demonstrated that Ki67 expression was negatively correlated with ER and age, and that breast cancer with negative hormone receptors has a stronger proliferation ability. Yoshioka et al. reported [14] that patients with negative hormone receptors and a high expression level of Ki67 were more sensitive to chemotherapy. This research also found a strong proliferation of Ki67 in young patients. Ki67 expression was positively correlated with breast tumor, and a higher Ki-67 expression level correlated to a greater tumor diameter. It is noteworthy that the expression level of Ki67 was positively related with the menstrual status of patients; there was a greater expression of Ki67 and stronger cancer proliferation activity in premenopausal patients than in postmenopausal patients [15]. No significant correlation between Ki67 and HER2 was found in our research, which is inconsistent with the positive correlation previously reported [16], possibly because no comparison was made between molecular subtypes. The Ki67 expression level decreased in 84.0% (137/164) of cases, but in 16.0% (26) it did not change after neoadjuvant chemotherapy. The higher PCR rate in the patient group in which Ki67 expression decreased suggested a correlation of Ki67 with the treatment efficacy.

Conclusions

The Ki67 expression level is a significant indicator for NCT therapeutic efficacy, with the optimum critical value being 30%. Patients with a high expression of Ki67 had a better short-term response to chemotherapy. A correlation between Ki67 levels and some clinicopathological characteristics was found, which can be used as an indicator to predict the likely efficacy of chemotherapy.

Table 1. Area under the ROC curve, standard error, *P*-value, sensitivity, specificity and the 95% CI of Ki67 for PCR prediction

Test variable	AUC	Cut-off value	SE	*P*-value	95% CI		Sensitivity	Specificity
					Lower	Upper		
Ki67 rate	0.729	30%	0.054	0.000	0.623	0.834	70.8%	66.2%

AUC: Area under the curve.

Table 2. Correlation between Ki67 expression, NCT efficacy and clinicopathological characteristics

Clinicopathological characteristics	Samples	Ki67 expression		Correlation coefficient	*P*-value
		Low expression ≤ 30% (n = 99)	High expression > 30% (n = 64)		
ER					
Negative	40	16 (16.2)	24 (37.5)	-0.242	0.002
Positive	123	83 (83.8)	40 (62.5)		
PR					
Negative	30	15 (15.2)	15 (23.4)	-0.104	0.185
Positive	133	84 (84.8)	49 (76.6)		
HER2					
Negative	106	62 (62.6)	44 (68.8)	-0.063	0.426
Positive (overexpression)	57	37 (37.4)	20 (32.1)		
Tumor diameter					
> 5 cm	33	15 (15.2)	18 (28.1)	0.158	0.044
≤ 5 cm	130	84 (84.8)	46 (71.9)		
Age					
> 40	126	85 (85.9)	41 (64.1)	-0.254	0.001
≤ 40	37	14 (14.1)	23 (35.9)		
Menstrual status					
Premenopausal	104	56 (56.6)	48 (75.0)	0.187	0.017
Postmenopausal	59	43 (43.4)	16 (25.0)		
Axillary lymph node metastasis					
Yes	143	86 (86.9)	57 (89.1)	0.033	0.679
No	20	13 (13.1)	7 (10.9)		
Efficacy					
PCR	25	8 (8.1)	17 (26.6)	0.25	0.001
Non-PCR	138	91 (91.9)	47 (73.4)		

Acknowledgements

We thank the patients who participated in this study and their families. We are grateful to the doctors in the Department of Pathology for their technical assistance.

Disclosure of conflict of interest

None.

Address correspondence to: Binghe Xu, Department of Medical Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences & Peking Union Medical College, No. 17, Panjiayuannanli, Chaoyang District, Beijing 100021, China. Tel: +86-10-87788826; Fax: +86-10-87715711; E-mail: xubingheBM@163.com

References

[1] Lian ZQ, He JH, Wang X and Tang J. Clinical features and survival analysis of molecular subtyping of breast cancer. Chin J Breast Dis 2009.

[2] Bo Z and Fei X. Molecular subtypes as predictive factors for response to neoadjuvant chemotherapy in breast cancer patients. Chin J Clin Oncol 2009.

[3] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ and Panel M. Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the St. Gallen inter-

national expert consensus on the primary therapy of early breast cancer 2011. Ann Oncol 2011; 22: 1736-1747.
[4] Bukholm IR, Bukholm G, Holm R and Nesland JM. Association between histology grade, expression of HsMCM2, and cyclin A in human invasive breast carcinomas. J Clin Pathol 2003; 56: 368-373.
[5] Cao DX, He JR, Shen KW, Ii YF, Chen WG, Zhu L and Han BS. Analysis of the value of hormone receptor, HER-2 and Ki-67 in predicting the efficacy of neoadjuvant chemotherapy in breast cancer. Chin J Cancer Prev Treat 2012; 19: 1000-1003.
[6] Montagna E, Bagnardi V, Rotmensz N, Viale G, Pruneri G, Veronesi P, Cancello G, Balduzzi A, Dellapasqua S, Cardillo A, Luini A, Zurrida S, Gentilini O, Mastropasqua MG, Bottiglieri L, Iorfida M, Goldhirsch A and Colleoni M. Pathological complete response after preoperative systemic therapy and outcome: relevance of clinical and biologic baseline features. Breast Cancer Res Treat 2010; 124: 689-699.
[7] Kong X, Moran MS, Zhang N, Haffty B and Yang Q. Meta-analysis confirms achieving pathological complete response after neoadjuvant chemotherapy predicts favourable prognosis for breast cancer patients. Eur J Cancer 2011; 47: 2084-2090.
[8] Williams DJ, Cohen C, Darrow M, Page AJ, Chastain B and Adams AL. Proliferation (Ki-67 and phosphohistone H3) and oncotype DX recurrence score in estrogen receptor-positive breast cancer. Appl Immunohistochem Mol Morphol 2011; 19: 431-436.
[9] Singhai R, Patil VW and Patil AV. Status of HER-2/neu receptors and Ki-67 in breast cancer of Indian women. Int J Appl Basic Med Res 2011; 1: 15-19.
[10] Li XR, Liu M, Zhang YJ, Wang JD, Zheng YQ, Li J, Ma B and Song X. ER, PgR, HER-2, Ki-67, topoisomerase IIalpha, and nm23-H1 proteins expression as predictors of pathological complete response to neoadjuvant chemotherapy for locally advanced breast cancer. Med Oncol 2011; 28 Suppl 1: S48-54.
[11] Ohno S, Chow L, Sato N, Masuda N, Sasano H, Takahashi F, Bando H, Iwata H, Morimoto T, Kamigaki S, Nakayama T, Nakamura S, Kuroi K, Aogi K, Kashiwaba M, Yamashita H, Hisamatsu K, Ito Y, Yamamoto Y and Toi M. Randomized trial of preoperative docetaxel with or without capecitabine after 4 cycles of 5-fluorouracil-epirubicin-cyclophosphamide (FEC) in early-stage breast cancer: exploratory analyses identify Ki67 as a predictive biomarker for response to neoadjuvant chemotherapy. Breast Cancer Res Treat 2013; 142: 69-80.
[12] Ács B, Zámbó V, Vízkeleti L, Szász AM, Madaras L, Szentmártoni G, Tőkés T, Molnár BÁ, Molnár IA, Vári-Kakas S, Kulka J and Tőkés AM. Ki-67 as a controversial predictive and prognostic marker in breast cancer patients treated with neoadjuvant chemotherapy. Diagn Pathol 2017; 12: 20.
[13] Fasching PA, Heusinger K, Haeberle L, Niklos M, Hein A, Bayer CM, Rauh C, Schulz-Wendtland R, Bani MR, Schrauder M, Kahmann L, Lux MP, Strehl JD, Hartmann A, Dimmler A, Beckmann MW and Wachter DL. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer 2011; 11: 486.
[14] Yoshioka T, Hosoda M, Yamamoto M, Taguchi K, Hatanaka KC, Takakuwa E, Hatanaka Y, Matsuno Y and Yamashita H. Prognostic significance of pathologic complete response and Ki67 expression after neoadjuvant chemotherapy in breast cancer. Breast Cancer 2015; 22: 185-191.
[15] Li J, Wu JH and Shi GQ. Different level expression of Ki-67 and its correlative study in breast cancer. Chin J Modern Med 2014; 24: 50-54.
[16] Jia M. The expression and progrosis analysis of cell proliferation marker Ki-67 in breast cancer. Hebei Med Univ 2011; 3: 13-15.