Predicting Response to Neoadjuvant Chemotherapy in Women with Locally Advanced Breast Cancer in Kenya: Utility of Ki67

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Abstract

Background: Ki67 levels have been shown to have good predictive value in breast cancer treatment. There is paucity of data on Ki67 levels in predicting response to neoadjuvant chemotherapy (NACT) in Kenya. This study evaluated the utility of Ki67 in predicting response to NACT. **Methods:** This was a prospective observational study carried out at Kenyatta National Hospital between December 2017 and January 2019 on patients with locally advanced breast cancer. We recruited 61 women through consecutive sampling technique. Data collected included patient demographics, pre-treatment tumor size, Ki67 levels and tumor biology. After 3 cycles of first-line chemotherapy, ultrasonography was used to determine response. Data were analyzed by SPSS for proportion of change in tumor size. The response was correlated with tumor biology and pretreatment levels of Ki67 using chi-square at a 95% confidence interval. A p-value <0.05 was considered statistically significant. **Results:** The response rate after 3 cycles of NACT was 39.4%, sensitivity and specificity of Ki67 levels were 70.8% and 43.2% respectively with a cut-off value of 32.5%. **Conclusions:** Ki67 was found to predict response in our context at a rate of 39.4% at 20% cutoff after 3 cycles.

Keywords: Ki67, Breast cancer, Neoadjuvant chemotherapy

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Introduction

Breast cancer contributes a significant proportion of morbidity and mortality globally. Data from GLOBACAN show it is the 2nd in incidence and 5th cause of mortality globally (1). Data from Nairobi Cancer Registry reveal a high burden of breast cancer: it is the leading malignancy with a prevalence of 51.7 per 100,000 of all cancers in women in Kenya (2). Locally advanced breast cancer (LABC) is the commonest stage accounting for between 50.7% and 60% of breast cancer cases at first presentation (3). Currently in Kenyatta National Hospital, approximately 3–4 patients receive neoadjuvant chemotherapy weekly, or an average of 12–16 patients per month and 144–192 patients per year (unpublished data). Treatment of LABC involves a multidisciplinary approach with neoadjuvant chemotherapy (NACT), surgery, i.e. breast conserving surgery or modified radical mastectomy for operable patients, plus radiation therapy, depending on patient selection (4).

LABC poses difficulties in achieving tumor-free resection margins and low chance of breast-conserving surgeries, results in higher recurrence rate and poses challenges in wound closure (5). NACT therefore enables the surgeon to achieve the acceptable resection margins, increases operability and increases the chances of wound closure (6). DNA microarrays have shown that some breast cancers are resistant to chemotherapy: this could result in some patients receiving unnecessary chemotherapy. DNA microarrays have been used to predict response to chemotherapy, but they are expensive and their utility is not universally adopted (7). The methods that have been used to monitor treatment are physical examination, imaging studies, pathologic response, NACT and biomarkers (8). Clinical methods have been shown to be inadequate on their own in predicting response to therapy (9).

Ki67 is a nuclear antigen expressed in actively dividing cells. It is a marker of cellular proliferation (10). It is produced in large amounts in all cancerous tissues and therefore can be used to predict response to treatment (11). It is recommended to measure levels of Ki67 before initiating neoadjuvant therapy in breast cancer (12). Values of Ki67 levels of more than 25% have been shown to have a favorable response to NACT (13). There is however paucity of data on its utility in locally advanced breast cancer in Kenya, and the data available lack uniformity of Ki67 cutoff levels. Ki67 applied with good accuracy has been shown to have good prediction values (13). This study therefore aims at determining the utility of Ki67 in predicting response to neoadjuvant chemotherapy in LABC.

Methods

This was a prospective observational study carried out at Kenyatta National Hospital surgical wards, surgical outpatient clinic, oncology clinics, radiology department and histopathology laboratory. The study was conducted between December 2017 and January 2019. Inclusion criteria were women with locally advanced breast cancer, who had no systemic metastasis, T3 disease on ultrasonography and chemotherapy naïve patients. Staging was done by clinical examination and radiological assessment using CT scan chest or plain chest x-ray, and abdominal ultrasound. Only patients who were negative for systemic metastases and fully staged were included in the study. Ultrasonography was performed by qualified radiologist as per hospital policy. Patients were recruited through convenient sampling procedure after informed consent was administered. Data were collected using the data collection sheet. Data collected included patients' demographics, pretreatment Ki67 levels, pretreatment tumor size, tumor biology (tumor grade, lymphovascular invasion and immunohistochemistry: ER, PR and HER2). Tumor size was determined by ultrasound prior to therapy and at the end of 3 cycles. Patients were assessed using Ultrasound Machine Aplio 400 with a high frequency linear probe of 12 MHz to measure the longest tumour diameter. Standard neoadjuvant first line chemotherapy was given for 3 cycles. Every third week the regime given was Adriamycin 60 mg/m^2 and cyclophosphamide 600 mg/m^2.

Sample size was calculated using Daniel's formula with finite population correction for standard distribution with 95% confidence interval and a desired precision of 0.05. A repeat breast ultrasound was performed after the 3 cycles of chemotherapy, and sizes recorded. The evaluation of response to NACT was performed using the response evaluation criteria in solid tumours (RECIST).

Data were entered into MS Excel sheet, cleaned and transferred to SPSS (version 21.0, Chicago-Illinois) for analysis. Analysis was performed for mean and range of age, mean tumor size change from pretreatment to after 3 cycles of NACT, proportions of patient with high or low Ki67 according to St Galen classification, which used 20% as the cutoff, and proportion of patients who had response as per RECIST. Chi-square was used to analyze the association between Ki67 level to response and other aspects of tumor biology: molecular subtype, tumor grade, and perineural and lymphovascular invasion. Receiver operator curve (ROC) was drawn using the Ki67 levels against response to NACT. This was then used to determine the sensitivity, specificity, cutoffs, and area under the curve of Ki67 for our population. p values and 95% confidence intervals (CIs) were

calculated as applicable, and p value <0.05 was considered statistically significant.

This study commenced after approval from the Department of Surgery and the UoN-KNH ERC, under approval number P247/05/2017.

Results

Sixty-one patients were recruited for this study. Their mean age was 45.9 (SD=10.4) years while the minimum age was 28 years and maximum 73 years; 27 (44.3%) patients were 41–50 years of age. All patients in this study had invasive ductal carcinoma and most (63.9%) had tumor grade II and 34.4% Luminal A molecular subtype (Table 1).

Table 1: Patient and tumor characteristics

	Frequency *n* (%)	
Age (years)		
≤30	4 (6.6)	
31–40	15 (24.6)	
41–50	27 (44.3)	
51–60	8 (13.1)	
61–70	6 (9.8)	
71–80	1 (1.6)	
Tumor biology		
	<20%*	>20%*
Grade		
I	2	8
II	9	30
III	3	9
Molecular type		
Luminal A	3	18
Luminal B	3	12
Basal like	5	14
HER2-enriched	3	3
Ki67	%	Frequency
	<20%	23.3%
	>20%	76.7%

**Cut-off point*

Figure 1 and Table 2 show the correlation between Ki67 levels and response to NACT based on sensitivity and specificity, cutoff, and area under the curve for Ki67. Point marked pink on the graph is the cutoff point, giving sensitivity and specificity for prediction of response.

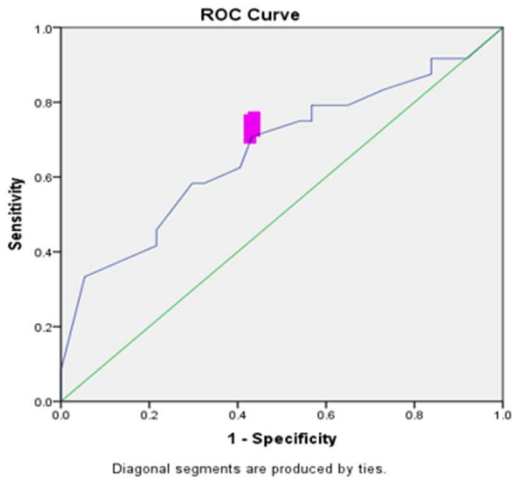

Figure 1: The receiver operator curve. Point marked pink on the graph is the cutoff point, giving sensitivity and specificity for predicting response.

The paired sample mean change in tumor size on ultrasonography from pretreatment to posttreatment was 1.24 (SD=2.19) with confidence interval of 0.68–1.80, $t(60)=4.420$, $p<0.001$. A dependent samples t-test was performed to test the hypothesis that the size of the breast mass (cm) by ultrasonography at week 0 (M=5.95, SD=4.34) and the size of the breast mass (cm) greatest dimensions following 3 cycles of NACT (M=4.71, SD=4.07) were equal. The correlation between the two conditions was estimated at $r=0.866$, $p<0.001$, suggesting that the dependent samples t-test is appropriate in this case. The null hypothesis of equal breast mass means was rejected $t(60)=4.42$, $p<0.001$. Thus, the mean of size of breast mass (cm) greatest dimensions at the end of 3 cycles of NACT was statistically significantly lower than the mean of size of breast mass (cm) by ultrasonography at week 0.

Table 2: Result of Ki67 cutoff levels, sensitivity, specificity and area under the curve.

Ki67 levels prior to therapy	Value
Area	0.671
Standard error	0.074
Asymptotic significance	0.025 (0.536-0.816)
Diagnostic accuracy (cut-off =32.5)	
Specificity	0.708
Sensitivity	0.432

Correlation of Ki67 levels with tumor biological characteristics

The pathological response after 3 cycles of chemotherapy was seen in 24 (39.4%) patients, most 31 (50.8%) remained stable while only 6 (9.8%) had progression of their disease while on chemotherapy. When considered against the cutoff agreed at Galen for Ki67, there was no significant difference between those above or below 20%.

A chi-square test for proportion was conducted between the Ki67 levels and response. There were no statistically significant differences in the proportions between Ki67 levels and responders and non-responders, $\chi2(1)=0.884$, p=.347 (Table 3).

Table 3: Proportions between Ki67 levels and responders and non-responders

Cut-off	Non-responders	Responders	OR (95% CI)	p-value
≤20	10 (27.0)	4 (16.7)	1.9 (0.5–6.8)	0.347
>20	27 (73.0)	20 (83.3)		

Response rates in relation to receptor status

HER2+ had a higher proportion of responders at 37.0%, followed by ER+ at 31.4% and PR+ at 20.7%. Results are shown in Table 4.

Table 4: Response rates in relation to receptor status

Receptor status	Non-responders	Responders	Total
ER+	24 (68.6)	11 (31.4)	35/61(57.4)
PR+	23 (79.3)	6 (20.7)	29/61(47.5)
HER2+	17 (63.0)	10 (37.0)	27/61(44.3)

Relationship between Ki67 and perineural and lymphovascular invasion

Perineural invasion after 3 cycles of chemotherapy was seen in 24 patients (39.4%), most 31 (50.8%) remained stable while only 6 (9.8%) had progression of disease while on chemotherapy (Table 5). When considered against the cutoff agreed at Galen for Ki67, difference between those above or below 20% was not significant.

A chi-square test for association was conducted between the lymphovascular invasion and perineural invasion with the Ki67 levels. There was no statistically significant association between lymphovascular invasion and Ki67 levels, $\chi2(1)=2.198$, p=.138, but association was statistically significant between perineural invasion and higher Ki67 levels, $\chi2(1)=10.509$, p =0.005.

Table 5: Ki67 levels in relation to lymphovascular and perineural invasion

	≤20*	>20*	OR (95% CI)	p-value
Lympho-vascular invasion				
Present	4 (14.3)	24 (85.7)	0.4 (0.1–1.4)	0.138
Absent	10 (30.3)	23 (69.7)		
Perineural invasion				
Present	5 (71.4)	2 (28.6)	12.5 (2.1–74.8)	0.005
Absent	9 (16.7)	45 (83.3)		

**Cutoff*

Discussion

This study included 61 women all with locally advanced ductal carcinoma. The mean age at diagnosis was 45.9±10.4 years; the median age at diagnosis is 48.5 years in Kenya and 64.1 years among US-born Americans (14). A study conducted in Aga Khan University, Nairobi, concluded that the median age at diagnosis was 47.5 years (15).The mean age of breast cancer diagnosis in African population is 46 years±6.2 SD with age range of 35–49 years. (16) Our study findings on age and commonest age group at diagnosis are within the findings of these studies.

Levels of Ki67 have been documented to correlate with both clinical and pathologic response to NACT and can help in selecting patients who will benefit from NACT. However, the cutoff values vary widely with different patient populations and the type of NACT used (17). The value in our study is close to that reported by Jain et al. who conducted a prospective observational study with 134 patients with stage II/III breast cancer between February 2014 and March 2016; in our study all patients were at stage III. The study reported Ki67 as an independent predictive factor for response and suggested 35% as best cutoff for Ki67 expression in predicting response to NACT and achieving pathologic complete response. The sensitivity was 68.7% and

specificity 71.6%. While the sensitivity is comparable to our study, Jain et al. had a higher specificity. However, in their study, the clinical complete response rate was lower at 26.1% than that of this study (39.4%). Jain et al. study in the Indian population had twice our sample size, and patients completed all the cycles of NACT(18). Kim Ki et al. in Gachon University in South Korea conducted a nine-year retrospective study of three large centres with 9,321 records retrieved; the study demonstrated a cutoff value of 25% (sensitivity 71%, specificity 77%) especially in ER- and HER2+ tumors, with Ki67 being the only independent predictor (13). A cohort study conducted by Acs et al. in Hungary between 2002 and 2013 with a sample size of 120 patients with invasive ductal carcinoma reported that NACT is more efficient in tumors with at least Ki67 20%, with a 95.7% sensitivity and 54.3% specificity, a cutoff value lower than our study. A similar cutoff value of 20% was reported by another study (19). Ki67 cutoff values therefore vary in different studies. A study by Denkert et al. suggested that Ki67 levels are a continuum variable and proposed that cutoff points are in the context dependent on two groups of tumors: tumors with low levels of Ki67 have good outcome and those with high levels of Ki67 have good outcomes only when the tumor responded to NACT (20).

This study also looked at other biological characteristics of the tumor in relation to Ki67 levels. Association between lymphovascular invasion and Ki67 levels was not statistically significant (21). Contrary to these findings, a study by Kilickap et al. reported that there was no relationship between perineural invasion and Ki67 positivity. However, they reported that higher Ki67 levels are associated with unfavorable prognostic factors including higher grade, ER negativity, HER2 positivity and axillary lymph node involvement (22). The differences in our study could be the result of a smaller sample size, hence further studies will be needed. This study showed no statistically significant association between Ki67 levels and tumor grade. A study by Awadelkarim et al. in Sudan suggested positive correlation between Ki67 and tumor differentiation, but it wasn't linked to any other variables tested including receptor type and tumor size (23). A similar study by Haroon et al. among 194 cases of newly diagnosed breast cancer with an aim of correlating Ki67 expression with other prognostic markers including tumor grade, reported that Ki67 is positively correlated with histological grade. The study also elucidated that high Ki67 >30% shows a lower HER2-neu expression and lymph node metastasis, hence may have worse prognosis (24).

Inter-observer variability during ultrasonography may introduce limitations in the study, but this was delimited by use of standard machine and use of two radiologists in reading the ultrasonographic report as part of quality assurance.

Conclusion

Ki67 was found to predict response in our context with a response rate of 39.4% using the St. Galen's cutoff of 20%. Using ROC, sensitivity was found to be 70.8% and specificity 43.2% at cutoff of 32.5%.

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Conflict of interest

The principal author or co-authors have no conflict of interest. The funder had no role in the design, collection, analysis and interpretation of the data.

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