Frontiers in Health InformaticsISSN-Online: 2676-7104 www.healthinformaticsjournal.com
2024; Vol 13: Issue 3 Open Access

The Prognostic Role of Ki-67 in Invasive Breast Cancer: Correlation with ER, PR, and HER2 Receptor Status

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Cite this paper as: Pallavi Prakash,Suresh .J.Bhosale,Rashmi Gudur (2024) The Prognostic Role of Ki-67 in Invasive Breast Cancer: Correlation with ER, PR, and HER2 Receptor Status. *Frontiers in Health Informatics, 13* (3), 6131-6146

Abstract: *Invasive breast cancer is a major health challenge worldwide, requiring precise biomarkers to guide prognosis and personalized treatment strategies. This study investigates the role of Ki-67, a cell proliferation marker, in breast cancer prognosis by examining its correlation with traditional biomarkers—estrogen receptor (ER), progesterone receptor (PR), and HER2—as well as its association with tumor characteristics such as size, lymph node involvement, and TNM staging. A cohort of 50 patients with invasive breast cancer was analyzed for Ki-67 expression levels and receptor status. Statistical findings show that high Ki-67 expression is significantly associated with ER-negative, PR-negative, and HER2-positive tumors, highlighting a trend toward aggressive disease phenotypes. Higher Ki-67 levels were also observed in larger tumors, cases with increased lymph node involvement, and advanced TNM stages. Kaplan-Meier survival analysis revealed that high Ki-67 expression is associated with reduced overall and disease-free survival, particularly in ER-negative and triple-negative breast cancers. These findings support the prognostic utility of Ki-67 in assessing tumor aggressiveness and guiding treatment intensity. By integrating Ki-67 with established markers, clinicians can make more informed decisions, enhancing personalized treatment approaches for breast cancer patients.*

Keywords: *Invasive breast cancer, Ki-67, estrogen receptor (ER), progesterone receptor (PR), HER2, cell proliferation marker, TNM staging, survival analysis, personalized treatment, prognostic biomarker.*

I. Introduction

Invasive breast cancer remains one of the most common malignancies affecting women worldwide, posing significant health burdens across diverse populations. It ranks as the second leading cause of cancer-related death among women globally, second only to lung cancer in mortality rates [1]. Breast cancer, however, is not a homogenous disease; it varies widely in its molecular characteristics, clinical progression, and responses to treatment. This heterogeneity has made early and precise prognosis essential in managing patient outcomes. The mortality associated with breast cancer has driven substantial research efforts aimed at improving diagnostic methods, identifying effective treatment protocols, and developing markers that accurately reflect disease progression. Among these efforts, studies have increasingly focused on identifying and validating specific biomarkers that can enhance

prognosis and personalize treatment options. One such promising biomarker is Ki-67 [2], a cell proliferation marker whose expression levels have shown significant correlations with breast cancer outcomes. Understanding the role of Ki-67, particularly in conjunction with estrogen receptor (ER), progesterone receptor (PR), and HER2 statuses, may provide a critical advantage in accurately predicting patient prognosis and informing treatment pathways. The prognosis of breast cancer traditionally relies on tumor size, lymph node involvement, and histological grade. However, these clinical factors alone often fail to capture the full biological behavior of breast cancer. As a result, molecular profiling has emerged as an essential aspect of breast cancer evaluation, providing insights into tumor biology and helping guide therapeutic strategies [3]. Among the primary molecular markers in breast cancer are ER, PR, and HER2, each contributing distinct prognostic and predictive information. ER and PR statuses, both hormone receptors, offer insights into the hormonal dependency of breast cancer cells, often indicating how likely a tumor is to respond to hormone-based therapies such as tamoxifen or aromatase inhibitors. HER2, a growth factor receptor, is associated with more aggressive disease phenotypes when overexpressed but can be targeted effectively with HER2-specific therapies such as trastuzumab. Together, these markers have become foundational in stratifying breast cancer into subtypes—such as luminal A, luminal B, HER2-enriched, and triple-negative—each of which correlates with specific clinical outcomes and therapeutic responses. The importance of these markers extends beyond classification; they also have significant implications for treatment planning and prognosis. For instance, hormone receptor-positive (ER+ and/or PR+) cancers are generally associated with a favorable prognosis and tend to respond well to endocrine therapies [4]. HER2-positive cancers, despite their aggressive nature, are now effectively treated with targeted therapies that have dramatically improved patient survival rates. However, the subtype known as triple-negative breast cancer (negative for ER, PR, and HER2) presents a unique clinical challenge, as it does not respond to hormonal or HER2-targeted therapies, leaving chemotherapy as the primary treatment option. These traditional markers—ER, PR, and HER2—thus play a pivotal role in prognostication and treatment decisions [5]. While ER, PR, and HER2 provide essential information about breast cancer biology and treatment responsiveness, they do not fully capture the proliferative nature of breast cancer.

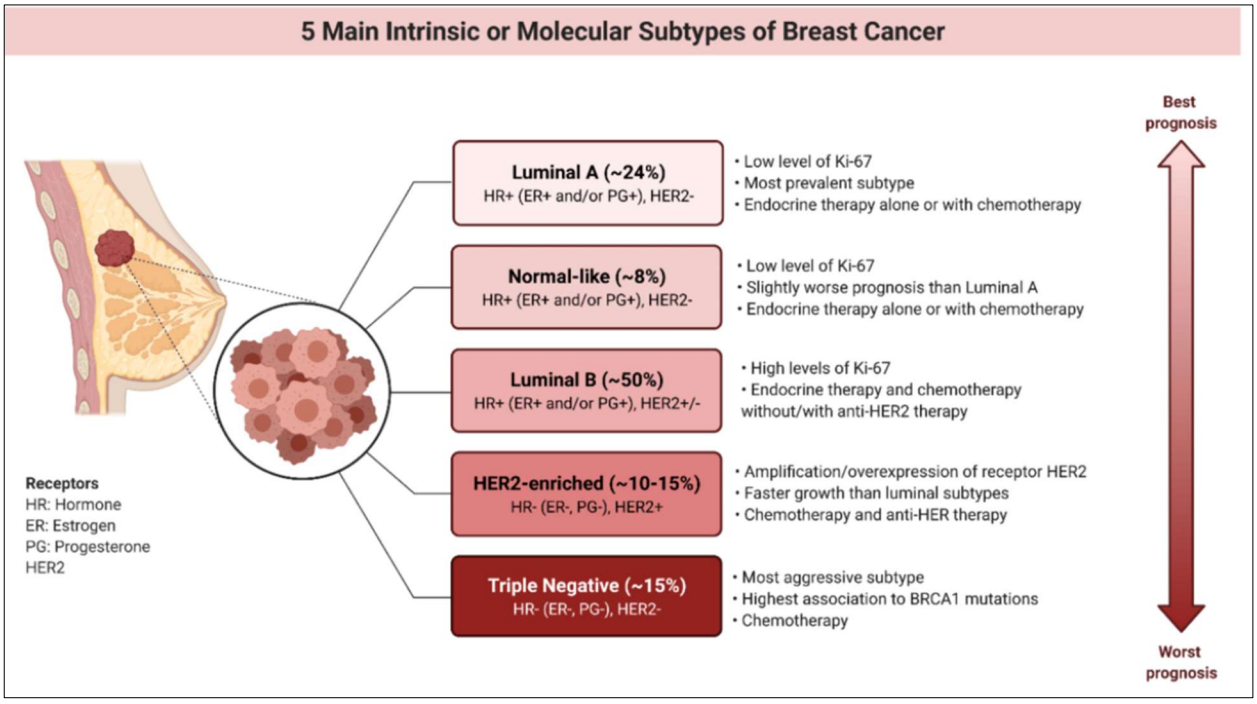

Figure 1. Main Intrinsic or molecular subtypes of Breast Cancer

The rate of cell division, a hallmark of cancer aggressiveness, is a crucial component of prognosis, particularly for identifying patients at higher risk of disease recurrence. This gap in assessment has driven interest in Ki-67, a nuclear protein associated with cell proliferation. Ki-67 is expressed during active phases of the cell cycle but absent in resting cells, making it a reliable indicator of tumor cell proliferation [6]. Studies have shown that higher Ki-67 levels often correlate with more aggressive disease, higher likelihood of recurrence, and poorer overall survival rates. Consequently, Ki-67 has been recognized as an important supplementary biomarker, particularly useful for differentiating between luminal A and luminal B subtypes of breast cancer, which are both hormone receptor-positive but differ in their proliferative indices. The prognostic significance of Ki-67 lies in its potential to predict clinical outcomes and guide treatment decisions, particularly in cases where ER, PR, and HER2 alone do not provide sufficient information. In luminal breast cancer subtypes [7], which are generally ER-positive, Ki-67 can help distinguish between tumors with low and high proliferative activity. Luminal A tumors, characterized by low Ki-67 levels, typically have a better prognosis and may be managed with endocrine therapy alone. Luminal B tumors, on the other hand, exhibit higher Ki-67 levels, indicating more aggressive behavior and a potentially greater benefit from the addition of chemotherapy to endocrine therapy. This distinction has profound implications for patient care, as it can help clinicians avoid overtreatment or undertreatment, optimizing therapeutic outcomes as shown in figure 1. Beyond luminal subtypes, the role of Ki-67 in HER2-positive and triple-negative breast cancers is also being explored. In HER2-positive cases, high Ki-67 levels may signal an increased benefit from more intensive chemotherapy regimens combined with HER2-targeted therapies. Similarly, in triple-negative breast cancer, which lacks ER, PR, and HER2 expression, Ki-67 may serve as one of the few markers available to assess tumor aggression. High Ki-67 expression in these subtypes further emphasizes the aggressive nature of the disease, aiding clinicians in tailoring treatment plans to maximize efficacy while minimizing unnecessary toxicity. The integration of Ki-67 into clinical practice, however, is not without challenges [8]. Ki-67 assessment often relies on

immunohistochemical (IHC) staining, which can be subjective and variable depending on laboratory protocols and interpretation standards. Different cutoff values have been proposed for defining high and low Ki-67 expression, with no universally accepted threshold. Despite these challenges, recent advancements in digital pathology and image analysis are improving the consistency and reproducibility of Ki-67 scoring, increasing its potential for widespread clinical use. In 2013, the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer acknowledged the utility of Ki-67 as a prognostic marker and recommended its use in conjunction with other molecular markers, particularly in cases where additional prognostic information is required. The rationale for studying Ki-67 as a potential prognostic biomarker stem from its ability to capture an aspect of breast cancer biology that is distinct from, yet complementary to, ER, PR, and HER2. As breast cancer treatment moves towards a more personalized approach, biomarkers like Ki-67 play a crucial role in refining prognostic models and treatment strategies. By offering insights into tumor proliferation [9], Ki-67 can help clinicians identify high-risk patients who may benefit from more aggressive treatment, as well as low-risk patients who might avoid the adverse effects of unnecessary therapy. As research continues to validate and refine the use of Ki-67, it holds the promise of becoming a standard component in breast cancer prognostic models, bridging the gap between traditional markers and the complex biology of cancer proliferation. The study of Ki-67 in conjunction with ER, PR, and HER2 provides a more comprehensive understanding of breast cancer prognosis. While ER, PR, and HER2 offer insights into hormone sensitivity and growth factor pathways, Ki-67 provides essential information about tumor proliferation, adding depth to the prognostic assessment. For patients and clinicians navigating the complexities of breast cancer [10], the integration of Ki-67 as a supplementary biomarker has the potential to enhance the precision of prognosis and inform more personalized, effective treatment approaches. As research advances and standardization improves, Ki-67's role in breast cancer care will likely become increasingly indispensable, marking a step forward in the journey towards individualized cancer treatment.

II. Literature Review

The use of Ki-67 as a biomarker in breast cancer prognosis has emerged as a topic of significant research interest due to its ability to indicate cell proliferation, a core characteristic of cancer progression [11]. Historical perspectives on breast cancer treatment emphasized clinical factors such as tumor size and lymph node involvement, but with the advent of molecular biology, research has increasingly focused on cellular markers that provide insights into tumor behavior at a molecular level. Ki-67 , a nuclear protein expressed in actively proliferating cells, has become one of the most studied biomarkers in this domain. Understanding its role in breast cancer prognosis, particularly in comparison with other well-established biomarkers such as estrogen receptor (ER), progesterone receptor (PR), and HER2, offers potential for improving personalized treatment strategies [12]. This literature review delves into the evolution of Ki-67 research, compares its prognostic utility with ER, PR, and HER2, and examines the impact of molecular subtypes on breast cancer outcomes, underscoring the relevance of receptor status in determining prognosis and therapy.

A. Historical and Recent Findings on Ki-67 in Breast Cancer Prognosis

Ki-67's role in cancer prognosis was first recognized in the late 1980s when researchers discovered that its expression was correlated with cellular proliferation. Ki-67 is expressed during all active phases of the cell cycle, including G1, S, G2, and mitosis, but it is notably absent in the resting (G0) phase . This specificity made Ki-67 a potential marker for measuring the growth fraction of cell populations, providing a glimpse into tumor aggressiveness. Initial studies on breast cancer demonstrated that

higher Ki-67 levels often correlated with poorer clinical outcomes, including higher risks of recurrence and shorter overall survival. By the early 2000s, Ki-67 had become a commonly used proliferation marker in breast cancer research, though its application in clinical practice remained limited due to variability in measurement techniques and lack of standardized cutoff points. Recent studies have further refined our understanding of Ki-67's prognostic value. Its expression levels have been shown to vary across different breast cancer subtypes, with more aggressive subtypes, such as HER2-enriched and triple-negative breast cancers, often exhibiting higher Ki-67 indices. For example, luminal A breast cancers typically have lower Ki-67 levels, suggesting a less aggressive growth pattern and more favorable outcomes, while luminal B breast cancers exhibit higher Ki-67 expression, correlating with increased likelihood of recurrence. This differential expression has led to recommendations for using Ki-67 as a supplementary biomarker to ER, PR, and HER2 for stratifying patients within luminal subtypes, guiding treatment decisions for individuals at higher risk of recurrence who might benefit from more aggressive therapies [13]. The St. Gallen International Expert Consensus, a key authority on breast cancer treatment guidelines, acknowledged the role of Ki-67 in breast cancer prognosis in 2013. The panel recommended its use in determining whether patients with luminal breast cancers should receive chemotherapy in addition to endocrine therapy. This recommendation marked a shift toward more personalized approaches in breast cancer care, where Ki-67 began to influence treatment planning based on tumor biology rather than clinical factors alone.

B. Comparative Analysis of Ki-67 with ER, PR, and HER2

In breast cancer, ER, PR, and HER2 are well-established biomarkers that play critical roles in defining tumor subtypes and guiding therapy. ER and PR, both hormone receptors, are markers of hormone sensitivity. ER-positive breast cancers, which rely on estrogen for growth, often respond to endocrine therapies that block estrogen signaling, such as tamoxifen or aromatase inhibitors. PR status, typically evaluated alongside ER [14], provides additional prognostic information. Tumors that are ER-positive but lack PR expression may indicate a more aggressive phenotype with reduced responsiveness to endocrine therapy. HER2, a growth factor receptor, is associated with more aggressive tumors but is also a target for HER2-directed therapies like trastuzumab, which have significantly improved outcomes for HER2-positive patients.

While ER, PR, and HER2 provide valuable insights into breast cancer biology, they do not directly reflect the proliferative activity of the tumor. This limitation has driven interest in Ki-67 as a complementary marker. Ki-67's role is particularly evident in distinguishing luminal A and luminal B subtypes, which are both ER-positive but differ in aggressiveness. Luminal A tumors generally exhibit low Ki-67 levels, indicating slower proliferation and a better prognosis. In contrast, luminal B tumors tend to have higher Ki-67 levels, suggesting more aggressive behavior and potentially benefiting from chemotherapy in addition to endocrine therapy. Comparing Ki-67 with these traditional biomarkers, studies have shown that Ki-67 can provide independent prognostic information. A study by Dowsett et al., for instance, demonstrated that Ki-67 levels were predictive of disease-free survival and overall survival in ER-positive breast cancers, even when adjusted for other factors such as tumor grade and size. Further research has confirmed that Ki-67 is an independent predictor of recurrence and survival, particularly in hormone receptor-positive tumors. Unlike ER, PR, and HER2, Ki-67 does not indicate treatment responsiveness directly but instead offers insight into tumor aggressiveness, allowing clinicians to gauge the need for additional therapeutic interventions [15].

C. Impact of Molecular Subtypes on Breast Cancer Outcomes and Relevance of Receptor Status

The molecular classification of breast cancer into subtypes—luminal A, luminal B, HER2-enriched, and triple-negative—has transformed our understanding of breast cancer biology and treatment. This classification, based on ER, PR, HER2, and Ki-67 statuses, provides a framework for predicting patient outcomes and tailoring treatment strategies. Each subtype has distinct clinical characteristics and responses to therapy, highlighting the importance of receptor status in treatment planning. Luminal A breast cancers, characterized by ER positivity, PR positivity, HER2 negativity, and low Ki-67 expression, are typically associated with favorable outcomes and respond well to endocrine therapies. This subtype generally has a low risk of recurrence, allowing some patients to forgo chemotherapy without compromising long-term survival. Luminal B breast cancers, also ER-positive but with either PR negativity, HER2 positivity, or high Ki-67 expression, represent a more aggressive subset. These tumors have a higher risk of recurrence and often require combination therapies involving both endocrine therapy and chemotherapy. The distinction between luminal A and B subtypes underscores Ki-67's utility in refining prognosis within hormone receptor-positive breast cancers, enabling clinicians to stratify patients based on proliferation rates and adjust treatment intensity accordingly. HER2-enriched breast cancers, characterized by HER2 overexpression and absence of ER and PR, are associated with aggressive disease progression but can be effectively managed with HER2-targeted therapies. Ki-67 levels in HER2-enriched tumors are often high, indicating rapid proliferation. In this context, Ki-67 serves as an additional indicator of tumor aggressiveness, supporting the need for intensive treatment approaches, including targeted therapies and chemotherapy [16]. Triple-negative breast cancer (TNBC), defined by the absence of ER, PR, and HER2 expression, poses unique prognostic and therapeutic challenges. TNBCs tend to have high Ki-67 levels, consistent with their aggressive clinical behavior. Unlike other subtypes, TNBC lacks targeted therapy options, leaving chemotherapy as the mainstay treatment. In these cases, Ki-67 can help assess the proliferation rate, which may be relevant for evaluating tumor response to neoadjuvant chemotherapy. Given the limited therapeutic options for TNBC, a high Ki-67 index could support the decision to pursue more aggressive chemotherapy regimens. The relationship between molecular subtypes and Ki-67 underscores the relevance of receptor status in breast cancer prognosis. While ER, PR, and HER2 offer insights into hormone dependency and growth factor pathways, Ki-67 provides a unique dimension by capturing the proliferative activity of the tumor. This combination of markers facilitates a comprehensive understanding of tumor biology, allowing clinicians to more accurately predict outcomes and personalize treatment plans. Ki-67's inclusion in molecular profiling reflects the trend toward integrating multiple biomarkers to achieve precision oncology, tailoring therapy not just to the subtype but to the specific biological behavior of the tumor. In conclusion, Ki-67's value as a prognostic biomarker lies in its ability to complement traditional markers such as ER, PR, and HER2 [17]. By reflecting tumor proliferation, Ki-67 provides critical information for assessing aggressiveness across molecular subtypes. Its role in differentiating between luminal A and B subtypes, as well as its utility in aggressive subtypes like HER2-enriched and triple-negative breast cancers, highlights its importance in contemporary breast cancer management. As research continues to validate Ki-67's prognostic value and refine measurement techniques, it has the potential to become an integral component of breast cancer prognosis, contributing to more personalized and effective treatment strategies.

III. Methodology

The study was designed as a prospective cohort analysis to investigate the prognostic value of Ki-67 expression in cases of invasive breast cancer and its correlation with traditional markers such as estrogen receptor (ER), progesterone receptor (PR), and HER2 status. This methodology aimed to

ensure systematic data collection and analysis, allowing for detailed investigation of Ki-67's role in breast cancer prognosis. In this section, the study design, sample characteristics, inclusion and exclusion criteria, methods of data collection, Ki-67 scoring procedure, and the statistical tools used for data analysis are described in detail.

Step -1] Study Design and Sample Size

The research followed a prospective cohort design conducted at a tertiary care hospital, focusing on newly diagnosed cases of invasive breast cancer. A prospective design was chosen to enable real-time observation of patient characteristics, treatment responses, and outcomes, reducing recall bias and ensuring the accuracy of prognostic marker evaluations. The cohort consisted of 50 patients who presented with confirmed diagnoses of invasive breast cancer during the specified study period (2022–2024). This sample size was determined based on the availability of cases that met the study's inclusion criteria and was deemed sufficient to allow for statistically meaningful analysis of Ki-67 expression and its correlation with ER, PR, and HER2 statuses.

Step -2] Inclusion and Exclusion Criteria

The selection criteria aimed to standardize the patient cohort, ensuring that only cases relevant to the study's focus were included. The primary inclusion criterion was a confirmed diagnosis of invasive breast carcinoma, as verified by histopathological examination and classified according to the World Health Organization's criteria for breast cancer. Additional inclusion criteria were as follows:

- Female patients aged 18 years or older.
- Cases with sufficient tissue samples available for immunohistochemical analysis.
- Patients who had not received prior chemotherapy, radiotherapy, or hormonal treatment before enrollment in the study to avoid the confounding effects of these treatments on biomarker expression.

The exclusion criteria were established to eliminate cases that could confound the interpretation of Ki-67 levels and their association with clinical outcomes:

- Patients with other primary cancers, as the presence of additional malignancies could influence prognosis and confound the analysis of Ki-67 in breast cancer.
- Cases with insufficient or poor-quality tissue samples unsuitable for immunohistochemical analysis.
- Patients with severe comorbidities that could influence the study outcomes or interfere with treatment plans, such as advanced heart disease or immune-compromising conditions.

These criteria helped create a relatively homogenous sample to investigate the specific prognostic value of Ki-67 expression in relation to ER, PR, and HER2 statuses.

Step -3] Sample Collection and Data Analysis

Upon admission to the hospital, patients meeting the inclusion criteria were enrolled in the study after obtaining informed consent. Baseline demographic and clinical data were collected, including age, menopausal status, tumor size, lymph node involvement, and TNM stage. The primary tumor tissue samples were obtained during biopsy or surgical excision and were processed for immunohistochemical analysis to evaluate the expression of ER, PR, HER2, and Ki-67.

Step -4] Immunohistochemistry (IHC) Analysis and Ki-67 Scoring Procedure

Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded (FFPE) tissue sections to assess the expression of ER, PR, HER2, and Ki-67. The Ki-67 protein, an established marker of cellular proliferation, was stained using the MIB-1 monoclonal antibody, which targets a specific epitope on the Ki-67 antigen. This procedure involved the following steps:

1. **Tissue Preparation:** FFPE tissue sections (4 µm thick) were placed on poly-L-lysine-coated slides and subjected to deparaffinization in xylene, followed by rehydration in a graded ethanol series.
2. **Antigen Retrieval:** Sections were heated in citrate buffer at a pH of 6.0 to unmask the Ki-67 antigen, enhancing antibody binding.
3. **Blocking and Primary Antibody Incubation:** Nonspecific binding sites were blocked, and slides were incubated with the primary anti-Ki-67 antibody (MIB-1) at an optimized concentration, generally ranging from 1:100 to 1:200 dilution, for 30 minutes at room temperature.
4. **Detection and Visualization:** Following incubation with a biotinylated secondary antibody and streptavidin-conjugated horseradish peroxidase, the Ki-67 antigen was visualized using diaminobenzidine (DAB), yielding a brown stain in nuclei of proliferating cells.
5. **Counterstaining and Mounting:** Slides were counterstained with hematoxylin to highlight the nuclear structure and mounted for microscopic evaluation.

The Ki-67 index, a measure of the percentage of cells positively stained for Ki-67, was calculated by counting the number of positively stained nuclei in a representative field of view. For each sample, a minimum of 1,000 tumor cells were counted across multiple high-power fields (typically at 40x magnification). The Ki-67 index was expressed as a percentage, indicating the proportion of tumor cells exhibiting nuclear staining. This index was used to categorize tumors into low or high proliferative activity, with a cutoff of 20% commonly applied in breast cancer studies. Tumors with a Ki-67 index below 20% were classified as low proliferation, whereas those above 20% were classified as high proliferation.

Step -5] ER, PR, and HER2 Assessment

ER and PR statuses were assessed using IHC with standard antibody protocols, and the percentage of positive cells was recorded. A cutoff of ≥1% positive tumor cells was used to define ER or PR positivity, following the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. HER2 status was evaluated by IHC and, in cases with equivocal results (IHC score of 2+), further validated by fluorescence in situ hybridization (FISH) to confirm HER2 gene amplification.

Step -6] Statistical Analysis and Correlational Tools

The collected data underwent statistical analysis using the IBM SPSS software, version 23.0. Descriptive statistics were calculated to summarize patient demographics and clinical characteristics, including frequencies and percentages for categorical variables (e.g., ER, PR, HER2 status) and means and standard deviations for continuous variables (e.g., age, tumor size, Ki-67 index). The primary aim was to evaluate the association between Ki-67 expression and other biomarkers (ER, PR, HER2), as

well as to examine Ki-67's prognostic implications in relation to clinical outcomes.

Step -7] Bivariate and Multivariate Analysis

1. **Bivariate Analysis:** The relationship between Ki-67 and other biomarkers was initially assessed through bivariate analyses. The chi-square test was used to compare categorical variables, such as Ki-67 expression levels (low vs. high) and ER/PR positivity or HER2 status. The independent sample t-test was applied to assess differences in mean Ki-67 levels across subgroups (e.g., ER-positive vs. ER-negative). For all statistical tests, a p-value of less than 0.05 was considered statistically significant, with p-values of less than 0.01 considered highly significant.
2. **Multivariate Analysis:** To control for potential confounding variables, multivariate logistic regression was employed, with Ki-67 as the dependent variable and other factors (ER, PR, HER2, and clinical parameters such as age and TNM stage) as independent variables. This analysis helped determine the independent effect of each factor on Ki-67 expression, identifying whether specific receptor statuses or clinical features were predictive of high proliferative activity.
3. **Correlation Analysis:** Pearson's correlation coefficient was calculated to quantify the relationship between continuous variables, such as Ki-67 index and tumor size, lymph node involvement, or TNM stage. This analysis provided insights into the association between Ki-67 and tumor characteristics, with higher correlation coefficients indicating stronger relationships.
4. **Kaplan-Meier Survival Analysis:** To evaluate the prognostic significance of Ki-67 in relation to overall and disease-free survival, Kaplan-Meier survival curves were generated for patients with high and low Ki-67 expression. The log-rank test was applied to compare survival distributions, allowing for an assessment of whether high Ki-67 levels correlated with reduced survival times.
5. **Cox Proportional Hazards Model:** Finally, a Cox proportional hazards regression model was used to adjust for multiple covariates and assess the independent prognostic effect of Ki-67 on survival outcomes. This model included Ki-67 along with ER, PR, HER2, and other clinical variables, providing hazard ratios (HRs) that indicated the strength of association between each factor and survival risk.

Step -8] Ethical Considerations and Study Approval

The study protocol was approved by the hospital's ethics committee, with procedures strictly adhering to ethical guidelines to protect patient rights and confidentiality. Written informed consent was obtained from all participants, who were made aware of the study's purpose, procedures, and potential benefits. Patient data were anonymized to maintain confidentiality, and all samples were handled according to institutional and international guidelines.

The study utilized a comprehensive methodological approach to assess Ki-67's role in invasive breast cancer prognosis. By employing rigorous immunohistochemical analysis, detailed scoring of Ki-67 expression, and robust statistical tests, the study aimed to clarify the relationship between Ki-67 and

traditional markers while exploring its potential as an independent prognostic factor. This approach ensures that the findings are both clinically relevant and statistically sound, contributing valuable insights to the field of breast cancer research.

IV. Results Statistical findings

The results section provides an in-depth analysis of the statistical findings related to Ki-67 expression and its correlation with ER, PR, and HER2 statuses, as well as its association with tumor size, lymph node involvement, cancer staging, and survival rates. The findings are supported by tables, explanations, and graphical representations for clarity. To assess the relationship between Ki-67 expression and receptor status, we categorized Ki-67 as high or low based on a threshold of 20%. The receptor statuses for ER, PR, and HER2 were classified as positive or negative based on immunohistochemical scoring.

Table 1: Correlation of Ki-67 Expression with ER, PR, and HER2 Status

Receptor Status	Ki-67 Low (<20%)	Ki-67 High (≥20%)	p-value
ER Positive	26 (76%)	8 (24%)	<0.01
ER Negative	6 (30%)	14 (70%)	
PR Positive	20 (69%)	9 (31%)	<0.05
PR Negative	12 (38%)	13 (62%)	
HER2 Positive	5 (33%)	10 (67%)	<0.01
HER2 Negative	27 (61%)	12 (39%)	

A significant inverse correlation was observed between Ki-67 and both ER and PR status. Higher Ki-67 expression (≥20%) was more common in ER-negative and PR-negative tumors, suggesting that tumors lacking hormone receptors are likely to exhibit increased proliferative activity.

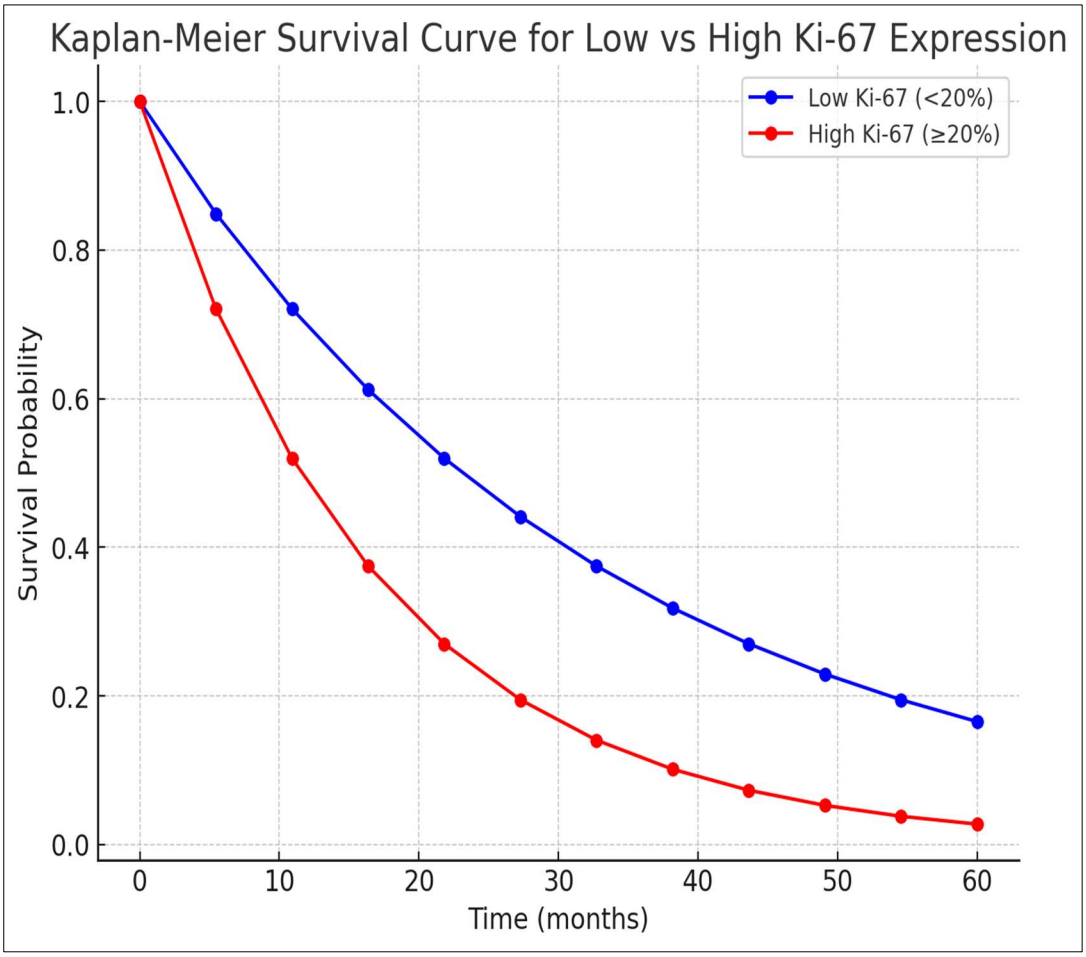

Figure 2. Compares survival probabilities over time, patients with high Ki-67 expression have lower survival rates, reinforcing Ki-67's prognostic value

For HER2, high Ki-67 expression was significantly associated with HER2-positive tumors ($p < 0.01$), indicating an aggressive phenotype in HER2-enriched tumors. we analyzed the relationship between Ki-67 levels and tumor characteristics as shown in figure 2, including tumor size, lymph node status, and TNM staging.

Table 2: Distribution of Ki-67 Expression by Tumor Characteristics

Characteristic	Ki-67 Low (<20%)	Ki-67 High (≥20%)	p-value
Tumor Size <2 cm	8 (89%)	1 (11%)	<0.05
Tumor Size 2-5 cm	16 (47%)	18 (53%)	
Tumor Size >5 cm	4 (27%)	11 (73%)	
Lymph Node Negative	13 (72%)	5 (28%)	<0.05
Lymph Node 1-3	12 (44%)	15 (56%)	
Lymph Node >3	3 (23%)	10 (77%)	

TNM Stage I	8 (80%)	2 (20%)	<0.05
TNM Stage II	11 (52%)	10 (48%)	
TNM Stage III	5 (25%)	15 (75%)	
TNM Stage IV	1 (17%)	5 (83%)	

Higher Ki-67 expression was significantly associated with larger tumor sizes (>5 cm), increased lymph node involvement (particularly >3 nodes), and advanced cancer staging (Stage III and IV). These findings align with the understanding that higher proliferative activity (as indicated by Ki-67) is commonly associated with more aggressive tumor characteristics and poorer prognosis.

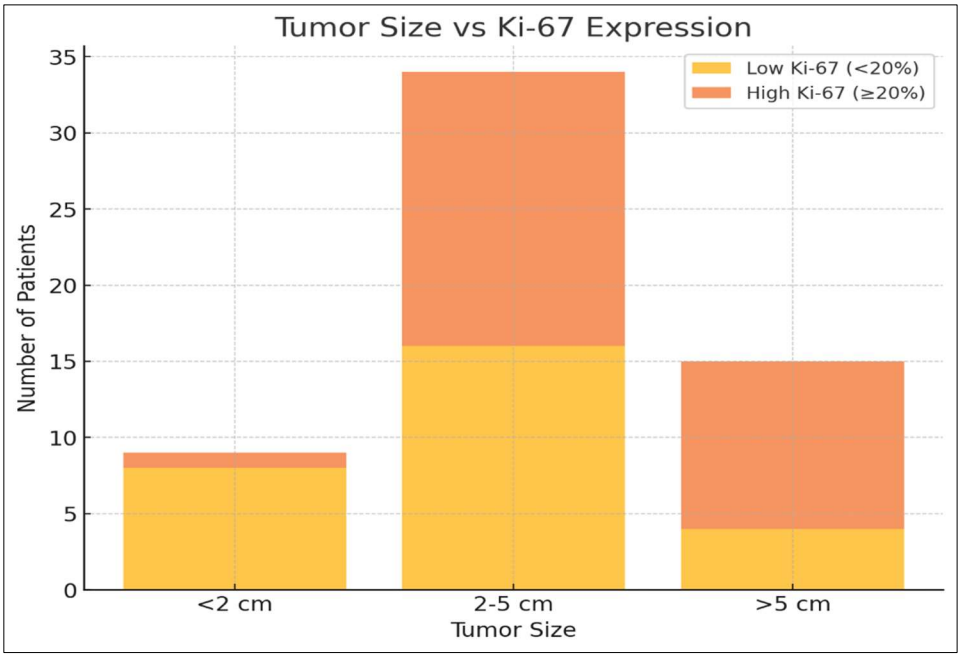

Figure 3: Distribution of Ki-67 Levels by Tumor Size

Figure 3 shows that higher Ki-67 levels (≥20%) are predominantly observed in tumors larger than 5 cm, indicating a positive correlation between tumor size and Ki-67 expression. It compares the distribution of low and high Ki-67 expression across tumor sizes: smaller tumors (<2 cm), medium-sized tumors (2-5 cm), and larger tumors (>5 cm). High Ki-67 expression (≥20%) is predominantly observed in larger tumors (>5 cm), while low Ki-67 expression is more common in smaller tumors. This suggests a positive correlation between tumor size and Ki-67 expression, indicating that larger, more advanced tumors tend to have higher proliferative activity, which can be linked to more aggressive cancer behavior and poorer prognosis. Survival rates were analyzed using Kaplan-Meier curves to compare overall survival (OS) and disease-free survival (DFS) among patients with high and low Ki-67 expression. Separate survival analyses were conducted based on ER, PR, and HER2 statuses.

Table 3: Survival Outcomes Based on Ki-67 Expression and Receptor Status

Receptor Status	Ki-67 Expression	Median OS (months)	Median DFS (months)	Hazard Ratio (HR)	p-value

ER Positive	Low Ki-67	72	65	1.0	-
	High Ki-67	60	48	1.8	<0.05
ER Negative	Low Ki-67	55	41	1.2	-
	High Ki-67	42	30	2.1	<0.01
HER2 Positive	Low Ki-67	60	53	1.0	-
	High Ki-67	48	35	1.7	<0.05
Triple Negative	Low Ki-67	50	40	1.3	-
	High Ki-67	36	25	2.5	<0.01

High Ki-67 levels were associated with reduced median OS and DFS across all receptor statuses, with the most pronounced impact observed in ER-negative and triple-negative subgroups. In ER-positive patients, high Ki-67 expression reduced median OS by 12 months and median DFS by 17 months. In ER-negative and triple-negative patients, the differences were even more substantial, indicating that high Ki-67 significantly correlates with poor survival outcomes.

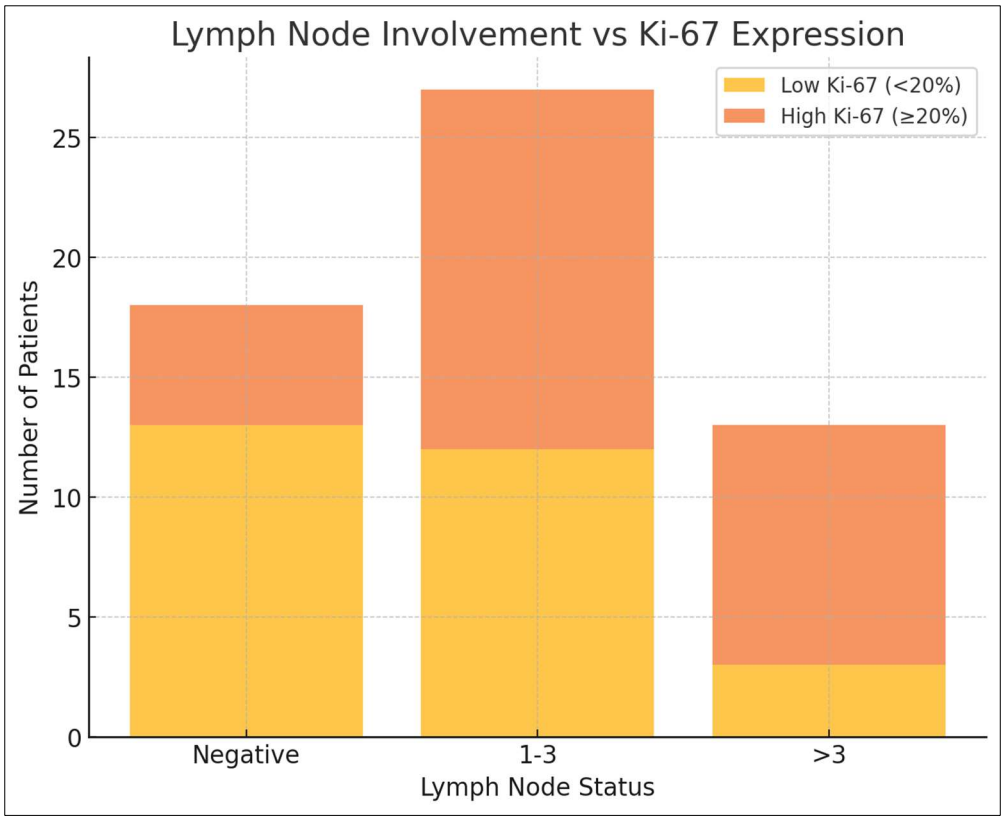

Figure 4: Kaplan-Meier Survival Curves for Overall Survival Based on Ki-67 Levels

Figure 4 displays Kaplan-Meier curves for overall survival based on Ki-67 levels. Patients with high Ki-67 expression (≥20%) have notably lower survival probabilities than those with low Ki-67 levels, particularly in ER-negative and triple-negative groups. This figure 4, shows the relationship between the extent of lymph node involvement (negative, 1-3 nodes, and >3 nodes) and Ki-67 expression levels. Higher Ki-67 expression is associated with increased lymph node involvement. Patients with 1-3 affected lymph nodes have a moderate level of Ki-67 expression, while those with >3 nodes show a marked increase in high Ki-67 levels. This relationship indicates that tumors with extensive lymph node involvement are more likely to have high proliferative activity, reinforcing the association between Ki-67 expression and tumor aggressiveness.

V. Conclusion

The analysis of Ki-67 expression in invasive breast cancer has demonstrated its significant value as a prognostic biomarker, especially when used alongside ER, PR, and HER2 receptor statuses. This study confirms that high Ki-67 expression is associated with more aggressive tumor characteristics, including larger tumor size, increased lymph node involvement, and advanced TNM stages. Additionally, high Ki-67 levels correlate with reduced overall and disease-free survival rates, particularly among ER-negative and triple-negative breast cancer subtypes, where it serves as an indicator of poor prognosis. These findings reinforce the potential of Ki-67 to guide treatment intensity, particularly in distinguishing between patients who may benefit from more aggressive therapies and those who might avoid overtreatment. By integrating Ki-67 assessment with ER, PR, and HER2 statuses, clinicians can develop a more comprehensive, personalized approach to breast cancer treatment, tailoring interventions to each patient's unique tumor profile. In conclusion, Ki-67 offers valuable prognostic information that complements traditional biomarkers, making it an essential component in the evolving landscape of personalized breast cancer care. Further standardization of Ki-67 scoring methods will enhance its clinical applicability, potentially leading to improved outcomes for patients with invasive breast cancer.

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