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Original Article

Assessment of the Prognostic Role of Ki-67 and Its Optimal Cutoff Value in Early Breast Cancer: A Retrospective Analysis

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Abstract

Background: Breast cancer is a heterogenous group of diseases classified into the biological subtypes luminal A, luminal B, HER2-enriched and triple negative. These subtypes have different treatment response patterns and survival rates. Ki-67 is the most commonly used proliferative marker in breast cancer and is used for the distinction between luminal A and B subtypes.

Methods: A retrospective study that included patients with early breast cancer diagnosed between 2010 and 2016 and treated in a single cancer center.

Results: The medical records of 498 patients were retrospectively reviewed. The median age of patients was 51 years (range: 21 – 81) and the median value of Ki-67 level among them was 20% (interquartile range: 10-30%). Ki-67 was significantly higher in younger (<35 years) and premenopausal patients (p=0.0002 and 0.0055, respectively). Higher Ki-67 level associated significantly with higher T stage, estrogen and progesterone receptors-negativity, HER2-positivity and higher grade (p=0.0256, <0.0001, <0.0001, =0.0001 and =0.0031; respectively). Univariate Cox regression analysis showed that the ≥14% and ≥20% cutoff values of Ki-67 level are associated with poorer disease-free survival (DFS) (HR=1.989 [95%CI: 1.163-3.402, p=0.0121] and HR=1.616 [95%CI: 1.001-2.61, p=0.0496], respectively). On stratifying patients according to the Ki-67 proliferation index into three strata, <14%, ≥14%--<20% and ≥20%; DFS differed significantly between them (p=0.0394). The 5-year DFS rate for the three strata was 82.2%, 64.7% and 64.8%; respectively.

Conclusion: Early breast cancer patients with lower Ki-67 levels have significantly better DFS. A Ki-67 cutoff value of ≥14% appears to correlate better with DFS than the newer cutoff value of ≥20%.

Keywords: Biological subtypes, Cutoff value, Early breast cancer, Ki-67, Prognosis

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Introduction

Breast cancer is the most common malignancy in females affecting 38.8% of female patients diagnosed with cancer yearly, with an incidence rate of 35.8 for every 100,000 Egyptian females [1]. There are many factors with prognostic significance in breast cancer that have been investigated thoroughly in the last decades including patients' age, tumor size, nodal status, lymphovascular invasion, hormone receptor status and HER2 expression [2]. Breast cancer is considered a heterogenous disease. Based on hormonal receptors status and human epithelial growth factor receptor 2 (HER2) expression, it is classified into four biological subtypes: luminal A and B, HER2-enriched and basal-like [2]. The luminal subtype (HR-positive) demonstrates an indolent disease with prolonged disease-free survival (DFS), low recurrence rates and sluggish response to chemotherapy. However, the emergence of Ki-67 proliferative index differentiated between two luminal subtypes; luminal A and luminal B breast cancer. These two subtypes showed different responses to chemotherapy and hormonal

treatment as well as different patterns of recurrence and disease progression [3]. Ki-67 is a non-histone nuclear protein expressed in all phases of the cell cycle except for the G0-phase i.e. a marker of cell proliferation [3]. In a large meta-analysis, Ki-67 expression in early breast cancer has been shown to be a significant prognostic indicator for DFS and overall survival (OS) when comparing node-negative / node-positive and untreated patients with Ki-67-positive expression regardless of the treatment options [4]. However, the Ki-67 proliferative index remains a controversial point in breast cancer prognosis of DFS and progression-free survival, especially with regards to its cutoff value of significance. Many studies have offered a cutoff value for significant Ki-67 level ranging from 14% to 20 % [3, 4].

Another challenge with the routine use of Ki-67 in clinical practice is the high inter- and intra-observer variability observed in testing. In spite of advanced immunohistochemical (IHC) methods, there is variation in human visual assessment of Ki-67 level during the examination of specimens [3, 5].

The aim of this study was to evaluate the prognostic impact of Ki-67 on the outcome of early breast cancer as regards DFS and OS, and to determine which cutoff value (14% vs. 20%) correlates better with outcome.

Methods

This retrospective study was conducted at a single Egyptian Cancer Center; Kasr Al-Ainy Center of Clinical Oncology and Nuclear Medicine (NEMROCK), Kasr Al-Ainy School of Medicine, Cairo University.

Patients

The medical records of patients treated at NEMROCK in the period from January 2010 to December 2016 were searched for breast cancer patients (identified through ICD-10 codes).

The criteria for inclusion in the study were: age > 18, female gender, pathologically proven breast cancer, non-metastatic disease at presentation (stages I-III), available IHC results for estrogen receptors (ER), progesterone receptors (PR), HER2 and Ki-67, available baseline and follow-up clinical, radiologic and pathologic data and a minimum follow-up duration of 6 months. Patients with male gender, incomplete medical records or metastatic (stage IV) disease were excluded. Patients with no exact Ki-67 measurement and/or unknown HER2 status were also excluded.

For T staging, the clinical T for patients who received neoadjuvant treatment and the pathological T for other patients were taken into consideration.

HER2-negative breast cancer patients with weak ER/PR expression levels by IHC have survival outcomes similar to those with negative ER/PR expression [6]; consequently, they were considered as triple-negative subtype.

Statistical Analysis

Statistical analysis was performed using MedCalc version 19.4.0 (software for Windows MedCalc Software Ltd, Ostend, Belgium).

Categorical variables were described as number and percentage and abnormally distributed continuous variables as median and interquartile range (IQR). The Mann-Whitney U test was used to test the difference in Ki-67 expression value between two groups and the Kruskal–Wallis test for more than two groups.

The Kaplan-Meier method was used to estimate survival and to generate survival curves. Disease-free survival was calculated from the date of diagnosis to the date of recurrence or death. Cox regression analysis or log-rank test was performed to test the association between variables and DFS. A p value <0.05 was considered significant.

Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of Kasr Al-Ainy Center of Clinical Oncology and Nuclear Medicine (NEMROCK), Kasr Al-Ainy School of Medicine, Cairo University.

Results

From 2,456 records of breast cancer patients in the specified time period, the data of 498 patients was complete and were eligible for analysis.

Patients' and Disease Characteristics

The median age of included patients was 51 years (range: 21 – 81) and 241 (48.4%) of them were premenopausal at diagnosis. Disease characteristics are mentioned in Table 1. One hundred and three (20.7%) patients received neoadjuvant chemotherapy before surgery and 395 (79.3%) received adjuvant therapy. The type of surgery was

breast conservative surgery in 269 (54%) patients and modified radical mastectomy in 227 (45.6%). The type of surgery was missing in two patients.

Table 1: Disease characteristics

Characteristic	No.	%
T stage		
T1	105	21.1
T2	274	55
T3	46	9.2
T4	30	6
Missing	43	8.6
N stage		
N0	223	44.8
N1	128	25.7
N2	76	15.3
N3	56	11.2
Missing	15	3
Grade		
1	3	0.6
2	333	66.9
3	42	8.4
Missing	120	24.1
Lymphovascular invasion		
No	229	46
Yes	135	27.1
Missing	134	26.9
Estrogen receptor status		
Negative	126	25.3
Weakly positive	34	6.8
Moderately positive	73	14.7
Strongly positive	265	53.2
Progesterone receptor status		
Negative	167	33.5
Weakly positive	50	10
Moderately positive	97	19.5
Strongly positive	184	36.9
HER2		
Negative	385	77.3
Positive	113	22.7
Ki-67		
<14	191	38.4
≥14-<20	55	11
≥20	252	50.6

In our sample, Ki-67 measured in surgical specimens had a median of 20% (IQR: 10-30%). The distribution of Ki-67 measurements is shown in Figure 1.

Correlation Between Ki-67 and Clinico-Pathological Variables

The relation between Ki-67 and the other variables is shown in Table 2. Ki-67 level was significantly higher in premenopausal and younger patients. Higher T-stage, estrogen and progesterone-negativity, HER2-positivity and higher grade were also associated with significantly higher Ki-67.

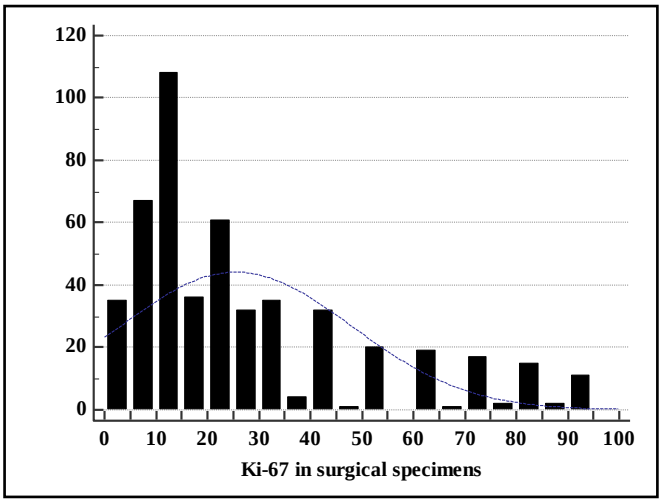

Figure 1: Histogram showing the distribution of Ki-67 expression in 498 early breast cancer patients

Survival Analysis

After a median follow up time of 24.6 months (95%CI: 19.93-67.57), 71 (14.3%) had relapsed disease and only 6 (1.2%) patients died.

The estimated mean OS was 66.3 months (95%CI: 65.24-67.36) and the estimated median was not reached. No further OS analysis was performed because of the very few number of events (deaths). The estimated mean DFS was 56 months (95%CI: 53.58-58.41) and the median was 67.57 months (95%CI: 67.57-67.57).

Univariate DFS analysis using Cox proportional hazards model is shown in Table 3. Being young (< 35 years) and premenopausal were associated with significantly worse DFS. This was also true for nodal positivity, higher grade, lymphovascular invasion, and ER and PR negativity. Although higher T stage and HER2 positivity were associated with worse DFS, the association was not statistically significant.

Cox proportion hazards model was used after stratification of patients according to the Ki-67 level using the cutoff value of 14%. Using the 14% cutoff value for Ki-67 (≥14% vs. <14%), there was a significant correlation to DFS (HR=1.989 [95%CI: 1.163-3.402]; p=0.0121) (Figure 2). The 5-year DFS rate was 65% for Ki-67 ≥ 14% and 82.2% for Ki-67 < 14%.

When 20% was used as a cutoff value for Ki-67, there was also a significant correlation to DFS

(HR=1.616 [95%CI: 1.001-2.61], p=0.0496) (Figure 3). The 5-year DFS rate was 64.8% for Ki-67 ≥ 20% and 78.3% for Ki-67 < 20%. On stratifying the Ki-67 proliferation index into three strata i.e. < 14%, ≥ 14% --< 20% and ≥ 20%, DFS showed a statistically significant difference between the 3 strata (p=0.0394) (Figure 4).

Table 2: Correlation between Ki-67 and clinico-pathological variables

Variable	Ki-67 Median (IQR)	P-value
Age		
< 35	30 (15-60)	0.0002
≥ 35	18 (10-30)	
Menopause		
Premenopausal	20 (10-40)	0.0055
Postmenopausal	15 (10-30)	
T stage		
1-2	18 (10-30)	0.0256
3-4	20 (11-40)	
N stage		
0	17 (9-38.8)	0.4367
1-3	20 (10-30)	
Estrogen receptor		
Negative	27.5 (14-60)	< 0.0001
Weakly positive	22.5 (10-60)	
Moderately positive	18 (10-30)	
Strongly positive	15 (10-25)	
Progesterone receptor		
Negative	25 (14-50)	< 0.0001
Weakly positive	16.5 (10-30)	
Moderately positive	15 (10-30)	
Strongly positive	12 (9.5-25)	
HER2		
Negative	15 (10-30)	0.0001
Positive	20 (14-40)	
Grade		
1-2	17 (10-30)	0.0031
3	25 (15-60)	

IQR: Interquartile range

The 5-year DFS rate for the three categories was 82.2%, 64.7% and 64.8%; respectively. As shown in Figure 4, the DFS curve of the Ki-67 ≥ 14% --< 20% group was overlapping with the ≥ 20% group curve but not the < 14% group curve.

In multivariate Cox regression analysis that included significant variables in Table 3, Ki-67 cutoff value of 14% did not associate significantly with DFS (HR=1.354 [95%CI: 0.663-2.762], p=0.405). The same was true for the cutoff value of 20% (HR=1.376 [95%CI: 0.723-2.619], p=0.33). In the multivariate analysis, age, nodal involvement and ER and PR status maintained significance.

Table 3: Univariate analysis for disease-free survival

Variable	HR (95%CI)	P-value
Age		
< 35	Ref.	<0.0001
≥ 35	0.282 (0.159-0.499)	
Menopausal status		
Postmenopausal	Ref.	0.0092
Premenopausal	1.917 (1.175-3.126)	
T stage		
1-2	Ref.	0.6917
3-4	1.155 (0.566-2.359)	
N stage		
0	Ref.	<0.0001
1-3	3.479 (1.925-6.288)	
Grade		
1-2	Ref.	0.0313
3	2.211 (1.074-4.552)	
Lymphovascular invasion		
No	Ref.	0.0087
Yes	2.006 (1.193-3.374)	
Estrogen receptor		
Negative	Ref.	0.0038
Positive	0.49 (0.302-0.795)	
Progesterone receptor		
Negative	Ref.	0.0456
Positive	0.619 (0.387-0.991)	
HER2 amplification		
No	Ref.	0.2766
Yes	1.334 (0.794-2.243)	

HR: Hazard ration; **CI**: Confidence interval

Discussion

To our knowledge, this is the biggest study of the prognostic impact of Ki-67 in breast cancer performed in Egypt. Multiple prognostic and predictive markers of breast cancer outcome have been identified over the last few decades. Among these, Ki-67 arises as an important prognostic factor currently integrated into routine clinical practice.

Despite of that, a lot of controversy is raised around the routine use of Ki-67 especially with the lack of consistent evidence of its predictive value regarding the use of adjuvant systemic treatment.

The technical challenges and high discordance rate of Ki-67 reporting among pathologists (inter- and intra - observer variation) poses another

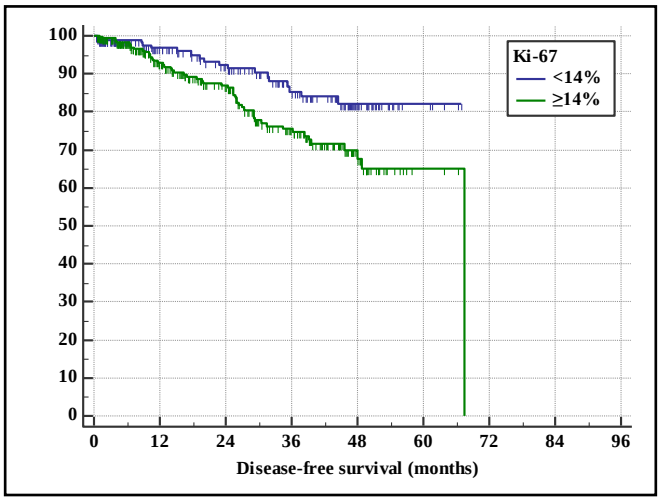

Figure 2: Kaplan-Meier disease-free survival estimates according to Ki-67 cutoff value of 14%

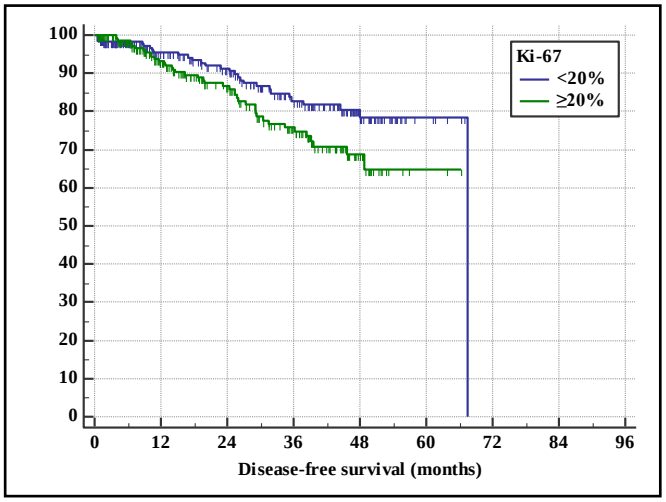

Figure 3: Kaplan-Meier disease-free survival estimates according to Ki-67 cutoff value of 20%

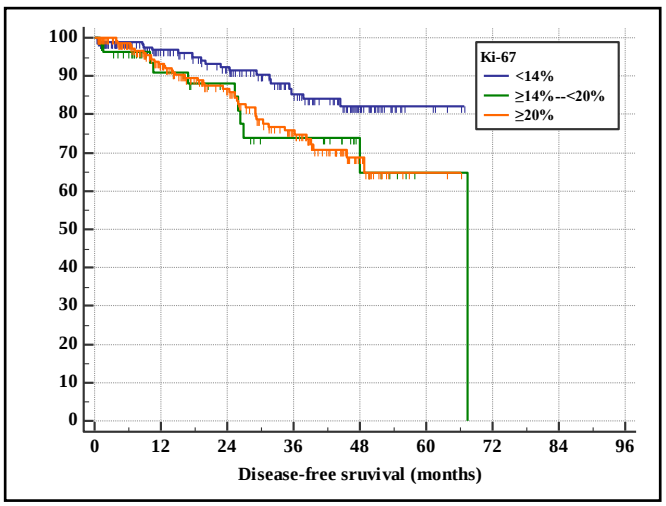

Figure 4: Kaplan-Meier disease-free survival estimates according to Ki-67 stratified into 3 categories

challenge for the routine use of Ki-67 in clinical practice.

Furthermore, the debate regarding the optimum Ki-67 cutoff value of clinical significance is another challenge facing the use of Ki-67 in clinical practice. The use of Ki-67 was first suggested in the St. Gallen consensus meeting in 2009 to identify highly proliferating tumors within Luminal breast cancers. In 2011, the consensus meeting suggested a cutoff value of 14% based on the data reported by Cheang et al [2]; however, in 2013 the suggested cutoff value was changed to 20%.

In the current study, we aimed to evaluate the prognostic impact of Ki-67 on breast cancer outcome, as well as to correlate different cutoff levels of Ki-67 to survival to elucidate the optimum cutoff level of clinical significance at our institute, especially with the limited use of gene expression profiling due to financial restrictions.

In the current study, Ki-67 did correlate neither to tumor size nor to nodal status. This is consistent with the data reported by Inwald et al revealing no correlation between Ki-67 and T-stage, while it did correlate to N-stage [7]. However, many studies demonstrated the reverse; with a positive correlation between Ki-67 and both [4]. Kremani et al found a marginal significant relationship between lymph node status and Ki-67 expression [8].

On the other hand, in our study Ki-67 correlated significantly to ER, PR and HER2 status. Mirmalek et al stated in their study that age, ER and PR status correlated negatively with Ki-67, while HER2 correlated positively [9]. Estrogen receptor and PR had an inverse relationship with Ki-67 in the study of Sheikhpour et al [10]. Our study results showed a significant correlation to younger age (<35 years), and accordingly to being premenopausal, similar to other studies done across Egypt [1].

When we used a cutoff value of <14% to evaluate the impact of Ki-67 on breast cancer outcome, a positive correlation to DFS was statistically significant. This is consistent with the results of Hugh et al who studied 1,350 patients using the same cutoff value of 14% and found a significant improvement in 3-year DFS rate in patients with Ki-67 less than 14% [11]. Cheang et al [2], Chung et al [12], Thangarajah et al [13] and Yang et al [14] also reported similar results with the use of the same cutoff value of 14%.

When we performed the analysis using a Ki-67 cutoff value of 20%, similar results were obtained with significant correlation to DFS. This concords with the data reported by Bustreo et al on 1,577

patients where patients with Ki-67 > 20% had a poorer prognosis [3]. Accordingly, we stratified our patient cohort into three groups according to Ki-67 into those with an index of < 14%, ≥ 14% --< 20% and ≥ 20%. This resulted in a significant difference in DFS was between the three groups with patients with Ki-67 ≤14% showing the best outcome. This is consistent with the suggestion of Denkert et al [15] that Ki-67 can be considered as a continuous marker because of its prognostic significance using different cutoff values. The 5-year DFS rates of patients in the Ki-67 ≥ 14%--< 20% and ≥ 20% groups were similar and significantly lower than that of the < 14% group.

In our study, we faced some limitations such as incomplete medical records of patients who had not completed their treatment at our center and the incomplete panel of IHC. In addition, in many patients, high and low readings were interpreted according to the 14% cutoff value that was previously recommended by St. Gallen consensus in 2011. Furthermore, our study was a retrospective one from a single cancer center with limited follow-up duration.

Conclusion

The present study further confirms the prognostic impact of Ki-67 on early breast cancer outcome, and that a Ki-67 cutoff value of 14% correlates better with DFS.

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None.

Author's contribution

Conception or design: WM and SL; Acquisition, analysis or interpretation of data: SL; Drafting the manuscript or revising it: WM and SL; Final approval of the manuscript version to be published: WM and SL; Agreement to be accountable for all aspects of the work: WM and SL.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

Data availability

Deidentified individual participant data used to produce the results of this study are available from the corresponding author (WM) upon request.

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Study registration

None.

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