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Original Article

KI-67 As A Prognostic Marker According to Breast Cancer Molecular Subtypes and Its Relationship with Other Prognostic Factors

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ABSTRACT

Introduction: - IHC evaluation of Ki-67 is widely used for estimation of tumour proliferation in breast cancer which aids in the management of the breast cancer patients.
Objectives: - To find the prognostic importance of Ki 67 and to analyse its correlation with other prognostic markers.
Material and Methods: - A cross-sectional study was done at Baroda Medical College, India between 2019 to 2021. Total of 85 histologically proven breast carcinomas were studied; histopathological grading was done. IHC for ER, PR, HER2, Ki-67 and Molecular subtyping was done. Association of Ki-67 with other prognostic markers like tumour grade and molecular subtypes of breast cancer was evaluated. Ki-67 was subdivided in < 14% and > 14%.
Results: - Out of 85 patients, a total of 83(97.6 %) were diagnosed as Invasive Ductal Carcinoma (IDC), 48(56.5 %) cases were of histological grade II and 28(33 %) cases were of Triple Negative subtype. No luminal A patients showed Ki-67 level higher than 14%. Ki-67 level >14% was observed in 44.4% of luminal B, 16.7% of HER2enriched and 38.9% of triple negative subtypes.
Conclusion: Proliferation acts as an important determinant of cancer outcome and Ki 67 can be used to objectively measure this. This study showed that high Ki-67 expression is associated with higher grade, invasive ductal carcinoma type, ER/PR and HER2/neu negativity and Luminal B subtype.

Keywords: *breast cancer, Ki67.*

INTRODUCTION

Breast cancer is the most common cancer in women worldwide and is also the leading cause of cancer death. It accounts for almost one in four cancer cases in women.[1] In India, the incidence of breast cancer has been increasing in recent years and it has replaced cervical cancer as the most common cancer in urban areas. In comparison to the western population, breast cancer occurs at a younger premenopausal age in India, and most of the patients present with locally advanced or metastatic disease.[2] The clinical feature of breast cancer is very heterogeneous because of the variable prognostic factors impact its behaviour [3]. To know prognostic factors may help to estimate the prognosis and to choose the most appropriate treatment modality. Age, histopathologic subtypes, tumour size, tumour grade, lymph node involvement, extracapsular extension (ECE), lymphovascular invasion (LVI), and hormonal receptor status are the most important conventional prognostic factors [4]. Breast cancer can be classified according to the histopathological type {World Health Organisation (WHO) 2019} [5], tumour grade (Elston Ellis modification of Scarff-Bloom-Richardson grading system) [6], tumour stage {American Joint Committee on Cancer (AJCC) 8th edition} [7]and different molecular subtypes of breast cancer (Luminal A, Luminal B, HER2 rich and Basal-like/Triple Negative) [8].

Four main breast cancer subtypes have been identified according to estrogen receptor (ER), progesterone receptor (PR), and HER2. These subtypes include luminal types A and B, basal-like, and HER2-enriched subtype [9]. Luminal A is the most common breast cancer subtype and characterized by ER+ and/or PR+/HER2− status, low-grade tumour, and good prognosis [10-12]. Luminal B subtype accounts for approximately 10% of all breast cancers and is distinguished by ER+ and/or PR/HER2− status [13]. Differentiation of luminal A from luminal B/HER2− breast cancers result in important

therapeutic implications. Hence, the Saint Gallen Guidelines recommended the assessment of the Ki-67 proliferation index [14]. Luminal B breast cancer should show a higher proliferation index than Luminal A [13]. Breast cancer subtypes with negative ER, PR, and HER2 status are typically called "triple-negative" breast cancers and approximate the basal like category. This subtype is also associated with high-grade tumours [10,12,15]. HER2-enriched subtype (HER2+/ER−/PR−) is less common but is similarly characterized by high-grade tumours and poor outcomes [10].

The Ki-67 antigen is also known as MKI67 or MIB-1 [16,17]. It is a non- histone protein [17]. Ki-67 is present within the nucleus in all active phases of cell cycle, except the Go phase that is the resting phase [18]. Ki 67 binding, as an objective measurement of cell proliferation aids significantly in the management of the breast cancer patient [19].

Aim:
The aim of the present study was to determine the Ki 67 in molecular subtypes, to find the prognostic importance of Ki 67 and to analyze its correlation with other prognostic markers.

MATERIALS AND METHODS:
The study included 85 cases of primary breast cancers retrospectively during January 2019 till January 2021. Specimens were of MRM, biopsy, lumpectomy and simple mastectomy. The breast tissue obtained in the form of Modified Radical Mastectomy (MRM), an excision and or a trucut biopsy was fixed in 10% of buffered formalin (formalin buffered to pH 7.0-7.4) for 12- 24 hours. Grossing of the specimen was done according to the standard protocols followed by paraffin embedding and staining with haematoxylin and eosin. Sections were studied to evaluate histologic type, histologic grade and lymph node status and lympho- vascular invasion. Histopathological grade was assessed using Bloom and Richardson's method, modified by Elston and Ellis. Age, sex and laterality was obtained from histopathology request form. A representative block with maximum tumour tissue and adjacent normal breast tissue was selected for IHC. Four serial sections were obtained on Poly-L-lysine coated slide. These sections were used for IHC staining of ER, PR, HER2 and Ki-67. Stains were performed according to defined protocol with positive & negative controls
The primary antibodies used for ER was clone EP1, for PR was clone EP2, for HER2 was clone EP3, and Ki-67 was Mib-1.
Immunohistochemical assessment of hormone receptors is performed by using two parameters, namely the number of positively stained tumour cell nuclei and the intensity of staining i.e. **Allred scoring system.**

Allred Score= Proportion Score (PS) + Intensity Score (IS)
The PS is the number of cells that are stained and IS is the intensity of staining that is pale or dark. The two scores were added together for the final score that is the Allred score. A total score of 0-2 was considered negative and a score of 3-8 was considered positive [20].

Score for proportion of positive nuclei	Percentage of stained tumour cell nuclei
0	No staining
1	<1%
2	1-10%
3	11-33%
4	34-66%
5	67-100%

Score for intensity	Intensity of staining
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

HER2 staining (ASCO and CAP guidelines) was considered positive (Score 3+) when complete, intense, circumferential membrane staining was seen in >10% of invasive tumours cells and it was negative (0 or 1+) when no or incomplete, faint membrane staining was seen in >10% of invasive tumour cells [21].

Staining Patterns	Score	HER2/Neu protein overexpression assessment
No staining is observed or membrane staining is observed in <10% of tumour cells	0	Negative
A faint/barely perceptible membrane staining is detected in >10% of tumour cells. The cells are only stained in part of their membrane	1+	Negative

A weak to moderate complete membrane staining is observed in more than 10% of tumour cells	2+	Weakly positive/ Equivocal
A Strong complete membrane staining is observed in more than 30% of tumour cells	3+	Strongly Positive

Ki-67 score or PI was evaluated as percentage of positively stained cells against the total number of tumour cells scored (0-100%). For Ki67, nuclear expression was recorded quantitatively, at least 1000 cells were assessed to calculate an average estimate and was categorized into <14% and ≥14%. Molecular subtyping was done.
Breast cancers were classified into four molecular subtypes as mentioned below [22]:
1. Luminal A (ER+ and/or PR+, HER2/neu negative, Ki67< 14%)
2. Luminal B with HER2/neu negative (ER+ and/or PR+, HER-2 negative, Ki-67≥14%) or
HER2/neu positive (ER+ and/or PR+, HER-2 positive, any Ki67)
3. HER-2 enriched (ER-, PR-, HER-2 positive)
4. Triple negative (ER-, PR- HER-2 negative)
For data analysis, Chi square test was applied. P value ≤ 0.005 was considered significant.

RESULTS:
This retrospective study included 85 patients with invasive breast cancer, with a mean age of 50.40 ± 13.38 years. Most cases were female (97.6%). Trucut biopsies accounted for 30 cases, while the remainder were excision specimens, predominantly obtained via MRM (59%).

Histopathologically, the vast majority were invasive ductal carcinoma, NOS (97.6%), with only one case each of invasive lobular carcinoma and medullary carcinoma (1.2% each). Most tumours were Grade II (56.5%), with a score of 6/9 being the most common (38%).

Table 1: Details of Specimen of type of tumour

Type of specimen	**Count**	**%**
MRM	**50**	**58.82**
biopsy	30	35.29
lumpectomy	3	3.53
simple mastectomy	2	2.35
Grade		
1	19	22.35
2	**48**	**56.47**
3	18	21.18
Score		
3/9.	1	1.18
4/9.	4	4.71
5/9.	14	16.47
6/9.	**32**	**37.65**
7/9.	16	18.82
8/9.	17	20.00
9/9.	1	1.18
HP diagnosis		
Invasive ductal carcinoma	**83**	**97.65**
Invasive lobular carcinoma	1	1.18
Medullary carcinoma	1	1.18

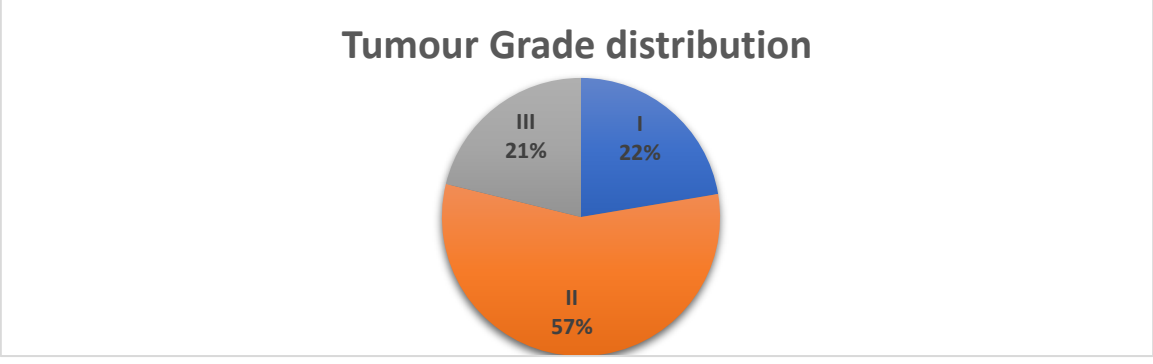
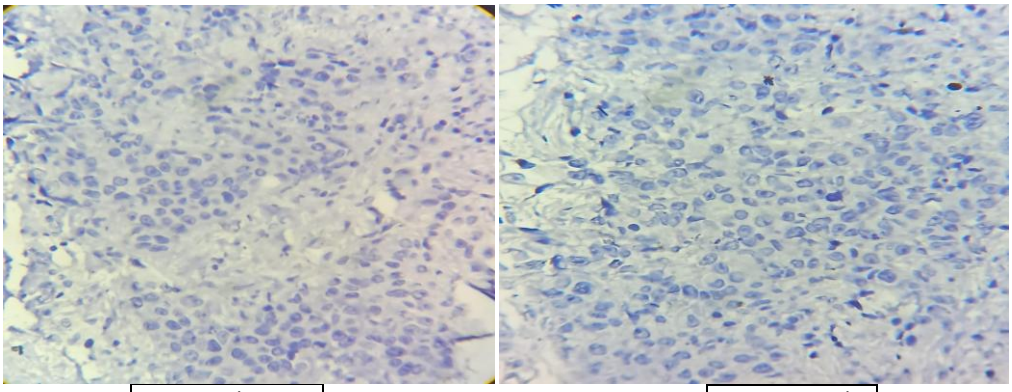


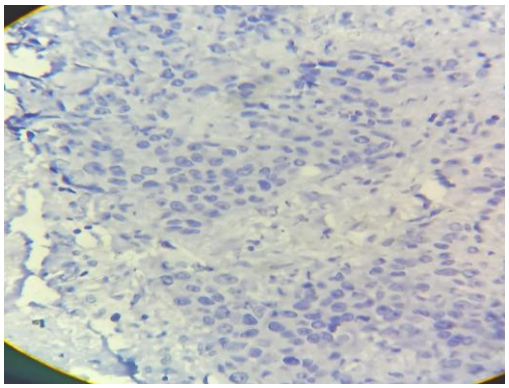
Figure1: Tumour Grade distribution

Table 2: Distribution of cases

Biological Markers	**% of cases**
ER & PR positive	49 %
HER2 positive	31 %
Ki 67 nuclear positivity of >14%	63.5 %
According to this phenotyping, the cases used in this study were classified as	
Luminal A	20 %
Luminal B	29.5 %
Her2 enriched	17.6 %
Triple negative	32.9 %

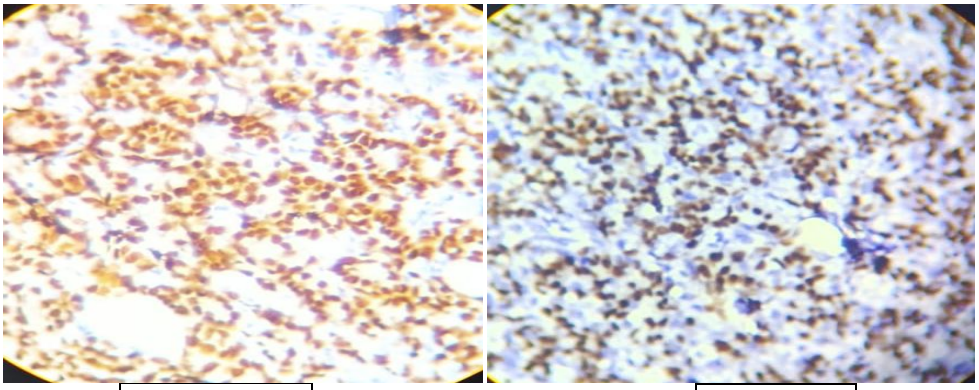


ER Negative PR Negative

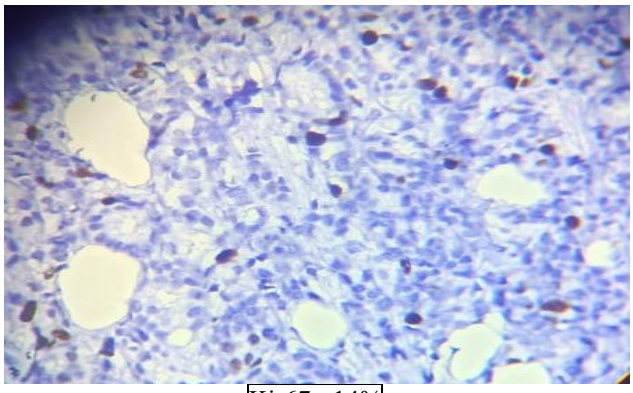


Her-2 neu Negative

Figure 2: Triple Negative subtype



ER Positive PR Positive



Ki-67 <14%

Figure 3: Luminal A subtype

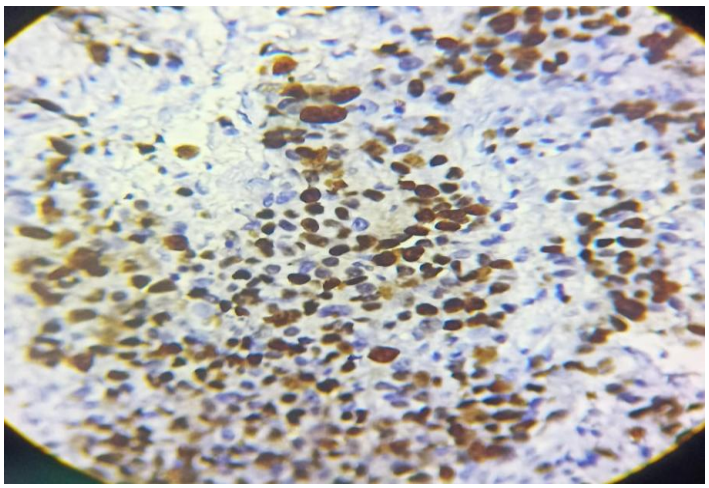


Figure 4: Ki67 >60%

Table 3: Ki67 as prognostic marker and its correlation with other prognostic marker

Chi square = 42.7, df=3
Cramer's V = **0.708**
The table shows that high Ki-67 (≥14%) was observed in 54 cases (63.5%), with 57.4% and 24.1% of these belonging to

	Ki67 <14%	**Ki67 ≥14%**	**P value**
Tumour Grade			0.453
I	9(47.4%)	10(52.6%)	
II	17(35.4%)	31(64.6%)	
III	5(27.8%)	13(72.2%)	
ER			0.227
Positive	18(42.9%)	24(57.1%)	
Negative	13(30.2%)	30(69.8%)	
PR			0.227
Positive	18(42.9%)	24(57.1%)	
Negative	13(30.2%)	30(69.8%)	
HER 2			0.225
Positive	7(26.9%)	19(73.1%)	
Negative	24(40.7%)	35(59.3%)	
Molecular subtype			**<0.001 (sig.)**
Her 2 Enriched			
Luminal A	6(40%)	9(60%)	
Luminal B	17(100%)	0	
Triple Negative	1(4%)	24(96%)	
	7(25%)	21(75%)	

Grades II and III, respectively. No significant association was found between the Ki-67 index and tumour grade or other prognostic markers (ER, PR, HER2).

Ki67 index is significantly associated with Molecular subtypes (Her 2 Enriched, Luminal A, Luminal B and Triple Negative) with Strong strength of association between the two (p= <0.001).

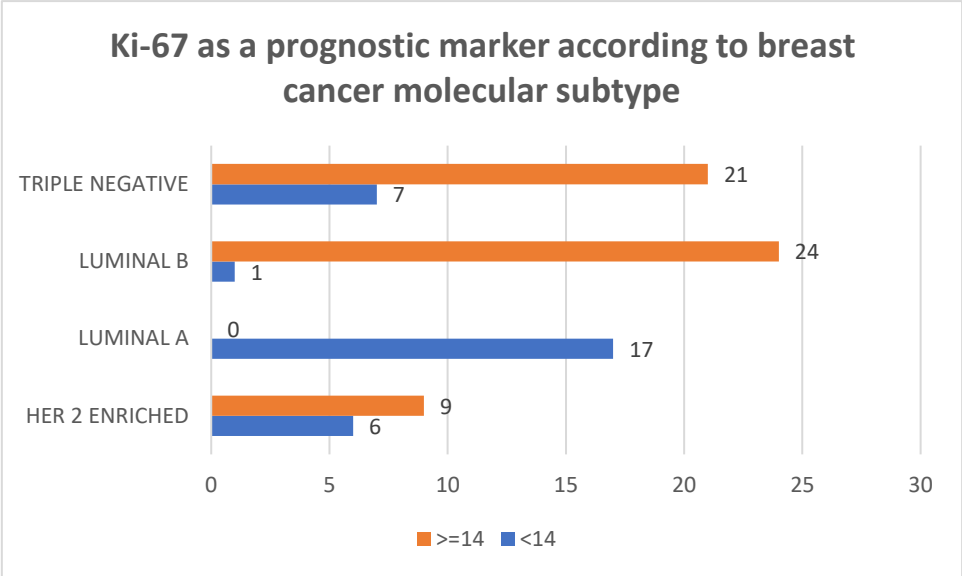


Figure 5: Ki-67 and molecular subtypes

Table 5: Characteristics of entire study with molecular subtypes and their association

Characteristics	Luminal A	Luminal B	Her2	Triple Negative	P
Age					0.83
<=50 years	9	13	7	17	
>50 years	8	12	8	11	
Tumour size, cm					
<2	0	0	0	1	0.38
≥2	10	11	11	18	
NA	7	14	4	9	
Lymph node					0.51
Positive	5	5	6	8	
Negative	1	1	4	6	
NA					
Grade					0.067
I	7	7	1	4	
II	9	15	8	16	
III	1	3	6	8	
Histological type					0.61
IDC	17	24	15	27	
ILC	0	1	0	0	
Medullary	0	0	0	1	
Ki67					**<0.0001**
<14%	17	1	6	7	
≥14%	0	24	9	21	
ER					**<0.0001**
Positive	17	25	0	O	
Negative	0	0	15	28	
PR					**<0.0001**
Positive	17	25	0	0	
Negative	0	0	15	28	
HER 2					**<0.0001**
Positive	0	11	15	0	
Negative	17	14	0	28	

- **Statistically significant association among molecular subtypes of cases with Ki 67 positivity, ER, PR And HER2 (<0.0001).**

- **No significant association was observed between molecular subtypes of the studied cases and age, tumour grade and histological subtype.**

Table 6: Molecular subtypes and Ki67

Molecular subtypes	Ki67 <14%	Ki67 >14%
Luminal A	100 %	0 %
Luminal B	4 %	96 %
Her2 Enriched	40 %	60 %
Triple Negative	25 %	75 %

DISCUSSION:

In our study, a cut-off of ≥14% was used to define Ki-67 positivity. A statistically significant association was observed between Ki-67 expression and molecular subtypes ($p \leq 0.001$), including ER, PR, and HER2 status (<0.0001). This finding is consistent with Al-Zawi et al. [23]

Higher Ki-67 indices were predominantly seen in Grade II tumors and in triple-negative and Luminal B subtypes. Similarly, Ahmed et al. [24] demonstrated higher Ki-67 indices in aggressive subtypes such as HER2-positive and triple-negative breast cancers, supporting the association of Ki-67 with tumour biology.

However, no statistically significant association was found between Ki-67 expression and tumor grade ($p = 0.453$), ER ($p = 0.227$), PR ($p = 0.227$), or HER2 ($p = 0.225$) in the present analysis.

In the current study according to scoring pattern (14%, ≥14%), majority of different tumour grades of breast tumour belonged to higher Ki-67 index. Majority of the different molecular subtypes had a higher Ki-67 PI, which was significantly higher in basal-like tumours.

Comparison of Present study with other studies:-

Ki-67 Expression Across Molecular Subtypes

1. **Triple-Negative Breast Cancer (TNBC):**
   Our study showed that 75% of TNBC cases had a Ki-67 index >14%, indicating high proliferative activity. This is consistent with findings by Hashmi et al. [25], who reported a mean Ki-67 of 50.9% ± 23.7%, and Soliman et al. [26] who observed Ki-67 >15% in 60% of TNBC cases, supporting the aggressive nature of this subtype.
2. **HER2-Positive Subtype:**
   Our study showed that 60% of HER2-positive cases had Ki-67 >14%, indicating high proliferative activity. This is consistent with Ahmed et al. [24], Hashmi et al. [25] and Soliman et al. [26]
3. **Luminal B Subtype:**
   Our study reveals that 96% of Luminal B breast cancer cases have a Ki-67 index greater than 14%, indicating high proliferative activity. This finding is consistent with Hashmi et al.[25] who reported a mean Ki-67 index of 34.9% ± 20.05% in Luminal B tumours, highlighting the higher proliferative potential of this subtype compared to Luminal A. Soliman et al. [26] did not provide specific data for Luminal B, their emphasis on Ki-67's role in assessing tumour aggressiveness supports our findings, underscoring the importance of Ki-67 in evaluating Luminal B breast cancer.
4. **Luminal A Subtype:**
   Our study found that all Luminal A breast cancer cases had a Ki-67 index less than 14%, indicating low proliferative activity. This finding is consistent with Hashmi et al. [25], who reported a mean Ki-67 index of 23.6% ± 19.7% in Luminal A tumours, suggesting relatively lower proliferative potential. However, Soliman et al. [26] noted variability in Ki-67 expression within Luminal A tumours, with 34% of cases having Ki-67 levels greater than 15%. These findings highlight the generally indolent nature of Luminal A breast cancer, but also suggest potential heterogeneity within this subtype.

Association with Prognostic Parameters

Our study demonstrated high Ki-67 expression across various tumour grades and molecular subtypes; however, no statistically significant association was observed with tumour grade. This is in concordance with Ahmed et al. [24] who similarly found no significant association between Ki-67 expression and tumour size or lymph node status. In contrast, Hashmi et al. [25] and Soliman et al. [26] reported a positive correlation between high Ki-67 and higher tumour grade, though without independent prognostic significance.

Age and Breast Cancer Subtypes

Our study found that younger patients were more likely to have TNBC, consistent with Hashmi et al. [25], who reported higher Ki-67 in younger patients, especially in TNBC and Luminal B subtypes. Soliman et al. [26] did not specifically address the relationship between age and Ki-67 expression but emphasized the importance of Ki-67 in assessing tumour aggressiveness.

Limitations in Establishing Prognostic Significance of Ki-67 Index

Our study observed that the Ki-67 index lacked independent prognostic significance, likely due to its limited association with other prognostic factors and the absence of follow-up data. Similarly, Hashmi et al. [25] and Soliman et al. [26] acknowledged the limitations of their studies due to the lack of long-term follow-up data on recurrence and survival outcomes.

Conclusion:

In conclusion, Ki-67 is a useful biomarker reflecting tumor aggressiveness and molecular subtypes in breast cancer. However, it did not show independent prognostic significance in our study, likely due to limited follow-up. Larger studies with long-term follow up are needed to better define its role in predicting outcomes such as tumour recurrence and disease-free survival, and guiding clinical practice.

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