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Role of KI67 in response rate of neoadjuvant chemotherapy in hormonal positive breast cancer

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***Abstract*---**Introduction: A pathological complete response (pCR) after neo-adjuvant chemotherapy indicates that the outlook for individuals with breast cancer is encouraging. identifying factors, for instance the growth biomarker Ki67, that could forecast pCR, can be useful for improving understanding how drug reaction affects outcome. This research aims to evaluate Ki67's prognostic as well as prediction utility in infiltrating breast cancer patients receiving neoadjuvant treatment. Methods: After fixation and embedding, the researchers performed Ki67 staining on core biopsies taken from 552 patients. They also evaluated HER2/neu, oestrogen, progesterone receptors, and graded the samples prior to therapy. They created both univariate and multivariate models to forecast pCR and prognosis using this data. They also divided the tumors into various molecular phenotypes in order to find possible subgroups in which Ki67 could be used to forecast pCR and prognosis. Results: The researchers discovered that Ki67 was a significant autonomous pCR predictor (OR 4; 94.7% CI, 1.3 , 9), overall survival (HR 9; 96% CI, 4 to 21), and survival without

illness (HR 3; 96% CI, 2 to 6) utilizing a cut-off value of > 14% positive neoplasic cell staining. In contrast to patients who did not achieve pCR, who had an mean Ki67 value of 27 23% positive carcinoma cell staining, patients who did achieve pCR had an average Ki67 value of 50.6 23.4%. Conclusions: Given its proven usefulness of prediction and prognosis, Ki67 is a valuable indicator in therapeutic practice. It has been demonstrated to improve the precision of prognosis and treatment response forecast in breast cancer patients receiving neoadjuvant therapy. Given that patients with pCR had very high mean Ki67 values, one might hypothesize that a higher cut-off value could help distinguish patients who would have a better outlook from those who would have lower Ki67 values. However, more extensive research will be needed to explore these results.

***Keywords*---**neoadjuvant chemotherapy, role KI67, breast cancer, multigene tests.

Introduction

Modern personalized medicine aims to identify patients with an unfavorable prognosis and, ideally, to pinpoint those who could benefit from targeted treatments to enhance their prognosis. Although there has been discussion about the suitability of the proliferation marker Ki67 for routine clinical practice, it is not yet considered ready for such use (Stuart-Harris R et.al 2008). Proliferation, however, significantly affects the calculations for the chance of recurrence in novel multigene tests. Ki67 is already a one of the elements of a multigene test used in clinical studies like TailorX and planB (Paik S et al., 2004). (Harbeck N, et.al 2010). According to one research, the prognostic value of combining the markers Ki67, ER, PR, and HER2/neu may be comparable to that of a multigene prognostic score (Cuzick et al., 2009). Regardless of the type of therapy, It has been demonstrated that Ki67 and the prognosis for breast cancer, suggesting that it may have both prognostic and predictive impacts (Yerushalmi et al., 2010). Ki67 has been studied in the neoadjuvant context as a prognostic and predictive factor. However, patients who progress have a greater proliferation rate than those who react during neoadjuvant chemotherapy (Caudle et al., 2010), suggesting a nonlinear effect of Ki67. Studies have found that a high Ki67 proliferation rate a higher incidence of a full pathological response is predicted (pCR) (Yerushalmi et al., 2005). Ki67 may be used to identify patients with a good prognosis despite a partial response to neoadjuvant treatment or those with a poorer prognosis even after a pCR because pCR is a surrogate marker for prognosis (Kuerer et al., 1999; Guarneri et al., 2006). Therefore, the purpose of the current research was to examine the prognostic value of Ki67 in patients who underwent neoadjuvant treatment for breast cancer and to compare the prognosis of different chemotherapy response groups using Ki67 expression (reference withheld).

Materials and Methods

Data collection

Between June 2020 to June 2022 patients with invasive breast cancer who received neoadjuvant treatment were seen at Hayatabad Medical Complex, Peshawar. The study was open to patients who received surgery after neoadjuvant chemotherapy and were at least 18 years old. The pretreatment assessment was used to gather data for the research on a number of parameters, including patient age, tumour size, HER2 state, progesterone status, oestrogen receptor status, grade, and proliferation status. Patients gave informed consent before participating in the research, whose data they provided.

Clinical data

A breast center must maintain thorough records of each instance of breast cancer it treats in order to be certified. According to institute regulations, this information must be recorded and includes details about the patient and tumor characteristics, treatment information, and some epidemiological information. After the original diagnosis, the facility must also provide follow-up data, including local recurrences, distant metastases, and deaths. The original pathological findings must be used to verify all histopathological information, including the size of the tumor, the condition of the axillary lymph nodes, the grade, and the oestrogen, progesterone, and HER2/neu state. As part of the ongoing certification process, yearly audits of breast centers' data quality are conducted. These procedures produced the data that were used in the analysis of this study. The definition of a full pathological response is the lack of any indications of tumor cells in the breast or axilla.

Histopathological data and pCR assessment

The initial pathology reports, which were examined by two researchers, provided the histopathological data that was used in the analysis. Since 1995, the breast center has kept regular records of the classification, tumor type, HER2/neu status, tumor proliferation, oestrogen receptor state, and progesterone receptor status that have been formalin-fixed, paraffin-embedded. If a specific proportion of the tumor cells reacted positively for the oestrogen and progesterone receptors, they were deemed positive. The HER2 status was determined using a kit with two distinct colored probes, and the Ki67 staining cutoff for positivity was set at more than 13% of positively stained cells. Histopathological reports were used to evaluate pCR, and patients were deemed to have done so if no residual tissue was discovered in the breast or nodes. A team of doctors scored the data gathered using a consistent methodology.

Table 1 lists the characteristics of the patient before therapy and correlations with pathological full remission that are univariate

	Total		Without PCR		With PCR		
	Average	Standard deviation	average	Standard deviation	average	Standard deviation	*P value*
Age	54	12	55.3	12.2	52.0	13.0	< 0.01
BMI	25	5.2	27.1	6.1	26.0	5.1	< 0.01
cT							
1	93	17	61	64.1	31	33.9	< 0.0001
2	370	68	293	77.9	82	22.3	
3	25	5	25	93.3	2.4	8.2	
4	62	12	54	92.0	6.1	9.0	
Grade							
1	28	5.7	25	94.9	1.9	6.9	< 0.00001
2	302	69.9	270	90	33	11.1	
3	173	35.1	95	53.9	77	46.1	
Histology							
Ductal	443	81.9	343	77.0	108	25.0	0.001
Lobular	81	15.0	77	94.1	5	5.3	
Other	27	5.0	17	70.0	9	31.1	
estrogen							
-ive	199	36.9	102	51.0	96	49.0	< 0.00001
+ive	357	66.1	328	91.9	26	7.2	
Progesterone							
-ive	257	45.9	158	61.5	101	40.4	< 0.00001
+ive	294	45.1	276	94.8	19	7.0	
HER2							
-ive	446	85.4	365	83.1	76	17.4	< 0.00001
+ive	103	17.6	63	54.5	44	41.3	
Ki67							
↓	166	23.3	155	95.7	8	5.6	< 0.00001
↑	393	74.7	277	73.0	114	30.0	
PO Radiation							
No	65	16.5	48	77.6	17	26.4	0.94
Yes	335	85.5	255	75.2	88	24.8	

Statistical considerations

The goal of the research was to compare the traits of patients who attained pCR to those who did not, as well as to evaluate the significance of Ki67 for prognosis and prediction, a biomarker used to gauge the rate at which cancer cells proliferate. Tests were performed for continuous data, categorical variables, and for ordinal categorical variables were all used to evaluate patient features. ORs were calculated, examining the connection between each risk factor and pCR, using logistic regression models.

Table 2: Multiple linear regression analysis prediction of pathological full remission with Ki67

Characteristic	OR	95% Cl	p value
pT			
1	1	-	-
3-5	0.35	0.19-0.69	< 0.01
Grade			
1-2	1	-	-
3	2.59	1.43-4.50	< 0.01
Estrogen receptor status			
-ive	1	-	-
+ive	0.30	0.17-0.59	< 0.01
Progesterone receptor status			
-ive	1	-	-
+ive	0.60	0.25-1.23	0.10
HER2 receptor			
-ive	1	-	-
+ive	2.40	1.34-4.23	< 0.01
Ki67			
↓	1	-	-
↑	3.52	1.44-10.9	0.01

Confidence ranges, odds ratios, and pathological tumour stage are all used (TNM classification).

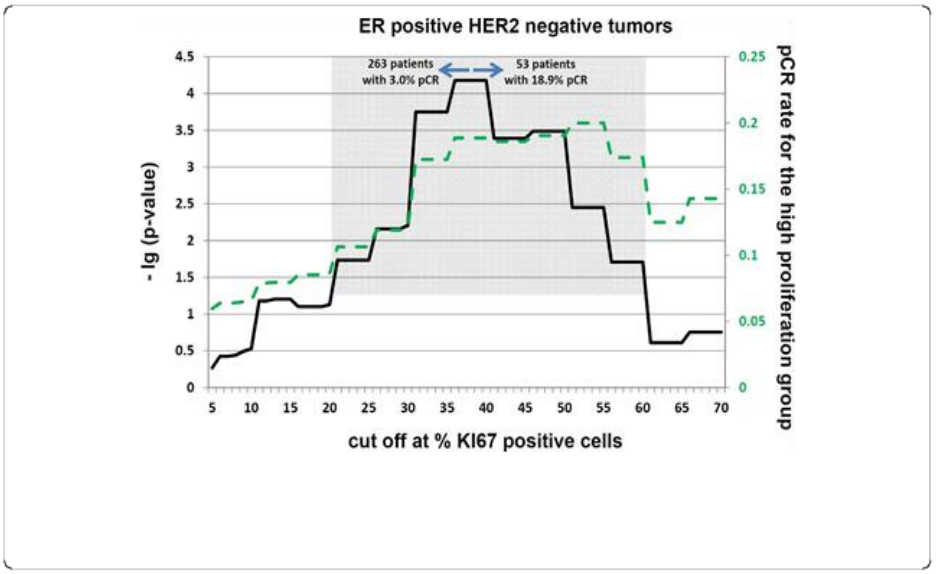
A model with numerous logistic regressions model, which included all risk variables but Ki67, was used to evaluate the predictive ability of Ki67. In order to find the optimal model, there was a reverse varying selection in step by step. The latest model also included the danger element for Ki67. The receiver operating characteristic graph and probability ratio test were used to evaluate the 2 models, and the AUC was used to assess the expanded final model's level of predictability. The Kaplan-Meier product limit method and Cox proportional hazard (PH) models were used to calculate HRs and predict survival rates, respectively, for prognosis analyses. The minimum P value method was used to determine an ideal cut-off point for Ki67. A P value of 0.05 or lower was deemed statistically significant for all two-sided tests. The R system for statistical analysis was used for all calculations. (version 2.11.1).

Results

552 patients with an average of 54.1 years and a mean BMI of 26.1 were enrolled in the research. Prior to receiving chemotherapy, the majority of patients (67.5%) had cT2 tumors (2–5 cm), followed by cT1 tumors (up to 2 cm, 16.7%), and cT4 tumors (11.1%). The most frequent groups seen were ductal tumors (80.8%) and tumors graded 1 or 2 (65.7%). Most tumors (71.8%) were categorized as having an

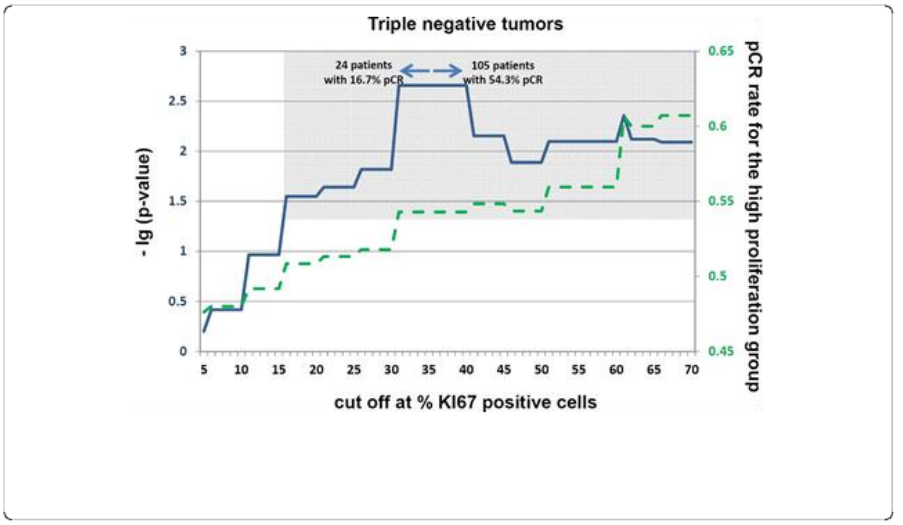
increased degree of high level of Ki67 using a cut-off value of >13%. During the 2.8-year median follow-up period, there were 67 fatalities, 77 distant tumors, and 33 localy tumors reappeared.

In terms of treatment, 328 patients (60%) received a taxane-free anthracycline-based treatment, while 102 patients (18%) received alternative therapies. 50 (49%) of the 102 HER2 +ive patients underwent trastuzumab treatment in conjunction with a taxane- and anthracycline-based protocol, while 26 of the 53 not-neoadjuvant-transtatumab patients underwent adjuvant trastuzumab therapy. The most popular treatment categories, such as anthracycline, taxane, and trastuzumab treatments, were not associated with the therapy of choice.



Univariate analysis for the association with pCR

The study found that 29% of patients with a high level of cancer cell proliferation (measured by Ki67) achieved a complete pathological response (pCR) when treated with chemotherapy and had more than 13% of their tumor cells staining positively. In contrast, only 4.3% of patients with a low level of cancer cell proliferation achieved pCR.



Using multiple variables to predict pCR

Each element that was part of the initial model continued to be a reliable indicator of pCR. With an OR of 4.2 and independent predictor status, Ki67 was also discovered to be a trustworthy predictor of pCR. According to bootstrap analysis, the model's confirmed sensitivity value is 83%, and its confirmed specificity value is 74%.

Discussion

This retrospective research looked at the usefulness of Ki67 as a predictor in connection to neoadjuvant treatment and possible outcomes (Liedtke et al., 2008; Straver et al., 2010; von Minckwitz et al., 2008). In all cases across all subtypes, an independent indicator of pathological full reactions and survival was found to be Ki67. (Andre et al., 2008; Liedtke et al., 2008). Despite having higher Ki67 proliferation rates patients with hormone receptor-positive, HER2-negative breast cancer or triple-negative breast cancer looked to have an improved outlook, when a pCR was obtained when comparing Ki67 values in various molecular subtypes. These findings might imply that in these subgroups. It might be necessary to put the Ki67 final readings for chemotherapy patients at a higher level in order to forecast how the chemotherapy will respond.

The pCR rate in the present study (48.1%) was within the range noted in previously published investigations (Liedtke et al., 2008; Straver et al., 2010). Patients with the hormone receptors + and HER2 -ive had a pCR rate of 5.7%, which was in line with other studies that had been reported (von Minckwitz et al., 2011). The current research also supports earlier findings that, in some molecular subgroups, pCR is linked to a better prognosis (Andre et al., 2008; Liedtke et al., 2008). Age, body mass index, tumor stage, type of histology, hormone receptor, and HER2 state were discovered to be associated with pCR, which is consistent with earlier research (von Minckwitz G et.al 2011, Litton JK et.al 2008). A logistic regression model's predictive power was independently increased by Ki67, which

additionally demonstrated a high correlation with pCR. According to the hormone receptor and HER2 status, this association remained true for all patients and for all molecular subtypes of breast cancer. The association was significant in the HER2 receptor-positive and hormone-positive subgroups with a cut-off value of 13%, but not in the triple-negative cohort. The cut-off number for the HER2-positive group ranged from 17% to 20%. Interpretation was challenging because the HER2-positive group included patients receiving and not receiving preoperative trastuzumab therapy.

Since there are usually more Ki67-positive cells in triple-negative tumors, there may be more differentiation between responsiveness groups. However, a cut-off of 13% was established in earlier molecular analysis to distinguish between luminal A and luminal B tumors in the hormone receptor-positive cohort (Cheang MC et.al 2009). The accuracy of this cut-off in forecasting chemotherapy response was subpar. Due to a successful therapeutic response, patients with tumors that proliferate more quickly may have a better outlook than those with lower Ki67 levels. Patients with pCR had a higher mean Ki67 value and a better prognosis in the current research, while the outlook was worse for individuals whose Ki67 number was lower. This could explain discrepancies in accounts about the prognostic significance of Ki67 (Yerushalmi R et.al 2010).

The Ki67 staining and evaluation techniques used in the research are commonplace in clinical practice, and given that the staining and assessment of entire portions may be a majority of published studies employ tissue microarrays. Fixation and staining procedures were carried out immediately after core biopsies were embedded in paraffin, potentially decreasing variability in studies using paraffin blocks of different ages. However, standard clinical evaluation calls for the use of various batches of chemicals and antibodies as well as various witnesses. Another issue is the arbitrary molecular categorization of tumors that was used (Liedtke C et.al 2010). According to Sotiriou C et al. (2006), Patients with luminal B tumors were included in the HER2-positive group in the present study because roughly 30% of these tumors are HER2-positive. Although confirmation of extra Ki67 cut-off values in a group of chemotherapy patients would have been optimal, the sample size in the present research seemed inadequate to achieve this objective.

Conclusions

A collection of breast cancer patients' overall prognosis and response to chemotherapy can be further and separately determined by using KBs as a marker. It is simple to combine it with other indicators that are frequently assessed in clinical settings. Based on the study's results, KBs may be able to identify patients who might not benefit from chemotherapy, such as those who have tumors that are HER2-negative and hormone receptor positive but have low proliferative rates. Determining which patients will unquestionably profit from chemotherapy and how to translate their response to a general prognosis, however, are more difficult. Taking into account the different molecular subgroups of breast cancer and including two cut-off points for prediction, more study is required in larger cohorts of chemotherapy patients.

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